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REVIEW

Effect of neutrophils on tumor immunity and immunotherapy resistance with underlying mechanisms

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Abstract

Neutrophils are key mediators of the immune response and play essential roles in the development of tumors and immune evasion. Emerging studies indicate that neutrophils also play a critical role in the immunotherapy resistance in cancer. In this review, firstly, we summarize the novel classification and phenotypes of

Abbreviations: TME, Tumor microenvironment; DCs, Dendritic cells; NK, Natural killer; APCs, Antigen-presenting cells; ROS, Reactive oxygen species; NETs, neutrophil extracellular traps; NF-κB, Nuclear factor kappa-B; PI3K, Phosphoinositide 3-kinase; AKT, protein kinase B; JAK, Janus kinase; STAT, Signal transducers and activators of transcription; TANs, Tumor associated neutrophils; IL, Interleukin; G-CSF, Granulocyte colony-stimulating factor; EMT, epithelial–mesenchymal transition; PMNs, Polymorphonuclear neutrophils; G-MDSCs, Granulocytic myeloid-derived suppressor cells; LDNs, Low-density neutrophils; TANs, Tumor-associated neutrophils; TGF-β, Transforming growth factor beta; CXCR, C-X-C motif chemokine receptor; IFN-β, Interferon Beta; SAT1, Spermidine/spermine acetyltransferase 1; HK3, Hexokinase 3; CRC, Colorectal cancer; HCC, Hepatocellular carcinoma; CXCL, C-X-C motif chemokine ligand; PFKFB2, Phosphofructo-2-Kinase/Fructose-2,6-Biphosphatase 2; OSM, Oncostatin-M; AA, Amino acid; PD-L1, Programmed death ligand 1; MCT1, Monocarboxylate transporter 1; COX-2, Cyclooxygenase-2; RA, Retinoic acid; ADH1, Alcohol dehydrogenase 1; ARG1, Arginase-1; Fn, Fusobacterium nucleatum; TLR, Toll-like receptor; NOD, Nucleotide binding oligomerization domain containing; HMGB1, High mobility group box 1; MMP-9, Matrix metalloproteinase 9; CCL, C-C motif chemokine ligand; PC, Pancreatic cancer; NE, Neutrophil elastase; HBV, Hepatitis B virus; S100A9, S100 calcium-binding protein A9; RAGE, Receptor for advanced glycation end products; GSH, Glutathione; GR, Glutathione reductase; GRP, Gastrin-releasing peptide; GRPR, Gastrin-releasing peptide receptor; SNS, Sympathetic nervous system; HPA, Hypothalamus-pituitary-adrenal; mTOR, mammalian target of rapamycin; RT, Radiotherapy; PDAC, Pancreatic ductal adenocarcinoma; RCC, Renal cell carcinoma; PD-1, Programmed death receptor 1; KP, Kras-Lox-STOP-Lox-G12D/p53 flox/flox; ISGs, Interferon-stimulated genes; BCG, Bacillus Calmette-Guerin; HSV, Herpes simplex virus; OV, Oncolytic virus; AR, Androgen receptor; TβRI, Transforming growth factor β receptor I; nMOF, Metal-organic framework; RT-RDT, Radiotherapy-radiodynamic therapy; ICD, Immunogenic cell death; HSV1, Herpes simplex virus type 1; PARC, Pathogen response-like chemokine; MyD88, Myeloid differentiation primary response protein 88; MDSCs, Myeloid-derived suppressor cells; THBS1, Thrombospondin-1; STAT3, Signal transducer and activator of transcription 3; FOXP3, Forkhead box protein 3; iNOS, Inducible nitric oxide synthase; STING, Stimulator of interferon genes; HGF/c-MET, Hepatocyte growth factor/cellular mesenchymal-epithelial transition; LAG-3, Lymphocyte activation gene-3; TIM3, T cell immunoglobulin and mucin domain 3; ZEB1, Zinc finger E-box binding homeobox 1; WTAP, Wilms' tumor 1-associating protein; ENO1, α-Enolase; ICIs, Immune checkpoint inhibitors; NSCLC, Non-small cell lung cancer; VISTA, T-cell-activated immunoglobulin inhibitor of structural domain V; TILs, Tumor-infiltrating T lymphocytes; Del-1, Developmental endothelial locus-1; LXR, Liver X receptor; LTB4, Leukotriene B4; SETD2-H3K36me3, SET domain containing 2-histone 3 lysine 36 trimethylation; IRAK-M, Interleukin-1 receptor-associated kinase M; PPM1D, Protein phosphatase $Mn2^{+}/Mg2^{+}$-dependent 1D; Wip1, Wild-type p53 induced phosphatase 1; hPSCs, Human pluripotent stem cells; CAR-NE, Chimeric antigen receptor-neutrophil; SIRPα, Signal-regulatory protein alpha; LILRB2, Leukocyte immunoglobulin-like receptor subfamily B member 2; TIGIT, T cell immunoreceptor with immunoglobulin and ITIM domain; NDEs, Neutrophil-derived exosomes; NFAT1, Nuclear factor of activated T-cells 1; FATP2, Fatty acid transporter protein 2; ATGL, Adipose triglyceride lipase; MPO, Myeloperoxidase; PAD4, Protein arginine deaminase type 4; PGC1a, Peroxisome proliferator-activated receptor gamma coactivator-1 alpha; PGE2, Prostaglandin E2.

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neutrophils and describe the regulatory relationships between neutrophils and tumor metabolism, flora microecology, neuroendocrine and tumor therapy from a new perspective. Secondly, we review the mechanisms by which neutrophils affect drug resistance in tumor immunotherapy from the aspects of the immune microenvironment, tumor antigens, and epigenetics. Finally, we propose several promising strategies for overcoming tumor immunotherapy resistance by targeting neutrophils and provide new research ideas in this area.

KEYWORDS
immune resistance, immunotherapy, neutrophil, tumor, Tumor microenvironment

1 | INTRODUCTION

The immune system surveillance within tumors involves a variety of innate and adaptive immune cells and molecules. With variations in tumor immunogenicity and the inhibition of human immune system by tumor microenvironment (TME), tumor cells eventually evade immune system surveillance and survive every stage of the antitumor immune response [1]. The purpose of tumor immunotherapy is to reactivate antitumor immune cells, overcome tumor immune evasion mechanisms, and fight tumors by controlling the immune system and restoring antitumor immunity [2]. However, the resistance of tumors to immunotherapy is still the main challenge addressed by many researchers. The regulation of immune response is particularly important for overcoming tumor immunotherapy resistance and improving the effectiveness of immunotherapy.

Neutrophils are the most dominant human leukocytes and key mediators of the innate immune response [3]. In recent years, there has been an increasing focus on the function of neutrophils within the immune system. Regarding innate immunity, neutrophils play a vital role in enhancing nonspecific immune responses by facilitating the recruitment, activation, and maturation of monocytes, macrophages, dendritic cells (DCs), and natural killer (NK) cells [4]. When it comes to adaptive immunity, neutrophils support specific T cell and B cell responses by encouraging the differentiation of monocytes and DCs into specialized antigen-presenting cells (APCs) [5, 6] (Figure 1). Nevertheless, the contribution of neutrophils to the immune response has not been extensively studied in recent decades, especially their role in tumor immunity, in part because their half-life is considered to be too short for them to exert their important functions [7]. Within the past ten years, an increasing number of researchers have connected neutrophils with tumor immunity and immune drug resistance and made many new discoveries and important conclusions.

The development, biological characteristics, and signal transduction of cancer-associated neutrophils have been described in many existed reviews [8, 9]. Herein, based on the research progress on neutrophils in TME, we review the novel neutrophil subsets in tumors and new regulatory mechanisms underlying tumor-associated neutrophils (TANs) activation and summarize the role of neutrophils in tumor immunotherapy resistance. We also explore the potential of neutrophils as a therapeutic approach to address tumor immune resistance and discuss the deficiencies of current studies, suggesting new avenues for future investigations.

2 | THE CLASSIFICATION AND FUNCTION OF TUMOR-ASSOCIATED NEUTROPHILS IN THE TUMOR MICROENVIRONMENT

Neutrophils are a crucial component of the immune system, typically serving as the first line of defense against infections [10]. Accumulated studies suggest that neutrophils in TME have a double-edged regulatory role [11–16]. Neutrophils can exert anti-tumor effects, directly eliminating tumor cells through phagocytosis, the release of reactive oxygen species (ROS) and antibody-dependent cell-mediated cytotoxicity [17, 18]. Conversely, Neutrophils can also promote cancer cell proliferation and metastasis, and tumor angiogenesis through cytokine and chemokine secretion, neutrophil extracellular traps (NETs) formation, and intercellular interactions [16]. Moreover, neutrophils participate in various signaling pathways, such as nuclear factor kappa-B (NF-κB) [19], phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) [20], and Janus kinase (JAK)/signal transducers and activators of transcription (STAT) [21], thereby contributing to tumor-associated inflammation, growth, immune evasion, and drug resistance. Neutrophils have both challenges and opportunities in cancer treatment. Some studies indicate

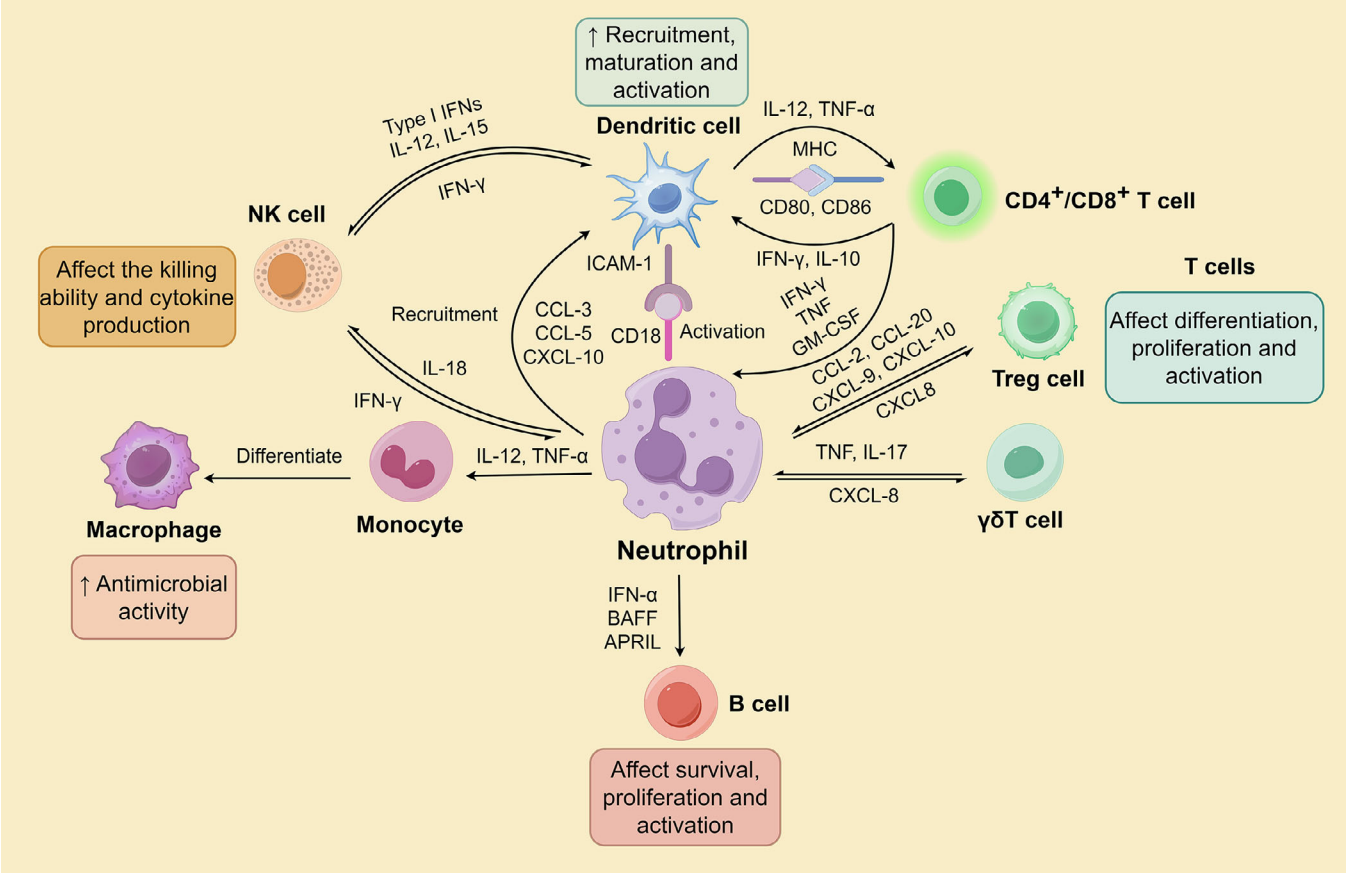

FIGURE 1 **Interactions between neutrophils and other immune cells**. Neutrophils can affect the proliferation and activity of B cells through BAFF and other mediators. IL-12 and other cytokines promote the differentiation of monocytes into macrophages and enhance their antibacterial activity. There is bidirectional regulation between neutrophils and T cells, and neutrophils can affect the activation, differentiation and proliferation of T cells. As for dendritic cells, neutrophils can not only directly affect their recruitment, activation and maturation, but also influence T cells and NK cells with dendritic cells as bridges. In addition, neutrophils can also directly affect the killing activity of NK cells and the production of cytokines through IL-18. Abbreviations: NK, natural killer; TNF, tumor necrosis factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; BAFF, B cell activating factor; APRIL, a proliferation-inducing ligand.

that TANs can reduce the efficacy of traditional therapies such as chemotherapy, radiotherapy and immunotherapy [22–24], while other studies suggest that increased neutrophil infiltration may enhance the efficacy of chemotherapy [25, 26]. Understanding and leveraging the dual roles of neutrophils in the TME could lead to more effective therapeutic strategies and improved patient outcomes.

When discussing the roles of neutrophils in cancer progression or cancer therapy, NETs formation is considered as a crucial biological process. NETs are mesh-like structures composed of DNA, histones, and antimicrobial proteins, which constitute a vital mechanism by which neutrophils regulate tumor progression [27–29]. On the one hand, various endogenous factors within the TME, such as Interleukin (IL)-8, granulocyte colony-stimulating factor (G-CSF), and other TME components can induce the formation of NETs [30–32]. On the other hand, NETs contribute to tumor progression, metastasis, and drug resistance by increasing tumor cell resistance to ferroptosis [33], promoting the epithelial–mesenchymal transition (EMT) process [19, 34], inducing angiogenesis [35], and activating cancer-associated fibroblasts [36]. Therefore, the interaction of tumor and NETs both deserves in-depth investigation. The diversity and plasticity of neutrophils contribute to their heterogeneity in the tumor microenvironment (TME) [16]. In tumors, different terms are used to depict neutrophils, such as polymorphonuclear neutrophils (PMNs), granulocytic myeloid-derived suppressor cells (G-MDSCs) or polymorphonuclear (PMN)-MDSCs, low-density neutrophils (LDNs) and tumor-associated neutrophils (TANs) [37]. These terms represent a heterogeneous group of neutrophils that are distinct but have overlapping phenotypes and functions [38]. In 2009, Fridlender *et al.* [39] categorized TANs into two phenotypes, N1 and N2, according to their polarization state. N1 TANs

exhibit an antitumor effect, while N2 TANs exhibit a protumor effect with their induction by transforming growth factor beta (TGF-β) signaling. However, the aforementioned classifications and phenotypes are not sufficient to summarize the complexity of neutrophils. Recently, Antuamwine *et al.* [40] have pointed out that the traditional N1 vs N2 paradigm is insufficient for capturing the full spectrum of neutrophil functional states in cancer and establishing a novel classification framework for neutrophils is in urgent need. Identification of novel neutrophil subsets with phenotypic markers and functional properties are essential for establishing a novel classification framework for neutrophils in cancer. With advancements in single-cell transcriptomics, new breakthroughs have led to a better understanding of the classification and phenotype of tumor neutrophils. Among different types of tumors, neutrophils have new and complex classifications that go beyond the traditional dichotomy. Currently, single-cell RNA sequencing studies have analyzed TANs in various tumor types, including lung cancer [41–45], liver cancer [46, 47], gastric cancer [48, 49], pancreatic ductal adenocarcinoma [50–52], breast cancer [53], melanoma [54] and brain-related tumors [55, 56]. Based on differential gene and marker expression profiles, TANs in these tumors were divided into different clusters. Each cluster had unique transcription profiles. They co-existed in the TME, with certain clusters associated with specific functions (Table 1), revealing the great phenotypic diversity and plasticity of TANs. Recently, based on the neutrophil single-cell transcriptome of 17 cancers, including liver cancer, bile duct cancer, and gallbladder cancer, a pan-cancer study systematically revealed the phenotype of neutrophils and revealed that human leukocyte antigen-DR$^+$ leukocyte differentiation antigen 74$^+$ neutrophils are positively correlated with patient prognosis in multiple cancers; they can induce T cell antigen-specific responses and promote the formation of a "hot tumor" microenvironment [57]. In addition, emerging studies have showed the heterogeneity of neutrophils during immunotherapy and suggested an immunosuppressive neutrophil phenotype [58, 59]. These studies showed that neutrophils in TME are diverse and heterogeneous, providing novel perspectives and insights to better understand the function of tumor-associated neutrophils and the mechanism of tumor immunology. However, the classification and phenotypic analysis of neutrophils based on surface markers using single-cell transcriptomics are complicated and challenging. Due to the diversity and tissue heterogeneity of TANs, a unified standard has not yet been reached and necessitates further research.

3 | NOVEL PROGRESS ON REGULATORY MECHANISMS OF TAN ACTIVATION AND FUNCTION

TANs are plastic and undergo adaptive changes in the TME, manifesting as changes in quantity and quality [12]. Tumor cells, immune cells, fibroblasts, and endothelial cells in the TME can secrete neutrophil chemotaxis-associated chemokines such as G-CSF, C-X-C motif chemokine receptor (CXCR) 2, and IL-8 to promote the activation and recruitment of neutrophils, resulting in changes in the number of tumor-associated neutrophils. Additionally, soluble factors derived from the TME, such as interferon beta (IFN-β) and TGF-β, affect the polarized phenotype of neutrophils, resulting in functional changes in neutrophils [12, 60]. Recent studies have increasingly demonstrated that, alongside the various factors released by the tumor microenvironment (TME) that impact neutrophils, cancer metabolism, tumor microbiota, neuroendocrine mediators, and tumor treatment can also regulate TANs (Figure 2).

3.1 | Cancer metabolism

A notable characteristic of tumor cell metabolism is its capacity to extract vital nutrients from environments that lack sufficient resources, utilizing these nutrients to sustain cell survival and generate new biomass [61]. The metabolic reprogramming associated with tumors can lead to alterations in both intracellular and extracellular metabolites, significantly influencing gene expression, cellular differentiation, and the TME [62]. The byproducts generated from tumor metabolism not only serve as the basis for material and energy production but also play a role in regulating gene and protein expression, thus mediate metabolic cross-talk with cells in the surrounding environment, affecting the behavior of non-transformed cells, including neutrophils [63].

Abnormal glucose metabolism in tumors can change the degree of neutrophil infiltration. The TME becomes acidic in pH due to increased glycolysis and H^+ and lactate secretion under hypoxic conditions. These acidic extracellular conditions can affect the metabolism in human cancer cell lines and xenograft model, leading to accumulation of N1-acetylspermidine in the polyamine pathway by upregulating spermidine/spermine acetyltransferase 1 (SAT1) expression, thereby stimulating the expression of inflammatory cytokines and thus recruiting neutrophils to infiltrate into tumors and promote tumor growth

TABLE 1 Overview of neutrophil heterogeneity revealed by single-cell RNA sequencing in human solid tumors.

Tumor type	Reference	Neutrophil subset	Phenotypic markers	Functional properties
Pan-cancer	[57]	$ARG1^+$	ARG1, CD163, H3-3A	Arginine & proline metabolism; systemic lupus erythematosus
		$CXCR2^+$	CXCR2, CXCR1, P2RY13	Chemokine signaling pathway; FcγR mediated phagocytosis
		$CXCL8^+IL1B^+$	CXCL8, IL1B, FRMD4B	Inflammatory response; Leishmania infection
		$HLA\text{-}DR^+CD74^+$	HLA-DRA, CD74, HLA-DQB1	Antigen processing and presentation; cell adhesion molecules
		$IFIT1^+ISG15^+$	IFIT1, ISG15, IFIT2	IFNγ response; IFNα response
		$MMP9^+$	MMP9, CD177, FCN1	Focal adhesion, hematopoietic cell lineage
		$NFKBIZ^+HIF1A^+$	NFKBIZ, HIF1A, MAPK6	TNFα signaling via NF-κB
		$S100A12^+$	S100A12, S100A6, S100A4	Leukocyte transendothelial migration; reactive oxygen species pathway
		$TXNIP^+$	TXNIP, MALAT1, IFITM2	Complement, hypoxia
		$VEGFA^+SPP1^+$	VEGFA, SPP1, CCL3	IL2 STAT5 signaling; angiogenesis
Lung cancer	[42]	TAN-1	IL1RN, CD44, RIPK2	Regulation of cell recruitment and adhesion
		TAN-2	HLA-DRA, CD74, HLA-DMB, HLA-DRB1	Immunogenic antigen-presenting
		TAN-3	C15orf48, CCL3, CCL4, CSTB, LGALS3	Neutrophil activation and emigration
	[43]	N1	S100A8, S100A9	A cluster of mature neutrophils
		N2	PLIN2, LRPAP1; VEGFA	Lipid metabolism; promoting angiogenesis and endothelial cell growth
		N3	CCL3, CCL4, CCL20, CCL3L1, CCL4L2, CXCL8, and CXCL2	Regulating the status of TANs and promoting tumor growth
		N4, N6	HSP90AB1, HSP90AA1, HSPA1A, HSPH1, CTSB, MMP12	Synthesis and/or decomposition of intra/extracellular proteins
		N5	ISG15, IFIT3, IFIT2, IFI6, RSAD2	Regulation of innate immune responses to viral infections
		N7	TDG, CDKI2, PRDXI, NOL8, DDX18, TRMT112, NARS	DNA repair process; RNA synthesis process; biological processes of cell growth
	[45]	N1	MMP8, MMP9, S100A8, S100A9, ADAM8	Canonical neutrophil
		N2	IFIT1, IRF7, RSAD2	Type I interferon response
		N3	CXCR2, JAML, NCOA1, EGLN1, CASS4	Transition subsets
		N4	NR4A2, CTSC, SERPINB9, SIGLEC10	
		N5	CCL3, CSF1, CSTB, CTSB, IRAK2	Tumor-specific and promote tumor growth
	[59]	Neu_OSM	$CD16^+CXCR2^{high}CXCR4^{low}$; OSM	Mature neutrophils; promotion of proinflammatory response; neutrophil activation, degranulation, and chemotaxis
		Neu_S100A12	$CD16^+CXCR2^{high}CXCR4^{low}$; S100A8, S100A9, S100A12	
		Neu_CCL3	$CD16^+CXCR2^{low}CXCR4^{high}$; CCL3, CCL4, CXCL8	Aged neutrophils; an immunomodulatory phenotype; interferon signaling, translational initiation, and response to interleukin-
		Neu_IFIT3	$CD16^+CXCR2^{low}CXCR4^{high}$; IFIT1-3, RSAD2, MX1, CD274, IDO1	
	[229]	TAN-0	HLA-DQB1, HLA-DPB1, CD1E, CD1C, FCER1A, HLA-DMB	Up-regulation of AP pathway activity
		TAN-1	TNFRSF9, IL15, BIRC3, FSCN1, CCL22, CCL19, CCL17, GBP1	IL-2 and IFN-γ-mediated signaling pathways with immunostimulating competence
		TAN-2	IL10, APOE, CXCL2, LGALS3, C5AR1, CSTB	Glycerolipid catabolic Process and positively modulating angiogenesis and macrophage proliferation
		TAN-3	S100A8, S100A9, THBS1, FCN1, CEBPB, S100A4	Regulating macrophage proliferation
		TAN-4	CXCR3, CDH1, LILRA4, PLD4, PLAC8	

(Continues)

TABLE 1 (Continued)

Tumor type	Reference	Neutrophil subset	Phenotypic markers	Functional properties
Liver cancer	[47]	Neu_09	IFIT1	Suppressing cytotoxic $CD8^+$ T cells
		Neu_11	CCL4	Recruiting macrophages
	[58]	NEU1	CD10, ALPL, CST7, IQGAP2, JAML, CD170	Expression of numerous immunosuppression-related molecules; immature; tumor-promoting
		NEU3, 4, 5, 6	IFIT2, CD74, SECTM1	Features of neutrophil activation and antitumor immunity
	[46]	Neu_HSPA1A	HSPA1A	Increased in tumor; a developmental differentiation terminal cluster
		Neu_TUBA4A	TUBA4A	Increased in tumor
		Neu_HOPX	HOPX	Starting point for neutrophil development
		Neu_AIF1	AIF1	Extensive communication with TME; promoting tumor progression
Gastric cancer	[49]	tsNeu3	$CD11b^+CD66b^+CD10^-CD54^+$	Negatively correlated with lymphatic invasion, lymph node metastasis, tumor size and tumor stage
		tsNeu4	$CD11b^+CD66b^+CD101^+CD54^+$	
	[48]	N0	LYZ	Paneth-like cells
		N1	PD-L1	Activated by tumor-derived GM-CF
		N2	CXCR4	Pro-angiogenic and pro-invasive
		N5	PLCG2	Recruited to the ovary metastasis site and inhibit tumor progression
	[230]	NC0	PPIF, PLAU, HCAR2, PLEK, IL1B, ATP2B1-AS1, IL1RN, CMTM2	Response to lipopolysaccharide and positive regulation of inflammatory response
		NC2	RPS29, RPL32, RPL13, RPS23, RPS12, RPS6, RPL10, RPL34, RPS8, RPL11	Cytoplasmic translation, ribosome biogenesis and oxidative; lower differentiated and more mature phosphorylation
Pancreatic ductal adenocarcinoma	[51]	TAN-1	VEGFA, PLAU, LGALS3	Beneficial for tumor progression; hyperactivated glycolytic activity
		TAN-2	NLRP3, PDE4B, CD69, IL1RN, ADM	An inflammatory subpopulation
		TAN-3	VNN2, SELL	Transitional stage from PMNs to TANs
		TAN-4	IFIT1, IFIT2, IFIT3, ISG15, RSAD2	Preferentially expressing interferon-stimulated genes
PDAC liver metastases	[52]	$S100A8^+$ neutrophils	IFITM2, S100A8, NAMPT, CXCR4, SRGN, CXCL8	Linked to the metastatic progression of PDAC
Breast cancer	[231]	N0	IFITM2, S100A9, CXCR2, XPO6, GCA, MME, RNF24, LIMK2, S100A12, S100P	Representing G-MDSCs
Melanoma brain metastases	[54]	IL-8 high neutrophils	CXCL8, VEGFA	Related to angiogenesis and EMT
		IFN-responsive neutrophils	IFI6, IFIT2, ISG15, TAP1	-
		calprotectin high neutrophils	S100A8, S100A9	Associated with NETosis
Glioma and lung cancer brain metastases	[56]	PMN-MDSCs	AGE1, CSTA	-
		mature neutrophils	CXCR2, MXD1	-
		activated neutrophils	ITGB2, ITGAM	-
		ROS-producing neutrophils	LDHA, VEGFR	Participation in tumor angiogenesis
		degranulated neutrophils	HSP90AA1, HSPA8, HSPA1A	Activation of antigen processing and presentation

Abbreviations: AP, antigen presentation; EMT, epithelial-mesenchymal transition; FcγR, Fcγ receptor; GM-CSF, granulocyte-macrophage colony-stimulating factor; G-MDSCs, granulocytic myeloid-derived suppressor cells; PDAC, pancreatic ductal adenocarcinoma; PMNs, polymorphonuclear leucocytes; ROS, reactive oxygen species; TANs, tumor-associated neutrophils; TME, tumor microenvironment.

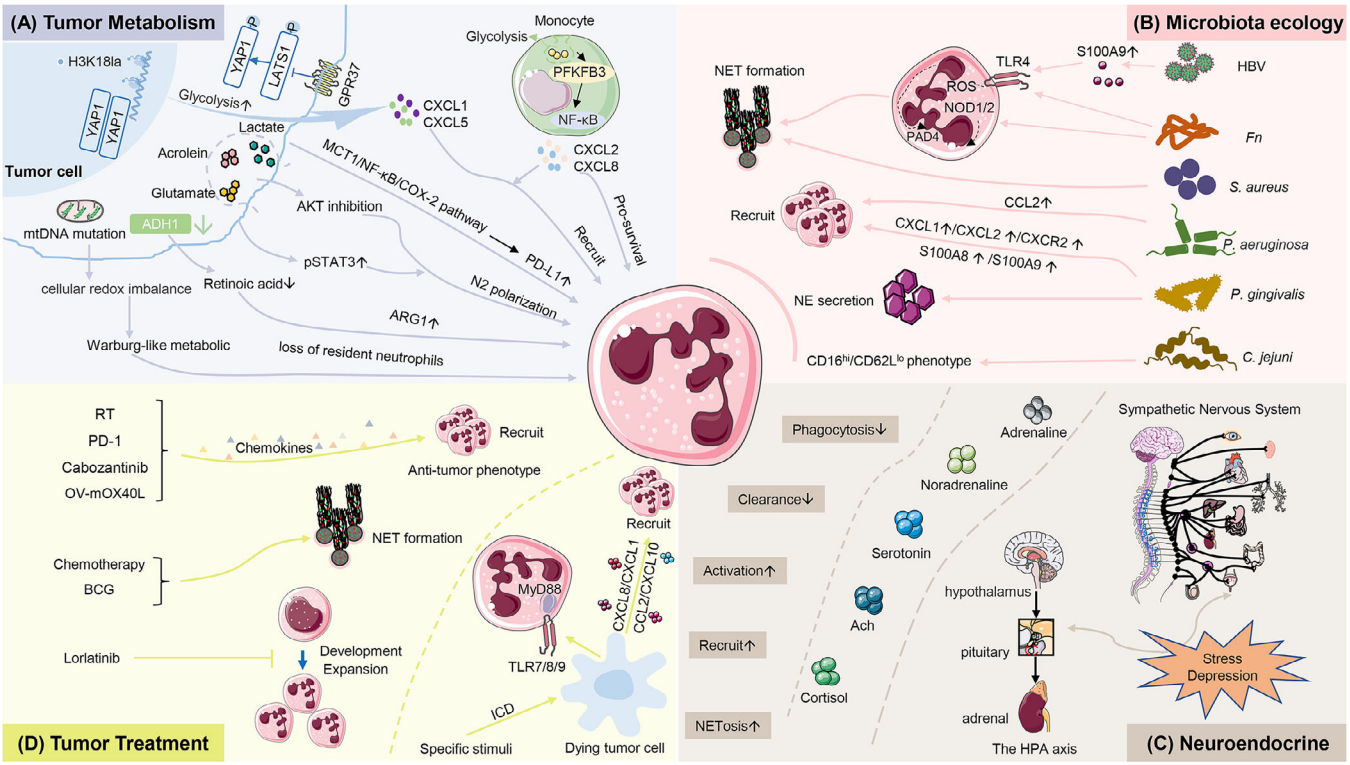

FIGURE 2 **Novel progress has been made in the research on the regulation of tumor-associated neutrophils**. (A) The tumor metabolism and certain intermediate metabolites regulate the secretion of crucial downstream molecules or cytokines through multiple signaling pathways, thereby influencing the infiltration, polarization, and expression of immunosuppressive signals in neutrophils. (B) The microbial ecology within tumors can directly induce neutrophils to secrete NE or form NETs, as well as cause changes in neutrophil phenotype. Furthermore, it can recruit neutrophils and enhance NETs formation through the secretion of inflammatory factors or via TLR4-ROS signal transduction. (C) The release of various neuroendocrine hormones, including adrenaline, noradrenaline, serotonin, ach, and cortisol, can impair the phagocytic and clearance abilities of neutrophils, while also facilitating the recruitment and activation of neutrophils. (D) Tumor treatment, either independently or through the induction of chemotactic factor release, can impact the quantity, phenotype, and NETs formation of neutrophils. Moreover, certain therapies can induce the recruitment and chemotaxis of neutrophils by affecting the immunogenic cell death of tumor cells, leading to the release of chemotactic factors. Abbreviations: NE, neutrophil elastase; NETs, neutrophil extracellular traps; TLR4, Toll-like receptor 4; ROS, Reactive oxygen species; mtDNA, mitochondrial DNA; HBV, Hepatitis B virus; *Fn*, *Fusobacterium nucleatum*; *S. aureus*, *Staphylococcus aureus*; *P. aeruginosa*, *Pseudomonas aeruginosa*; *P. gingivalis*, *Porphyromonas gingivalis*; *C. jejuni*, *Campylobacter jejuni*; HPA, hypothalamus-pituitary-adrenal; Ach, acetylcholine; RT, radiotherapy; OV, oncolytic virus; BCG, bacillus Calmette-Guerin; ICD, immunogenic cell death.

[64]. Hexokinase 3 (HK3) is a key enzyme in glycolytic pathway. Bioinformatics analysis and immunostaining of tumors from a mouse xenograft model showed that HK3 expression could increase the infiltration of neutrophils in gliomas [65]. G protein-coupled receptor 37 expression in tumor cells can activate the Hippo pathway to promote glycolysis in colorectal cancer (CRC) cells, thereby further promoting H3K18la lactylation and leading to neutrophil infiltration [66]. Monocytes in human hepatocellular carcinoma (HCC) preferentially perform aerobic glycolysis, which induces the expression of C-X-C motif chemokine ligand (CXCL) 8 and CXCL3 via the Phosphofructo-2-Kinase/Fructose-2, 6-Biphosphatase 2 (PFKFB2)/NF-κB pathway, and thereby recruits neutrophils. The accumulated neutrophils were then induced to produce pro-metastatic factor Oncostatin-M (OSM) to promote HCC metastasis both ex vivo and in vivo [67]. These findings highlight a critical link between tumor glucose metabolism and neutrophil infiltration, underscoring the profound impact of metabolic alterations on TME. Abnormal glucose metabolism, driven by heightened glycolysis and resultant acidification of the TME, significantly influences neutrophil dynamics within tumors.

In addition to glucose metabolism, nitrogen metabolism and some metabolic intermediates also have regulatory effects on neutrophils. Tumor cells systemically change their intracellular amino acid metabolism and extracellular amino acid distribution to meet their proliferative demands, resulting in metabolic reprogramming and TME remodeling. Glutamate released by tumor cells can

promote the phosphorylation of STAT3 in neutrophils converting them from a tumor-killing phenotype to an immunosuppressive phenotype both ex vivo and in vivo, considered as a switch from the N1 to N2 phenotype [68]. Tumor-derived lactate in patient samples, subcutaneous or orthotopic mouse models induces programmed death ligand 1 (PD-L1) expression on neutrophils through the Monocarboxylate transporter 1 (MCT1)/NF-κB/Cyclooxygenase-2 (COX-2) pathway, thereby inhibiting T cell toxicity [69]. Compared with those in adjacent colon tissues, expression of the genes and enzymes related to retinol metabolism were shown to be significantly reduced in human CRC tissues, and the tumors exhibited defects in retinoic acid (RA) synthesis. An alcohol dehydrogenase 1 (ADH1)-mediated reduction in retinoic acid was shown in cell lines and mouse CRC models to promote the production and inhibitory ability of PMN-MDSCs (reduced retinol metabolism was associated with weakened RA signaling and the accumulation of PMN-MDSCs in CRC tumors) [70]. Under hypoxia, acrolein produced in glioma cells inhibits neutrophil activation and induces neutrophil polarization to the N2 phenotype to produce Arginase-1 (ARG-1) in vitro, which promotes the progression of glioma by directly reacting with the Cys310 residue of AKT to inhibit AKT activity [71]. Furthermore, metabolic abnormalities in TANs also can influence tumor development and treatment [23, 72]. As for lipid metabolism, lipid homeostasis within neutrophils significantly impacts their development, infection response, and cancer metastasis [73, 74]. However, the influence of cancer cell lipid metabolism on neutrophils requires further investigation. Collectively, these findings underscore the pivotal role of metabolic intermediates in shaping neutrophil phenotype and function within the TME.

In summary, tumor-associated metabolic changes, particularly those related to glycolysis, orchestrate neutrophil infiltration and subsequent phenotype transformation. This comprehensive understanding of the interplay between tumor metabolism and neutrophil dynamics not only enhances our grasp of tumor biology but also opens new avenues for therapeutic strategies aimed at disrupting these maladaptive interactions in cancer.

3.2 | Tumor microbiota

Neutrophils are an important part of innate immunity. They are influenced by microbiota and their metabolites and participate in the control and regulation of microbiota. Microbiota and their metabolites can form a self-interest microenvironment by regulating the chemotaxis, activation and function of neutrophils. In recent years, it has been reported that the presence of microbiota in tumors can also regulate the activity and function of neutrophils, thereby affecting tumor growth and metastasis. One study compared neutrophil activity between gastric cancer patients without *Helicobacter pylori* infection and gastric cancer patients infected with *Helicobacter pylori* [75], and the results suggested an impact of tumor-associated microorganisms on neutrophils plasticity. *Fusobacterium nucleatum* (*Fn*) induces the production of large amounts of NETs by activating Toll-like receptor 4 (TLR4)-ROS and nucleotide binding oligomerization domain containing (NOD) 1/2 signaling in CRC neutrophils, thereby accelerating CRC growth and promoting CRC metastasis through angiogenesis, EMT-related migration, CRC cell capture and other mechanisms both in vitro and in vivo [76]. Chronic bacterial infection in the lungs increased the expression of C-C motif chemokine ligand (CCL) 2 in the lungs and subsequent recruitment of $MHCII^{hi}$ neutrophils with tumor-promoting effects into lung tissue, thereby promoting the metastasis of breast cancer cells to lung and facilitating their colonization in a spontaneous metastasis mouse model [77]. Furthermore, in the microenvironment of breast cancer lung metastasis, *Staphylococcus aureus* can recruit neutrophil infiltration and trigger autophagy-dependent NET formation in vitro and in vivo, thereby capturing circulating cancer cells and promoting new metastasis [78]. In *Porphyromonas gingivalis*-gavaged orthotopic pancreatic cancer (PC) model, *Porphyromonas gingivalis* promotes the progression of PC by increasing the release of neutrophil chemokines and the recruitment of neutrophil to form a proinflammatory TME and the secretion of neutrophil elastase (NE) [79]. *Campylobacter jejuni* induces human neutrophils to differentiate into the $CD16^{hi}/CD62L^{lo}$ subtype, delays apoptosis, increases arginase-1 expression, and increases ROS generation in vitro, resulting in the upregulation of tumor-promoting gene expression in CRC cells [80]. Hepatitis B virus (HBV)-induced S100 calcium-binding protein A9 (S100A9) activates TLR4/the receptor of advanced glycation end products (RAGE)-ROS signaling both in vitro and in vivo experiments, resulting in the formation of a large amount of NETs, thereby promoting HCC growth and metastasis [81]. The above findings show that tumor microbiota can recruit neutrophils and activate neutrophils to secrete NETs or NE to promote tumor progression. However, a recent study utilizing mouse models showed that the intratumor injection of microorganisms can lead to rapid and drastic alterations in the immune composition of tumors, especially a significant increase in activated neutrophils. Activated TANs transform into highly mobile neutrophils, aggregate to form neutrophil clusters, remodel the TME and exhibit a cytotoxic phenotype, inhibiting tumor growth [82]. Additionally, TANs can inhibit cancer progression by restricting bacterial burden

[83]. Moreover, some studies indicate that tumor microbial burden may modulate the tumor immune microenvironment, particularly T cell infiltration [84, 85]. However, research on the direct influence of the bacterial load on neutrophil regulation within tumors remains in its early stages. At present, although it is widely recognized that tumor microbiota affects tumor behavior through the regulation of neutrophils, the tumor-promoting/inhibiting effects and mechanisms are still unclear, and further study is still needed.

3.3 | Neuroendocrine system

The systemic environment of tumor patients is a complete ecosystem. The interaction between tumor cells and other cellular or noncellular components in the host environment is not limited to the local TME but also includes other distant organs, as well as the immune and neuroendocrine systems [86].

The accumulation and function of neutrophils are regulated by various hormones of the neuroendocrine system. Melatonin protects neutrophils from oxidative stress-triggered apoptosis by reducing ROS production and restores neutrophils function like phagocytosis, degranulation, and NETs in glutathione (GSH) and glutathione reductase (GR) activity-deficient neutrophils through regulating ROS levels both in vitro and in vivo [87]. The neuroendocrine system can secrete various hormones to regulate the accumulation and function of neutrophils. β-Endorphin released during stress may act on endothelial cells and stimulate neutrophils to migrate from the vasculature in vitro [88]. Furthermore, gastrin-releasing peptide (GRP) has selective effects on neutrophils in vivo and induces their migration through the activation of gastrin-releasing peptide receptor (GRPR) [89]. These findings highlight the intricate interplay between the neuroendocrine system and neutrophil function, offering new insights into how hormonal regulation can impact immune responses.

Recent studies have shown that neuroendocrine hormones can affect tumors through modulating neutrophil activity. The stress response pathway transmits signals from the brain through the sympathetic nervous system (SNS) and/or the hypothalamus-pituitary-adrenal (HPA) axis. When the SNS is activated, it triggers the release of hormones such as adrenaline and the neurotransmitter norepinephrine (from nerve endings of different organs). Stress can increase granulopoiesis and neutrophil efflux from the bone marrow to the periphery, as well as reduce the neutrophil clearance rate in mice, which can rapidly lead to neutrophilia, especially in senescent $CD62L^{lo}CXCR4^{hi}$ neutrophils [90]. In chronic stress responses and depression, the persistent activation of the HPA axis and the sympathetic-adrenal-medullary axis impairs the immune response, including high concentrations of circulating neutrophils and weakened neutrophil phagocytosis, resulting in the progression of various tumors from experimental animal models, human studies, and clinical evidence [91]. Stress hormones such as adrenaline, norepinephrine, cortisol and serotonin cause the rapid release of S100A8/A9 from neutrophils in mouse models, which mediates the reactivation of dormant tumor cells and promotes cancer recurrence [92]. Glucocorticoids induce mammalian target of rapamycin (mTOR) signaling in epithelial cells to recruit and activate innate immune cells, including neutrophils, thereby promoting the onset and progression of acute ulcerative colitis and colitis-associated cancers in mice [93]. In the lungs of patients with breast cancer, chronic stress induces nonepithelial cells to produce Ach to remodel lung immune cell subsets with striking increase of neutrophils, enhance NETs in lung and promote NETotic neutrophils to capture cancer cells to form premetastatic niches [94].

In conclusion, changes in the neuroendocrine system caused by systemic factors such as chronic stress and psychological stress can affect the infiltration, activation and migration of neutrophils through related hormones, thereby regulating neutrophil-mediated tumor innate immunity and promoting tumor progression processes.

3.4 | Cancer treatment

In 2019, Shaul *et al.* [9] reported that the mechanisms of conventional cancer treatment, such as chemotherapy and immunotherapy, may regulate the phenotype or function of tumor-associated neutrophils, providing potential targets for combination therapy. Recent studies have continuously revealed a new understanding of the regulation of neutrophils in tumor treatment.

Traditional treatment methods such as chemotherapy and radiotherapy have important effects on the phenotype and function of neutrophils [95–97]. For example, chemotherapy can promote neutrophil infiltration and the formation of NETs in vitro and in vivo, thereby activating the TGF-β signaling in tumor cells and inducing the EMT and chemotherapy resistance [96]. Radiotherapy (RT) recruits neutrophils and polarizes them into an antitumor phenotype through the release of CXCL1, CXCL2 and CCL5 and induces the mesenchymal-epithelial transition through inhibition of the ROS-mediated PI3K/Akt/Snail pathway, thereby improving antitumor immunity [95]. Conversely, in murine breast cancer, the composition and spatial distribution of neutrophil altered after RT.

Radiotherapy significantly reduced the abundance of neutrophils and decreased NETs level in serum, suggesting that RT can modulate neutrophil infiltration and their function to enhance therapeutic outcomes [98]. RT modulates the recruitment and function of neutrophils to impact tumor progression and the therapeutic response. Conventional therapies have dual roles in modifying neutrophil function, suggesting that therapeutic strategies aimed at optimizing neutrophil responses could enhance treatment efficacy and overcome resistance mechanisms in cancer treatment.

Targeted therapies, such as tyrosine kinase inhibitors, have become the first-line drugs for some tumors. In pre-clinical mouse models of pancreatic ductal adenocarcinoma (PDAC), lorlatinib inhibits neutrophil-induced tumor growth by inhibiting the development and release of neutrophils from bone marrow [99]. However, in HCC and renal cell carcinoma (RCC), cabozantinib treatment upregulates the expression of factors associated with neutrophil chemotaxis and migration, thereby increasing the intratumor infiltration and antitumor function of neutrophils [100, 101]. The differences may be related to the heterogeneity of neutrophils in tumors. The impact of targeted therapies on neutrophil dynamics suggests that tumor-specific factors and neutrophil heterogeneity may trigger differential responses. Understanding these nuances is crucial for optimizing therapeutic strategies and improving clinical outcomes, as the efficacy of targeted therapies may vary depending on the tumor type and the distinct role of neutrophils within the tumor microenvironment.

Immunotherapy can also regulate tumor neutrophils. Immune checkpoint inhibitors are currently important means of immunotherapy. In liver cancer and gastric cancer, anti-programmed death receptor 1(PD-1) therapy increases the infiltration of neutrophils into the TME and induces a phenotypic shift toward an antitumor state [101, 102]. In an orthotopic Kras-Lox-STOP-Lox-G12D/p53 flox/flox (KP)-based lung adenocarcinoma mouse model, anti-CD40 treatment caused intratumoral expanded neutrophils to be in the $Sell^{hi}$ state, with expression of cytotoxicity-related genes and a large amount of interferon-stimulated genes (ISGs) in this cell population. This finding suggested that anti-CD40 immunotherapy can induce neutrophils to develop into an anticancer phenotype [103]. Other immunotherapies, such as vaccines and oncolytic viruses, can also regulate neutrophil function. For example, the bacillus Calmette-Guerin (BCG) vaccine serves as a treatment for bladder cancer by promoting the development of neutrophil extracellular traps (NETs), demonstrating cytotoxic effects, and triggering both apoptosis and cell cycle arrest, which collectively impede the movement of bladder tumor cells [104]. Mouse OX40L was inserted into herpes simplex virus-1 (HSV-1) to construct an oncolytic virus (OV)-mOX40L oncolytic virus, which can induce neutrophil infiltration and increase antigen processing and presentation, cell killing, phagocytosis and the response to viral signaling pathways, reprogramming neutrophils to a more proinflammatory antitumor state [105]. These findings underline the versatility of immunotherapies in reprogramming neutrophils to support antitumor responses, highlighting their potential to improve therapeutic outcomes through tailored modulation of the immune landscape.

In addition, other treatments can also regulate neutrophil-associated antitumor immune responses. Androgen receptor (AR) inhibition suppresses the neutrophil antitumor immune response through TGF-β receptor I (TβRI). TβRI can be utilized as a possible target for enhancing neutrophil antitumor response during ADT (second-generation androgen deprivation therapy) [106]. In transplantable and autochthonous murine tumor models, nanoscale metal-organic framework (nMOF) - radiotherapy-radiodynamic therapy (RT-RDT) notably increases the infiltration of $CD11b^{+}Ly6G^{+}CD11c^{+}$ hybrid neutrophils and the expression of co-stimulatory molecules CD80 and CD86, along with major histocompatibility complex class (MHC) II molecules, thereby reprogramming tumor-associated neutrophils to serve as non-classical antigen-presenting cells and remodeling the TME toward an anti-tumor immune status [107]. The combining traditional and innovative therapies have the potential to modulate neutrophil functions and enhance antitumor immune responses, paving the way for more effective and tailored cancer treatments.

Immunogenic cell death refers to the process by which tumor cells undergo cell death in response to external stimuli and then turn from nonimmunogenic to immunogenic, mediating the collective generation of an antitumor immune response. In addition to the direct regulation of neutrophils by tumor treatment, specific treatment modalities, such as chemotherapeutic drugs, specific forms of radiotherapy and photodynamic therapy, can induce tumor cell immunogenic cell death (ICD) [108, 109], leading to the secretion of some chemokines, and the mobilization and activation of neutrophils to further kill tumor cells or improve the immune microenvironment. One study revealed that the treatment effect significantly improved after ICD induction chemotherapy combined with Herpes simplex virus type 1 (HSV-1) oncolytic virus therapy in mice. Immune cell infiltration analysis revealed that neutrophil infiltration in tumors significantly increased [110]. In addition, ICD caused by immunogenic treatment can lead to the secretion of CXCL8 to induce tumor inflammation, triggering the strong recruitment and chemotaxis of neutrophils [111]. Immunogenic dying tumor cells trigger the pathogen response-like chemokine

(PARC) signature, which is characterized by the corelease of CXCL1, CCL2 and CXCL10. This PARC signature preferentially recruits neutrophils. In addition, the key danger signals sent by these dying cells stimulate the interaction of purinergic receptors and TLR7/8/9-myeloid differentiation primary response protein 88 (MyD88) signaling to induce neutrophil activation, which eventually leads to H_2O_2- and NO-driven respiratory burst-mediated killing of live residual cancer cells [112]. Ferroptosis is a type of immunogenic cell death. Cisplatin can induce ferroptosis in tumor cells and further activate neutrophils to secrete CXCL9, CXCL10 and CXCL11 to promote T cell infiltration and enhance Th1 differentiation through the secretion of IL12A and IL12B in the TME, thereby remodeling "cold" tumors into "hot" tumors [113]. These findings highlight the potential of leveraging ICD and associated neutrophil responses to improve cancer immunotherapy outcomes, by not only enhancing direct tumor killing but also by remodeling the tumor microenvironment to better support immune-mediated tumor eradication.

In summary, important progress has been made in the study of the regulation of neutrophil phenotype and function by tumor treatment. These studies not only contribute to a more in-depth understanding of the function and mechanism of neutrophils in tumor immunity but also provide novel ideas and directions for the development of more effective tumor treatment methods.

4 | EFFECT AND MECHANISM OF NEUTROPHILS ON DRUG RESISTANCE TO TUMOR IMMUNOTHERAPY

4.1 | Neutrophils affect drug resistance by promoting an immunosuppressive microenvironment

The tolerance of tumors to immunotherapy is closely related to the formation of an immunosuppressive TME. Neutrophils play important roles (mediator release and surface immune checkpoint molecules) through interactions with immune cells (Figure 3), cytokines and chemokines in TME and the formation of NETs.

4.1.1 | Mediators released by neutrophils

From the perspective of endogenous factors, neutrophils can produce a number of mediators, such as thrombospondin-1 (THBS1), ROS, ARG1, and OSM, that inhibit the activation of tumor killer cells (T cells and NK cells) and promote their dysfunction (Figure 3). Pathologically activated neutrophils, such as PMN-MDSCs that express CD300, are key immunosuppressive factors that are necessary for the inhibition of T cell activation and tumor immune resistance [114]. Studies in mice have shown that this subgroup of neutrophils can also induce cytotoxic T cell exhaustion and CRC resistance to immune checkpoint inhibitors (ICIs) through THBS1 [115]. in vivo and in vitro studies of tumor patient-derived samples show that OSM released by TANs activates intracellular signal transducer and activator of transcription 3 (STAT3) signal transduction in the TME, upregulating the expression of transcription factor forkhead box protein 3 (FOXP3), further inhibiting the maturation of DCs and the activation of naive T cells and weakening the antitumor immune response of the body [38, 39]. TANs are recruited by the chemotaxis of tumor-derived CXCL5 and hinder the antitumor activity of CD8+ T cells. This type of neutrophil depletion promotes the proliferation of tumor-specific CD8+ T cells, facilitates their maturation into effector cells, and enables them to effectively eliminate tumor cells. Blocking TAN infiltration can overcome tumor resistance to ICIs. [116] In addition to the unidirectional action of neutrophils on T cells, T cells can also promote neutrophils to form an immunosuppressive microenvironment, which is also one of the main mechanisms underlying the immune drug resistance in tumors. Animal studies revealed that tumor-derived IL-1β activates $\gamma\delta$ T cells to produce IL-17. IL-17-producing $\gamma\delta$ T cells can promote G-CSF expression in breast tumors, resulting in changes in the neutrophil phenotype described above; these neutrophils can in turn produce inducible nitric oxide synthase (iNOS) and inhibit the activity of antitumor $CD8^+$ T cells, thereafter facilitating the formation of an immunosuppressive microenvironment in breast cancer [117].

Like $CD8^+$ T cells, NK cells could identify and kill tumor cells, and their reduction in number and functional inhibition are also related to tumor immune escape. Mouse studies have demonstrated that PMN-MDSCs inhibit NK cell infiltration, activation and adoptive immunotherapy effects through TGF-β, NO and H_2O_2 [118]. in vitro studies using human peripheral blood cells show that ARG1 released by TANs suppresses the ability of NK cells to produce antitumor factors such as IFN-γ [119].

Although most studies have proposed that neutrophils promote the formation of the immunosuppressive microenvironment through the inhibition of immune cell function, some studies have put forward a different point of view. For example, under IFN stimulation, neutrophils with high Ly6E expression participate in anti-PD-1 therapy through the stimulator of interferon genes (STING) signaling pathway and activate T cell cytotoxicity by secreting IL-12. Moreover, this type of neutrophil subset can predict the effectiveness of anti-PD-1 therapy [120]. This may be related to the phenotype of the neutrophils

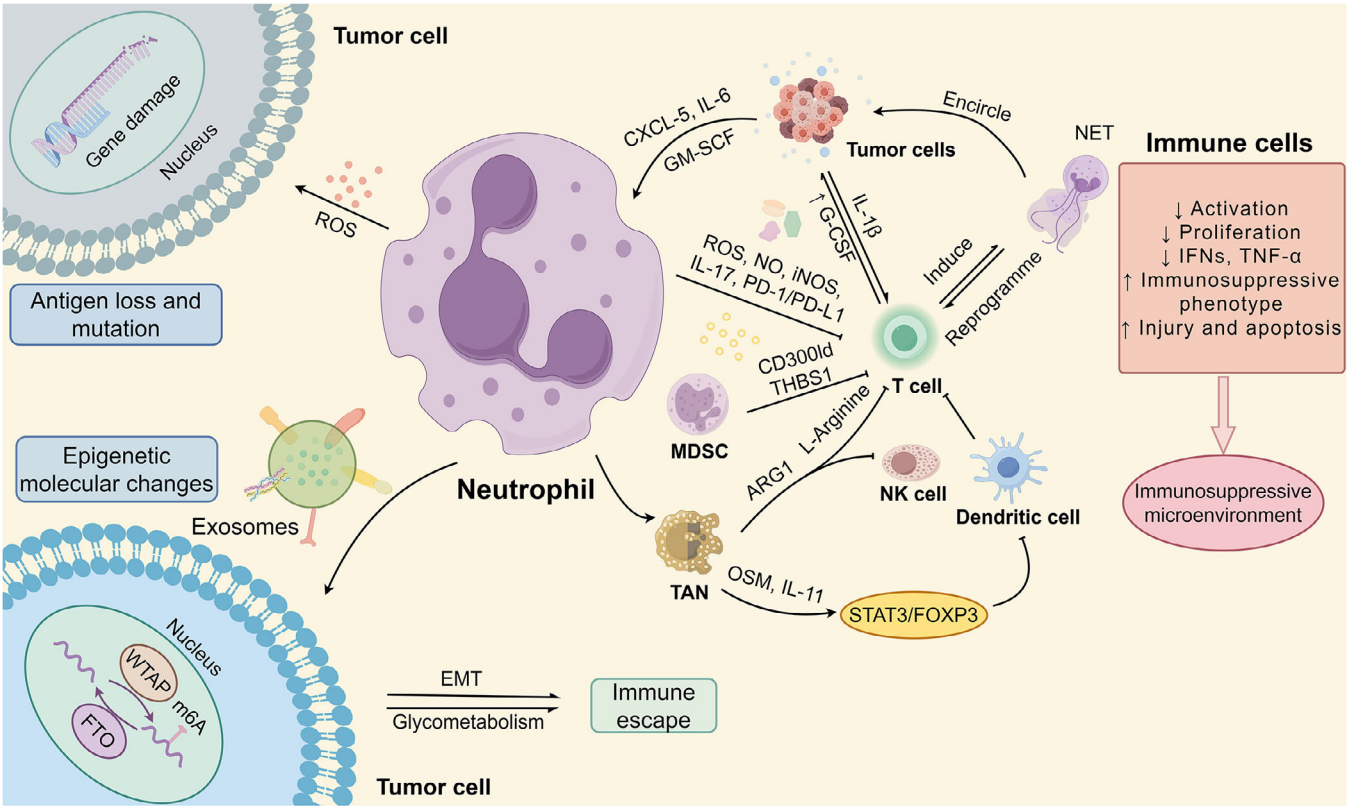

FIGURE 3 **Mechanisms of neutrophil-mediated tumor immunotherapy resistance**. From the aspect of promoting the formation of immunosuppressive microenvironment, neutrophils mainly inhibit the activation and proliferation of immune cells through some cytokines and signaling pathways, and promote their injury, apoptosis or transformation to immunosuppressive phenotype. From the aspect of affecting tumor antigens, neutrophils can damage tumor cell DNA by producing ROS, resulting in tumor antigen loss or mutation. In addition, neutrophils can also affect the expression of epigenetic molecules in tumor cells through exosomes, and promote the immune escape of tumors. Abbreviations: ROS, reactive oxygen species; iNOS, inducible nitric oxide synthase; GM-CSF, granulocyte-macrophage colony-stimulating factor, NETs, neutrophil extracellular traps; NK, natural killer; MDSC, myeloid-derived suppressor cell; EMT, epithelial-mesenchymal transition; OSM, oncostatin-M; ARG1, arginase-1; WTAP, wilms' tumor 1-associating protein; m6A, N6-methyladenosine; THBS1, thrombospondin-1; TAN, tumor associated neutrophil; TNF, tumor necrosis factor; STAT3, signal transducer and activator of transcription 3; FOXP3, forkhead box protein 3.

and the mediators producing the effect. However, further research is needed to improve and confirm these findings.

4.1.2 | Immune checkpoint molecules on the neutrophil surface

At present, the main cause of tumor immunotherapy resistance is the development of resistance to ICIs, especially those involving the PD-1/PD-L1 pathway. Neutrophils have been confirmed to be associated with tumor ICI resistance. For example, low-density neutrophils mediate the primary resistance of non-small cell lung cancer (NSCLC) to ICIs through the activation of the hepatocyte growth factor/cellular mesenchymal-epithelial transition (HGF/c-MET) pathway, which is the important signaling pathway mediating cancer cell-TME crosstalk [121]. PMN-MDSCs mediate CRC resistance to ICIs through THBS1 [115]. This is mainly attributed to the activation and dysfunction of T cells. In addition to endogenous mediators, neutrophils can also promote drug resistance in immunotherapy by inhibiting T cells through immune checkpoints on their surface (Figure 3).

Studies have shown that when the neutrophil/T-cell ratio increases, ICIs cannot exert their tumor suppressive effect, and the immune system cannot be rapidly activated [24]. Restoring the neutrophil-to-lymphocyte ratio to normal range can resolve neutrophil-induced ICI resistance [122]. in vitro experiments in breast cancer, PD-L1 on neutrophils has an inhibitory effect on the proliferation of T cells and the secretion of interferons [123]. in vitro experiments using patient HCC samples found that neutrophils stimulated with IL-6 and GM-CSF negatively regulate the T cell immune response through the PD-1/PD-L1 signaling pathway, thereby promoting immune escape [124, 125]. in vitro studies with ovarian cancer patient-derived

tissues show that the upregulation of PD-L1 in neutrophils can suppress the antitumor immunity of T cell, thereby enhancing the ability of ovarian cancer cells to evade immune surveillance [126]. In an in vitro study of samples from patients with gastric cancer, GM-CSF produced by tumors activates neutrophils and promotes the expression of PD-L1 via the JAK/STAT3 signaling pathway. These activated neutrophils can significantly inhibit T cell immunity in vitro, and blocking PD-L1 on these neutrophils can reverse this effect [127].

In addition to PD-L1, V-domain Ig suppressor of T cell activation (VISTA) is also an immune checkpoint expressed on the surface of neutrophils, which has costimulatory effects on the ICI pathway. Previous studies have evaluated the potential mechanism of acquired anti-PD-1 resistance in biopsies of patients with metastatic melanoma collected longitudinal, confirming that VISTA is an important target for acquired resistance to PD-1 therapy in melanoma patients, and enhancing the VISTA immune checkpoint pathway may be a promising therapeutic strategy for patients with metastatic melanoma [128]. In addition, mouse studies showed that the VISTA antagonist CA170 can decrease the infiltration of PMN-MDSCs and Tregs into tumors, increase the infiltration of $CD8^+$ T cells, and enhance the efficacy of the KRAS vaccine [129].

All of the above studies have shown that neutrophils can suppress T-cell immunity through immune checkpoints on their surface, contributing to tumor resistance to immunotherapy.

4.1.3 | NETs

A number of studies have suggested that the formation of NETs is associated with tumor immune evasion and that NETs are potential therapeutic targets.

Mouse studies have shown that the $CD4^+$ T cell-associated tumor immune microenvironment induces NET formation and exerts immunosuppressive effects through PD-1/PD-L1 [130]. NETs can also directly contact naïve $CD4^+$ T cells through TLR4 to promote Treg differentiation and hinder tumor immune surveillance [131]. NETs also have inhibitory effects on the number and function of $CD8^+$ T cells. In the NETs-rich TME, $CD8^+$ T lymphocytes exhibit a functionally exhausted phenotype and express high levels of exhaustion markers, such as PD-1, lymphocyte activation gene-3 (LAG-3) or T cell immunoglobulin and mucin domain 3 (TIM3, an inhibitory checkpoint protein highly expressed in tumor-infiltrating lymphocytes), and NETs inhibitors can reverse this phenotypic switch [132]. In addition, both mouse studies and cell experiments confirm that long-term exposure to NETs produced by neutrophils leads to T cell exhaustion, DC cell death, limited NK cell expansion, and NETs encapsulation of tumor cells and blocks contact between immune cells and surrounding target cells, protecting them from $CD8^+$ T cell- and NK cell-mediated cytotoxicity [133–135]. Neutrophils recruited by IL-17 develop NETs and inhibit $CD8^+$ T cells in tumors. IL-17 blockade can sensitize tumors to ICIs through the regulation of NETs [136]. Blocking NET formation can enhance the effect of adoptive NK cell therapy and prevent HCC recurrence after resection [137].

Collectively, the formation of NET prevents immune cells from exerting anti-tumor immune functions, leading to tumor immune escape and making immunotherapy much less effective.

4.2 | Neutrophils induce drug resistance by destroying tumor antigens

The effective antitumor immune response depends on two factors. The first is the expression of sufficient tumor antigens, and the second is the successful presentation of tumor antigens for $CD8^+$ T cells to recognize and kill [138]. Previous studies have shown that neutrophils can regulate tumor antigens by affecting these two processes.

The formation of tumor antigens is related to gene mutations and oncogene expression. Neutrophils have been confirmed to regulate the c-Myc gene [139]. In addition, studies using mouse tumor models and human tumor tissues have revealed that neutrophils can also regulate the genes and phenotype of tumor cells through microRNAs [140]. Active oxygen species released by neutrophils cause DNA damage resulting in gene mutation [141]. Notably, DNA damage is closely associated with immune checkpoint blockade, as it drives the production of tumor neoantigens and facilitates the immunotherapy in tumors [142] (Figure 3). In terms of antigen presentation, studies of mouse and human tumor tissues reported that in addition to mediating the loss, mutation and destruction of tumor antigens, neutrophils can also interact with T cell-mediated initial antitumor effects [143]. Researchers remodeled TANs to improve the efficiency of DC-based neoantigen nanovaccines for HCC [144]. This indicates that TANs may affect the process of tumor antigen uptake by DCs and the process of antigen cross-presentation to T cells. These studies may solve the problem of tumor vaccine tolerance and improve the immune efficacy of tumor vaccines.

From the above studies, it is evident that neutrophils not only promote the production of tumor neoantigens, but also facilitate the presentation of tumor antigens. Based on these findings, in-depth research should be important to develop new strategies for anti-tumor immunotherapy.

4.3 | Neutrophils affect the epigenetic regulation of drug resistance

Normally, a variety of key epigenetic molecules, such as histone deacetylases, histone methyltransferases, and DNA methyltransferases, can affect the primary or secondary drug resistance of tumors through the regulation of the TME [145, 146]. Neutrophils can not only induce genetic instability in cells through microRNAs [147] but also regulate the expression of some epigenetic molecules.

Studies of neutrophils isolated from tumor tissues and peripheral blood of patients revealed that the neutrophil-derived exosomal piRNA-17560 enhanced fat mass and the expression of obesity-related proteins (FTOs) in breast cancer cells. The upregulation of FTO expression further enhances the stability of zinc finger E-box binding homeobox 1 (ZEB1), an important transcription factor in EMT, and increases the ZEB1 transcripts by reducing m6A RNA methylation, which lead to the EMT in tumor cells and promotes tumor immune evasion [148] (Figure 3). Cell experiments reveal that C5aR1-positive neutrophils alter tumor glucose metabolism through the Wilms' tumor 1-associating protein (WTAP)-dependent m6A methylation of α-Enolase (ENO1), and changes in glucose metabolism in the TME are closely related to tumor immune evasion [149, 150]. In addition, a retrospective analysis proposed that the methylation status of FOXP1 could correlated with PD-L1 mutation, which affects the therapeutic response of NSCLC patients to anti-PD-1 treatment. The infiltration of various cells, including neutrophils, may be involved in this process, but the specific mechanism involved remains unclear [151].

The above studies suggest that neutrophils may contribute to immune escape from tumors by modulating epigenetic mechanisms. Therefore, targeting neutrophils is a promising way to control the immune drug resistance of tumors mediated by epigenetic molecules at the source.

5 | NOVEL STRATEGIES FOR TARGETING NEUTROPHILS TO OVERCOME DRUG RESISTANCE IN IMMUNOTHERAPY

5.1 | Overcoming drug resistance by inhibiting neutrophil activation and recruitment

The first step for neutrophils to produce biological effects is activation and recruitment. Some complement components and interleukins can activate neutrophils [152], and chemokines and their receptors can promote neutrophil recruitment [153]. Targeting these cytokines and receptors may be one of the strategies to overcome tumor immune resistance (Figure 4).

In terms of neutrophil activation, mouse and cell experiments have shown that in the absence of C5a receptor or the neutralization of C5a with an anti-C5 monoclonal antibody, the number of activated neutrophils is reduced, and tumor metastasis is severely impaired [154]. In addition, inhibitors of the C5a receptor antagonize the growth of subcutaneous tumors in various tumor types, modulate immunosuppressive mechanisms, and synergize with anti-PD-1 therapy [155]. IL-1 and IL-8 are also stimulators of neutrophils. IL-1 signaling is associated with high levels of activated neutrophils. In experiments with mice, inhibiting IL-1 signaling can counteract tumor immunosuppression work in tandem with anti-PD-1 therapy to eliminate tumors [156]. In patients with tumors, increased levels of IL-8 in the serum correlate with higher numbers of intratumoral neutrophils and a diminished effectiveness of immune checkpoint inhibitors (ICIs) [157]. High blood levels of IL-8 have been shown to serve as a reliable indicator of patients unlikely to gain advantages from checkpoint immunotherapy. Currently, the development of drugs to block IL-8 is in progress, and clinical trials assessing the efficacy of ICIs, both alone and in combination, are actively ongoing [158].

In terms of neutrophil recruitment, a number of studies have been performed to improve the tumor immune microenvironment by regulating neutrophil recruitment. Mouse studies have shown that PD-L1 antibody monotherapy increases the tumor infiltration of the immunosuppressive neutrophils, and combined neutrophil deprivation therapy can increase the proliferation of $CD8^+$ T lymphocytes in tumors by greatly reducing the recruitment of TANs [159]. Inhibition of the recruitment of TANs by developmental endothelial locus-1 (Del-1), a secreted protein that inhibits leukocyte-endothelial adhesion and inflammation initiation, can improve the efficacy of bacteria-mediated cancer immunotherapy and elicit a more powerful initial immune response [160]. TAN-infiltrating tumors with a high proportion of TAN infiltration have been shown to have very few tumor-infiltrating T lymphocytes (TILs). The removal of CXCL1 or CXCL5/CXCR2 signaling reduces TAN infiltration, increases the abundance of $PD\text{-}1^+CD8^+$ T cells, and sensitizes tumors to anti-CD40 and anti-CTLA-4 and anti-PD-1 combination immunotherapy [161]. in vivo studies of lung cancer have shown that TANs notably infiltrate in tumor tissues in mouse models, activate the CXCL/CXCR2 signaling pathway, and upregulate inhibitory molecules such as ARG-1 and TGF-β. The selective CXCR2 inhibitor SB225002 has shown good therapeutic effects; it can significantly reduce neutrophil recruitment, enhance antitumor T cell activity, and improve the efficacy of anti-PD-1 immunotherapy by

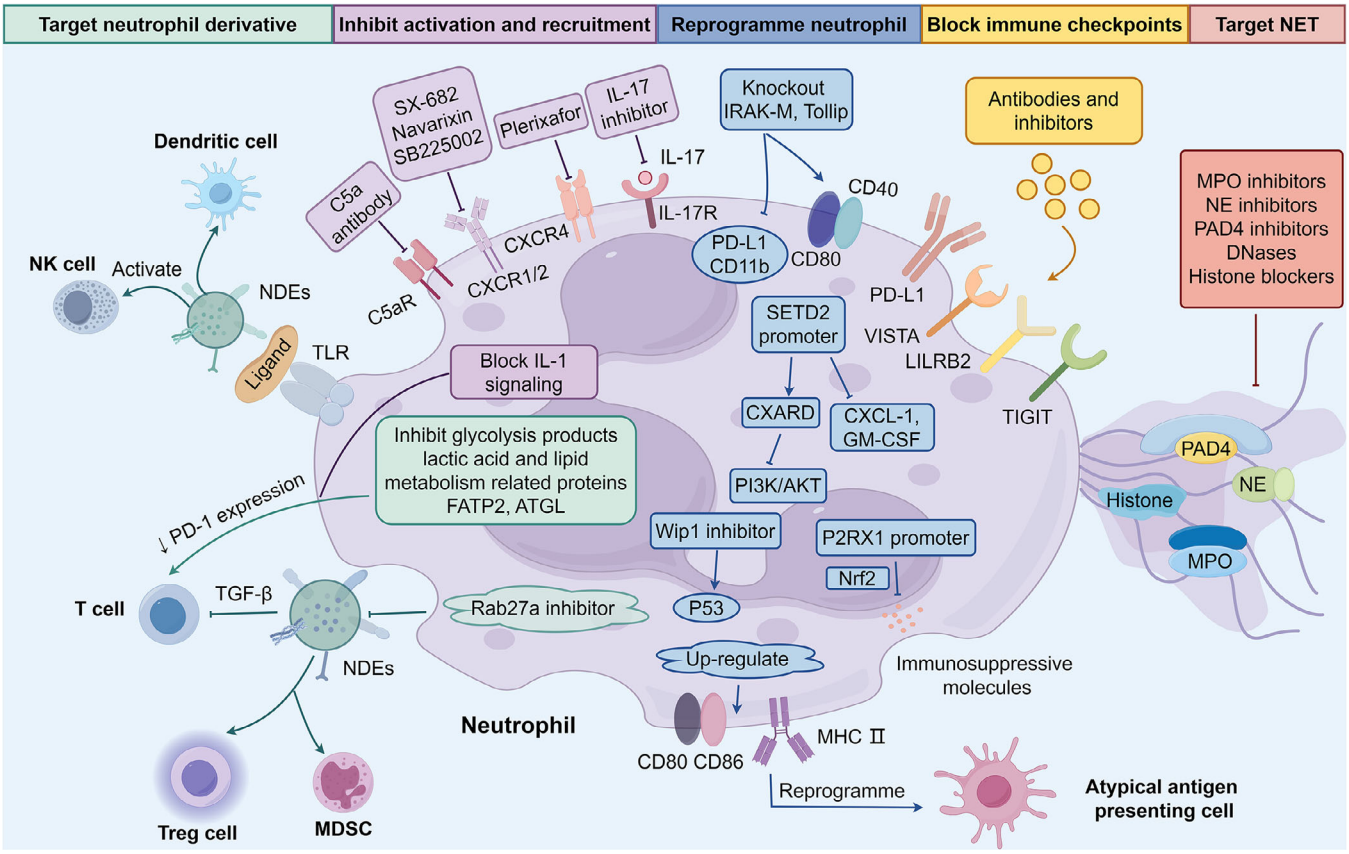

FIGURE 4 **Strategies for overcoming tumor immunotherapy resistance by targeting neutrophils**. Activation and recruitment of neutrophils can be inhibited by using related receptor inhibitors or blocking some signaling pathways, thus blocking subsequent reactions caused by neutrophils. From the perspective of targeting neutrophil derivatives, modifying neutrophil derived exosomes with specific ligands can activate surrounding immune cells to stimulate an immune response. In addition, the expression of PD-1 on T cell surface can be inhibited by targeting glycometabolites and lipid metabolism-related proteins of neutrophils. Reprogramming neutrophils by regulating their genes is also a strategy. For example, the elimination of IRAK-M and Tollip can inhibit PD-L1 expression. Promoting SETD2 expression can inhibit the production of chemokines and colony-stimulating factors. Upregulation of CD80, CD86, and MHC II can reprogram neutrophils into atypical antigen-presenting cells. Blocking the immune checkpoint of neutrophils with blockers can effectively enhance the antitumor immune responses. From the perspective of targeting NETs, on one hand, NETs formation can be inhibited by inhibiting proteins related to NETs formation, on the other hand, NETs can also be dissolved by DNA enzymes. Abbreviations: NDE, neutrophil-derived exosome; NETs, neutrophil extracellular traps; NK, natural killer; MDSC, myeloid-derived suppressor cell; TLR, Toll-like receptor; IRAK-M, interleukin-1 receptor-associated kinase M; VISTA, T-cell-activated immunoglobulin inhibitor of structural domain V; LILRB2, leukocyte immunoglobulin-like receptor subfamily B member 2; TIGIT, T cell immunoreceptor with immunoglobulin and ITIM domain; MPO, myeloperoxidase; NE, neutrophil elastase; PAD4, protein arginine deaminase type 4; SETD2, SET domain containing 2; GM-CSF, granulocyte-macrophage colony-stimulating factor, TGF, transforming growth factor; Wip1, wild-type p53 induced phosphatase 1.

promoting the activation of $CD8^+$ T cells [162, 163]. CXCR4 is also an important mediator in the regulation of neutrophil recruitment. CXCR4 related signaling axes, such as the CXCR4–CXCL12 axis, regulate the release of neutrophils and their ratio in the circulation and may be potential targets [164]. A cohort study confirmed that CXCR4 inhibitors combined with PD-1 inhibitors can reduce TAN infiltration and improve cytotoxic T cell function [165]. In addition to the chemokine pathway, the liver X receptor (LXR) agonist RGX-104 is also considered one of the most promising methods for inhibiting neutrophil accumulation in tumors and the periphery, suppressing TAN recruitment, and sensitizing tumors to PD-1 blockade in mouse models and human dose-escalation Phase I trials [166].

The above findings show that the inhibition of neutrophil activation and recruitment can enhance the antitumor immune response and can serve as a novel strategy for reversing resistance to immunosuppression. However, some studies put forward a different point of view. For example, activating neutrophils in vitro enables them to lyse human tumor cells. Mechanistically, activated neutrophils can induce rapid mobilization and tumor infiltration while activating complement in tumors. The

complement component C5a activates neutrophils to produce leukotriene B4 (LTB4), which stimulates ROS production through xanthine oxidase, resulting in oxidative damage and the T cell-independent clearance of various tumor types. These data confirm that neutrophils are effective antitumor immune mediators [167]. Therefore, the promotion or inhibition of neutrophil activation and recruitment depends on the mediators that specifically mediate neutrophil activation and recruitment.

5.2 | Reversal of drug resistance by reprogramming neutrophils

In general, neutrophils that produce tumor-promoting effects have an adverse phenotype. Many studies have shown that reprogramming neutrophils in tumors is expected to regulate the immune microenvironment of tumors, thus reversing the adverse neutrophil-mediated immune drug resistance.

In pancreatic cancer, the deletion of SET domain containing 2-histone 3 lysine 36 trimethylation (SETD2-H3K36me3), a key component of common tumor suppressor mechanisms, leads to the downregulation of Cxadr expression and the activation of PI3K-AKT pathway and the overexpression of CXCL1 and GM-CSF, thereby triggering neutrophil reprogramming and promoting the development of neutrophils toward an immunosuppressive phenotype, while the inhibition of $CD8^+$ T cells promoted immune evasion. The upregulation of SETD2 expression in neutrophils may delay this process [168]. Interleukin-1 receptor-associated kinase M (IRAK-M) plays a key role in the establishment of immunosuppressed neutrophils. In mouse and cell experiments, decreased levels of IRAK-M-deficient neutrophil inhibitory molecules PD-L1 and CD11b stimulate the expression of CD80 and CD40 and the ability to promote the proliferation and activation of effector T cells both in vitro and in vivo, effectively enhancing the antitumor immune response [169]. Protein phosphatase $Mn2^+/Mg2^+$-dependent 1D (PPM1D)/wild-type p53 induced phosphatase 1 (Wip1) is a negative regulator of the tumor suppressor p53. Chemical inhibition of Wip1 in neutrophils in mice can promote an antitumor phenotype, the p53-dependent expression of costimulatory ligands and the proliferation of cocultured cytotoxic T cells and enhance the antitumor immune response [170]. Tollip (a key innate immune cell regulator)-deficient neutrophils significantly increase T cell activation and enhance tumor immune surveillance by enhancing the expression of the costimulatory molecule CD80 and decreasing the expression of inhibitory molecules [171]. Studies using mouse tumor models demonstrate that radiotherapy can also induce the differentiation of $CD11b^+Ly6G^+CD11c^-$ neutrophils into a hybrid subpopulation of $CD11b^+Ly6G^+CD11c^+$ neutrophils-DCs and upregulate the expression of the costimulatory molecules CD80 and CD86 and MHC II molecules, reprogramming neutrophils into atypical antigen-presenting cells, which effectively cross-present tumor antigens to form an antitumor immune response to promote immune-mediated tumor regression [107]. In addition, recently, a research team used CRISPR-Cas9-mediated gene knock-in to genetically engineer human pluripotent stem cells (hPSCs) and constructed CAR-neutrophil cells (CAR-NEs) with optimal antitumor activity. Under in vitro TME-simulating conditions, CAR-NEs retained the antitumor phenotype, retained high antitumor activity, and produced more TNF-α, reflecting their potential for use in targeted immunotherapy. Moreover, this combination of CAR-NEs and nanomedicine can also enhance the targeted delivery of drugs [172]. These findings all provide novel ideas for reprogramming neutrophils (Figure 4).

5.3 | Neutrophils affect drug resistance through the blockade of immune checkpoints on the neutrophil surface

Immune checkpoints and immune checkpoint ligands expressed on neutrophils include PD-L1, VISTA, signal-regulatory protein alpha (SIRPα), leukocyte immunoglobulin-like receptor subfamily B member 2 (LILRB2), and T cell immunoreceptor with immunoglobulin and ITIM domain (TIGIT) (Figure 4). The blockade of PD-L1 on neutrophils enhances their cytotoxic activity against tumor cells [127, 173]. Mouse and cell experiments confirm that blocking VISTA turns DCs and monocytes into proinflammatory cells and promotes the T cell-mediated activation of antitumor immunity [174, 175]. In the mouse HCC model, CXCR2 antagonists combined with PD-1 antagonists increase the activation of DCs and the number of $CD8^+$ T cells in tumors and facilitate the reprogramming of TANs from the original tumor phenotype to the antitumor progenitor-like neutrophil phenotype, and reprogrammed TANs directly contact with $CD8^+$ T cells improving the HCC responses to ICIs [176]. Studies on primary neutrophils isolated from human tumor tissues and mouse models reveal that CD47-SIRPα checkpoint blockade can enhance the cytotoxic activity of neutrophils against HER2-mediated breast cancer cells in vitro [18]. In addition, when a SIRPα mAb is used in combination with other antitumor mAbs, the therapeutic effect is more robust [177]. in vitro and in vivo experiments in mice have shown that LILRB2 blockade combined with PD-L1 blockade inhibits the infiltration of immunosuppressive neutrophils and promotes antitumor

immunity [178]. Neutrophil TIGIT expression is associated with T cell inhibition and exhaustion and can predict clinical outcomes and anti-PD-1 responses in patients with follicular lymphoma [179, 180]. The combination of TIGIT inhibitors and anti-PD-L1 therapy is expected to expand stem cell-like memory T cell subsets and prevent or reverse T cell exhaustion [181]. Multi-group, randomized, and blind preclinical trials reveal that TIGIT/PD-1 co-blockade plus a CD40 agonist can also reactivate partially effective antitumor immune responses during immune evasion in PDAC [182].

To summarize, there is no doubt that blocking the immune checkpoints on the surface of neutrophils can enhance anti-tumor immune response. Combining other treatments on this basis could enhance the therapeutic effect and overcome tumor immunotherapy resistance.

5.4 | Regulation of drug resistance by targeting neutrophil derivatives

5.4.1 | Cytokines

The ability of neutrophils to exert subsequent biological effects largely depends on the cytokines released by neutrophils (such as IL-8, IL-10, IL-11, CCL17, and MMP-9). Therefore, neutrophil-mediated adverse effects and drug resistance may be able to be blocked by regulating the cytokines released by them.

In vitro studies of tissue samples derived from patients with tumors demonstrate that IL-8 is one of the factors driving tumor immune evasion and impairs the efficacy of adenoviral immunotherapy. The inhibition of IL-8 activity can improve the efficacy of oncolytic viruses [183]. Therefore, the inhibition of IL-8 secretion by neutrophils may be a promising way to promote the restoration of antitumor immune activity to overcome tumor immune resistance. IL-10 is an immunosuppressive factor that suppresses cellular immunity, and the neutralization of IL-10 can enhance the cytotoxicity of T cells in mouse studies [184]. IL-11 released by TANs can activate STAT3 signal transduction, inhibit DC maturation and T cell activation, and attenuate the antitumor immune response [185, 186]. TANs can also secrete large amounts of the Treg chemoattractant CCL17, which recruits a large number of Tregs to tumors and inhibits the T cell-mediated antitumor immune response in mice [187]. Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of progenitor cells and are a main component of the immunosuppressive TME. The secretion of MMP-9 by neutrophils can promote the expansion of this cell type and mediate the immune escape of tumors [188]. Blocking the release of IL-11, CCL17 and MMP-9 by neutrophils may reverse the above process.

5.4.2 | Exosomes

Neutrophil-derived exosomes (NDEs) contain a variety of bioactive molecules that can regulate the tumor immune response by regulating their contents. For example, in vivo and ex vivo studies in mice have confirmed that NDEs can induce T cell apoptosis, inhibit NK cell activity, and promote the expansion of Treg cells and MDSCs through different mediators to suppress the immune system [189]. Blocking TGF-β released by NDEs in the late tumor period can improve the immune system surveillance of tumors [190]. Rab27a and b (RAS oncogene family members) play a crucial role in the process of neutrophil granules docking and fusing with the plasma membrane. This process is essential for the exocytosis of neutrophil granzymes into the extracellular space. The suppression of Rab27a expression can lead to a decrease in the secretion of NDEs [191]. Notably, differences in neutrophil polarization can lead to differences in the production of exosomes derived from these cells. N1 neutrophils generate N1 NDEs, whereas N2 neutrophils produce N2 NDEs. The N1 NDEs are capable of fostering anti-tumor effects and may bolster the immune response against tumors, particularly through lymphocyte activation. In contrast, N2 NDEs contain components that may support tumor growth, enhance survival, promote angiogenesis, and aid in evading immune surveillance.

NDEs are modified with specific ligands to make them bind to receptors on tumor cells, increasing the probability of their absorption by these cells and boosts their effectiveness as a delivery system for tumor treatment [192]. The immune response can be stimulated by loading TLR ligands into NDEs. In a melanoma mouse model, NDEs loaded with TLR9 ligands were shown to activate DCs and induce antitumor immunity [193]. in vivo and ex vivo studies in mice have also shown that NDE surface molecules can bind to TLR4 ligands and TLR1/2 ligands to activate surrounding DCs and NK cells, increase the secretion of proinflammatory and immunoregulatory cytokines, and enhance TH1 polarization [194] (Figure 4).

Collectively, targeting NDEs, either by modulating their contents or by modifying them with specific ligands, is a promising strategy based on the critical role of neutrophils in the regulation of tumor immune response.

5.4.3 | Neutrophil metabolites

In tumors, neutrophils undergo metabolic changes in response to local changes in the microenvironment. These

metabolic changes or metabolites may be major factors leading to the heterogeneity of neutrophils in tumors and changes in the tumor immune microenvironment [195]. Therefore, targeting neutrophil-derived metabolites is also a promising strategy for overcoming tumor immune resistance.

An exploration of the heterogeneity of TANs revealed that tumor-promoting neutrophils are characterized by hyperglycolytic activity and induce immunosuppression [51]. ex vivo and in vivo studies in mice have shown that the product of glycolysis, lactic acid, can promote the translocation of nuclear factor of activated T-cells 1 (NFAT1, a master regulator of immune cell proliferation) into the nucleus of T cells, thereby enhancing PD-1 expression in Treg cells and inhibiting the expression of PD-1 in effector T cells. PD-1 blockade activates PD-1-expressing Treg cells, resulting in immunotherapy failure [196]. In addition, lactic acid in tumors is an effective inhibitor of the function and survival of T cells and NK cells. Lactate accumulation blunts immune surveillance by these two cell types, resulting in tumor immune evasion [197].

In addition to glucose metabolites, substances involved in lipid metabolism in neutrophils are also related to tumor immunity. For example, mouse studies have shown that neutrophils can upregulate fatty acid transporter 2 (FATP2) expression to promote immune suppression and tumor growth. Targeted pharmacological inhibition of FATP2 can abolish the immunosuppressive activity of neutrophils and can block tumor progression when used in conjunction with immune checkpoint inhibitors (ICIs) [198]. Suppressing adipose triglyceride lipase (ATGL) activity in neutrophils leads to the buildup of neutral lipids within these cells, which are then transferred to tumor cells to promote tumor proliferation and metastasis [199].

All of these studies have shown that tumor neutrophil metabolites inhibit anti-tumor immune responses, and targeting neutrophil metabolites has the potential to eliminate these adverse responses.

5.5 | Regulation of drug resistance by targeting NETs

NET formation is closely related to neutrophil phenotype switching and the neutrophil-mediated immune response. The inhibition of NET formation can bias neutrophils in mouse tumors toward an antitumor phenotype [31]. Strategies can be designed to overcome tumor immune resistance by inhibiting NET formation or promoting NET degradation (Figure 4).

In terms of the inhibition of NET formation, targeting proteins associated with NET formation (myeloperoxidase (MPO), NE and protein arginine deaminase type 4 (PAD4)) can be an approach to prevent NET formation or communication between cells. Targeting NETs-associated proteins suggest that the inhibition of NADPH oxidase-mediated ROS generation or the blockade of MPO-mediated chromatin decondensation may inhibit NET formation [200, 201]. NE inhibitors can also inhibit the formation of NETs. PAD4 not only plays a key enzymatic role in promoting chromatin decondensation and NET formation but is also associated with many immune-mediated pathological conditions [202]. Similar to mice that received daily injections of DNase I, PAD4 knockout mice injected with CRC and HCC cells exhibited reduced NE release from NETs, inhibited TLR4 activation and upregulated peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC1a, a pivotal transcriptional coactivator involved in the regulation of mitochondrial metabolism) expression on tumor cells, preventing tumor cells from escaping the immune system [203]. However, PAD4 could have other critical functions in immunity, and these functions may be impaired by inhibiting PAD4 [204]. In addition, targeting neutrophil intracellular signaling can also regulate NET formation. For example, gasdermin D promotes NET formation through the mtDNA cGAS-STING pathway [205]. Mouse studies have confirmed that gasdermin D (a protein mediating pyroptosis and inflammation) inhibitors can inhibit NET formation by affecting neutrophil intracellular signaling [206]. Prostaglandin E2 (PGE2) inhibits NET formation in a cAMP- and PKA-dependent manner [207]. The inhibition of NF-κB pathway activation or the downregulation of the expression of the transcription factor NFAT to inhibit the calcineurin pathway can also inhibit NET formation [208].

In terms of promoting NETs degradation, animal studies have shown that DNase I can degrade NETs. DNase I can reverse resistance to PD-1 blockade by improving the infiltration and cytotoxicity of $CD8^+$ T cells through the degradation of NETs [209]. Other engineered DNase proteins are also being developed for the dissolution of NETs [210]. However, DNase I injection might have off-target effects in vivo that would diminish the effects observed in vitro [211].

5.6 | Latest clinical trials targeting neutrophils

As a result of the theoretical foundation laid by previous studies, there are a few clinical studies evaluating the efficacy and safety of targeting the neutrophil-associated pathway for the treatment of tumors [8]. Herein, we summarized the latest research in recent years, which provide clues for reversing immune resistance by targeting neutrophils (Table 2).

TABLE 2 Clinical trials based on neutrophil-targeted cancer therapies.

Class of target	Agents	Cancer applications	Phase	Clinicaltrails.gov No
G-CSF	Pegfilgrastim	Breast cancer	III	NCT00035594 (Completed)
	F-627		III	NCT03252431 (Completed)
	Pegfilgrastim	Colorectal cancer	II	NCT00094809 (Completed)
	Filgrastim		II	NCT00541125 (Completed)
	YPEG-rhG-CSF	Non-small cell lung cancer	II	NCT02005458 (Completed)
	Pegfilgrastim		I	NCT01840579 (Completed)
IL-8/CXCL8	BMS-986253	Hepatocellular carcinoma	II	NCT04050462 (Active, not recruiting)
CXCR2	Navarixin	Solid tumors; Non-small cell lung cancer; Prostate cancer; Colorectal cancer	II	NCT03473925 (Completed)
CXCR1/CXCR2	SX-682	Non-small cell lung cancer	II	NCT05570825 (Recruiting)
		Pancreatic cancer	II	NCT05604560 (Recruiting)
		Pancreatic ductal adenocarcinoma; Pancreatic cancer	I	NCT04477343 (Recruiting)
CCR5	Vicriviroc	Colorectal cancer	II	NCT03631407 (Completed)
CCR2/5	BMS-813160	Colorectal cancer; Pancreatic cancer	I II	NCT03184870 (Completed)

Abbreviations: CCR, C-C chemokine receptor; CXCL, C-X-C motif chemokine ligand; CXCR, C-X-C motif chemokine receptor; G-CSF, granulocyte colony-stimulating factor.

G-CSF plays an integral role in neutrophil genesis and release. It has been clinically tested alone or in combination with chemotherapy in breast cancer (NCT00035594, NCT03252431), CRC (NCT00094809, NCT00541125), and NSCLC (NCT02005458, NCT01840579).

In addition to colony-stimulating factors, chemokines and their receptors play important roles in neutrophil recruitment, and most of the current clinical studies targeting neutrophils are also related to CXCL/CXCR inhibitors. BMS-986253, an IL-8/CXCL8 antibody, in combination with an anti-PD-1 antibody for the treatment of patients with advanced HCC has entered a phase II clinical trial (NCT04050462). Navarixin is a CXCR2 antagonist that has showed efficacy and safety in combination with Pembrolizumab in the treatment of adult solid tumors in a randomized trial (NCT03473925). SX-682 is an orally effective CXCR1/CXCR2 variant inhibitor that blocks pathologic neutrophil recruitment and enhances T-cell activation and antitumor immunity [212]. SX-682 is a potent oral CXCR1/CXCR2 variant inhibitor. Currently SX-682 is in several clinical trials (NCT05570825, NCT05604560, NCT04477343), but has not yet been concluded. Plerixafor, a reversible CXCR4 inhibitor, in combination with bevacizumab has demonstrated good tolerability and response in the treatment of patients with recurrent high-grade gliomas [213]. There are also trials evaluating whether the combination of the CCR5 inhibitor vicriviroc with pembrolizumab (NCT03631407) or the CCR2/5 inhibitor BMS-813160 with nivolumab (NCT03184870) is more effective than ICI alone in inhibiting TAN-mediated tumor promotion and immunosuppression.

6 | CHALLENGES AND PERSPECTIVES

Previous studies have shown that neutrophils exhibit significant heterogeneity and functional diversity and are important players in the immune response. They can mediate the immune response through multiple pathways, including regulating the TME, affecting tumor antigens, and influencing epigenetic molecules, and play important roles in the development of various tumors and drug resistance to immunotherapy.

Most recent tumor studies have focused only on the inhibition of the immune response by neutrophil subsets with adverse phenotypes and their ability to promote tumor immune resistance. In fact, regardless of their adverse phenotype or normal phenotype, neutrophils stimulatory effect on the immune response and their antitumor effect on the TME cannot be ignored. Neutrophils with tumor-promoting effects can also have antitumor effects. For example, TANs with tumor-promoting effects can directly kill tumor cells by releasing ROS, stimulating T cell response, facilitating antigen presentation, and suppressing early tumor formation and metastasis [214]. This seemingly contradictory situation suggests that the complex relationship between neutrophils and tumor progression may depend on a variety of factors,

such as the type of tumor, stage of tumor progression, endogenous influences, exogenous therapeutic interventions, and individual patient differences. Different types of tumors may produce different degrees of inflammation and microenvironments, resulting in differences in neutrophil recruitment and infiltration in tumor tissues. The regulation of tumor immunity and immune drug resistance by neutrophils is not only an underreaction or overreaction but also an obvious transformation from a protective phenotype to a harmful phenotype at different stages of tumor development. On the one hand, as the tumor progresses, competitive consumption of oxygen and nutrients by tumor cells usually impairs the metabolic adaptations of neutrophils. On the other hand, aberrant metabolites in tumors also have the potential to affect the function of neutrophils, contributing to a phenotypic shift in neutrophils [68]. Additionally, the subsequent effects caused by different phenotypes of neutrophils are complex; whether the final result is positive or negative may depend on which side performs a stronger effect. The influence of various endogenous factors on neutrophils are complex and diverse. Mediators from tumor cells and their surrounding stromal cells, such as IL-6, contribute to reprogramming neutrophils to a pro-tumorigenic phenotype. Genetic aberrations in tumors can alter the cytokine secretion profile and induce the accumulation of immunosuppressive neutrophils. Notably, microbes are also capable of modulating the functional heterogeneity of neutrophils. Local microbes not only prolong neutrophil lifespan under inflammatory conditions, but also involve in neutrophil-mediated inflammation and tumor growth [77]. Different therapeutic interventions such as surgery, chemotherapy, radiotherapy, and immunotherapy are also prone to affect the TME in which neutrophils are located, producing different effects. In addition, individual differences in patients (e.g., age, gender, physiological rhythms, presence of poor lifestyle habits, and obesity) can also have an impact on the functional status of neutrophils. All of these factors are intertwined so that the very same functional pathways may exert both pro- and anti-tumor effects. Therefore, when targeting neutrophils, the neutrophil phenotype must be therapeutically corrected, and the disruption of the immune response by negative factors must be inhibited, rather than simply activating or inhibiting neutrophils with a certain phenotype. In addition, with respect to modulating neutrophils, consideration should also be given to the influence on these cells, which play a central role in the host's immune defense. In tumor immunotherapy, targeting neutrophils has the potential to unintentionally affect other cells or anti-tumor neutrophils, disrupting the normal immune system and making patients more susceptible to adverse reactions such as infections [161]. In order to optimize the efficacy of targeted neutrophil therapy, it is necessary to develop methods that specifically target neutrophils in a way that ensures the normal physiological function of neutrophils while minimizing the effects on other cell types and reducing side effects.

In terms of targeting neutrophils, in addition to the strategies mentioned above, trained immunity should also be emphasized. Trained immunity is an emerging immunological concept that does not rely on T and B cells, but rather on the memory response of intrinsic immune cells, which allows the organism to show higher reactivity upon re-encounter after initial stimulation by a pathogen. Reprogramming can be performed at the genetic level or epigenetic level, which allows intrinsic immune cells to acquire long-term immune memory capacity and better resistance to pathogen invasion. Recently, neutrophils have been recognized as effectors of trained immunity [215]. In the presence of trained immunity agonists, neutrophils exhibit several hallmarks of trained immunity, including transcriptomic, epigenetic, and metabolic changes [216]. Trained immunity induced by bacillus calmette guerin (BCG) has been reported to induce long-term functional reprogramming of neutrophils in vivo via chromatin remodeling. Reprogrammed neutrophils were characterized by enhanced expression of activation markers and decreased expression of markers associated with immunosuppression [217]. Researchers found that the antitumor effects of β-glucan-induced trained immunity were associated with transcriptomic and epigenetic rewiring of granulocyte progenitors, reprogramming neutrophils to an antitumor phenotype. Transfer of trained neutrophils into hormonal mice significantly inhibited the tumor growth [218]. Therefore, in the future, it may be possible to interfere with neutrophil reprogramming by trained immunity, preventing the generation of pro-tumor phenotype neutrophils [219]. Additionally, adoptive transfer of trained neutrophils is a major direction.

Although neutrophil-related studies have provided new ways to overcome tumor immune resistance, there are still many challenges associated with the current neutrophil-based regulatory therapeutic effects on tumor immunity. Reprogramming of neutrophils is still at the initial stage of development. First of all, the efficiency of cell reprogramming is not guaranteed. Moreover, during the reprogramming process, the genes necessary for neutrophils to play a positive role may be mutated, leading to hazardous consequences. In addition, reprogrammed cells are likely to be recognized by the immune system as foreign cells and immune rejection may occur. Considering the risk of pulmonary toxicity associated with granulocyte infusion, safety needs to be assessed when infusing back exogenous reprogrammed neutrophils [220]. In terms of interfering with neutrophil mediators, attention needs

to be paid to the physiologic functions of those mediators. It is necessary to find a balance between increasing the anti-tumor immune effect and maintaining their physiological functions. In addition, a particular cellular mediator may be the crossroads of multiple signaling cascade. Thus, interfering with a single mediator may trigger compensatory alterations in other mediators or signaling pathways, resulting in adverse consequences. In terms of targeting NDEs, enhancing the specific delivery of NDE components between cells while ensuring NDE delivery efficiency is a major issue. On the one hand, some proteins and nucleic acids could be degraded or form insoluble nucleic acid aggregates during the loading process, leading to the incomplete transport of required substances to recipient cells. On the other hand, as the TME where NDEs are located is a mixture of various microenvironments, targeting a component does not necessarily ensure that the target effect will occur only in a certain environment [221]. In addition, there are few clinical trials on the use of NDEs for tumor treatment and more efforts are needed to translate relevant preclinical findings into clinical practice. The current in vitro isolation and purification methods for NDEs lack standardization, and the obtained exosomes are often mixed with other types of extracellular vesicles [222]. Therefore, more effective and standardized techniques for the in vitro isolation of NDEs need to be developed. In terms of blocking immune checkpoint molecules on the neutrophil surface, in addition to PD-L1 and VISTA, other immune checkpoint molecules including CD200R, paired immunoglobulin-like receptor α (PILRα), atypical chemokine receptor-2 (ACKR2), LILRB2 and SIRPα, are also highly expressed on neutrophils, but relevant studies are still lacking. These checkpoint molecules may be potential new targets for reprogramming neutrophils. For targeting NETs, a key limitation is that primary human neutrophils can not be transfected, and it is difficult to specifically inhibit those pathways promoting NET formation in vivo. In addition, we need to gain a more in-depth understanding of the biochemical and immunological mechanisms of NET formation and the multifaceted roles of NETs in tumor growth and progression and antitumor immunity. The stimulatory factors that lead to NET formation at different stages of tumors and in different tumor types need to be further studied. Finally, of the studies mentioned above, some focused only on the effect of neutrophils on the immune response; there is a lack of corresponding in-depth studies on the efficacy of immunotherapy. The results of such studies on neutrophils may help to generate new tumor immunotherapy methods and guide us in searching for new immunotherapy targets and new ways to overcome tumor resistance to immunotherapy.

7 | CONCLUSIONS

In conclusion, neutrophils play a critical role in tumor immunotherapy and immune drug resistance. Studies related to targeting neutrophils in combination with different immunotherapies have great clinical application potential for enhancing the effectiveness of tumor immunotherapy and reducing drug resistance. However, due to the enormous plasticity of neutrophils in the TME and some clinical challenges, more efforts are needed to fill the gaps in current research.

AUTHOR CONTRIBUTIONS

Jiali Yao and Linlin Ji drafted the manuscript in detail, generated and corrected the figures, and constructed and plotted the table. Guang Wang and Jin Ding critically revised the manuscript for important intellectual content.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

DATA AVAILABILITY STATEMENT

Not applicable.

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