REVIEW

The double-edged role of neutrophil heterogeneity in inflammatory diseases and cancers

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Abstract

Neutrophils are important immune cells act as the body's first line of defense against infection and respond to diverse inflammatory cues. Many studies have demonstrated that neutrophils display plasticity in inflammatory diseases and cancers. Clarifying the role of neutrophil heterogeneity in inflammatory diseases and cancers will contribute to the development of novel treatment strategies. In this review, we have presented a review on the development of the understanding on neutrophil heterogeneity from the traditional perspective and a high-resolution viewpoint. A growing body of evidence has confirmed the double-edged role of neutrophils in inflammatory diseases and tumors. This may be due to a lack of precise understanding of the role of specific neutrophil subsets in the disease. Thus, elucidating specific neutrophil subsets involved in diseases would benefit the development of precision medicine. Thusly, we have summarized the relevance and actions of neutrophil heterogeneity in inflammatory diseases and cancers comprehensively. Meanwhile, we also discussed the potential intervention strategy for neutrophils. This review is intended to deepen our understanding of neutrophil heterogeneity in inflammatory diseases and cancers, while hold promise for precise treatment of neutrophil-related diseases.

KEYWORDS
cancers, inflammatory diseases, neutrophil extracellular traps, neutrophil heterogeneity, single-cell sequencing

1 | INTRODUCTION

Neutrophils are important immune cells, defending against microbial infections and eliminating pathogenic bacteria.[1]

#These authors contributed equally to this work.

Differentiated and developed in the bone marrow, neutrophils would release and migrate into the blood or tissues, accounting for approximately 50−70% of circulating leukocytes.[2] Various antimicrobial enzymes are found in neutrophils, including myeloperoxidase, acid hydrolytic enzymes, and elastase.[3] As a result of being stimulated
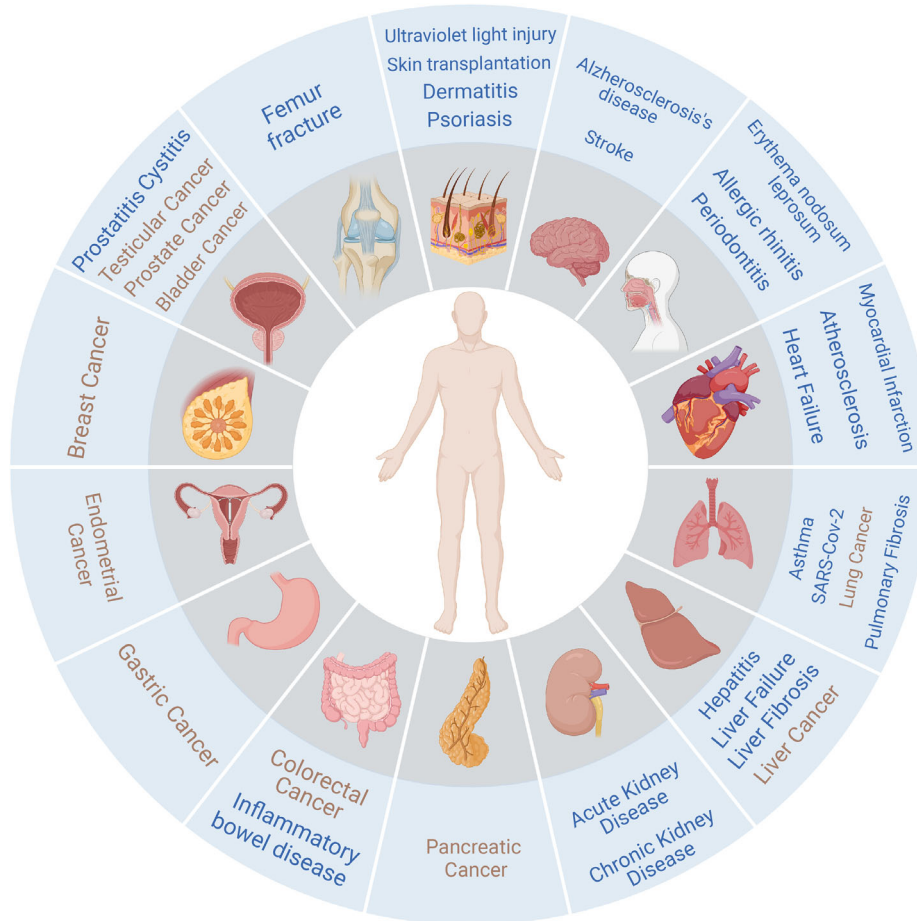
FIGURE 1 Schematic representation of the neutrophil heterogeneity in inflammatory diseases and cancers (in blue). This figure is created with BioRender.com.

by the products of pathogenic bacteria, neutrophils would produce antimicrobial enzymes and display their defensive capabilities. Aside from its important defensive role in combating infections, neutrophils could also trigger inflammatory responses at the site of infection, which in turn lead to immunopathological changes.[4,5] Therefore, it is important to clarify the double-edged role of neutrophils in the body.

Studies in mounting numbers have identified that phenotypically and functionally distinct neutrophil subtypes that exist in circulating or tissue neutrophils under physiological or pathological conditions.[6] In recent years, with the development of single-cell multiomics sequencing technology and mass cytometry, the accurate and unbiased classification of neutrophils has greatly facilitated our understanding of neutrophil heterogeneity.[7,8] A growing number of researchers are finding that the role of different neutrophil subsets in disease may be complex, as some subsets can contribute to the disease process while others would inhibit disease progression.[9] The dual role of neutrophil heterogeneity in inflammatory diseases and cancers has gained increasing attention.[10,11] It is hoped

that elucidating the role of neutrophil heterogeneity in diseases would lead to a new era of precision medicine in inflammation and cancer-related diseases.

Therefore, this review aims to highlight the double-edged role of neutrophils in diseases, systematically summarizing the important roles of neutrophil heterogeneity in inflammatory diseases and cancers. Meanwhile, the potential intervention strategy for neutrophils has been discussed. Overall, this review is intended to deepen our understanding of neutrophil heterogeneity in inflammatory diseases and cancers, holding promise for precise treatment of neutrophil-related diseases (Figure 1).

2 | THE FUNCTIONS AND THE DOUBLE-EDGED ROLE OF NEUTROPHILS

2.1 | Neutrophils in homeostasis

Neutrophils have long been considered as an important part of the innate immune system, playing a pivotal role in

controlling infectious diseases.[12] In physiological context, inactivated neutrophils move slowly in the circulation. The origin of neutrophils is derived from hematopoietic cords located at venous sinuses in the bone marrow and derive from a common committed myeloid progenitor cell.[1] The neutrophils development are stimulated by transcription factors, proteins, and receptors such as Egr1, HoxB7, S100A8, S100A9, N-formyl-methionyl-leucyl-phenylalanine receptor and granulocyte-macrophage colony stimulating factor receptor.[13–16] The neutrophil maturation process consists of the following steps: myeloblast→promyelocyte→myelocyte→metamyelocyte→band cell→polymorphonuclear segmented cell. In general, granulocyte colony stimulating factor (G-CSF) directs the commitment of progenitor cells to the myeloid lineage, promotes proliferation of neutrophil precursors, reduces the transit time through the compartment, and releases of mature neutrophils out of the bone marrow. Once maturation has been completed in the bone marrow and the pool of mature cells is released into the circulation, neutrophils circulate with a set of preformed adhesion and chemotactic receptors and effector proteins to rapidly migrate and respond to multiple microbial and sterile challenges.[17]

2.2 | Neutrophils in response to stimulation

Upon stimulation by external signals, these neutrophils move rapidly toward the injury site and effect in eliminating pathogenic bacteria and phagocytosis of injured cells.[18,19] Neutrophils and their derivatives, such as cytokines, reactive oxygen species (ROS), and neutrophils extracellular traps (NETs), are involved in both innate and adaptive immune responses, influencing the function of other immune cells in various ways and thus participate in the disease process.[18] Neutrophils and NETs could promote macrophage release cytokine while enhancing dendritic cells recruitment and antigen presentation.[20] Furthermore, by regulating cytokine and growth factor production, neutrophils would promote innate immune resolution and repair. In addition, neutrophils might trigger B cell expansion and antibody production.[21] Neutrophils and their derivatives could also affect T cell cytokine production and differentiation, thereby influencing T cell function.[22] During the process of infection or tissue damage, a diverse array of signals could mediate neutrophil activation and forward migration toward the site of damage. Many studies have found that blocked recruitment of neutrophils would lead to the spread of pathogens to the blood and vital organs, resulting in systemic infection and even death.[23–25] Important signals establishing chemoat-

tractant gradients that could take effect in neutrophil recruitment and migration include pathogen-associated molecular patterns and/or damage-associated molecular patterns (DAMPs), hydrogen peroxide (H_2O_2), fMet-Leu-Phe (fMLP), lipid mediators, chemokines, and so on.[4–6] As early signals, DAMPs have been reported to be responsible for early neutrophil recruitment. DAMPs could be sensed by pattern recognition receptors (PRRs), including Toll-like receptors (TLRs) and NOD-like receptors.[26,27] They would bind to and activate G-protein-coupled receptors (GPCRs) on neutrophils, thereby inducing resident cells to produce inflammatory mediators and to modulate neutrophil migration, function and behavior.[28] N-formyl peptides, such as fMLP, is another early signal which could trigger neutrophil chemotaxis and activation.[29–31] Derived from bacterial proteins or released from mitochondria after tissue damage, they would activate human neutrophils by binding to the GPCRs FPR1, FPR2, and FPR3. H_2O_2 could promote chemotaxis of human and mouse neutrophils.[32,33] At present, SRC family kinases (SFKs) have been reported to be a direct sensor to H_2O_2, required for neutrophils.[32] Interestingly, the peak of H_2O_2 production occurred around 30 min after injury, and the signal was completely abolished by myeloperoxidase activity of injury-associated neutrophils 1 h later.[34] Chemokines and lipid mediators usually cause long-term chemotactic signaling in order to facilitate more sustained recruitment and migration of neutrophils.[35–37] Many studies have reported the involvement of chemokines in neutrophil migration, including CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, and CXCL8.[38–40] These chemokines may be derived from immune and nonimmune cells during tissue infection and injury.[41,42] Neutrophils would sense these chemokines through the GPCRs, CXCR1, and CXCR2,[43–45] leading to downstream signaling activation of vasodilator-stimulated phosphoprotein, phosphoinositide 3-kinase, and SFKs, contributing to neutrophil-directed migration.[46,47] Lipid mediators, including leukotriene B4 (LTB4), 5-oxo-ETE, and 5-KETE, derived from arachidonic acid, could induce neutrophil chemotaxis.[48–50] During the process of chemotaxis, neutrophils must integrate multiple chemical signals, respond to physical constraints, and prioritize their directional decisions to generate an efficient immune response. A recent study reported that neutrophils were more likely to choose paths with the steepest chemoattractant gradient and the most direct approach angle, and the migration efficiency across planar chambers was inversely correlated with chamber diameter.[51] Many studies have focused on the effect of specific molecules on the migration efficiency of neutrophils. For example, G-protein-coupled receptor GPR35 was upregulated in activated neutrophils, and it promoted their migration.[52] Another study reported that CXCL1,

derived from endothelial cells, and CXCL2, which was derived from neutrophils, acted in a sequential manner to guide neutrophils through venular walls.[53] In addition, many other molecules, including filamin A, ICAM-1, IL-8, G protein signaling 5 (RGS5), Glia maturation factor-gamma, and MMP2, have also been reported to be involved in affecting the efficiency of neutrophil migrate.[54–58] At the site of damaged or infected tissue, neutrophils would fight against pathogens mainly through phagocytosis and production of ROS, bactericidal granulocytes, and NETs.[59] We would focus on the specific effects of neutrophils in the context of different diseases. After the acute inflammatory phase, neutrophil resolution has been shown to be critical in preventing tissue damage and transition to chronic wounds.[60] Neutrophil resolution would occur through neutrophil apoptosis or necrosis, with subsequent clearance by macrophages.[61] Interestingly, some studies have reported that neutrophils would leave the site of damaged or infected tissue in a process termed neutrophil reverse migration.[5,61] Wang et al.[62] reported that neutrophils can migrate from the site of inflammation to the blood vessels, and then subsequently return to the bone marrow to promote apoptosis. This process has been known as reverse migration and could also occur through reverse transendothelial migration.[63] Neutrophils could leave the wound in other ways as well, such as metastasis from adjacent tissues and lymphatic metastasis, and eventually spread to other parts of the body.[64] The reverse migration of neutrophils played a crucial role in accelerating the resolution of inflammation.[65] However, another study reported that the return of neutrophils from the wound site to the blood vessel might contribute to the spread of systemic inflammation.[66] This contradiction may be caused by different disease backgrounds. The possible mechanisms of neutrophil reverse migration involved adhesion molecule C (JAM-C)-mediated abnormal endothelial cell function, HIF signaling pathway mechanism, lipid mediating mechanism, and CXCL12/CXCR4 signal axis mechanism.[67–70] In addition, macrophages could also promote neutrophil reverse migration.[71] Moreover, a recent study highlighted the importance of vascular permeability in neutrophil reverse migration.[72] Several studies have described the important role of neutrophil swarming in diseases.[73,74] Neutrophil swarming is the formation of a population of neutrophils in infected tissues. The coordination of this population response is essential for the removal of bacteria.[75] As reported, a cell-intrinsic stop mechanism for the self-organization of collective behavior suggested the crucial role of GPCR desensitization in attenuating the self-organized swarming dynamics of neutrophils. In detail, when neutrophils sense high concentrations of swarm-secreted attractants (LTB4 and CXCL2), the GPCR kinase GRK2 desensitizes

the corresponding GPCRs to induce migration arrest.[73] This is a critical step for tissue repair after infection. To date, however, the exact mechanism of neutrophil reverse migration remains unclear. Further research is required to determine the effect of neutrophil reverse migration on different diseases and how to accurately intervene.

2.3 | The double-edged role of neutrophils

Neutrophils play a complex role in diseases, with the ability to both promote and inhibit disease progression. They can promote tumor growth and metastatic progression through the secretion of tumor-promoting cytokines, enhancement of tumor angiogenesis, modulation of the extracellular matrix, support of tumor cell dissemination, priming of the premetastatic niche, and suppression of antitumor immune responses.[76–78] However, neutrophils can also limit tumor growth and metastatic progression through direct cytotoxicity, antibody-dependent cell-mediated cytotoxicity, and inducing adaptive antitumor immunity.[79–82] The double-edged role of neutrophils in diseases, both promoting and suppressing diseases, has garnered attention from researchers, with most recent studies highlighting the existence of different subsets of neutrophils with varying functions in blood or tissues.[83,84] Neutrophils exhibit heterogeneity in both physiological and pathological conditions.[83] Specific signals at different developmental stages or disease backgrounds would drive neutrophil heterogeneity.[9] We will discuss this in detail in the context of different diseases. Clarification of neutrophil heterogeneity may help explain the dual role of neutrophils in diseases, and understanding the complicated role of neutrophils might benefit the development of treatment for a great many diseases. Therefore, in this review, we focus on how to analyze neutrophil heterogeneity and its double-sided role in the course of inflammatory diseases and tumors.

3 | METHODS OF STUDYING NEUTROPHIL HETEROGENEITY

The advent of high-resolution methods coupled with investigations of traditional methods in recent years has helped us to reveal the presence of heterogeneity in neutrophils, as presented in various phenotypes, under physiological and pathological contexts[83,85,86] (Figure 2). Neutrophils from different subpopulations function diversely in inflammatory diseases and cancers, which merits further exploration.[87–89]
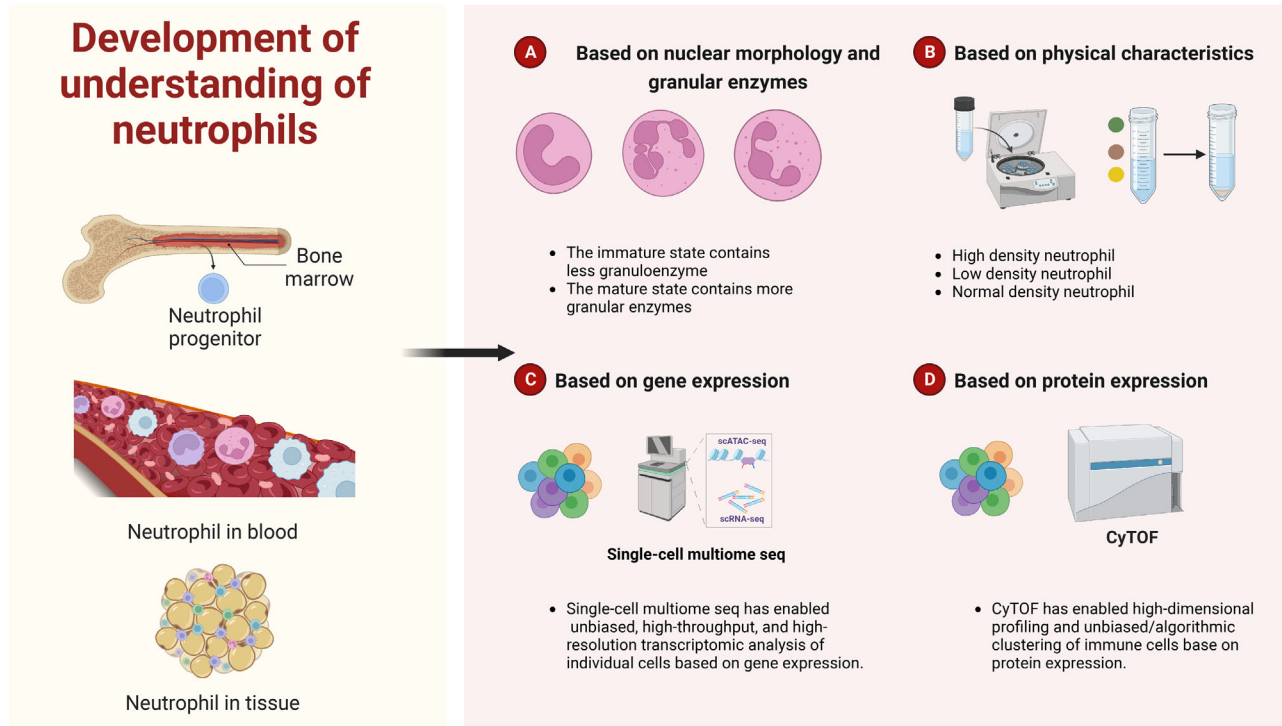
Development of understanding of neutrophils

Bone marrow

Neutrophil progenitor

Neutrophil in blood

Neutrophil in tissue

A Based on nuclear morphology and granular enzymes

- The immature state contains less granuloenzyme
- The mature state contains more granular enzymes

B Based on physical characteristics

- High density neutrophil
- Low density neutrophil
- Normal density neutrophil

C Based on gene expression

scATAC-seq

scRNA-seq

Single-cell multiome seq

- Single-cell multiome seq has enabled unbiased, high-throughput, and high-resolution transcriptomic analysis of individual cells based on gene expression.

D Based on protein expression

CyTOF

- CyTOF has enabled high-dimensional profiling and unbiased/algorithmic clustering of immune cells base on protein expression.

FIGURE 2 Development of understanding of neutrophils. This figure is created with BioRender.com.

3.1 | Studies on neutrophils by the traditional methods

Neutrophil heterogeneity has historically been defined as high- and low-density neutrophils (HDNs and LDNs) through its physical properties.[90–93] This easy classification of cells has resulted in the retention of this widely accepted definition, despite the lack of information at high resolution.[90,93] It is generally accepted that LDNs are immature neutrophils containing few granule enzymes, whereas HDNs are mature neutrophils with higher levels of granule enzymes.[10,83,94–96] However, complex transitions between these two subpopulations occur in pathological scenarios.[97,98] Neutrophils often undergo changes in their nucleus and surface marker genes as they mature and develop.[10,99] There are three neutrophil subsets in the mouse bone marrow, including a committed proliferative precursor terming preneutrophils, which would sequentially differentiate into nonproliferating immature and mature neutrophils. Preneutrophils express CD177, whereas differential expression of C-X-C chemokine receptor 4 (CXCR4), CXCR2 and CD101 allows for discrimination between immature neutrophils (which are CXCR2$^-$CD101$^-$) and mature neutrophils (which are CXCR2$^+$CD101$^+$).[99–101] As neutrophils mature, surface expression of markers gradually increases, such as CD62L, CD101, or glycosylphosphatidylinositol-linked receptor Ly6G in mice.[83,94] Physiologically, neutrophils

would release into the circulation and enter into tissues within a few days, often relying on specific adhesion molecules and regulated by circadian rhythm.[98,102–106] While under pathological conditions, neutrophils behave in a more complex manner. To be specific, neutrophils die in inflamed or damaged tissues and are even recruited back to the bone marrow by regulating the expression of related receptors.[62,107] Attempts have also been made to differentiate the functional differences of neutrophils in cancers according to their density.[96–98,108,109] Even so, this relies on non-high-resolution information, and it often appears to be inaccurate. As an example, some studies have demonstrated that immature LDNs have the ability to promote cancer progression or immunosuppression.[110–112] While some other studies have suggested that mature neutrophils may also possess these same properties.[97,98,113] Consequently, researches on neutrophil heterogeneity under non-high-resolution conditions have been of limited precision and have substantially limited our understanding.

3.2 | Studies on neutrophils by high-resolution methods

Recently, the development of novel technologies, including single-cell transcriptome sequencing, single-cell assay for transposase-accessible chromatin sequencing and mass cytometry, has greatly promoted the accurate and

unbiased classification of neutrophils, thus contributing to the advancement of neutrophil heterogeneity.[114–117] In humans, mice, non-human primates, and even other mammals, researchers have examined the heterogeneity of neutrophils derived from bone marrow in depth, laying the foundation for understanding how neutrophils develop.[101,118–122] Studies have revealed that in healthy individuals, 45−65% of circulating neutrophils are CD177^{+}, and about 20−25% of circulating neutrophils express the glycoprotein olfactomedin 4 (OLFM4).[10,123] The presence of other circulating neutrophil subpopulations, for example, those expressing the T cell receptor $\alpha\beta$ (TCR$\alpha\beta$)[+] or proangiogenic CD49d^{+}CXCR4^{+} vascular endothelial growth factor (VEGFR1)[+], is also observed in healthy individuals.[124,125] Further evidence has been provided that these circulating neutrophils presented in mice.[126] In addition, the proportion of CXCR4^{−}CD62L^{+} and CXCR4^{+}CD62L^{−} circulating neutrophils in healthy individuals followed the diurnal regime. The immature CD66b^{+}CD10^{−} neutrophils would generate antibacterial granules, ready to enter into the circulation in response to external signals.[127] These neutrophils, despite their "immature state," are capable of performing innate immune functions and display functional plasticity, as well as immunoregulatory properties.[128,129] Physiologically, heterogeneity of circulating neutrophils and neutrophils in tissue is more complex. A more comprehensive discussion would be conducted in the following context relating to multiple diseases. Researchers have developed computational methods to infer cell differentiation trajectories, however, experimental validation has yet to be achieved.[130,131] In addition, a further study of the heterogeneity of neutrophils across species is also worthwhile.

4 | NEUTROPHIL HETEROGENEITY IN DISEASES

4.1 | Neutrophil heterogeneity in inflammation-related diseases

In both acute and chronic inflammation, the genetic structures of neutrophils appear to undergo a reprogramming process due to the stimulation of external signals, resulting in dynamic transitions between subpopulations.[132–134] In inflammatory diseases, infection or ischemia can induce the production of G-CSF, GM-CSF, or other myelopoietic factors, which contribute to different functional neutrophils.[135–137] We will discuss what factors would contribute to neutrophil heterogeneity in the context of diseases. There has been evidence that neutrophil plasticity and heterogeneity are involved in the process of various inflammatory diseases, both physiologically

and pathologically.[60] In addition, neutrophils would not only present proinflammation activity via proinflammation neutrophils, neutrophil extracellular traps (NETs), and different neutrophil subsets linked with inflammation process, but also hold anti-inflammation capacities through various pathways, including anti-inflammation neutrophils, NETs, and neutrophil subsets that would inhibit inflammation process (Figure 3). Therefore, analyzing neutrophil heterogeneity is of great significance in the field of inflammation-related diseases, including liver diseases, kidney diseases, respiratory diseases, cardiovascular diseases, and inflammatory bowel diseases (IBDs).

4.1.1 | Liver diseases

Liver diseases, including alcoholic liver injury, nonalcoholic fatty liver disease, liver fibrosis, and liver failure, pose serious threats to human life. Neutrophils function vitally in the above-mentioned diseases.[138,139] The accumulation of neutrophils in liver tissue promotes liver injury has been reported by multiple research teams.[140–142] Senger and coworkers[143] found that blocking dipeptidase-1 on endothelial cells could reduce the recruitment of neutrophils to liver, improving inflammation in liver tissues. Thus, reducing the accumulation of neutrophils in liver tissues could alleviate liver injury, offering new hope for clinical treatment of liver injury. Hwang et al.[144] also reported that the overexpression of C-X-C motif chemokine ligand 1 (CXCL1) in mice liver drive steatosis-to-nonalcoholic steatohepatitis (NASH) progression through neutrophil-derived ROS and activation of stress kinases, and this process could be reversed by IL-22 treatment. Interestingly, another study showed that the absence of neutrophils contributes to resolution of liver inflammation, a process associated with neutrophil-induced proinflammatory macrophage function.[145] It is likely that neutrophils play different roles for they function distinctively in varied disease stages. HDNs and LDNs exist in liver tissues from clinical samples and alcoholic liver injury mice. Alcohol could induce HDNs to form NETs, thereby leading LDNs to reside in the liver and escape been cleaned-up by macrophages, causing chronic inflammation.[146] Kolodziejczyk et al.[147] reported the presence of two neutrophil subpopulations in the liver tissue of a mouse model of acute liver failure, among which CXCL2^{+} neutrophils may be involved in disease progression through their proinflammatory function. In addition, the progression of fibrosis in patients with chronic viral hepatitis has been shown to be associated with IL-17(+) neutrophils.[148] At present, the liaison between some neutrophil subpopulations and liver-related diseases has been analyzed. though, the causal relationship between these specific neutrophil

Pro-inflammation activities of neutrophils　Anti-inflammation capacities of neutrophils

(A) Pro-inflammation neutrophil

Induce pro-inflammatory macrophage function
Exhibit higher levels of Na+ influx and ROS
Lower levels of apoptosis
Stronger proinflammatory ability

(B) Neutrophil extracellular traps (NETs)

Induce tissue cell to
release TNF-α and IL-1β

(C) Different neutrophil subsets
associated with inflammation
process
RANKL1+ neutrophils;
CD49d+CysLTR1+ neutrophils;
CD64+ neutrophils etc.

(D) Anti-inflammation neutrophil

Phagocytic pathogen
Clear senescent cells

(E) Neutrophil extracellular traps (NETs)

Release neutrophil
elastase, myeloperoxidase

(F) Different neutrophil subsets
inhibit inflammation process

DEspR+CD11b+ neutrophils;
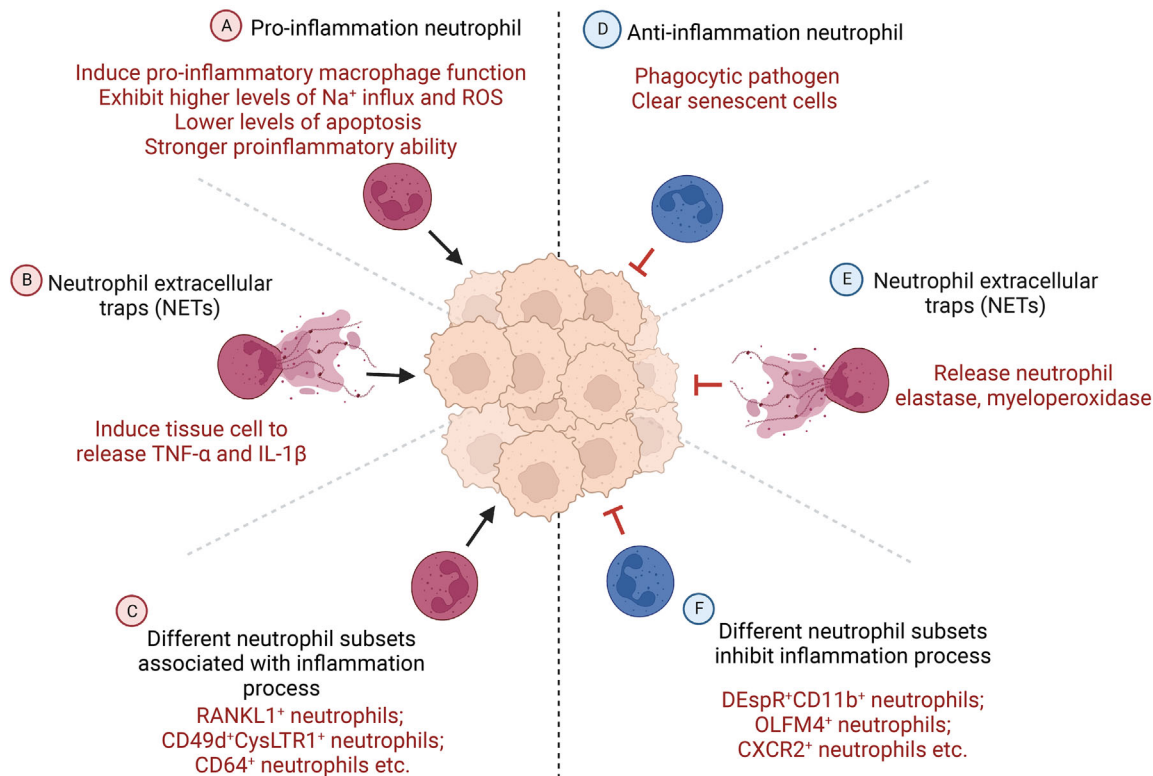OLFM4+ neutrophils;
CXCR2+ neutrophils etc.

FIGURE 3 Neutrophils show both proinflammation activity or anti-inflammation capacities. This figure is created with BioRender.com.

subpopulations and diseases remains unclear, warranting further investigation.

4.1.2 | Kidney diseases

Glomerulonephritis is a common concomitant of systemic lupus erythematosus (SLE).[149,150] The proportion of neutrophils peripheral blood neutrophils and in kidneys is significantly increased in SLE patients and mice.[151] Among them, LDNs exhibited higher activity.[152] Inhibition of infiltrating neutrophils in the kidney ameliorated lupus nephritis progression in mice.[152] Neutrophils also play an important role in other acute and chronic nephritis progression.[153,154] Neutrophil subpopulations differ functionally in various kidney diseases. To be specific, the presence of OLFM4+ neutrophils have a negative impact on sepsis-induced kidney injury, which is associated with renal cell apoptosis and increased plasma creatinine levels.[155] Skopelja-Gardner et al.[156] reported that acute skin exposure to ultraviolet light drives kidney injury via Ly6G+GFP+ICAM1hiCXCR1lo neutrophils. Another study showed an increase in the proportion of CD14−CD16−CD15+ neutrophils in the peripheral blood of patients with chronic nephritis, and the function of this subset of neutrophils is mainly related to vascular

calcification.[157] These findings have further confirmed the significance of neutrophil heterogeneity in nephritis.[89,158] Targeting neutrophil elastase has been proven to alleviate chronic nephritis.[159] Nevertheless, the deficiency of efficient drugs that target particular neutrophil subsets exists, requiring additional exploration. There are relatively few studies on neutrophil heterogeneity in renal tissues, and the lack of functional experimental studies on specific neutrophil subsets also warrant further investigation.

4.1.3 | Respiratory diseases

The essential role of neutrophils in diseases, including pneumonia, asthma, and chronic obstructive pulmonary disease (COPD), has gradually aroused researchers' attention.[160–162] During acute lung infection, neutrophils are the first to be recruited in large numbers to the infection site among all cell types, thereby participating in the initial clearance of infected cells. However, a recent study reported that impairing alveoli outnumber alveolar macrophages chemotaxis toward bacteria would induce superfluous neutrophil recruitment, leading to inappropriate inflammation and injury.[163] This illustrates the double-edged role of neutrophils in respiratory disease. An increasing number of studies in recent years have

established the importance of neutrophil heterogeneity in respiratory diseases.[164,165] Specifically, the phenotype of neutrophils alters as a disease progresses.[166] The number of CD177^{+} neutrophils, CD64^{+} neutrophils and OLFM4^{+} neutrophils is significantly increased in the peripheral blood of patients with sepsis and asthma, which has been confirmed by several studies.[167–171] The CD16^{high} CD62L^{dim} neutrophils were unveiled to enhance the response to bradykinin in both human isolated small airways and murine tracheae.[172] In hemorrhagic shock and sepsis mouse, inhibition of OLFM4 attenuated lung inflammation in mice.[155,173] Acute and chronic respiratory inflammation would mediate the emergence of neutrophil subsets.[174,175] Another study revealed the impaired activation and phagocytosis functions of CD123^{+} neutrophils and PD-L1^{+} neutrophils in the peripheral blood of sepsis patients. Subsets that highly express PD-L1 exerted an immunosuppressive effect, including the inhibition of T cell activation, induction of T cell apoptosis and trans-differentiation, while the number of CD123^{+} neutrophils correlated with the severity of the disease.[176,177] In a sepsis mouse model, extracellular cold-inducible RNA-binding protein (eCIRP) can induce the formation of the proinflammatory phenotype Ly6G^{+} CD11b^{hi} of LDNs through TLR4.[178] In a mice model of chronic granulomatous pneumonia, G-CSF mobilized immature CD101^{neg} neutrophil subsets released from the marrow with a proinflammatory phenotype.[179] From the single-cell scale, researchers have systematically described the heterogeneous population and transcriptome dynamics of neutrophils in the process of maturation, differentiation, and fate determination in both homeostatic and inflammatory states. In the inflammatory state, the heterogenous population of neutrophils did not change, while the proportion, functional characteristics, expression of the transcription factor, and the transformation pathway altered dramatically between populations. This provides basic information for the precise investigation of neutrophil heterogeneity.[180,181] It was reported that, compared with normal lung tissue, two distinct neutrophil subsets were generated in *Cryptococcus neoformans*-infected lung tissue, and these two subsets highly expressed inflammation-related genes and oxidative stress-related genes, respectively. Neutrophils highly expressing inflammation-related genes can modulate cell-cell communication with dendritic cells and alveolar macrophages.[182] CD49d^{+}CysLTR1^{+} neutrophils were present in the nasal lavage fluid from patients with viral respiratory tract infections.[183] A significantly increased amount of CD63^{+} neutrophils was detected in the blood of cystic fibrosis patients.[184] Both PD-L1^{hi} and PD-L1^{lo} neutrophils were present in sputum from cystic fibrosis patients, while in healthy control, only PD-L1[hi] neutrophils were present.[185] Moreover, RANKL1^{+} neu-

trophils were found specifically present in the peripheral blood of COPD patients.[186] The pivotal role of neutrophil heterogeneity in Coronavirus disease 2019 (COVID-19) has lately received increasing attention.[187–189] In a recent study, interferon (IFN)-stimulated gene (ISG) neutrophils were shown absent in the lung tissue of patients with severe COVID-19.[190,191] Immature and PD-L1^{+} neutrophils were, in contrast, significantly increased.[192] Other subpopulations, such as CXCR4^{high}/CD62L^{low} neutrophils and CD16^{dim}/CD62L^{bright} neutrophils, have also been reported to be involved in the pathogenesis of COVID-19, which can suppress T cell function.[113,193] The beneficial effects of dexamethasone during COVID-19 were mainly through the downregulation of IFN-responsive genes and activation of IL-1R2[+] neutrophils.[194] Unlike COVID-19 infection, the proportion of ICAM-1^{+} neutrophils in the peripheral blood of SARS-CoV-2-infected patients varied vastly.[195] This phenotype may be induced by CIRP.[196] Carstensen et al.[197] demonstrated that inhibition of the DEspR^{+}CD11b^{+} neutrophil subset could alleviate multiple respiratory diseases.[198] While targeting the intervention of CD11b^{+}CD18^{+} neutrophils would aggravate influenza-induced lung damage, the specific mechanism of which was related to affecting functions of T cells.[199] Some researchers have speculated that neutrophil differentiation in humans and mice are similar before and after lung inflammation through computational analysis.[200] This, to some extent, indicates that conclusions from mouse-based studies could provide support for clinical research. The progression of inflammatory diseases usually results from a combination of interactions between neutrophils and other immune cells. At present, the interactions between specific subsets of neutrophils and other immune cells are relatively rare, which is worthy of further investigation.

4.1.4 | Cardiovascular diseases

The study of neutrophils, especially neutrophil heterogeneity, is of great momentousness in the field of cardiovascular and cerebrovascular diseases.[201–203] The normal-density neutrophils (NDNs) and LDN subpopulations in the peripheral blood of patients with hypertension-associated chronic inflammation exhibited higher levels of Na^{+} influx and ROS, along with lower levels of apoptosis and stronger proinflammatory ability, thereby promoting the chronic inflammatory response associated with hypertension.[204] Some research teams identified that Ly6G^{+}SiglecF^{+} (Myc^{+}NFκB^{+}) neutrophils served as proinflammatory cells in the progression of myocardial infarction (MI).[205,206] The CD33^{high}CD16^{low} neutrophils were the main effector neutrophil subset in the peripheral blood of MI patients.[207] Interestingly, compared with

young male mice, reduced neutrophil proinflammatory gene expression were found in the infarct region of young females.[208] This suggested that sex differences existed in the function of neutrophils. The proportion of IFN-responsive/ISG-expressing neutrophils in the peripheral blood of MI patients was increased. With the help of experiments on mouse models, the researchers indicated that this group of neutrophils originated from the bone marrow and was negatively regulated by Nrf2 activation.[209] In the mouse model of heart failure, two neutrophil subpopulations with different expression patterns were found within the myocardial. Despite the significant increase in the proportion and both having proinflammatory function, the two populations have functional differences in antigen presentation.[210] Patients with ischemic stroke had higher percentages of the overactive senescent (CXCR4^{bright}/CD62L^{dim}) neutrophil subset and neutrophils with a reverse transendothelial migration (CD54^{high}CXCR1^{low}) phenotype in their peripheral blood than controls, and this was significantly correlated with disease progression.[211] Similarly, the ratio of CXCR4^{high}/CD62L^{low} neutrophils in the peripheral blood of Parkinson's patients could also reflect the disease process.[212] Neutrophil-specific deletion of Syk would improve cognitive dysfunction after traumatic injury to the brain.[213] Trivedi et al.[213] reported the existence of a proinflammatory neutrophil subset (antigen-presenting cell-like neutrophils) in both hyperlipidemic patients and atherosclerotic mice. This unique phenotype of neutrophils had the ability to activate the adaptive immune response, thereby promoting atherosclerosis progression. Moreover, a positive correlation between the presence of neutrophils and CD3$^+$ T cells was observed.[214] Despite the fact that many studies have confirmed the diversity of neutrophils in peripheral blood in cardiovascular and cerebrovascular diseases, there is insufficient evidence for the heterogeneity of neutrophils in situ in tissues, which may be attributed to the difficulty in obtaining clinical samples.

4.1.5 | Inflammatory bowel diseases

IBDs are chronic inflammatory conditions affecting the digestive system.[215] Abnormal function of neutrophils significantly affects the progression of IBDs.[216] Neutrophils and NETs play a pivotal role in IBD.[217] There was upregulation of NETs in the tissues of patients with ulcerative colitis (UC), and NETs could activate the UC lamina propria mononuclear cells to activate extracellular signal-regulated kinase-1/2 (ERK1/2), thereby enhancing TNF-α and interleukin-1β (IL-1β) production.[218] Neutrophils exhibit both cytotoxicity and the potential to cause severe tissue damage to the colon tissue. They

are also capable of defensive functions. NETs in UC tissues, for instance, have been shown to be advantageous in terms of immune thrombus formation and the prevention of colonic bleeding.[219] The remarkable effect of improving IBD by affecting neutrophil function has gradually attracted researchers' interest.[220] The artemisinin analogue SM934 or the Annickia polycarpa extract ameliorated UC in mice by inhibiting neutrophil activation or recruitment.[221,222] CD177$^+$ neutrophils played a role in protecting intestinal barrier function in IBD by increasing bactericidal activity and IL-22 production.[223,224] Therefore, targeting CD177$^+$ neutrophils may benefit the treatment of IBD. IL-17$^+$ neutrophils in the colons of mice with dextran sulfate sodium-induced colitis might contribute to the progression of the disease.[225] Levinsky et al.[226] demonstrated that OLFM4$^+$ neutrophils centrally participated in the pathologic pathway leading to intestinal ischemia/reperfusion injury in mice through experiments involving adoptive transfer of bone marrow. In the realm of IBD drug therapy, the existence of certain neutrophil subsets was also essential. Due to the presence of CXCR2$^+$ neutrophils, patients with UC often appeared insensitive to ustekinumab.[227] Thus, interventions targeting specific neutrophil subsets in colon tissue are expected to pave the way for precise treatment for IBD.

4.1.6 | Other inflammatory diseases

In addition to the above-mentioned prevalent acute and chronic inflammatory diseases mentioned above, neutrophil heterogeneity is also observed in other inflammatory diseases. Hypodense neutrophils and the ability to form NETs in peripheral blood and gastrointestinal tissues in patients with idiopathic inflammatory myopathy and vasculitis were greatly upregulated.[228,229] Neutrophil subpopulations also have fundamental effects on periodontal health regulation, as CD177$^+$ neutrophils were recruited to gingival crevicular fluid in periodontitis preferentially.[230–232] Some researchers have identified specific neutrophil subsets, such as CD14$^+$Ly6G^{low} neutrophils, that could secrete growth factors NGF and IGF-1, thereby promoting neuronal survival.[233,234] Ly-6G$^+$SiglecF$^+$ neutrophils specifically exist in the allergic mouse nasal mucosa, exhibiting an activated phenotype.[235] IL-10R1$^+$ neutrophils were found in the peripheral blood of patients with erythema nodosum leprosum. Senescent CD10^{neg}CD16^{low}CD11b^{low} and immature CD10^{neg}CD16^{neg}CD11b^{neg} neutrophils in psoriatic skin tissue induced more IL-17 expression.[236] Induction of CD49d$^+$ neutrophil production favors wound closure after skin transplantation.[237] CD11b^{high}/CD11a^{high} neutrophils specifically appeared in peripheral blood

Neutrophil Heterogeneity in Inflammatory Diseases

Respiratory diseases
Ly6G$^+$CD11b^{hi} neutrophils;
CD11b$^+$CD18$^+$ neutrophils;
CD16^{high}CD62L^{dim} neutrophils;
CD49d$^+$CysLTR1$^+$ neutrophils;
CXCR4^{high}/CD62L^{low} neutrophils etc.

Cardiovascular diseases
CD33^{high}CD16^{low} neutrophils;
CD54^{high}CXCR1^{low} neutrophils;
CXCR4^{bright}/CD62L^{dim} neutrophils;
CXCR4^{high}/CD62L^{low} neutrophils etc.

Liver diseases
Cxcl2$^+$ neutrophils;
IL-17$^+$ neutrophils.

Kidney diseases
CD14$^-$CD16$^-$CD15$^+$ neutrophils;
Ly6G$^+$GFP$^+$ICAM1^{hi}CXCR1^{lo} neutrophils;
OLFM4$^+$ neutrophils.

IBD
CD17$^+$ neutrophils;
CD177$^+$ neutrophils;
CXCR2$^+$ neutrophils;
OLFM4$^+$ neutrophils.

Other inflammatory diseases
CD49d$^+$ neutrophils;
CD177$^+$ neutrophils
CD14$^+$Ly6G^{low} neutrophils;
IL-10R1$^+$ neutrophils;
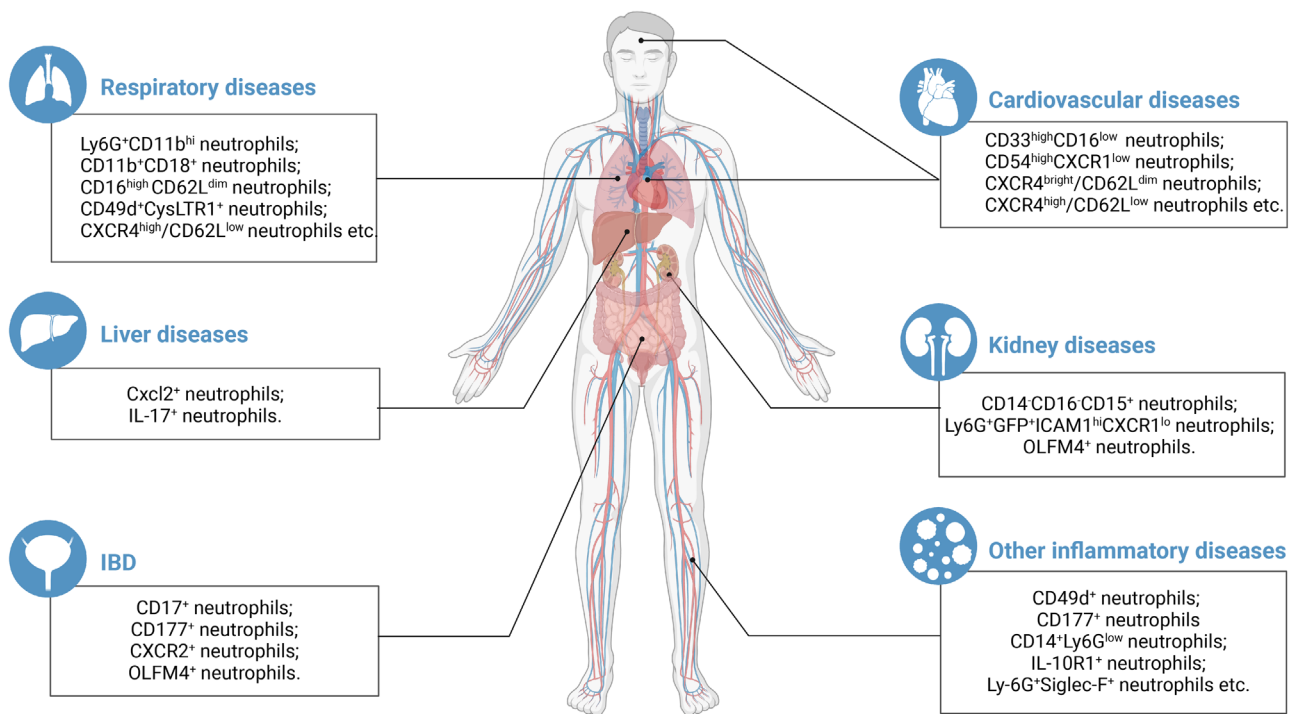Ly-6G$^+$Siglec-F$^+$ neutrophils etc.

FIGURE 4 Neutrophil heterogeneity in inflammatory diseases. This figure is created with BioRender.com.

in a mouse fracture model.[238] In addition, common IFN-responsive neutrophils played crucial roles in viral infection and other diseases. In summary, numerous studies have elucidated the essential function of neutrophil heterogeneity in both acute and chronic inflammatory diseases, including liver diseases, kidney diseases, respiratory diseases, cardiovascular and cerebrovascular diseases, and colon diseases[4,18,239] (Figure 4). However, there is currently limited research on the detailed functions of neutrophil subsets in disease development, especially the lack of functional verification of neutrophil subsets in vivo. This represents a potential avenue for future research (Table 1).

4.2 | Neutrophile heterogeneity in cancer-related diseases

Over years past, accumulating evidence has demonstrated that neutrophils perform dual roles, exhibiting both pro- and antitumor activity. Specific mechanisms are associated with tumor cell proliferation, apoptosis, and the immune microenvironment.[240,241] As study advances, researchers have shown that neutrophils undergo phenotype and functional remodulation under the influence of tumors and

their microenvironment, playing a complicated role in tumor development and metastasis.[242–244] Besides protumor activity through protumor neutrophils, NETs, and various subsets associated with cancer process, neutrophils also exert antitumor functions via antitumor neutrophils, other derivatives, and subsets inhibiting cancer process (Figure 5).

4.2.1 | Colorectal cancer

Colorectal cancer (CRC) is one of the most prevalent malignancies globally, accounting for 10% of all cancer cases. The incidence of CRC ranked third among malignant tumors, making it the third leading cause of cancer mortality.[245,246] In recent years, several studies have shown that the double role of neutrophils in inflammation-related diseases, particularly in the development and metastasis of tumors, has been gradually recognized and has garnered growing attention.[247,248] Neutrophils and NETs might promote CRC development and metastasis in part by the mediation of tumor metastasis by NETs via binding to CCDC25 or CEACAM1 on cancer cells.[247,249–252] Inhibition of NETs release attenuated colitis as well as colitis-associated tumorigenesis.[253,254]

TABLE 1 Neutrophil heterogeneity in inflammation-related diseases.

Diseases	Species	Samples	Neutrophil heterogeneity	Functions	References
Alcohol-associated hepatitis	Human/mice	Liver tissues	High-density neutrophils (HDNs); low-density neutrophils (LDNs)	HDNs contribute to NETs; LDNs lead to persistent inflammation	[146]
Acute liver failure	Mice	Liver tissues	$CXCL2^+$ neutrophils	Regulatory cell infiltration	[147]
Chronic viral hepatitis	Human	Liver tissues	$IL-17^+$ neutrophils	The number of cells increases significantly in the fibrotic stage	[148]
Sepsis-related kidney injury	Human/mice	Blood, kidney tissues	$OLFM4^+$ neutrophils	Juvenile OLFM4-null mice are protected from sepsis-related injury	[155,173]
Kidney inflammation	Mice	Kidney	$Ly6G^+GFP^+ICAM1^{hi}CXCR1^{lo}$ neutrophils	Promote inflammation	[156]
Chronic kidney disease	Human	Blood	$CD14^-CD16^-CD15^+$ neutrophils	Regulatory vascular calcification	[157]
Septic shock	Human	Blood	$OLFM4^+$ neutrophils	The number of cells increases significantly in patients	[167,171]
Septic shock	Human	Blood	$CD177^+$ neutrophils	The number of cells increases significantly in patients	[168,169]
Airway hyperreactivity	Human/mice	Blood	$CD16^{high}\ CD62L^{dim}$ neutrophils	To enhance the response to bradykinin in both human isolated small airways and murine tracheae	[172]
Sepsis	Mice	Bone marrow	$PD-L1^+$ neutrophils	Play an immunosuppressive role in infection	[176]
Sepsis	Human	Blood	$PD-L1^+$ neutrophils, $CD123^+$ neutrophils	The proportion of $CD123^+$ neutrophils correlated with clinical severity	[177]
Sepsis	Mice	Blood	$Ly6G^+CD11b^{hi}$ neutrophils	Promote inflammation	[178]
Chronic granulomatous disease	Mice	Bone marrow	$CD101^{neg}$ neutrophils	Trafficked to the lung and acquired a significantly more proinflammatory transcriptome	[179]
Bacterial infection	Mice; human	Spleen; blood	Interferon-stimulated gene (ISG) neutrophils; $Ly6G^{low}CXCR4^{hi}$ cells; $Ly6G^{hi}CXCR4^{hi}$ cells	The number of ISG neutrophils increases significantly in spleen of human and mice during infection; the number of $Ly6G^{low}CXCR4^{hi}$ cells and $Ly6G^{hi}CXCR4^{hi}$ cells increases significantly in blood and spleen during infection	[180]
SARS-CoV-2 infection	Non-human primates	Lung tissue, bronchoalveolar lavage fluid	$CD11b^+$ neutrophils	Reflect the severity of the disease	[181]
Cryptococcus neoformans (Cn) infection	Mice	Lung	Ox-PMN; Cyt-PMN	Ox-PMNs interact with the fungus and generate ROS; Cyt-PMNs respond to cytokines to modulate cell-cell communication with dendritic cells and alveolar macrophages	[182]
Acute onset of upper respiratory symptoms	Human	Nasal lavage	$CD49d^+CysLTR1^+$ neutrophils	The number of cells increases significantly in patients	[183]

(Continues)

TABLE 1 (Continued)

Diseases	Species	Samples	Neutrophil heterogeneity	Functions	References
Cystic fibrosis	Human	Blood	CD63$^+$ neutrophils	The number of cells increases significantly in patients	[184]
Cystic fibrosis	Human	Sputum	PD-L1^{high} neutrophils	T cell suppression	[185]
COPD	Human	Blood	RANKL1$^+$ neutrophils	Correlate with IL-1β, IL-6 and IL-8 in plasma in COPD	[186]
SARS-CoV-2 infection	Human	Bronchoalveolar lavage fluid	CD16^{Int} neutrophils	Promote inflammation	[187]
SARS-CoV-2 infection	Human	Blood	DEspR$^+$ neutrophils	Correlate with elevated circulating CCL23, increased NETosis, and the severity of the disease	[189,198]
SARS-CoV-2 infection	Human	Blood	ISG neutrophils	The number of cells decreases significantly in patients with severe disease	[190]
SARS-CoV-2 infection	Human	Blood	CD16^{Int} low-density neutrophils	Promote T cell proliferation	[191]
SARS-CoV-2 infection	Human	Blood	PD-L1$^+$ neutrophils	Immunosuppressive function	[192]
SARS-CoV-2 infection	Human	Blood	CXCR4^{high}/CD62L^{low} neutrophils	Promote inflammation	[193]
SARS-CoV-2 infection	Human	Blood	IL2$^+$ neutrophils	Anti-inflammation	[194]
SARS-CoV-2 infection	Human	Blood	ICAM-1$^+$ neutrophils	The number of cells increases significantly in patients	[195]
Acute lung injury	Human, rhesus macaque, rat	Bronchoalveolar lavage fluid	DEspR$^+$CD11b$^+$/CD66b$^+$ neutrophils	The number of cells increases significantly in disease	[197]
Severe influenza virus infection	Mice	Lung	CD11b$^+$CD18$^+$ neutrophils	Regulate T cell function	[199]
Hypertension	Human	Blood	Normal-density neutrophils (NDN), low-density neutrophils (LDN)	Promote inflammation	[204]
Myocardial infarction	Mice	Blood, bone marrow, heart	Ly6G$^+$SiglecF$^+$ neutrophils; ISG neutrophils	Promote inflammation; provide a clinical biomarker	[205,206,209]
Myocardial infarction	Human	Blood	CD33^{high}CD16^{low} neutrophils	An increase in the frequency of hyperactivated	[207]
Heart failure	Mice	Heart	CCR2$^+$ neutrophils, CXCR2$^+$ neutrophils	Promote inflammation; antigen presentation	[210]
Ischemic stroke	Human	Blood	CXCR4^{bright}/CD62L^{dim} neutrophils, CD54^{high}CXCR1^{low} neutrophils	Reflect the severity of the disease	[211]
Alzheimer's disease	Human	Blood	CXCR4^{high}/CD62L^{low} neutrophils	Reflect the severity of the disease	[212]
Atherosclerosis	Human	Blood	APC-like neutrophils	Activate the adaptive immune response to promote disease progression	[214]
IBD	Human	Blood, colon tissue	CD177$^+$ neutrophils	Play a protective role in IBD through increased bactericidal activity and IL-22 production	[223,224]
IBD	Mice	Colon tissue	CD17$^+$ neutrophils	Promote inflammation	[225]

(Continues)

TABLE 1 (Continued)

Diseases	Species	Samples	Neutrophil heterogeneity	Functions	References
Ischemia/ reperfusion injury (IR)	Mice	Colon tissue	OLFM4$^+$ neutrophils	Lead to intestinal damage and mortality after IR injury	[226]
Ulcerative colitis	Human	Colon tissue	CXCR2$^+$ neutrophils	Enrich in nonresponders to ustekinumab therapy	[227]
Periodontitis	Human	Blood	CD177$^+$ neutrophils	Preferentially recruited to the gingival crevice of periodontitis patients	[230]
Optic nerve and spinal cord injury	Mice	Optic nerve and spinal cord	CD14$^+$Ly6G^{low} neutrophils	Neuroprotective role by secreting NGF and IGF-1	[233,234]
Allergic rhinitis	Mice	Nasal mucosa	Ly-6G$^+$SiglecF$^+$ neutrophils	Exhibit an activated phenotype and enhance effector functions	[235]
Erythema nodosum leprosum	Human	Blood	IL-10R1$^+$ neutrophils	Release inflammatory cytokines	[166]
Psoriasis	Human	Skin tissue	CD10^{neg}CD16^{low}CD11b^{low} neutrophils; CD10^{neg}CD16^{neg}CD11b^{neg} neutrophils	Induce IL-17 and IFN-γ production by T cells	[236]
Wound closure after skin transplantation	Mice	Blood; Skin tissue	CD49d$^+$ neutrophils	Play a role in local vessel remodeling during inflammation	[237]
Femur fracture	Rat	Blood	CD11b^{high}/CD11a^{high} neutrophils	The number of cells increases significantly in diseases.	[238]

The dual role of neutrophils in diseases ought to get sufficient consideration. On the one hand, as innate immune phagocytes, neutrophils function pivotally in immune defense. On the other hand, specific neutrophil subpopulations would promote disease progression. Extensive and comprehensive research of the functional heterogeneity of neutrophils is envisaged to enable precision intervention on neutrophil subpopulations and achieve precise treatment of inflammation-related diseases.
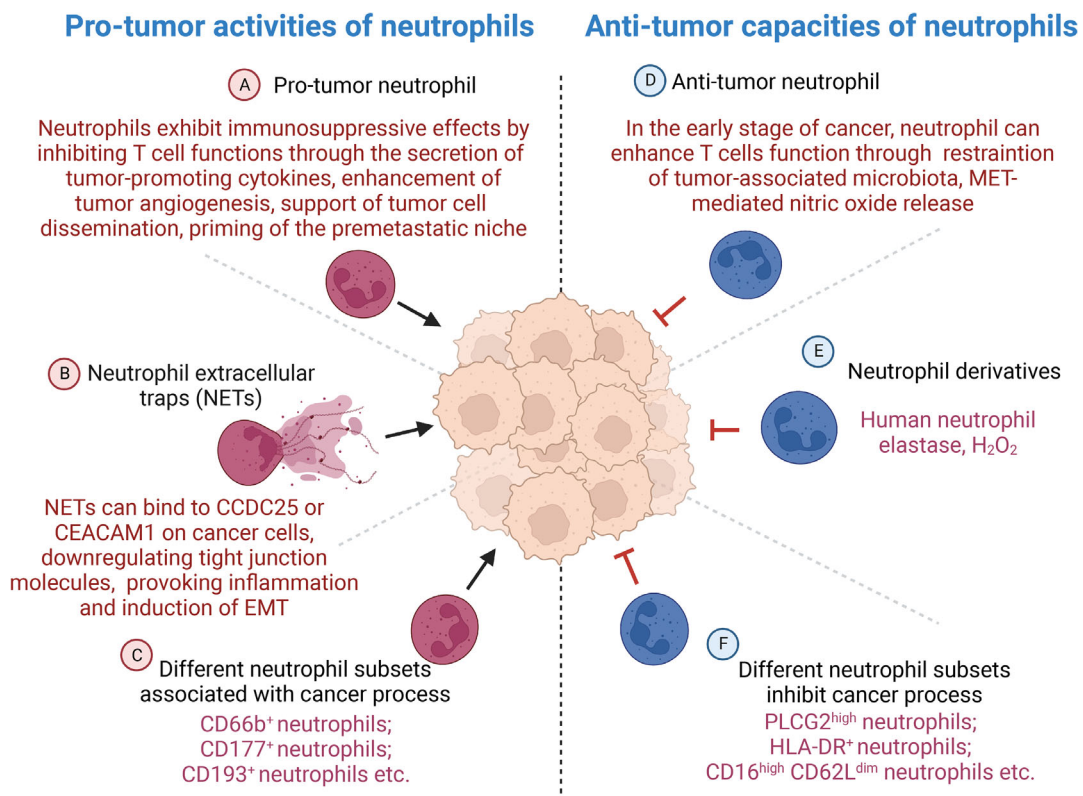

Pro-tumor activities of neutrophils

Ⓐ Pro-tumor neutrophil

Neutrophils exhibit immunosuppressive effects by inhibiting T cell functions through the secretion of tumor-promoting cytokines, enhancement of tumor angiogenesis, support of tumor cell dissemination, priming of the premetastatic niche

Ⓑ Neutrophil extracellular traps (NETs)

NETs can bind to CCDC25 or CEACAM1 on cancer cells, downregulating tight junction molecules, provoking inflammation and induction of EMT

Ⓒ Different neutrophil subsets associated with cancer process
CD66b$^+$ neutrophils; CD177$^+$ neutrophils; CD193$^+$ neutrophils etc.

Anti-tumor capacities of neutrophils

Ⓓ Anti-tumor neutrophil

In the early stage of cancer, neutrophil can enhance T cells function through restraintion of tumor-associated microbiota, MET-mediated nitric oxide release

Ⓔ Neutrophil derivatives
Human neutrophil elastase, H$_2$O$_2$

Ⓕ Different neutrophil subsets inhibit cancer process
PLCG2^{high} neutrophils; HLA-DR$^+$ neutrophils; CD16^{high} CD62L^{dim} neutrophils etc.

FIGURE 5 Neutrophils show both protumor activity or antitumor capacities. This figure is created with BioRender.com.

In addition, neutrophils seemed to be protective against the advancement of CRC. Neutrophil elastase has been proven to be inhibitory effective against 35 kinds of cancer cells, including CRC cells.[255] Neutrophils restrained tumor-associated microbiota and decreased colon tumor development and invasion in an inflammatory-induced CRC mice model, while neutrophil depletion would in contrast made tumors more aggressive.[256] The role of neutrophils in preventing bacterial invasion may be mediated by IL-1β.[257] These findings indicated the intricacy of tumor-associated neutrophil (TAN) involvement in CRC progression. What is more, neutrophils from CRC patients could also exhibit immunosuppressive effects by inhibiting T cell functions through TGFβ.[258] CD177^{+} neutrophils and CD66b^{+} neutrophils suppress epithelial cell tumorigenesis in colitis-associated cancer and became a predictor for a better prognosis in CRC.[259,260] In the early stages of CRC, the number of CD66b^{+} neutrophils were often associated with a favorable prognostic factor. Specific inductions would stimulate the generation of particular neutrophil subsets.[261] For instance, the addition of IL-21 into bone marrow cell culture increased the amount of CD193^{+} neutrophils, which migrated readily into the duodenum.[262] Moreover, in a genetic CRC model, suppression of neutrophil-dependent angiogenesis abrogated resistance to anti-VEGF antibody.[263] Taken together, these studies suggested that targeting neutrophils is of great significance for CRC treatment.

4.2.2 | Hepatocellular carcinoma

The important role of neutrophils in liver cancer has gradually attracted accelerated interest in past few years.[264–267] According to the present studies, NETs could promote the development from NASH to hepatocellular carcinoma (HCC).[268] Besides, NETs promoted HCC metastasis by downregulating tight junction molecules on adjacent endothelial cells or provoking inflammation.[269,270] Neutrophils, especially type-2 neutrophils (N2), could mediate the formation of an immunosuppressive microenvironment in liver tissue, and neutrophil clearance in mouse models would impede tumor progression.[271–273] The production of neutrophils for specific functions is usually influenced by a variety of factors. For instance, in several models of liver autoimmunity, pharmacologically induced autoantigen-specific T regulatory type-1 (TR1) cells and TR1-cell-induced B regulatory (Breg) cells used five immunoregulatory cytokines to coordinately recruit neutrophils into the liver and program their transcriptome to generate regulatory neutrophils.[272] Additionally, PD-L1[+] neutrophils have been shown to promote the growth of tumor cells in tissues from HCC patients by inhibiting the proliferation and activation of T cells.[274] The generation of PD-L1[+] neutrophils was likely attributed to the induction of cancer-associated fibroblasts through the IL6-STAT3 pathway.[275] Another study also confirmed that, in addition to PD-L1[+] neutrophils, both CCL4^{+} TANs presented immunosuppressive activity.[276] Neutrophils also have a significant impact on the treatment of HCC.[277] Tumor-derived lactate would inhibit the efficacy of Lenvatinib in HCC by modulating PD-L1 expression on neutrophils.[278] The mechanism of action of cabozantinib combined with PD-1 antibody to enhance antitumor immunity through neutrophil-based immune responses favored this approach for HCC therapy.[279] Furthermore, in HCC immunotherapy, PD-L1[+] neutrophils would be directly activated by IFN-γ and subsequently prompting inflammatory response.[280] Therefore, precise targeting of specific neutrophil subsets might reduce the occurrence of immune-related adverse events to a significant extent. Although the tumor-killing effect of neutrophils has been reported in other tumors, it has not been reported in liver cancer, which may be a future research direction.

4.2.3 | Gastric cancer

With high morbidity and mortality, gastric cancer (GC) is a ubiquitous malignancy worldwide.[281,282] Heretofore, surgical treatment remains the first-line strategy to providing a cure. It is expected that analyzing the molecular events taken place during GC progression would cast new light on GC treatment.[283] Tumor-derived exosomes could induce neutrophil activation through the HMGB1/TLR4/NF-κB signaling pathway during GC progression.[284] As well, neutrophils were able to promote GC progression by inducing Th17 cell polarization or inhibiting CD8^{+} T cell function.[285,286] Xia et al.[287] reported that NETs could promote the metastasis of postoperative abdominal infectious complications in GC patients. Induction of epithelial–mesenchymal transition (EMT) has been verified to be another way for NETs to promote GC metastasis.[288,289] Five subpopulations of neutrophils were identified in tissues from GC patients. Among them, CXCR4^{+} neutrophils have proangiogenic and proinvasive properties; while PLCG2^{high} neutrophils can inhibit the invasion and migration of tumor cells.[290] Through single-cell RNA sequencing, researchers from another team mapped the lymph node metastasis landscape of GC and found that lipocalin 2 (LCN2)[+] neutrophils served as a promotor in lymph node metastasis. This suggested that neutrophil subsets might drive metastatic in specific tumor sites.[291] The number of PD-L1^{+} neutrophils and CD66b^{+} neutrophils are associated with the survival of GC patients.[292–294] Interestingly,

the number of FasL$^+$PD-L2$^+$ neutrophils was reported to be associated with poor prognosis of GC in another study. These neutrophils owned immunosuppressive functions on tumor-specific CD8$^+$ T cells and promoted the growth and progression of human GC tumors both in vitro and in vivo. Targeting the removal of FasL$^+$PD-L2$^+$ neutrophils could hinder GC progression.[295] The advancement of medical therapy for GC has been quite sluggish thus far. Consequently, the development of targeted drugs for the heterogeneity of neutrophil function is anticipated to yield novel insights for the therapeutic treatment of GC.

4.2.4 | Lung cancer

Lung cancer is among the world's most prevalent malignant tumors. The incidence and mortality rate have grown dramatically over the past 50 years, and males were reported to have the highest morbidity and mortality of all malignant tumors.[296,297] The proportion of neutrophils in lung cancer patients' tissue or peripheral blood can be utilized to predict the prognosis of lung cancer.[298,299] Studies have demonstrated that neutrophils and NETs they generate play a critical role in fostering lung tumor development and metastasis.[300–302] Among these, senescent TANs (CXCR4$^+$CD62L^{low}) are more likely to form NETs and facilitate metastasis.[303] It has been reported by Tyagi et al.[304] that N2 neutrophils could release LCN2 which would in turn facilitate lung cancer metastasis. The heterogeneity of neutrophils in the peripheral blood of non-small cell lung cancer patients were greater, and the proportion of LDNs was remarkably upregulated.[305,306] Faget et al.[307] reported that Gr1$^+$ neutrophils could favor tumor progression by altering angiogenesis, leading to hypoxia, and sustaining Snail expression. Through MET-mediated nitric oxide release, neutrophils can also abate tumor growth and metastasis.[79] In recent years, a group of TANs with high expression of SiglecF (sialic acid-binding immunoglobulin-like lectin F) has gradually attracted researchers' attention. SiglecF^{high} neutrophils could selectively upregulate the expression of genes associated with tumor-processes, including angiogenesis, myeloid cell differentiation and recruitment, extracellular matrix remodeling, suppression of T cell responses, and tumor cell proliferation and growth.[308] In the early stages of lung cancer, TANs could enhance the T cell activity to display an antitumor effect.[309] This unique neutrophil population was more mature with a longer lifespan.[98,106,111] Its function in promoting lung cancer, however, was dependent on the number of osteoblastic cells, suggesting that the function of SiglecF^{high} neutrophils was infected by the ratio of cell types.[308] Interestingly, another study on mouse lung cancer showed that, similar to SiglecF^{high}

neutrophils, neutrophils with Glut1 high expression could live longer in the tumor microenvironment, and could promote the high expression of SiglecF and MMP9, facilitating tumor progression.[310,311] However, whether Glut1^{high} neutrophils and SiglecF^{high} neutrophils are the same population of neutrophils remains unclear. Another research team identified a group of ISG neutrophils in mouse lung tissue, and the number of ISG neutrophils was significantly reduced in tumor tissue.[312] A subset of tumor-associated HLA-DR$^+$ neutrophils was also identified in early-stage human lung cancer tumor tissue, which can cross-present antigens to CD8$^+$ T cells, triggering antitumor T cell immune responses.[313] A group of CD66b$^+$/CD10^{low}/CXCR4$^+$/PDL1^{inter} neutrophils were identified in the peripheral blood of advanced lung cancer patients, the existence of which was limited in the advanced stage of lung cancer.[90] At present era, neutrophils have promising potential as effector cells in the treatment of lung cancer. By modifying neutrophil infiltration, for instance, the CXCR2 selective inhibitor SB225002 can boost the therapeutic effect of cisplatin in the treatment of lung cancer.[314] To sum up, neutrophil heterogeneity is extremely important in the prognosis, progression, and treatment of lung cancer. Unfortunately, studies of neutrophil heterogeneity in specific types of lung cancer are limited. For example, it has been reported that neutrophil to lymphocyte ratio predicts the prognosis of extensive small-cell lung cancer.[315] However, there are few studies on neutrophil heterogeneity in small cell lung cancer.

4.2.5 | Breast cancer

Breast cancer is the most common malignancy in women and one of the three most common cancers worldwide, along with lung and colon cancer.[316] Numerous studies have demonstrated that neutrophils could promote the growth and metastasis of breast cancer via the secretion of cytokines and other pathways,[317–319] as well as facilitate breast cancer metastasis by altering other cell functions, such as inhibiting the antimetastasis function of NK cells or mediating the immunosuppressive microenvironment.[320,321] Tumor-derived factors such as G-CSF and stem cell factor could in turn responsible for activity of immunosuppressive neutrophils, thereby contributing to the advancement of breast cancer.[109,322] Interestingly, during the premetastatic stage of breast cancer, tumor-entrained neutrophils in the lung aggregated and, by generating H$_2$O$_2$, might limit metastatic seeding in the lungs.[80] This partly explains the functional differences of neutrophils at the primary site and the metastatic site. Neutrophils could also play a protumor

role by affecting the lipid metabolism of tumor cells. In the context of a low glucose supply in the tumor microenvironment of 4T1 tumor-bearing mice, neutrophils would engage in fatty acid oxidation, supporting ROS production and amplifying T cell suppression.[109] In addition, a separate study reported that lipids stored within lung neutrophils can be transported to metastatic tumor cells through a macropinocytosis–lysosome pathway, endowing tumor cells with augmented survival and proliferative capacities.[301] Already published is the discovery that neutrophils could exert a mode of destruction of breast cancer cells has already been reported, and the specific mode was related to the regulation function of neutrophils toward CD47-SIRPα checkpoint through the trogoptosis mechanism.[323,324] It was identified by Veillette et al.[324] that C5aR1$^+$ neutrophils would predict worse prognosis of BC patients, which could mechanistically be explained by the ability of C5aR1$^+$ neutrophils in inducing breast cancer glycolysis via increasing ERK1/2-WTAP-dependent m6A methylation of ENO1.[325] Moreover, Coffelt et al.[326] reported that in mammary tumor-bearing $K14^{cre};Cdh1^{F/F};Trp53^{F/F}$ (KEP) mice, the tumor microenvironment favors a unique population of metastasis-inducing neutrophils with upregulation of genes including $cKit$, $Nos2$, $Prok2$, $S100a8$, and $S100a9$. Using a panel of 16 distinct genetically engineered mouse models for breast cancer, another analysis also identified the significant enrichment of cKIT$^+$ neutrophils was more likely to potentiates metastatic progression.[327] In previous studies, neutrophil Cathepsin G was found to interact with RAGE (receptor for advanced glycation end products), displaying its antitumor activity.[328] By releasing metastasis-promoting factors including forming NETs, CXCR4^{hi}CD62L^{lo} aged neutrophils could enhance metastasis of breast cancer cells to the liver or lung.[303,329] In summary, neutrophils play a dual role in breast cancer progression. The distinction of which may be attributed to the various phases of the disease and the heterogeneity of neutrophils. which merit additional investigation.

4.2.6 | Pancreatic cancer

Pancreatic cancer is one of the most aggressive tumors of the digestive system. The mortality rate is rising annually due to the its stealthy onset, fast development, and ineffective treatment.[330,331] Multiple research teams have proven that neutrophils and NETs may greatly enhance the growth and metastasis of pancreatic cancer, and that inhibiting NETs can somewhat delay pancreatic cancer metastasis.[332–334] In an orthotopic pancreatic cancer mouse model, the presence of neutrophils was shown to be associated with functional suppression of the CD8$^+$

T cells.[334] In the pancreatic tumor microenvironment, TAN subsets with various functions exist, among which BHLHE40$^+$ neutrophils have protumor and immunosuppression functions.[335] A recent research revealed that P2RX1^{neg} neutrophils were highly immunosuppressive, hence provoking pancreatic cancer liver metastasis.[335] CD13^{hi} neutrophil-like myeloid-derived suppressor cells exert immune suppression in pancreatic ductal adenocarcinoma by influencing the expression of Arginase 1.[336] Targeting neutrophils has also showed encouraging effectiveness in the treatment of pancreatic cancer.[337] Lorlatinib attenuates pancreatic cancer growth by suppressing TAN amount and functions.[338] The therapeutic effect of nivolumab in pancreatic cancer was in association with CD11b$^+$ neutrophil degranulation.[339] Steele et al.[340] revealed that mediating the expression of CXCR2 on neutrophils could improve the efficacy of PD-1 antibodies in a pancreatic cancer mouse model. This may be due to the fact that high expression of CXCR2 on neutrophils would mediate immunosuppressive microenvironment, effecting T cell entry.[340,341] Targeting tumor-associated CXCR2$^+$ neutrophils and CCR2$^+$ macrophages was reported to improve chemotherapeutic responses in pancreatic ductal adenocarcinoma.[342] In a nutshell, a series of studies have shown that it is necessary to investigate neutrophil heterogeneity in the context of pancreatic cancer treatment.[266–343] For subsequent investigation, the inductive factors of the emergence of diverse neutrophil subsets may be probed, enabling a brand-new viewpoint for the treatment of pancreatic cancer.

4.2.7 | Other cancers

Researchers are also intrigued by the role of neutrophil heterogeneity in other cancers. Neutrophils could regulate the function of CD47-SIRPα checkpoint through trogoptosis, presenting tumor-suppressive property in acute promyelocytic leukemia.[344] Meanwhile, combination of SIRPα antibody with CD70 antibody could, to some extent, enhance the antitumor effect of neutrophils.[345] Whether this process is dependent on neutrophils remain obscure. In addition, neutrophils also presented tumoricidal effect in neuroblastoma and endometrial cancer, the mechanism of which might be correlated with the induction of MMP9 expression and ROS generation.[346–348] The ROS-dependent antitumor effect of neutrophils has also been demonstrated in a mesothelioma mouse model.[349] However, another study showed that CXCR2$^+$CD11b$^+$Ly6G^{hi} myeloid-derived suppressor cells (mainly expressing neutrophil marker genes) affected the efficacy of PD-1 antibodies in rhabdomyosarcoma mice.[350] Three neutrophil subsets were identified in head and neck squamous
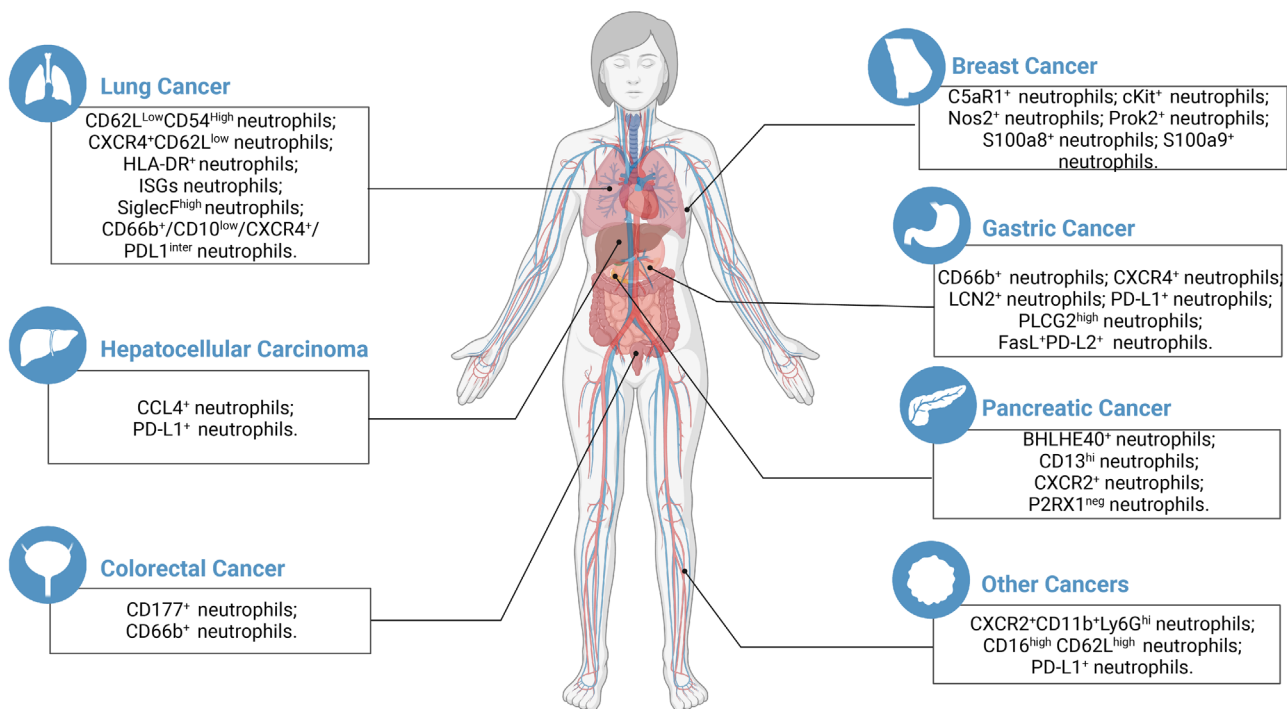
Neutrophil Heterogeneity in Cancers

Lung Cancer
CD62L^{Low}CD54^{High} neutrophils;
CXCR4$^+$CD62L^{low} neutrophils;
HLA-DR$^+$ neutrophils;
ISGs neutrophils;
SiglecF^{high} neutrophils;
CD66b$^+$/CD10^{low}/CXCR4$^+$/
PDL1^{inter} neutrophils.

Hepatocellular Carcinoma
CCL4$^+$ neutrophils;
PD-L1$^+$ neutrophils.

Colorectal Cancer
CD177$^+$ neutrophils;
CD66b$^+$ neutrophils.

Breast Cancer
C5aR1$^+$ neutrophils; cKit$^+$ neutrophils;
Nos2$^+$ neutrophils; Prok2$^+$ neutrophils;
S100a8$^+$ neutrophils; S100a9$^+$
neutrophils.

Gastric Cancer
CD66b$^+$ neutrophils; CXCR4$^+$ neutrophils;
LCN2$^+$ neutrophils; PD-L1$^+$ neutrophils;
PLCG2^{high} neutrophils;
FasL$^+$PD-L2$^+$ neutrophils.

Pancreatic Cancer
BHLHE40$^+$ neutrophils;
CD13^{hi} neutrophils;
CXCR2$^+$ neutrophils;
P2RX1^{neg} neutrophils.

Other Cancers
CXCR2$^+$CD11b$^+$Ly6G^{hi} neutrophils;
CD16^{high} CD62L^{high} neutrophils;
PD-L1$^+$ neutrophils.

FIGURE 6 Neutrophil heterogeneity in cancers. This figure is created with BioRender.com.

cell carcinoma (HNSCC) patients: CD16^{dim} CD62L^{high}, CD16^{high} CD62L^{high} and CD16^{high} CD62L^{dim} neutrophils. Among them, CD16^{high} CD62L^{dim} neutrophils could inhibit the migration and proliferation of HNSCC cells, as well as inducing cell apoptosis.[351] Another study showed that immunosuppressive neutrophil subsets, such as CD16^{high} CD62L^{high} neutrophils, might be induced by tumor-produced TGF-β/IL-10.[352] Six phenotypically distinct human blood neutrophil populations were identified. The proportion and function of neutrophil subsets were shown to be correlated with melanoma stage, and their changes would favor the differentiation of different melanoma stages.[353,354] The profound study of neutrophils has also given hope for the treatment of melanoma. A recent study showed that neutrophils, especially TAN1, were isolated from mice underwent innate immune training by exposure to β-glucan hold a stronger antitumor effect.[355] The activity of alemtuzumab against B-cell chronic lymphocytic leukemia was reported to be dependent on neutrophil-mediated cytotoxicity.[356] Combination of CTLA-4 and PD-L1 blockade in patients with chemotherapy-naive metastatic castration-resistant prostate cancer was shown to be associated with the modulation of neutrophil subsets in the bone microenvironment.[357] Collectively, neutrophil het-

erogeneity has been observed in a variety of cancers (Figure 6). The subsets of neutrophils, however, seem to vary from tissue to tissue, requiring researchers to follow up with investigations in specific tumors (Table 2).

5 | OUTLOOK

In this review, we have provided a comprehensive summary of the significance of neutrophil heterogeneity in inflammatory and tumor-associated diseases. The presence of neutrophils with different phenotypes in blood, peripheral blood, or tissue has been confirmed by conventional or high-resolution methods. However, two major challenges regarding neutrophil heterogeneity exist currently. One is to understand the underlying causes of neutrophil heterogeneity, while the other is how neutrophil subsets are defined. There are two potential mechanisms for neutrophil heterogeneity. The first is the intrinsic heterogeneity of neutrophils present in the bone marrow and blood. The second involves exposure to extrinsic factors, either locally or systemically, that modify neutrophil properties.[83] It is noteworthy that the factors inducing neutrophil heterogeneity vary between different diseases, as previously discussed in the context of inflammatory

TABLE 2 Neutrophil heterogeneity in cancer-related diseases.

Diseases	Species	Samples	Neutrophil heterogeneity	Functions	References
Colorectal cancer	Human	Colon tissues	CD177$^+$ neutrophils	Suppress epithelial cell tumorigenesis	[262]
Colorectal cancer	Human	Colon tissues	CD66b$^+$ neutrophils	A favorable prognostic factor in early stages of colon cancer	[263]
Hepatocellular carcinoma	Mice	Liver tissues	N2 neutrophils	Immunosuppressive function	[275]
Hepatocellular carcinoma	Human, mice	Liver tissues	PD-L1$^+$ neutrophils	Immunosuppressive function	[277,278]
Hepatocellular carcinoma	Human, mice	Liver tissues	CCL4$^+$ neutrophils	Recruit macrophages	[279]
Hepatocellular carcinoma	Mice	Liver tissues	PD-L1$^+$ neutrophils	Promote inflammatory response	[283]
Gastric cancer	Human	Gastric tissues	CXCR4$^+$ neutrophils; PLCG2^{high} neutrophils	Proangiogenesis; inhibit tumor cell invasion and migration	[293]
Gastric cancer	Human	Gastric tissues; metastatic lymph nodes	LCN2$^+$ neutrophils	Contribute to lymph node metastasis	[294]
Gastric cancer	Human	Gastric tissues	PD-L1$^+$ neutrophils	Associated with disease progression and reduced GC patient survival	[295]
Gastric cancer	Human	Gastric tissues	CD66b$^+$ neutrophils	Predict the poor disease special survival	[296,297]
Gastric cancer	Human	Gastric tissues	FasL$^+$PD-L2$^+$ neutrophils	Immunosuppressive function	[298]
Breast cancer lung metastasis	Mice; Human	Lung tissues	CXCR4$^+$CD62L^{low} neutrophils	Contribute to breast cancer lung and liver metastasis	[306,331]
Breast cancer lung metastasis	Mice; Human	Lung tissues	N2 neutrophils	Contribute to breast cancer lung metastasis	[307]
Lung cancer	Human	Blood	Low-density neutrophils	The number of cells increases significantly in patients	[79]
Lung cancer	Mice	Lung tissues	Gr1$^+$ neutrophils	Immunosuppressive function	[309]
Lung cancer	Human	Lung tissues	CD62L^{Low}CD54^{High} neutrophils	Release inflammatory cytokines	[312]
Lung cancer	Human	Lung tissues	SiglecF^{high} neutrophils	Promote tumor growth by inducing Vegfa, Ctsb and Tgfb1	[311]
Lung cancer	Human	Lung tissues	ISG neutrophils	The number of cells decreases significantly in patients.	[317]
Lung cancer	Human	Blood	HLA-DR$^+$ neutrophils	Enhance immune response	[318]
Lung cancer	Human	Blood	CD66b$^+$/CD10^{low}/CXCR4$^+$/PDL1^{inter} neutrophils	Relate to advanced lung cancer diagnosis	[90]
Breast cancer	Human	Tumor tissues	C5aR1$^+$ neutrophils	Induce breast cancer glycolysis and promote tumor growth	[328]
Breast cancer	Human	Tumor tissues	Neutrophils (cKit$^+$, Nos2$^+$, Prok2$^+$, S100a8$^+$, S100a9$^+$)	Promote tumor metastasis	[329]
Breast cancer	Mice	Tumor tissues	cKit$^+$ neutrophils	Promote tumor metastasis	[330]
Pancreatic ductal adenocarcinoma	Human	Blood, tumor tissues	BHLHE40$^+$ neutrophils	Immunosuppressive function	[338]

(Continues)

TABLE 2 (Continued)

Diseases	Species	Samples	Neutrophil heterogeneity	Functions	References
Pancreatic ductal adenocarcinoma	Human	Tumor tissues	P2RX1^{neg} neutrophils	Immunosuppressive function	[339]
Pancreatic ductal adenocarcinoma	Human	Tumor tissues	CD13^{hi} neutrophils	Immunosuppressive function	[340]
Pancreatic ductal adenocarcinoma	Human	Tumor tissues	CXCR2$^+$ neutrophils	Immunosuppressive function	[345–347]
Rhabdomyosarcom	Mice	Tumor tissues	CXCR2$^+$CD11b$^+$Ly6G^{hi} neutrophils	Immunosuppressive function	[354]
Neck squamous cell carcinoma	Human	Blood	CD16^{high} CD62L^{high} neutrophils	Correlate with increased survival rate	[355]
Melanoma	Human	Blood	PD-L1$^+$ neutrophils	Correlate with decreased survival rate	[358]

A comprehensive study of neutrophil heterogeneity would favor us in acquiring a deeper understanding of disease mechanisms and may yield novel insights into cancer treatment.

diseases and tumors. It remains difficult to ascertain whether the occurrence of neutrophil heterogeneity is due to a single signal or a combination of multiple factors. This requires more detailed exploration. Additionally, the correlation between neutrophil functions and the phenotypes identified by surface markers is an important issue that warrants discussion. For example, neutrophils with immunosuppressive function and high expression of PD-L1 would be defined as PD-L1$^+$ neutrophils.[276] Although some high-resolution assays such as RNA-seq have helped in providing functional information of neutrophil subsets and specific expression of maker genes, the relationship between the neutrophil functions and surface makers is still unclear. This represents an important direction of future research.

Despite the fact that extensive evidence has supported the importance of neutrophils as effector cells for disease treatment, many uncertainties remain to be addressed. Currently, studies of neutrophil heterogeneity have been concentrated on the association level. In the upcoming investigation, functions of multiple neutrophil subpopulations should be evaluated based on various transgenic mice. Simultaneously, comprehensive and accurate definition of neutrophil subpopulations along with factors promoting their formation deserve further investigation. Neutrophils exhibit species specificity, and human and mouse neutrophils might function differently in some respects.[312,358] Cui et al.[255] reported that human neutrophil elastase selectively killed tumor cells, whereas similar effects did not occur with mouse neutrophil elastase. This indicated that our research of neutrophil heterogeneity in mice did not immediately correlate with clinical cases. In addition, the role of neutrophils also varies widely across different tumors. Neutrophils with high MET expression had a great antitumor effect in the lung cancer model. In the melanoma model, however, the expression of MET

would greatly limit the effect of immunotherapy.[79,359] Neutrophils function based on the tissue type, the tissue microenvironment, and the stage of disease. Even within the same disease situation, it is possible for neutrophils to perform conflict functions. To be specific, neutrophils showed an antitumor effect by inducing ferroptosis in glioblastoma through MPO; however, the presence of neutrophils in glioblastoma was often seen as a prediction of poor prognosis.[360] The function of neutrophil subsets in peripheral blood from volunteers of different sexes also differs.[361] Differences in neutrophil heterogeneity across species, genders, and disease types have brought great challenges to our study of neutrophil heterogeneity, and will continue to be the focus and difficulty of future research.

From a therapeutic standpoint, investigations targeting specific neutrophil subpopulations are being refined. It is of great significance to develop drugs that target particular neutrophil subpopulation for treatment of inflammation and cancer-related diseases. Though, approaches that would target particular neutrophil subpopulation remain limited and warrant additional investigation. Presently, some neutrophil-targeted nanoparticles and nanoformulations targeting specific neutrophil subpopulations have shown favorable alleviative effects.[362,363] This may be due to innate ability possessed by cells to sense, integrate and respond to the dynamic environment within the body, rendering them suitable as carriers of therapeutic drugs. In recent years, the design of drug delivery system regarding neutrophils has become a prominent research direction in the field of drug delivery.[364,365] There are two types of drug delivery systems: neutrophils as carriers and neutrophil-membrane-derived nanovesicles.[364] Several studies have demonstrated that neutrophil-based drug delivery systems can improve current therapies for inflammatory disorders and cancers. To be specific, Zhang and

coworkers[366] prepared a tumor-penetrating neotype neutrophil cytopharmaceutical (NEs@STING-Mal-NP) that conjugated liposomal STING agonists on the surface of neutrophils, inheriting the merits of neutrophils such as proactive tumor vascular extravasation and tissue penetration, significantly boosting the tumor penetration of STING agonists. However, one challenge with neutrophil-based drug delivery systems is determining the optimal time to deliver nanoparticles for better targeting of infiltrated neutrophils. After all, the time course of neutrophil infiltration is heavily reliant on the pathogenesis of the disease and its stages. The use of effector neutrophil subsets in specific disease context to design drug-delivery systems may offer a novel approach.

Overall, we have provided information on the fundamental function of neutrophils, the double-edged role of neutrophils in diseases, and the important function of neutrophilic heterogeneity in inflammatory diseases and tumors. Additionally, potential future research directions for neutrophils have also been discussed. We hope that this review would provide a comprehensive understanding of neutrophils.

AUTHOR CONTRIBUTION

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

ETHICS STATEMENT

Not applicable.

DATA AVAILABILITY STATEMENT

Not applicable.

REFERENCES

1. Liew PX, Kubes P. The neutrophil's role during health and disease. *Physiol Rev*. 2019;99(2):1223-1248.
2. Margraf A, Ley K, Zarbock A. Neutrophil recruitment: from model systems to tissue-specific patterns. *Trends Immunol*. 2019;40(7):613-634.
3. Cassatella MA, Ostberg NK, Tamassia N, Soehnlein O. Biological roles of neutrophil-derived granule proteins and cytokines. *Trends Immunol*. 2019;40(7):648-664.
4. Metzemaekers M, Gouwy M, Proost P. Neutrophil chemoattractant receptors in health and disease: double-edged swords. *Cell Mol Immunol*. 2020;17(5):433-450.
5. de Oliveira S, Rosowski EE, Huttenlocher A. Neutrophil migration in infection and wound repair: going forward in reverse. *Nat Rev Immunol*. 2016;16(6):378-391.
6. Filippi MD. Neutrophil transendothelial migration: updates and new perspectives. *Blood*. 2019;133(20):2149-2158.
7. Spitzer MH, Nolan GP. Mass cytometry: single cells, many features. *Cell*. 2016;165(4):780-791.
8. Vandereyken K, Sifrim A, Thienpont B, Voet T. Methods and applications for single-cell and spatial multi-omics. *Nat Rev Genet*. 2023:1-22.
9. Silvestre-Roig C, Hidalgo A, Soehnlein O. Neutrophil heterogeneity: implications for homeostasis and pathogenesis. *Blood*. 2016;127(18):2173-2181.
10. Silvestre-Roig C, Fridlender ZG, Glogauer M, Scapini P. Neutrophil diversity in health and disease. *Trends Immunol*. 2019;40(7):565-583.
11. Giese MA, Hind LE, Huttenlocher A. Neutrophil plasticity in the tumor microenvironment. *Blood*. 2019;133(20):2159-2167.
12. Rosales C. Neutrophils at the crossroads of innate and adaptive immunity. *J Leukoc Biol*. 2020;108(1):377-396.
13. Dahl R, Walsh JC, Lancki D, et al. Regulation of macrophage and neutrophil cell fates by the PU.1:c/EBPalpha ratio and granulocyte colony-stimulating factor. *Nat Immunol*. 2003;4(10):1029-1036.
14. Nerlov C, Graf T. PU.1 induces myeloid lineage commitment in multipotent hematopoietic progenitors. *Genes Dev*. 1998;12(15):2403-2412.
15. Rosmarin AG, Yang Z, Resendes KK. Transcriptional regulation in myelopoiesis: hematopoietic fate choice, myeloid differentiation, and leukemogenesis. *Exp Hematol*. 2005;33(2):131-143.
16. Sasmono RT, Ehrnsperger A, Cronau SL, et al. Mouse neutrophilic granulocytes express mRNA encoding the macrophage colony-stimulating factor receptor (CSF-1R) as well as many other macrophage-specific transcripts and can transdifferentiate into macrophages in vitro in response to CSF-1. *J Leukoc Biol*. 2007;82(1):111-123.
17. Sadik CD, Kim ND, Luster AD. Neutrophils cascading their way to inflammation. *Trends Immunol*. 2011;32(10):452-460.
18. Burn GL, Foti A, Marsman G, Patel DF, Zychlinsky A. The neutrophil. *Immunity*. 2021;54(7):1377-1391.
19. Allen LA. The role of the neutrophil and phagocytosis in infection caused by Helicobacter pylori. *Curr Opin Infect Dis*. 2001;14(3):273-277.
20. Phillipson M, Kubes P. The healing power of neutrophils. *Trends Immunol*. 2019;40(7):635-647.
21. Dwivedi N, Radic M. Neutrophil activation and B-cell stimulation in the pathogenesis of Felty's syndrome. *Pol Arch Med Wewn*. 2012;122(7-8):374-379.
22. Kvedaraite E. Neutrophil-T cell crosstalk in inflammatory bowel disease. *Immunology*. 2021;164(4):657-664.
23. Alves-Filho JC, Spiller F, Cunha FQ. Neutrophil paralysis in sepsis. *Shock*. 2010;34(1):15-21.
24. Sonego F, Castanheira FV, Ferreira RG, et al. Paradoxical roles of the neutrophil in sepsis: protective and deleterious. *Front Immunol*. 2016;7:155.

25. Bouma G, Ancliff PJ, Thrasher AJ, Burns SO. Recent advances in the understanding of genetic defects of neutrophil number and function. *Br J Haematol*. 2010;151(4):312-326.

26. Gong T, Liu L, Jiang W, Zhou R. DAMP-sensing receptors in sterile inflammation and inflammatory diseases. *Nat Rev Immunol*. 2020;20(2):95-112.

27. Zindel J, Kubes P. DAMPs, PAMPs, and LAMPs in immunity and sterile inflammation. *Annu Rev Pathol*. 2020;15:493-518.

28. Pittman K, Kubes P. Damage-associated molecular patterns control neutrophil recruitment. *J Innate Immun*. 2013;5(4):315-323.

29. Li L, Chen K, Xiang Y, et al. New development in studies of formyl-peptide receptors: critical roles in host defense. *J Leukoc Biol*. 2016;99(3):425-435.

30. Raoof M, Zhang Q, Itagaki K, Hauser CJ. Mitochondrial peptides are potent immune activators that activate human neutrophils via FPR-1. *J Trauma*. 2010;68(6):1332-1334. 1328-32; discussion.

31. McDonald B, Pittman K, Menezes GB, et al. Intravascular danger signals guide neutrophils to sites of sterile inflammation. *Science*. 2010;330(6002):362-366.

32. Yoo SK, Starnes TW, Deng Q, Huttenlocher A. Lyn is a redox sensor that mediates leukocyte wound attraction in vivo. *Nature*. 2011;480(7375):109-112.

33. Klyubin IV, Kirpichnikova KM, Gamaley IA. Hydrogen peroxide-induced chemotaxis of mouse peritoneal neutrophils. *Eur J Cell Biol*. 1996;70(4):347-351.

34. Pase L, Layton JE, Wittmann C, et al. Neutrophil-delivered myeloperoxidase dampens the hydrogen peroxide burst after tissue wounding in zebrafish. *Curr Biol*. 2012;22(19):1818-1824.

35. Scapini P, Lapinet-Vera JA, Gasperini S, Calzetti F, Bazzoni F, Cassatella MA. The neutrophil as a cellular source of chemokines. *Immunol Rev*. 2000;177:195-203.

36. Norling LV, Perretti M. Proresolving lipid mediators enhance PMN-mediated bacterial clearance. *Proc Natl Acad Sci USA*. 2020;117(17):9148-9150.

37. Traidl-Hoffmann C, Kasche A, Jakob T, et al. Lipid mediators from pollen act as chemoattractants and activators of polymorphonuclear granulocytes. *J Allergy Clin Immunol*. 2002;109(5):831-838.

38. Rajarathnam K, Schnoor M, Richardson RM, Rajagopal S. How do chemokines navigate neutrophils to the target site: dissecting the structural mechanisms and signaling pathways. *Cell Signal*. 2019;54:69-80.

39. Matsushima K, Yang D, Oppenheim JJ. Interleukin-8: an evolving chemokine. *Cytokine*. 2022;153:155828.

40. Petri B, Sanz MJ. Neutrophil chemotaxis. *Cell Tissue Res*. 2018;371(3):425-436.

41. Zlotnik A, Yoshie O. The chemokine superfamily revisited. *Immunity*. 2012;36(5):705-716.

42. Charo IF, Ransohoff RM. The many roles of chemokines and chemokine receptors in inflammation. *N Engl J Med*. 2006;354(6):610-621.

43. Proost P, Struyf S, Van Damme J, Fiten P, Ugarte-Berzal E, Opdenakker G. Chemokine isoforms and processing in inflammation and immunity. *J Autoimmun*. 2017;85:45-57.

44. Russo RC, Garcia CC, Teixeira MM, Amaral FA. The CXCL8/IL-8 chemokine family and its receptors in inflammatory diseases. *Expert Rev Clin Immunol*. 2014;10(5):593-619.

45. Kobayashi Y. The role of chemokines in neutrophil biology. *Front Biosci*. 2008;13:2400-2407.

46. Sai J, Raman D, Liu Y, Wikswo J, Richmond A. Parallel phosphatidylinositol 3-kinase (PI3K)-dependent and Src-dependent pathways lead to CXCL8-mediated Rac2 activation and chemotaxis. *J Biol Chem*. 2008;283(39):26538-26547.

47. Neel NF, Barzik M, Raman D, et al. VASP is a CXCR2-interacting protein that regulates CXCR2-mediated polarization and chemotaxis. *J Cell Sci*. 2009;122(11):1882-1894.

48. Gelfand EW. Importance of the leukotriene B4-BLT1 and LTB4-BLT2 pathways in asthma. *Semin Immunol*. 2017;33:44-51.

49. O'Flaherty JT, Kuroki M, Nixon AB, et al. 5-Oxo-eicosanoids and hematopoietic cytokines cooperate in stimulating neutrophil function and the mitogen-activated protein kinase pathway. *J Biol Chem*. 1996;271(30):17821-17828.

50. Konya V, Blattermann S, Jandl K, et al. A biased non-Galphai OXE-R antagonist demonstrates that Galphai protein subunit is not directly involved in neutrophil, eosinophil, and monocyte activation by 5-oxo-ETE. *J Immunol*. 2014;192(10):4774-4782.

51. Ellett F, Marand AL, Irimia D. Multifactorial assessment of neutrophil chemotaxis efficiency from a drop of blood. *J Leukoc Biol*. 2022;111(6):1175-1184.

52. De Giovanni M, Tam H, Valet C, Xu Y, Looney MR, Cyster JG. GPR35 promotes neutrophil recruitment in response to serotonin metabolite 5-HIAA. *Cell*. 2022;185(5):815-830. e19.

53. Girbl T, Lenn T, Perez L, et al. Distinct compartmentalization of the chemokines CXCL1 and CXCL2 and the atypical receptor ACKR1 determine discrete stages of neutrophil diapedesis. *Immunity*. 2018;49(6):1062-1076. e6.

54. Roth H, Samereier M, Begandt D, et al. Filamin A promotes efficient migration and phagocytosis of neutrophil-like HL-60 cells. *Eur J Cell Biol*. 2017;96(6):553-566.

55. Tonetti MS, Imboden MA, Lang NP. Neutrophil migration into the gingival sulcus is associated with transepithelial gradients of interleukin-8 and ICAM-1. *J Periodontol*. 1998;69(10):1139-1147.

56. Chan EC, Ren C, Xie Z, et al. Regulator of G protein signaling 5 restricts neutrophil chemotaxis and trafficking. *J Biol Chem*. 2018;293(33):12690-12702.

57. Aerbajinai W, Liu L, Zhu J, Kumkhaek C, Chin K, Rodgers GP. Glia maturation factor-gamma regulates monocyte migration through modulation of beta1-integrin. *J Biol Chem*. 2016;291(16):8549-8564.

58. Monaco S, Sparano V, Gioia M, et al. Enzymatic processing of collagen IV by MMP-2 (gelatinase A) affects neutrophil migration and it is modulated by extracatalytic domains. *Protein Sci*. 2006;15(12):2805-2815.

59. Nathan C. Neutrophils and immunity: challenges and opportunities. *Nat Rev Immunol*. 2006;6(3):173-182.

60. Herrero-Cervera A, Soehnlein O, Kenne E. Neutrophils in chronic inflammatory diseases. *Cell Mol Immunol*. 2022;19(2):177-191.

61. Hind LE, Huttenlocher A. Neutrophil reverse migration and a chemokinetic resolution. *Dev Cell*. 2018;47(4):404-405.

62. Wang J, Hossain M, Thanabalasuriar A, Gunzer M, Meininger C, Kubes P. Visualizing the function and fate of neutrophils in sterile injury and repair. *Science*. 2017;358(6359):111-116.

63. Nourshargh S, Renshaw SA, Imhof BA. Reverse migration of neutrophils: where, when, how, and why. *Trends Immunol*. 2016;37(5):273-286.

64. de Castro Pinho J, Forster R. Lymph-derived neutrophils primarily locate to the subcapsular and medullary sinuses in resting and inflamed lymph nodes. *Cells*. 2021;10(6).

65. Mathias JR, Perrin BJ, Liu TX, Kanki J, Look AT, Huttenlocher A. Resolution of inflammation by retrograde chemotaxis of neutrophils in transgenic zebrafish. *J Leukoc Biol*. 2006;80(6):1281-1288.

66. Wu D, Zeng Y, Fan Y, et al. Reverse-migrated neutrophils regulated by JAM-C are involved in acute pancreatitis-associated lung injury. *Sci Rep*. 2016;6:20545.

67. Colom B, Bodkin JV, Beyrau M, et al. Leukotriene B4-neutrophil elastase axis drives neutrophil reverse transendothelial cell migration in vivo. *Immunity*. 2015;42(6):1075-1086.

68. Elks PM, van Eeden FJ, Dixon G, et al. Activation of hypoxia-inducible factor-1alpha (Hif-1alpha) delays inflammation resolution by reducing neutrophil apoptosis and reverse migration in a zebrafish inflammation model. *Blood*. 2011;118(3):712-722.

69. Gewirtz AT, McCormick B, Neish AS, et al. Pathogen-induced chemokine secretion from model intestinal epithelium is inhibited by lipoxin A4 analogs. *J Clin Invest*. 1998;101(9):1860-1869.

70. Isles HM, Herman KD, Robertson AL, et al. The CXCL12/CXCR4 signaling axis retains neutrophils at inflammatory sites in zebrafish. *Front Immunol*. 2019;10:1784.

71. Tauzin S, Starnes TW, Becker FB, Lam PY, Huttenlocher A. Redox and Src family kinase signaling control leukocyte wound attraction and neutrophil reverse migration. *J Cell Biol*. 2014;207(5):589-598.

72. Marki A, Ley K. Leaking chemokines confuse neutrophils. *J Clin Invest*. 2020;130(5):2177-2179.

73. Kienle K, Lammermann T. Neutrophil swarming: an essential process of the neutrophil tissue response. *Immunol Rev*. 2016;273(1):76-93.

74. Hopke A, Scherer A, Kreuzburg S, et al. Neutrophil swarming delays the growth of clusters of pathogenic fungi. *Nat Commun*. 2020;11(1):2031.

75. Serhan CN, Levy BD. Resolvins in inflammation: emergence of the pro-resolving superfamily of mediators. *J Clin Invest*. 2018;128(7):2657-2669.

76. Granot Z, Jablonska J. Distinct functions of neutrophil in cancer and its regulation. *Mediators Inflamm*. 2015;2015:701067.

77. Jablonska J, Leschner S, Westphal K, Lienenklaus S, Weiss S. Neutrophils responsive to endogenous IFN-beta regulate tumor angiogenesis and growth in a mouse tumor model. *J Clin Invest*. 2010;120(4):1151-1164.

78. Wu CF, Andzinski L, Kasnitz N, et al. The lack of type I interferon induces neutrophil-mediated pre-metastatic niche formation in the mouse lung. *Int J Cancer*. 2015;137(4):837-847.

79. Finisguerra V, Di Conza G, Di Matteo M, et al. MET is required for the recruitment of anti-tumoural neutrophils. *Nature*. 2015;522(7556):349-353.

80. 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):300-314.

81. Lopez-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):1752-1760.

82. van Egmond M, Bakema JE. Neutrophils as effector cells for antibody-based immunotherapy of cancer. *Semin Cancer Biol*. 2013;23(3):190-199.

83. Ng LG, Ostuni R, Hidalgo A. Heterogeneity of neutrophils. *Nat Rev Immunol*. 2019;19(4):255-265.

84. Deniset JF, Kubes P. Neutrophil heterogeneity: bona fide subsets or polarization states. *J Leukoc Biol*. 2018;103(5):829-838.

85. Chatfield SM, Thieblemont N, Witko-Sarsat V. Expanding neutrophil horizons: new concepts in inflammation. *J Innate Immun*. 2018;10(5-6):422-431.

86. Fukushima K, Nabeshima H, Kida H. Revealing the diversity of neutrophil functions and subsets. *Cell Mol Immunol*. 2021;18(4):781-783.

87. Bongers SH, Chen N, van Grinsven E, et al. Kinetics of neutrophil subsets in acute, subacute, and chronic inflammation. *Front Immunol*. 2021;12:674079.

88. Marzano AV, Borghi A, Wallach D, Cugno M. A comprehensive review of neutrophilic diseases. *Clin Rev Allergy Immunol*. 2018;54(1):114-130.

89. Prame Kumar K, Nicholls AJ, Wong CHY. Partners in crime: neutrophils and monocytes/macrophages in inflammation and disease. *Cell Tissue Res*. 2018;371(3):551-565.

90. Shaul ME, Eyal O, Guglietta S, et al. Circulating neutrophil subsets in advanced lung cancer patients exhibit unique immune signature and relate to prognosis. *FASEB J*. 2020;34(3):4204-4218.

91. Hacbarth E, Kajdacsy-Balla A. Low density neutrophils in patients with systemic lupus erythematosus, rheumatoid arthritis, and acute rheumatic fever. *Arthritis Rheum*. 1986;29(11):1334-1342.

92. Pember SO, Barnes KC, Brandt SJ, Kinkade JM. Density heterogeneity of neutrophilic polymorphonuclear leukocytes: gradient fractionation and relationship to chemotactic stimulation. *Blood*. 1983;61(6):1105-1115.

93. Hassani M, Hellebrekers P, Chen N, et al. On the origin of low-density neutrophils. *J Leukoc Biol*. 2020;107(5):809-818.

94. Ley K, Hoffman HM, Kubes P, et al. Neutrophils: new insights and open questions. *Sci Immunol*. 2018;3(30).

95. 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.

96. Mackey JBG, Coffelt SB, Carlin LM. Neutrophil maturity in cancer. *Front Immunol*. 2019;10:1912.

97. Sagiv JY, Michaeli J, Assi S, et al. Phenotypic diversity and plasticity in circulating neutrophil subpopulations in cancer. *Cell Rep*. 2015;10(4):562-573.

98. Pfirschke C, Engblom C, Gungabeesoon J, et al. Tumor-promoting Ly-6G(+) SiglecF(high) cells are mature and long-lived neutrophils. *Cell Rep*. 2020;32(12):108164.

99. Coffelt SB, Wellenstein MD, de Visser KE. Neutrophils in cancer: neutral no more. *Nat Rev Cancer*. 2016;16(7):431-446.

100. Martin C, Burdon PC, Bridger G, Gutierrez-Ramos JC, Williams TJ, Rankin SM. Chemokines acting via CXCR2 and CXCR4 control the release of neutrophils from the bone

marrow and their return following senescence. *Immunity*. 2003;19(4):583-593.

101. Evrard M, Kwok IWH, Chong SZ, et al. Developmental analysis of bone marrow neutrophils reveals populations specialized in expansion, trafficking, and effector functions. *Immunity*. 2018;48(2):364-379. e8.

102. Nemeth T, Sperandio M, Mocsai A. Neutrophils as emerging therapeutic targets. *Nat Rev Drug Discov*. 2020;19(4):253-275.

103. Adrover JM, Del Fresno C, Crainiciuc G, et al. A neutrophil timer coordinates immune defense and vascular protection. *Immunity*. 2019;50(2):390-402. e10.

104. Casanova-Acebes M, Pitaval C, Weiss LA, et al. Rhythmic modulation of the hematopoietic niche through neutrophil clearance. *Cell*. 2013;153(5):1025-1035.

105. Ballesteros I, Rubio-Ponce A, Genua M, et al. Co-option of neutrophil fates by tissue environments. *Cell*. 2020;183(5):1282-1297. e18.

106. Boivin G, Ancey PB, Vuillefroy de Silly R, et al. Anti-Ly6G binding and trafficking mediate positive neutrophil selection to unleash the anti-tumor efficacy of radiation therapy. *Oncoimmunology*. 2021;10(1):1876597.

107. Barkaway A, Rolas L, Joulia R, et al. Age-related changes in the local milieu of inflamed tissues cause aberrant neutrophil trafficking and subsequent remote organ damage. *Immunity*. 2021;54(7):1494-1510. e7.

108. Hsu BE, Tabaries S, Johnson RM, et al. Immature low-density neutrophils exhibit metabolic flexibility that facilitates breast cancer liver metastasis. *Cell Rep*. 2019;27(13):3902-3915. e6.

109. Rice CM, Davies LC, Subleski JJ, et al. Tumour-elicited neutrophils engage mitochondrial metabolism to circumvent nutrient limitations and maintain immune suppression. *Nat Commun*. 2018;9(1):5099.

110. Condamine T, Dominguez GA, Youn JI, et al. Lectin-type oxidized LDL receptor-1 distinguishes population of human polymorphonuclear myeloid-derived suppressor cells in cancer patients. *Sci Immunol*. 2016;1(2).

111. Veglia F, Perego M, Gabrilovich D. Myeloid-derived suppressor cells coming of age. *Nat Immunol*. 2018;19(2):108-119.

112. Wu WC, Sun HW, Chen HT, et al. Circulating hematopoietic stem and progenitor cells are myeloid-biased in cancer patients. *Proc Natl Acad Sci USA*. 2014;111(11):4221-4226.

113. Pillay J, Kamp VM, van Hoffen E, et al. A subset of neutrophils in human systemic inflammation inhibits T cell responses through Mac-1. *J Clin Invest*. 2012;122(1):327-336.

114. Chappell L, Russell AJC, Voet T. Single-cell (multi)omics technologies. *Annu Rev Genomics Hum Genet*. 2018;19:15-41.

115. Han X, Zhou Z, Fei L, et al. Construction of a human cell landscape at single-cell level. *Nature*. 2020;581(7808):303-309.

116. Han X, Wang R, Zhou Y, et al. Mapping the mouse cell atlas by Microwell-Seq. *Cell*. 2018;172(5):1091-1107 e17.

117. Qu J, Yang F, Zhu T, et al. A reference single-cell regulomic and transcriptomic map of cynomolgus monkeys. *Nat Commun*. 2022;13(1):4069.

118. Dinh HQ, Eggert T, Meyer MA, et al. Coexpression of CD71 and CD117 identifies an early unipotent neutrophil progenitor population in human bone marrow. *Immunity*. 2020;53(2):319-334. e6.

119. Calzetti F, Finotti G, Tamassia N, et al. CD66b(-)CD64(dim)CD115(-) cells in the human bone marrow represent neutrophil-committed progenitors. *Nat Immunol*. 2022;23(5):679-691.

120. Weisgrau KL, Vosler LJ, Pomplun NL, et al. Neutrophil progenitor populations of rhesus macaques. *J Leukoc Biol*. 2019;105(1):113-121.

121. Berdnikovs S. The twilight zone: plasticity and mixed ontogeny of neutrophil and eosinophil granulocyte subsets. *Semin Immunopathol*. 2021;43(3):337-346.

122. Rambault M, Doz-Deblauwe E, Le Vern Y, et al. Neutrophils encompass a regulatory subset suppressing T cells in apparently healthy cattle and mice. *Front Immunol*. 2021;12:625244.

123. Alder MN, Mallela J, Opoka AM, Lahni P, Hildeman DA, Wong HR. Olfactomedin 4 marks a subset of neutrophils in mice. *Innate Immun*. 2019;25(1):22-33.

124. Puellmann K, Kaminski WE, Vogel M, et al. A variable immunoreceptor in a subpopulation of human neutrophils. *Proc Natl Acad Sci USA*. 2006;103(39):14441-14446.

125. Massena S, Christoffersson G, Vagesjo E, et al. Identification and characterization of VEGF-A-responsive neutrophils expressing CD49d, VEGFR1, and CXCR4 in mice and humans. *Blood*. 2015;126(17):2016-2026.

126. Christoffersson G, Phillipson M. The neutrophil: one cell on many missions or many cells with different agendas? *Cell Tissue Res*. 2018;371(3):415-423.

127. Lawrence SM, Corriden R, Nizet V. The ontogeny of a neutrophil: mechanisms of granulopoiesis and homeostasis. *Microbiol Mol Biol Rev*. 2018;82(1).

128. van Grinsven E, Textor J, Hustin LSP, Wolf K, Koenderman L, Vrisekoop N. Immature neutrophils released in acute inflammation exhibit efficient migration despite incomplete segmentation of the nucleus. *J Immunol*. 2019;202(1):207-217.

129. Leliefeld PHC, Pillay J, Vrisekoop N, et al. Differential antibacterial control by neutrophil subsets. *Blood Adv*. 2018;2(11):1344-1355.

130. La Manno G, Soldatov R, Zeisel A, et al. RNA velocity of single cells. *Nature*. 2018;560(7719):494-498.

131. Qiu X, Mao Q, Tang Y, et al. Reversed graph embedding resolves complex single-cell trajectories. *Nat Methods*. 2017;14(10):979-982.

132. DeSouza-Vieira T. Metamorphosis of neutrophil transcriptional programme during Leishmania infection. *Parasite Immunol*. 2022;44(6):e12922.

133. Chan L, Morovati S, Karimi N, et al. Neutrophil functional heterogeneity and implications for viral infections and treatments. *Cells*. 2022;11(8).

134. Wigerblad G, Kaplan MJ. Neutrophil extracellular traps in systemic autoimmune and autoinflammatory diseases. *Nat Rev Immunol*. 2022:1-15.

135. Manz MG, Boettcher S. Emergency granulopoiesis. Emergency granulopoiesis. *Nat Rev Immunol*. 2014;14(5):302-314.

136. Anzai A, Choi JL, He S, et al. The infarcted myocardium solicits GM-CSF for the detrimental oversupply of inflammatory leukocytes. *J Exp Med*. 2017;214(11):3293-3310.

137. Boettcher S, Gerosa RC, Radpour R, et al. Endothelial cells translate pathogen signals into G-CSF-driven emergency granulopoiesis. *Blood*. 2014;124(9):1393-1403.

138. Hosseini N, Shor J, Szabo G. Alcoholic hepatitis: a review. *Alcohol Alcohol*. 2019;54(4):408-416.

139. Nati M, Chung KJ, Chavakis T. The role of innate immune cells in nonalcoholic fatty liver disease. *J Innate Immun*. 2022;14(1):31-41.

140. Koyama Y, Brenner DA. Liver inflammation and fibrosis. *J Clin Invest*. 2017;127(1):55-64.

141. Ren R, He Y, Ding D, et al. Aging exaggerates acute-on-chronic alcohol-induced liver injury in mice and humans by inhibiting neutrophilic sirtuin 1-C/EBPalpha-miRNA-223 axis. *Hepatology*. 2022;75(3):646-660.

142. Zhao X, Yang L, Chang N, et al. Neutrophil recruitment mediated by sphingosine 1-phosphate (S1P)/S1P receptors during chronic liver injury. *Cell Immunol*. 2021;359:104243.

143. Choudhury SR, Babes L, Rahn JJ, et al. Dipeptidase-1 is an adhesion receptor for neutrophil recruitment in lungs and liver. *Cell*. 2019;178(5):1205-1221. e17.

144. Hwang S, He Y, Xiang X, et al. Interleukin-22 ameliorates neutrophil-driven nonalcoholic steatohepatitis through multiple targets. *Hepatology*. 2020;72(2):412-429.

145. Calvente CJ, Tameda M, Johnson CD, et al. Neutrophils contribute to spontaneous resolution of liver inflammation and fibrosis via microRNA-223. *J Clin Invest*. 2019;129(10):4091-4109.

146. Cho Y, Bukong TN, Tornai D, et al. Neutrophil extracellular traps contribute to liver damage and increase defective low-density neutrophils in alcohol-associated hepatitis. *J Hepatol*. 2023;78(1):28-44.

147. Kolodziejczyk AA, Federici S, Zmora N, et al. Acute liver failure is regulated by MYC- and microbiome-dependent programs. *Nat Med*. 2020;26(12):1899-1911.

148. Macek Jilkova Z, Afzal S, Marche H, et al. Progression of fibrosis in patients with chronic viral hepatitis is associated with IL-17(+) neutrophils. *Liver Int*. 2016;36(8):1116-1124.

149. Maria NI, Davidson A. Protecting the kidney in systemic lupus erythematosus: from diagnosis to therapy. *Nat Rev Rheumatol*. 2020;16(5):255-267.

150. Rovin BH. Systemic autoimmune diseases and the kidney. *Kidney Int*. 2020;97(2):222-225.

151. Saisorn W, Saithong S, Phuengmaung P, et al. Acute kidney injury induced lupus exacerbation through the enhanced neutrophil extracellular traps (and apoptosis) in Fcgr2b deficient lupus mice with renal ischemia reperfusion injury. *Front Immunol*. 2021;12:669162.

152. Deng Y, Zheng Y, Li D, et al. Expression characteristics of interferon-stimulated genes and possible regulatory mechanisms in lupus patients using transcriptomics analyses. *EBioMedicine*. 2021;70:103477.

153. Cahilog Z, Zhao H, Wu L, et al. The role of neutrophil NETosis in organ injury: novel inflammatory cell death mechanisms. *Inflammation*. 2020;43(6):2021-2032.

154. Nakazawa D, Marschner JA, Platen L, Anders HJ. Extracellular traps in kidney disease. *Kidney Int*. 2018;94(6):1087-1098.

155. Stark JE, Opoka AM, Mallela J, et al. Juvenile OLFM4-null mice are protected from sepsis. *Am J Physiol Renal Physiol*. 2020;318(3):F809-F816.

156. Skopelja-Gardner S, Tai J, Sun X, et al. Acute skin exposure to ultraviolet light triggers neutrophil-mediated kidney inflammation. *Proc Natl Acad Sci USA*. 2021;118(3).

157. Rodriguez-Carrio J, Carrillo-Lopez N, Ulloa C, et al. A subset of low density granulocytes is associated with vascular calcification in chronic kidney disease patients. *Sci Rep*. 2019;9(1):13230.

158. Nishi H, Mayadas TN. Neutrophils in lupus nephritis. *Curr Opin Rheumatol*. 2019;31(2):193-200.

159. Bronze-da-Rocha E, Santos-Silva A. Neutrophil elastase inhibitors and chronic kidney disease. *Int J Biol Sci*. 2018;14(10):1343-1360.

160. Snelgrove RJ, Patel DF, Patel T, Lloyd CM. The enigmatic role of the neutrophil in asthma: friend, foe or indifferent? *Clin Exp Allergy*. 2018;48(10):1275-1285.

161. Heeb LEM, Egholm C, Impellizzieri D, Ridder F, Boyman O. Regulation of neutrophils in type 2 immune responses. *Curr Opin Immunol*. 2018;54:115-122.

162. Nakamura K, Kageyama S, Kupiec-Weglinski JW. The evolving role of neutrophils in liver transplant ischemia-reperfusion injury. *Curr Transplant Rep*. 2019;6(1):78-89.

163. Neupane AS, Willson M, Chojnacki AK, et al. Patrolling alveolar macrophages conceal bacteria from the immune system to maintain homeostasis. *Cell*. 2020;183(1):110-125. e11.

164. Zhang Y, Wang Q, Mackay CR, Ng LG, Kwok I. Neutrophil subsets and their differential roles in viral respiratory diseases. *J Leukoc Biol*. 2022;111(6):1159-1173.

165. Jenkins AR, Holden NS, Jones AW. Inflammatory responses to acute exercise during pulmonary rehabilitation in patients with COPD. *Eur J Appl Physiol*. 2020;120(10):2301-2309.

166. Laucirica DR, Schofield CJ, McLean SA, et al. Pseudomonas aeruginosa modulates neutrophil granule exocytosis in an in vitro model of airway infection. *Immunol Cell Biol*. 2022;100(5):352-370.

167. Alder MN, Opoka AM, Lahni P, Hildeman DA, Wong HR. Olfactomedin-4 is a candidate marker for a pathogenic neutrophil subset in septic shock. *Crit Care Med*. 2017;45(4):e426-e432.

168. Demaret J, Venet F, Plassais J, et al. Identification of CD177 as the most dysregulated parameter in a microarray study of purified neutrophils from septic shock patients. *Immunol Lett*. 2016;178:122-130.

169. Schreiber A, Jannerjahn A, Kettritz R. CD177/NB1 receptor expression is dynamically regulated in sepsis patients. *Immunohematology*. 2015;31(3):128-129.

170. Mortaz E, Alipoor SD, Adcock IM, Mumby S, Koenderman L. Update on neutrophil function in severe inflammation. *Front Immunol*. 2018;9:2171.

171. Kangelaris KN, Clemens R, Fang X, et al. A neutrophil subset defined by intracellular olfactomedin 4 is associated with mortality in sepsis. *Am J Physiol Lung Cell Mol Physiol*. 2021;320(5):L892-L902.

172. Ekstedt S, Safholm J, Georen SK, Cardell LO. Dividing neutrophils in subsets reveals a significant role for activated neutrophils in the development of airway hyperreactivity. *Clin Exp Allergy*. 2019;49(3):285-291.

173. Kassam AF, Levinsky NC, Mallela JP, et al. Olfactomedin 4-positive neutrophils are upregulated after hemorrhagic shock. *Am J Respir Cell Mol Biol*. 2021;64(2):216-223.

174. Cortjens B, Ingelse SA, Calis JC, et al. Neutrophil subset responses in infants with severe viral respiratory infection. *Clin Immunol*. 2017;176:100-106.

175. Xie Y, Abel PW, Casale TB, Tu Y. T(H)17 cells and corticosteroid insensitivity in severe asthma. *J Allergy Clin Immunol*. 2022;149(2):467-479.

176. Qi X, Yu Y, Sun R, et al. Identification and characterization of neutrophil heterogeneity in sepsis. *Crit Care*. 2021;25(1):50.

177. Meghraoui-Kheddar A, Chousterman BG, Guillou N, et al. Two new neutrophil subsets define a discriminating sepsis signature. *Am J Respir Crit Care Med*. 2022;205(1):46-59.

178. Takizawa S, Murao A, Ochani M, Aziz M, Wang P. Frontline Science: extracellular CIRP generates a proinflammatory Ly6G(+) CD11b(hi) subset of low-density neutrophils in sepsis. *J Leukoc Biol*. 2021;109(6):1019-1032.

179. Song Z, Bhattacharya S, Huang G, et al. NADPH oxidase 2 limits amplification of IL-1beta-G-CSF axis and an immature neutrophil subset in murine lung inflammation. *Blood Adv*. 2023;7(7):1225-1240.

180. Xie X, Shi Q, Wu P, et al. Single-cell transcriptome profiling reveals neutrophil heterogeneity in homeostasis and infection. *Nat Immunol*. 2020;21(9):1119-1133.

181. Fahlberg MD, Blair RV, Doyle-Meyers LA, et al. Cellular events of acute, resolving or progressive COVID-19 in SARS-CoV-2 infected non-human primates. *Nat Commun*. 2020;11(1):6078.

182. Deerhake ME, Reyes EY, Xu-Vanpala S, Shinohara ML. Single-cell transcriptional heterogeneity of neutrophils during acute pulmonary cryptococcus neoformans infection. *Front Immunol*. 2021;12:670574.

183. Cheung DS, Sigua JA, Simpson PM, et al. Cysteinyl leukotriene receptor 1 expression identifies a subset of neutrophils during the antiviral response that contributes to postviral atopic airway disease. *J Allergy Clin Immunol*. 2018;142(4):1206-1217 e5.

184. Tirouvanziam R, Gernez Y, Conrad CK, et al. Profound functional and signaling changes in viable inflammatory neutrophils homing to cystic fibrosis airways. *Proc Natl Acad Sci USA*. 2008;105(11):4335-4339.

185. Ingersoll SA, Laval J, Forrest OA, et al. Mature cystic fibrosis airway neutrophils suppress T cell function: evidence for a role of arginase 1 but not programmed death-ligand 1. *J Immunol*. 2015;194(11):5520-5528.

186. Hu X, Sun Y, Xu W, Lin T, Zeng H. Expression of RANKL by peripheral neutrophils and its association with bone mineral density in COPD. *Respirology*. 2017;22(1):126-132.

187. Morrissey SM, Geller AE, Hu X, et al. A specific low-density neutrophil population correlates with hypercoagulation and disease severity in hospitalized COVID-19 patients. *JCI Insight*. 2021;6(9).

188. Hazeldine J, Lord JM. Neutrophils and COVID-19: active participants and rational therapeutic targets. *Front Immunol*. 2021;12:680134.

189. deKay JT, Emery IF, Rud J, et al. DEspR(high) neutrophils are associated with critical illness in COVID-19. *Sci Rep*. 2021;11(1):22463.

190. Combes AJ, Courau T, Kuhn NF, et al. Global absence and targeting of protective immune states in severe COVID-19. *Nature*. 2021;591(7848):124-130.

191. McLeish KR, Shrestha R, Vashishta A, et al. Differential functional responses of neutrophil subsets in severe COVID-19 patients. *Front Immunol*. 2022;13:879686.

192. Schulte-Schrepping J, Reusch N, Paclik D, et al. Severe COVID-19 is marked by a dysregulated myeloid cell compartment. *Cell*. 2020;182(6):1419-1440 e23.

193. Loyer C, Lapostolle A, Urbina T, et al. Impairment of neutrophil functions and homeostasis in COVID-19 patients: association with disease severity. *Crit Care*. 2022;26(1):155.

194. Sinha S, Rosin NL, Arora R, et al. Dexamethasone modulates immature neutrophils and interferon programming in severe COVID-19. *Nat Med*. 2022;28(1):201-211.

195. Rebillard RM, Charabati M, Grasmuck C, et al. Identification of SARS-CoV-2-specific immune alterations in acutely ill patients. *J Clin Invest*. 2021;131(8).

196. Ode Y, Aziz M, Wang P. CIRP increases ICAM-1(+) phenotype of neutrophils exhibiting elevated iNOS and NETs in sepsis. *J Leukoc Biol*. 2018;103(4):693-707.

197. Carstensen S, Muller M, Tan GLA, et al. Rogue″ neutrophil-subset [DEspR+CD11b+/CD66b+] immunotype is an actionable therapeutic target for neutrophilic inflammation-mediated tissue injury - studies in human, macaque and rat LPS-inflammation models. *Front Immunol*. 2022;13:1008390.

198. Herrera VLM, Walkey AJ, Nguyen MQ, et al. A targetable 'rogue' neutrophil-subset, [CD11b+DEspR+] immunotype, is associated with severity and mortality in acute respiratory distress syndrome (ARDS) and COVID-19-ARDS. *Sci Rep*. 2022;12(1):5583.

199. Tak T, Rygiel TP, Karnam G, et al. Neutrophil-mediated suppression of influenza-induced pathology requires CD11b/CD18 (MAC-1). *Am J Respir Cell Mol Biol*. 2018;58(4):492-499.

200. Grieshaber-Bouyer R, Radtke FA, Cunin P, et al. The neutrotime transcriptional signature defines a single continuum of neutrophils across biological compartments. *Nat Commun*. 2021;12(1):2856.

201. Kologrivova I, Shtatolkina M, Suslova T, Ryabov V. Cells of the immune system in cardiac remodeling: main players in resolution of inflammation and repair after myocardial infarction. *Front Immunol*. 2021;12:664457.

202. Rolfes L, Riek-Burchardt M, Pawlitzki M, et al. Neutrophil granulocytes promote flow stagnation due to dynamic capillary stalls following experimental stroke. *Brain Behav Immun*. 2021;93:322-330.

203. Sim TM, Mak A, Tay SH. Insights into the role of neutrophils in neuropsychiatric systemic lupus erythematosus: current understanding and future directions. *Front Immunol*. 2022;13:957303.

204. Cerecedo D, Martinez-Vieyra I, Lopez-Villegas EO, Hernandez-Cruz A, Loza-Huerta ADC. Heterogeneity of neutrophils in arterial hypertension. *Exp Cell Res*. 2021;402(2):112577.

205. Calcagno DM, Zhang C, Toomu A, et al. SiglecF(HI) marks late-stage neutrophils of the infarcted heart: a single-cell transcriptomic analysis of neutrophil diversification. *J Am Heart Assoc*. 2021;10(4):e019019.

206. Vafadarnejad E, Rizzo G, Krampert L, et al. Dynamics of cardiac neutrophil diversity in murine myocardial infarction. *Circ Res*. 2020;127(9):e232-e249.

207. Yiu JYT, Hally KE, Larsen PD, Holley AS. Increased levels of low density neutrophils (LDNs) in myocardial infarction. *Acta Cardiol*. 2022:1-8.

208. DeLeon-Pennell KY, Mouton AJ, Ero OK, et al. LXR/RXR signaling and neutrophil phenotype following myocardial infarction classify sex differences in remodeling. *Basic Res Cardiol*. 2018;113(5):40.

209. Calcagno DM, Ng RP, Toomu A, et al. The myeloid type I interferon response to myocardial infarction begins in bone marrow and is regulated by Nrf2-activated macrophages. *Sci Immunol*. 2020;5(51).

210. Martini E, Kunderfranco P, Peano C, et al. Single-cell sequencing of mouse heart immune infiltrate in pressure overload-driven heart failure reveals extent of immune activation. *Circulation*. 2019;140(25):2089-2107.

211. Weisenburger-Lile D, Dong Y, Yger M, et al. Harmful neutrophil subsets in patients with ischemic stroke: association with disease severity. *Neurol Neuroimmunol Neuroinflamm*. 2019;6(4):e571.

212. Dong Y, Lagarde J, Xicota L, et al. Neutrophil hyperactivation correlates with Alzheimer's disease progression. *Ann Neurol*. 2018;83(2):387-405.

213. Trivedi A, Tercovich KG, Casbon AJ, Raber J, Lowell C, Noble-Haeusslein LJ. Neutrophil-specific deletion of Syk results in recruitment-independent stabilization of the barrier and a long-term improvement in cognitive function after traumatic injury to the developing brain. *Neurobiol Dis*. 2021;157:105430.

214. Zhao T, Jiang Q, Li W, et al. Antigen-presenting cell-like neutrophils foster T cell response in hyperlipidemic patients and atherosclerotic mice. *Front Immunol*. 2022;13:851713.

215. Chang JT. Pathophysiology of inflammatory bowel diseases. *N Engl J Med*. 2020;383(27):2652-2664.

216. Therrien A, Chapuy L, Bsat M, et al. Recruitment of activated neutrophils correlates with disease severity in adult Crohn's disease. *Clin Exp Immunol*. 2019;195(2):251-264.

217. Butin-Israeli V, Bui TM, Wiesolek HL, et al. Neutrophil-induced genomic instability impedes resolution of inflammation and wound healing. *J Clin Invest*. 2019;129(2):712-726.

218. Dinallo V, Marafini I, Di Fusco D, et al. Neutrophil extracellular traps sustain inflammatory signals in ulcerative colitis. *J Crohns Colitis*. 2019;13(6):772-784.

219. Leppkes M, Lindemann A, Gosswein S, et al. Neutrophils prevent rectal bleeding in ulcerative colitis by peptidyl-arginine deiminase-4-dependent immunothrombosis. *Gut*. 2022;71(12):2414-2429.

220. Trilleaud C, Gauttier V, Biteau K, et al. Agonist anti-ChemR23 mAb reduces tissue neutrophil accumulation and triggers chronic inflammation resolution. *Sci Adv*. 2021;7(14).

221. Yan YX, Shao MJ, Qi Q, et al. Artemisinin analogue SM934 ameliorates DSS-induced mouse ulcerative colitis via suppressing neutrophils and macrophages. *Acta Pharmacol Sin*. 2018;39(10):1633-1644.

222. Lartey NL, Vargas-Robles H, Guerrero-Fonseca IM, et al. Annickia polycarpa extract attenuates inflammation, neutrophil recruitment, and colon damage during colitis. *Immunol Lett*. 2022;248:99-108.

223. Zhou G, Yu L, Fang L, et al. CD177(+) neutrophils as functionally activated neutrophils negatively regulate IBD. *Gut*. 2018;67(6):1052-1063.

224. Jin M, Zhang H, Wu M, et al. Colonic interleukin-22 protects intestinal mucosal barrier and microbiota abundance in severe acute pancreatitis. *FASEB J*. 2022;36(3):e22174.

225. Li Y, Zhu L, Chu Z, et al. Characterization and biological significance of IL-23-induced neutrophil polarization. *Cell Mol Immunol*. 2018;15(5):518-530.

226. Levinsky NC, Mallela J, Opoka AM, et al. The olfactomedin-4 positive neutrophil has a role in murine intestinal ischemia/reperfusion injury. *FASEB J*. 2019;33(12):13660-13668.

227. Pavlidis P, Tsakmaki A, Pantazi E, et al. Interleukin-22 regulates neutrophil recruitment in ulcerative colitis and is associated with resistance to ustekinumab therapy. *Nat Commun*. 2022;13(1):5820.

228. Seto N, Torres-Ruiz JJ, Carmona-Rivera C, et al. Neutrophil dysregulation is pathogenic in idiopathic inflammatory myopathies. *JCI Insight*. 2020;5(3).

229. Carmona-Rivera C, Khaznadar SS, Shwin KW, et al. Deficiency of adenosine deaminase 2 triggers adenosine-mediated NETosis and TNF production in patients with DADA2. *Blood*. 2019;134(4):395-406.

230. Dahlstrand Rudin A, Amirbeagi F, Davidsson L, et al. The neutrophil subset defined by CD177 expression is preferentially recruited to gingival crevicular fluid in periodontitis. *J Leukoc Biol*. 2021;109(2):349-362.

231. Hirschfeld J. Neutrophil subsets in periodontal health and disease: a mini review. *Front Immunol*. 2019;10:3001.

232. Sansores-Espana LD, Melgar-Rodriguez S, Vernal R, Carrillo-Avila BA, Martinez-Aguilar VM, Diaz-Zuniga J. Neutrophil N1 and N2 subsets and their possible association with periodontitis: a scoping review. *Int J Mol Sci*. 2022;23(20).

233. Sas AR, Carbajal KS, Jerome AD, et al. A new neutrophil subset promotes CNS neuron survival and axon regeneration. *Nat Immunol*. 2020;21(12):1496-1505.

234. Jerome AD, Atkinson JR, McVey Moffatt AL, Sepeda JA, Segal BM, Sas AR. Characterization of zymosan-modulated neutrophils with neuroregenerative properties. *Front Immunol*. 2022;13:912193.

235. Matsui M, Nagakubo D, Satooka H, Hirata T. A novel Siglec-F(+) neutrophil subset in the mouse nasal mucosa exhibits an activated phenotype and is increased in an allergic rhinitis model. *Biochem Biophys Res Commun*. 2020;526(3):599-606.

236. Rodriguez-Rosales YA, Langereis JD, Gorris MAJ, et al. Immunomodulatory aged neutrophils are augmented in blood and skin of psoriasis patients. *J Allergy Clin Immunol*. 2021;148(4):1030-1040.

237. Turner TC, Sok MCP, Hymel LA, et al. Harnessing lipid signaling pathways to target specialized pro-angiogenic neutrophil subsets for regenerative immunotherapy. *Sci Adv*. 2020;6(44).

238. Teuben MPJ, Hofman M, Greven J, et al. Altered cell surface receptor dynamics and circulatory occurrence of neutrophils in a small animal fracture model. *Pathol Res Pract*. 2020;216(10):153108.

239. Mouillot P, Witko-Sarsat V, Wislez M. [Neutrophil plasticity: a new key in the understanding of onco-immunology] La plasticité des neutrophiles : nouvel élément de compréhension en onco-immunologie. *Rev Mal Respir*. 2022;39(7):587-594.

240. Cerezo-Wallis D, Ballesteros I. Neutrophils in cancer, a love-hate affair. *FEBS J*. 2022;289(13):3692-3703.

241. Furumaya C, Martinez-Sanz P, Bouti P, Kuijpers TW, Matlung HL. Plasticity in pro- and anti-tumor activity of neutrophils: shifting the balance. *Front Immunol*. 2020;11:2100.

242. Lecot P, Sarabi M, Pereira Abrantes M, et al. Neutrophil heterogeneity in cancer: from biology to therapies. *Front Immunol*. 2019;10:2155.

243. Rawat K, Syeda S, Shrivastava A. Neutrophil-derived granule cargoes: paving the way for tumor growth and progression. *Cancer Metastasis Rev*. 2021;40(1):221-244.

244. Lecot P, Ardin M, Dussurgey S, et al. Gene signature of circulating platelet-bound neutrophils is associated with poor prognosis in cancer patients. *Int J Cancer*. 2022;151(1):138-152.

245. Dekker E, Tanis PJ, Vleugels JLA, Kasi PM, Wallace MB. Colorectal cancer. *Lancet*. 2019;394(10207):1467-1480.

246. Biller LH, Schrag D. Diagnosis and treatment of metastatic colorectal cancer: a review. *JAMA*. 2021;325(7):669-685.

247. Mizuno R, Kawada K, Itatani Y, Ogawa R, Kiyasu Y, Sakai Y. The role of tumor-associated neutrophils in colorectal cancer. *Int J Mol Sci*. 2019;20(3).

248. Ruland J. Colon cancer: epithelial Notch signaling recruits neutrophils to drive metastasis. *Cancer Cell*. 2019;36(3):213-214.

249. Stehr AM, Wang G, Demmler R, et al. Neutrophil extracellular traps drive epithelial-mesenchymal transition of human colon cancer. *J Pathol*. 2022;256(4):455-467.

250. Khan U, Chowdhury S, Billah MM, Islam KMD, Thorlacius H, Rahman M. Neutrophil extracellular traps in colorectal cancer progression and metastasis. *Int J Mol Sci*. 2021;22(14).

251. Yang L, Liu Q, Zhang X, et al. DNA of neutrophil extracellular traps promotes cancer metastasis via CCDC25. *Nature*. 2020;583(7814):133-138.

252. Rayes RF, Vourtzoumis P, Bou Rjeily M, et al. Neutrophil extracellular trap-associated CEACAM1 as a putative therapeutic target to prevent metastatic progression of colon carcinoma. *J Immunol*. 2020;204(8):2285-2294.

253. Li T, Wang C, Liu Y, et al. Neutrophil extracellular traps induce intestinal damage and thrombotic tendency in inflammatory bowel disease. *J Crohns Colitis*. 2020;14(2):240-253.

254. Deng H, Lin C, Garcia-Gerique L, et al. A novel selective inhibitor JBI-589 targets PAD4-mediated neutrophil migration to suppress tumor progression. *Cancer Res*. 2022;82(19):3561-3572.

255. Cui C, Chakraborty K, Tang XA, et al. Neutrophil elastase selectively kills cancer cells and attenuates tumorigenesis. *Cell*. 2021;184(12):3163-3177. e21.

256. Triner D, Devenport SN, Ramakrishnan SK, et al. Neutrophils restrict tumor-associated microbiota to reduce growth and invasion of colon tumors in mice. *gastroenterology*. 2019;156(5):1467-1482.[crossref]

257. Dmitrieva-Posocco O, Dzutsev A, Posocco DF, et al. Cell-type-specific responses to interleukin-1 control microbial invasion and tumor-elicited inflammation in colorectal cancer. *Immunity*. 2019;50(1):166-180. e7.

258. Germann M, Zangger N, Sauvain MO, et al. Neutrophils suppress tumor-infiltrating T cells in colon cancer via matrix metalloproteinase-mediated activation of TGFbeta. *EMBO Mol Med*. 2020;12(1):e10681.

259. Zhou G, Peng K, Song Y, et al. CD177+ neutrophils suppress epithelial cell tumourigenesis in colitis-associated cancer and predict good prognosis in colorectal cancer. *Carcinogenesis*. 2018;39(2):272-282.

260. Wikberg ML, Ling A, Li X, Oberg A, Edin S, Palmqvist R. Neutrophil infiltration is a favorable prognostic factor in early stages of colon cancer. *Hum Pathol*. 2017;68:193-202.

261. Chadwick JW, Fine N, Khoury W, et al. Tissue-specific murine neutrophil activation states in health and inflammation. *J Leukoc Biol*. 2021;110(1):187-195.

262. Takeda Y, Kato T, Nemoto N, et al. Augmentation of the expression of the eotaxin receptor on duodenal neutrophils by IL-21. *Cytokine*. 2018;110:194-203.

263. Itatani Y, Yamamoto T, Zhong C, et al. Suppressing neutrophil-dependent angiogenesis abrogates resistance to anti-VEGF antibody in a genetic model of colorectal cancer. *Proc Natl Acad Sci USA*. 2020;117(35):21598-21608.

264. Chen H, Zhou XH, Li JR, et al. Neutrophils: driving inflammation during the development of hepatocellular carcinoma. *Cancer Lett*. 2021;522:22-31.

265. Tsilimigras DI, Brodt P, Clavien PA, et al. Liver metastases. *Nat Rev Dis Primers*. 2021;7(1):27.

266. Lu C, Rong D, Zhang B, et al. Current perspectives on the immunosuppressive tumor microenvironment in hepatocellular carcinoma: challenges and opportunities. *Mol Cancer*. 2019;18(1):130.

267. Geh D, Leslie J, Rumney R, Reeves HL, Bird TG, Mann DA. Neutrophils as potential therapeutic targets in hepatocellular carcinoma. *Nat Rev Gastroenterol Hepatol*. 2022;19(4):257-273.

268. van der Windt DJ, Sud V, Zhang H, et al. Neutrophil extracellular traps promote inflammation and development of hepatocellular carcinoma in nonalcoholic steatohepatitis. *Hepatology*. 2018;68(4):1347-1360.

269. Jiang ZZ, Peng ZP, Liu XC, et al. Neutrophil extracellular traps induce tumor metastasis through dual effects on cancer and endothelial cells. *Oncoimmunology*. 2022;11(1):2052418.

270. Yang LY, Luo Q, Lu L, et al. Increased neutrophil extracellular traps promote metastasis potential of hepatocellular carcinoma via provoking tumorous inflammatory response. *J Hematol Oncol*. 2020;13(1):3.

271. Xu X, Ye L, Zhang Q, et al. Group-2 innate lymphoid cells promote HCC progression through CXCL2-neutrophil-induced immunosuppression. *Hepatology*. 2021;74(5):2526-2543.

272. Umeshappa CS, Sole P, Surewaard BGJ, et al. Liver-specific T regulatory type-1 cells program local neutrophils to suppress hepatic autoimmunity via CRAMP. *Cell Rep*. 2021;34(13):108919.

273. Zhou Z, Hu Y, Wu Y, et al. The immunosuppressive tumor microenvironment in hepatocellular carcinoma-current situation and outlook. *Mol Immunol*. 2022;151:218-230.

274. He G, Zhang H, Zhou J, et al. Peritumoural neutrophils negatively regulate adaptive immunity via the PD-L1/PD-1 signalling pathway in hepatocellular carcinoma. *J Exp Clin Cancer Res*. 2015;34:141.

275. Cheng Y, Li H, Deng Y, et al. Cancer-associated fibroblasts induce PDL1+ neutrophils through the IL6-STAT3 pathway that foster immune suppression in hepatocellular carcinoma. *Cell Death Dis*. 2018;9(4):422.

276. Xue R, Zhang Q, Cao Q, et al. Liver tumour immune microenvironment subtypes and neutrophil heterogeneity. *Nature*. 2022;612(7938):141-147.

277. Wang Y, Zhao Q, Zhao B, et al. Remodeling tumor-associated neutrophils to enhance dendritic cell-based HCC neoantigen nano-vaccine efficiency. *Adv Sci (Weinh)*. 2022;9(11):e2105631.

278. Deng H, Kan A, Lyu N, et al. Tumor-derived lactate inhibit the efficacy of lenvatinib through regulating PD-L1 expression on neutrophil in hepatocellular carcinoma. *J Immunother Cancer*. 2021;9(6).

279. Esteban-Fabro R, Willoughby CE, Pique-Gili M, et al. Cabozantinib enhances anti-PD1 activity and elicits a neutrophil-based immune response in hepatocellular carcinoma. *Clin Cancer Res*. 2022;28(11):2449-2460.

280. Siwicki M, Gort-Freitas NA, Messemaker M, et al. Resident Kupffer cells and neutrophils drive liver toxicity in cancer immunotherapy. *Sci Immunol*. 2021;6(61).

281. Smyth EC, Nilsson M, Grabsch HI, van Grieken NC, Lordick F. Gastric cancer. *Lancet*. 2020;396(10251):635-648.

282. Machlowska J, Baj J, Sitarz M, Maciejewski R, Sitarz R. Gastric cancer: epidemiology, risk factors, classification, genomic characteristics and treatment strategies. *Int J Mol Sci*. 2020;21(11).

283. Tan Z. Recent advances in the surgical treatment of advanced gastric cancer: a review. *Med Sci Monit*. 2019;25: 3537-3541.

284. Zhang X, Shi H, Yuan X, Jiang P, Qian H, Xu W. Tumor-derived exosomes induce N2 polarization of neutrophils to promote gastric cancer cell migration. *Mol Cancer*. 2018;17(1):146.

285. Shan ZG, Chen J, Liu JS, et al. Activated neutrophils polarize protumorigenic interleukin-17A-producing T helper subsets through TNF-alpha-B7-H2-dependent pathway in human gastric cancer. *Clin Transl Med*. 2021;11(6):e484.

286. Nagaoka K, Shirai M, Taniguchi K, et al. Deep immunophenotyping at the single-cell level identifies a combination of anti-IL-17 and checkpoint blockade as an effective treatment in a preclinical model of data-guided personalized immunotherapy. *J Immunother Cancer*. 2020;8(2).

287. Xia X, Zhang Z, Zhu C, et al. Neutrophil extracellular traps promote metastasis in gastric cancer patients with postoperative abdominal infectious complications. *Nat Commun*. 2022;13(1):1017.

288. Zhu T, Zou X, Yang C, et al. Neutrophil extracellular traps promote gastric cancer metastasis by inducing epithelial-mesenchymal transition. *Int J Mol Med*. 2021;48(1).

289. Li S, Cong X, Gao H, et al. Tumor-associated neutrophils induce EMT by IL-17a to promote migration and invasion in gastric cancer cells. *J Exp Clin Cancer Res*. 2019;38(1):6.

290. Jiang H, Yu D, Yang P, et al. Revealing the transcriptional heterogeneity of organ-specific metastasis in human gastric cancer using single-cell RNA Sequencing. *Clin Transl Med*. 2022;12(2):e730.

291. Qian Y, Zhai E, Chen S, et al. Single-cell RNA-seq dissecting heterogeneity of tumor cells and comprehensive dynamics in tumor microenvironment during lymph nodes metastasis in gastric cancer. *Int J Cancer*. 2022;151(8):1367-1381.

292. Wang TT, Zhao YL, Peng LS, et al. Tumour-activated neutrophils in gastric cancer foster immune suppression and disease progression through GM-CSF-PD-L1 pathway. *Gut*. 2017;66(11):1900-1911.

293. Li S, Sun S, Sun H, et al. A risk signature with inflammatory and immune cells infiltration predicts survival and efficiency of chemotherapy in gastric cancer. *Int Immunopharmacol*. 2021;96:107589.

294. Cong X, Zhang Y, Zhu Z, et al. CD66b(+) neutrophils and alpha-SMA(+) fibroblasts predict clinical outcomes and benefits from postoperative chemotherapy in gastric adenocarcinoma. *Cancer Med*. 2020;9(8):2761-2773.

295. Shan ZG, Zhao YL, Zhang JY, et al. FasL(+) PD-L2(+) identifies a novel immunosuppressive neutrophil population in human gastric cancer that promotes disease progression. *Adv Sci (Weinh)*. 2022;9(5):e2103543.

296. Thai AA, Solomon BJ, Sequist LV, Gainor JF, Heist RS. Lung cancer. *Lancet*. 2021;398(10299):535-554.

297. Hirsch FR, Scagliotti GV, Mulshine JL, et al. Lung cancer: current therapies and new targeted treatments. *Lancet*. 2017;389(10066):299-311.

298. Yang R, Wang X, Ma C, et al. Tumor-infiltrating neutrophils and peripheral neutrophil-to-lymphocyte ratio conversely predicted the prognosis of patients with non-small cell lung cancer. *Cell Immunol*. 2022;379:104588.

299. Zhu X, Chen Y, Cui Y. Absolute neutrophil count and mean platelet volume in the blood as biomarkers to detect lung cancer. *Dis Markers*. 2020;2020:1371964.

300. Xiao Y, Cong M, Li J, et al. Cathepsin C promotes breast cancer lung metastasis by modulating neutrophil infiltration and neutrophil extracellular trap formation. *Cancer Cell*. 2021;39(3):e7.

301. Li P, Lu M, Shi J, et al. Lung mesenchymal cells elicit lipid storage in neutrophils that fuel breast cancer lung metastasis. *Nat Immunol*. 2020;21(11):1444-1455.

302. Nolan E, Bridgeman VL, Ombrato L, et al. Radiation exposure elicits a neutrophil-driven response in healthy lung tissue that enhances metastatic colonization. *Nat Cancer*. 2022;3(2):173-187.

303. Yang C, Wang Z, Li L, et al. Aged neutrophils form mitochondria-dependent vital NETs to promote breast cancer lung metastasis. *J Immunother Cancer*. 2021;9(10).

304. Tyagi A, Sharma S, Wu K, et al. Nicotine promotes breast cancer metastasis by stimulating N2 neutrophils and generating pre-metastatic niche in lung. *Nat Commun*. 2021;12(1):474.

305. Valadez-Cosmes P, Maitz K, Kindler O, 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.

306. Pang J, Yu Q, Chen Y, Yuan H, Sheng M, Tang W. Integrating Single-cell RNA-seq to construct a neutrophil prognostic model for predicting immune responses in non-small cell lung cancer. *J Transl Med*. 2022;20(1):531.

307. Faget J, Groeneveld S, Boivin G, et al. Neutrophils and snail orchestrate the establishment of a pro-tumor microenvironment in lung cancer. *Cell Rep*. 2017;21(11):3190-3204.

308. Engblom C, Pfirschke C, Zilionis R, et al. Osteoblasts remotely supply lung tumors with cancer-promoting SiglecF(high) neutrophils. *Science*. 2017;358(6367).

309. Eruslanov EB, Bhojnagarwala PS, Quatromoni JG, et al. Tumor-associated neutrophils stimulate T cell responses in early-stage human lung cancer. *J Clin Invest*. 2014;124(12):5466-5480.

310. Ancey PB, Contat C, Boivin G, et al. GLUT1 expression in tumor-associated neutrophils promotes lung cancer growth and resistance to radiotherapy. *Cancer Res*. 2021;81(9):2345-2357.

311. Deryugina EI, Zajac E, Juncker-Jensen A, Kupriyanova TA, Welter L, Quigley JP. Tissue-infiltrating neutrophils constitute the major in vivo source of angiogenesis-inducing MMP-9 in the tumor microenvironment. *Neoplasia*. 2014;16(10):771-788.

312. Zilionis R, Engblom C, Pfirschke C, et al. Single-cell transcriptomics of human and mouse lung cancers reveals conserved myeloid populations across individuals and species. *Immunity*. 2019;50(5):1317-1334. e10.

313. Singhal S, Bhojnagarwala PS, O'Brien S, 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):120-135.

314. Cheng Y, Mo F, Li Q, et al. Targeting CXCR2 inhibits the progression of lung cancer and promotes therapeutic effect of cisplatin. *Mol Cancer*. 2021;20(1):62.

315. Drpa G, Sutic M, Baranasic J, et al. Neutrophil-to-lymphocyte ratio can predict outcome in extensive-stage small cell lung cancer. *Radiol Oncol*. 2020;54(4):437-446.

316. Harbeck N, Gnant M. Breast cancer. *Lancet*. 2017;389(10074):1134-1150.

317. Wculek SK, Malanchi I. Neutrophils support lung colonization of metastasis-initiating breast cancer cells. *Nature*. 2015;528(7582):413-417.

318. Szczerba BM, Castro-Giner F, Vetter M, et al. Neutrophils escort circulating tumour cells to enable cell cycle progression. *Nature*. 2019;566(7745):553-557.

319. Benevides L, da Fonseca DM, Donate PB, et al. IL17 promotes mammary tumor progression by changing the behavior of tumor cells and eliciting tumorigenic neutrophils recruitment. *Cancer Res*. 2015;75(18):3788-3799.

320. Spiegel A, Brooks MW, Houshyar S, et al. Neutrophils suppress intraluminal NK cell-mediated tumor cell clearance and enhance extravasation of disseminated carcinoma cells. *Cancer Discov*. 2016;6(6):630-649.

321. Qi M, Xia Y, Wu Y, et al. Lin28B-high breast cancer cells promote immune suppression in the lung pre-metastatic niche via exosomes and support cancer progression. *Nat Commun*. 2022;13(1):897.

322. Casbon AJ, Reynaud D, Park C, et al. Invasive breast cancer reprograms early myeloid differentiation in the bone marrow to generate immunosuppressive neutrophils. *Proc Natl Acad Sci USA*. 2015;112(6):E566-75.

323. Matlung HL, Babes L, Zhao XW, et al. Neutrophils kill antibody-opsonized cancer cells by trogoptosis. *Cell Rep*. 2018;23(13):3946-3959. e6.

324. Veillette A, Chen J. SIRPalpha-CD47 immune checkpoint blockade in anticancer therapy. *Trends Immunol*. 2018;39(3):173-184.

325. Ou B, Liu Y, Yang X, Xu X, Yan Y, Zhang J. C5aR1-positive neutrophils promote breast cancer glycolysis through WTAP-dependent m6A methylation of ENO1. *Cell Death Dis*. 2021;12(8):737.

326. Coffelt SB, Kersten K, Doornebal CW, et al. IL-17-producing gammadelta T cells and neutrophils conspire to promote breast cancer metastasis. *Nature*. 2015;522(7556):345-348.

327. Wellenstein MD, Coffelt SB, Duits DEM, et al. Loss of p53 triggers WNT-dependent systemic inflammation to drive breast cancer metastasis. *Nature*. 2019;572(7770):538-542.

328. 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.

329. Peng Z, Liu C, Victor AR, et al. Tumors exploit CXCR4(hi)CD62L(lo) aged neutrophils to facilitate metastatic spread. *Oncoimmunology*. 2021;10(1):1870811.

330. Klein AP. Pancreatic cancer epidemiology: understanding the role of lifestyle and inherited risk factors. *Nat Rev Gastroenterol Hepatol*. 2021;18(7):493-502.

331. Park W, Chawla A, O'Reilly EM. Pancreatic cancer: a review. *JAMA*. 2021;326(9):851-862.

332. Deng J, Kang Y, Cheng CC, et al. DDR1-induced neutrophil extracellular traps drive pancreatic cancer metastasis. *JCI Insight*. 2021;6(17).

333. Kajioka H, Kagawa S, Ito A, et al. Targeting neutrophil extracellular traps with thrombomodulin prevents pancreatic cancer metastasis. *Cancer Lett*. 2021;497:1-13.

334. Zhang Y, Chandra V, Riquelme Sanchez E, et al. Interleukin-17-induced neutrophil extracellular traps mediate resistance to checkpoint blockade in pancreatic cancer. *J Exp Med*. 2020;217(12).

335. Wang L, Liu Y, Dai Y, et al. Single-cell RNA-seq analysis reveals BHLHE40-driven pro-tumour neutrophils with hyperactivated glycolysis in pancreatic tumour microenvironment. *Gut*. 2022.

336. Zhang J, Xu X, Shi M, et al. CD13(hi) neutrophil-like myeloid-derived suppressor cells exert immune suppression through arginase 1 expression in pancreatic ductal adenocarcinoma. *Oncoimmunology*. 2017;6(2):e1258504.

337. Peng H, Shen J, Long X, et al. Local release of TGF-beta inhibitor modulates tumor-associated neutrophils and enhances pancreatic cancer response to combined irreversible electroporation and immunotherapy. *Adv Sci (Weinh)*. 2022;9(10):e2105240.

338. Nielsen SR, Strobech JE, Horton ER, et al. Suppression of tumor-associated neutrophils by lorlatinib attenuates pancreatic cancer growth and improves treatment with immune checkpoint blockade. *Nat Commun*. 2021;12(1):3414.

339. Li K, Tandurella JA, Gai J, et al. Multi-omic analyses of changes in the tumor microenvironment of pancreatic adenocarcinoma following neoadjuvant treatment with anti-PD-1 therapy. *Cancer Cell*. 2022;40(11):1374-1391. e7.

340. Steele CW, Karim SA, Leach JDG, et al. CXCR2 inhibition profoundly suppresses metastases and augments immunotherapy in pancreatic ductal adenocarcinoma. *Cancer Cell*. 2016;29(6):832-845.

341. Chao T, Furth EE, Vonderheide RH. CXCR2-dependent accumulation of tumor-associated neutrophils regulates T-cell immunity in pancreatic ductal adenocarcinoma. *Cancer Immunol Res*. 2016;4(11):968-982.

342. Nywening TM, Belt BA, Cullinan DR, et al. Targeting both tumour-associated CXCR2(+) neutrophils and CCR2(+) macrophages disrupts myeloid recruitment and improves chemotherapeutic responses in pancreatic ductal adenocarcinoma. *Gut*. 2018;67(6):1112-1123.

343. Tintelnot J, Xu Y, Lesker TR, et al. Microbiota-derived 3-IAA influences chemotherapy efficacy in pancreatic cancer. *Nature*. 2023;615(7950):168-174.

344. Bouti P, Zhao XW, Verkuijlen P, et al. Kindlin3-Dependent CD11b/CD18-integrin activation is required for potentiation of neutrophil cytotoxicity by CD47-SIRPalpha checkpoint disruption. *Cancer Immunol Res*. 2021;9(2):147-155.

345. Ring NG, Herndler-Brandstetter D, Weiskopf K, et al. Anti-SIRPalpha antibody immunotherapy enhances neutrophil and macrophage antitumor activity. *Proc Natl Acad Sci*. 2017;114(49):E10578-E10585.

346. Martinez Sanz P, van Rees DJ, van Zogchel LMJ, et al. G-CSF as a suitable alternative to GM-CSF to boost dinutuximab-mediated neutrophil cytotoxicity in neuroblastoma treatment. *J Immunother Cancer*. 2021;9(5).

347. Mahiddine K, Blaisdell A, Ma S, Crequer-Grandhomme A, Lowell CA, Erlebacher A. Relief of tumor hypoxia

unleashes the tumoricidal potential of neutrophils. *J Clin Invest*. 2020;130(1):389-403.

348. Blaisdell A, Crequer A, Columbus D, et al. Neutrophils oppose uterine epithelial carcinogenesis via debridement of hypoxic tumor cells. *Cancer Cell*. 2015;28(6):785-799.

349. Fridlender ZG, Sun J, Kim S, et al. Polarization of tumor-associated neutrophil phenotype by TGF-beta: "N1" versus "N2" TAN. *Cancer Cell*. 2009;16(3):183-194.

350. Highfill SL, Cui Y, Giles AJ, et al. Disruption of CXCR2-mediated MDSC tumor trafficking enhances anti-PD1 efficacy. *Sci Transl Med*. 2014;6(237).

351. Millrud CR, Kagedal A, Kumlien Georen S, 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):2557-2567.

352. Podaza E, Risnik D, Colado A, et al. Chronic lymphocytic leukemia cells increase neutrophils survival and promote their differentiation into CD16(high) CD62L(dim) immunosuppressive subset. *Int J Cancer*. 2019;144(5):1128-1134.

353. Zhu YP, Eggert T, Araujo DJ, Vijayanand P, Ottensmeier CH, Hedrick CC. CyTOF mass cytometry reveals phenotypically distinct human blood neutrophil populations differentially correlated with melanoma stage. *J Immunother Cancer*. 2020;8(2).

354. Cristinziano L, Modestino L, Capone M, et al. PD-L1(+) neutrophils as novel biomarkers for stage IV melanoma patients treated with nivolumab. *Front Immunol*. 2022;13:962669.

355. Kalafati L, Kourtzelis I, Schulte-Schrepping J, et al. Innate immune training of granulopoiesis promotes anti-tumor activity. *Cell*. 2020;183(3):771-785. e12.

356. Siders WM, Shields J, Garron C, et al. Involvement of neutrophils and natural killer cells in the anti-tumor activity of alemtuzumab in xenograft tumor models. *Leuk Lymphoma*. 2010;51(7):1293-1304.

357. Subudhi SK, Siddiqui BA, Aparicio AM, et al. Combined CTLA-4 and PD-L1 blockade in patients with chemotherapy-naive metastatic castration-resistant prostate cancer is associated with increased myeloid and neutrophil immune subsets in the bone microenvironment. *J Immunother Cancer*. 2021;9(10).

358. Eruslanov EB, Singhal S, Albelda SM. Mouse versus human neutrophils in cancer: a major knowledge gap. *Trends Cancer*. 2017;3(2):149-160.

359. Peinado H, Aleckovic M, Lavotshkin S, et al. Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. *Nat Med*. 2012;18(6):883-891.

360. Yee PP, Wei Y, Kim SY, et al. Neutrophil-induced ferroptosis promotes tumor necrosis in glioblastoma progression. *Nat Commun*. 2020;11(1):5424.

361. Gupta S, Nakabo S, Blanco LP, et al. Sex differences in neutrophil biology modulate response to type I interferons and immunometabolism. *Proc Natl Acad Sci USA*. 2020;117(28):16481-16491.

362. Bachmaier K, Stuart A, Singh A, et al. Albumin nanoparticle endocytosing subset of neutrophils for precision therapeutic targeting of inflammatory tissue injury. *ACS Nano*. 2022;16(3):4084-4101.

363. Zhao J, Lu H, Xu D, et al. Neutrophil membrane-coated nanoparticles for enhanced nanosecond pulsed electric field treatment of pancreatic cancer. *Int J Hyperthermia*. 2022;39(1):1026-1035.

364. Chu D, Dong X, Shi X, Zhang C, Wang Z. Neutrophil-based drug delivery systems. *Adv Mater*. 2018;30(22):e1706245.

365. Han Y, Zhao R, Xu F. Neutrophil-based delivery systems for nanotherapeutics. *Small*. 2018;14(42):e1801674.

366. Hao M, Zhu L, Hou S, et al. Sensitizing tumors to immune checkpoint blockage via STING agonists delivered by tumor-penetrating neutrophil cytopharmaceuticals. *ACS Nano*. 2023.