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ORIGINAL ARTICLE

Neutrophils and neutrophil extracellular traps in cancer: promising targets for engineered nanomaterials

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Abstract

Neutrophils are the most abundant white blood cells in circulation and constitute up to 60% of circulating leukocytes. Neutrophils play a significant role in host defense against pathogens through various mechanisms, including phagocytosis, production of antimicrobial proteins, and formation of neutrophil extracellular traps (NETs). Recently, the role of neutrophils and NETs in cancer has generated significant interest, as accumulating evidence suggests that neutrophils and NETs contribute to cancer progression and are associated with adverse patient outcomes. In this review, we will first highlight the roles of neutrophils and NETs in cancer progression and metastasis and discuss new drug delivery approaches to target and modulate neutrophils and NETs for cancer therapeutics.

Keywords Neutrophil extracellular trap · Neutrophils · Nanoparticle · Cancer

Introduction

Neutrophils are the first responder cells to sites of acute inflammation and constitute a major part of the host innate immune defense. Since their identification more than 100 years ago, neutrophils are significant in host immunity, as illustrated in neutropenic hosts with life-threatening infections [1, 2]. Also, mutations in genes associated with neutrophil function often result in susceptibility to opportunistic pathogens or the occurrence of rare and life-threatening diseases [3]. For several decades, our knowledge of neutrophil function in immunity was limited to phagocytosis and initiation of acute inflammation. Developments in research methodologies have enabled the investigation of neutrophil functions in vivo and highlighted previously unknown functions of neutrophils in shaping the immune response through their activity or by interaction with other immune cells. These discoveries have sparked renewed interest in neutrophil biology. Of significant interest is the role of neutrophils in determining the resolution or progression of several pathologies from inflammation to cancer.

As a critical part of the innate immune defense, neutrophils express all known Toll-like receptors (TLRs) except TLR3. The principal function of neutrophils is antimicrobial defense, and microbes or microbial products activate neutrophil signaling pathways, leading to phagocytosis. Neutrophils engulf microbes to form a phagosome that fuses with the granules distributed throughout the cytoplasm [4]. Lysozymes and granular proteins, including elastase, myeloperoxidase (MPO), cathelicidins, and defensins, all facilitate microbial killing and degradation.

Recently, neutrophils have been shown to form neutrophil extracellular traps (NETs) as a newly discovered method of microbial capture and to prevent the dissemination of microbes (Fig. 1). NETs are web-like extrusions of neutrophil cytoplasmic contents, composed mainly of processed chromatin and granular proteins [5]. The size of the microbe may be a deciding factor in the deployment of NETs by neutrophils. Small microbes that can be readily ingested

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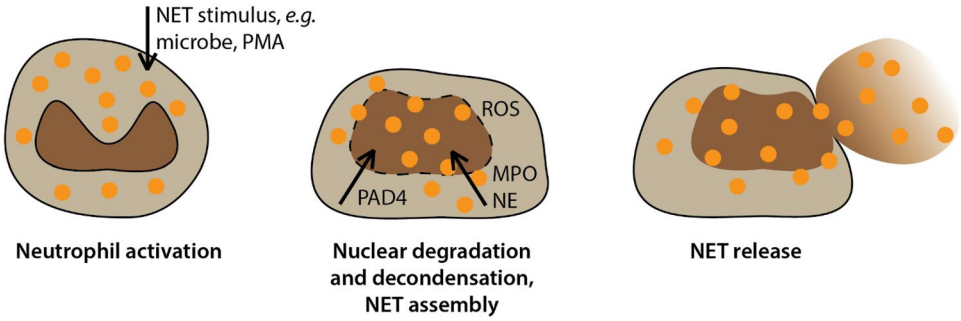

Fig. 1 Neutrophil activation leads to the formation of neutrophil extracellular traps (NETs)

trigger phagocytosis by neutrophils, whereas larger organisms induce neutrophils to form NETs [6]. The molecular mechanisms of NET formation are not fully understood, but prior research efforts have unraveled critical pathways necessary for NET formation. NET formation was initially described as a process involving the death of neutrophils and occurring through a unique cell death pathway termed NETosis [7]. NETosis progresses through several stages involving the collapse of the nuclear envelope, chromatin decondensation, plasma membrane rupture, and expulsion of cellular contents with granular proteins attached to the chromatin framework (Fig. 1). Several studies have since shown that neutrophils can form NETs in the absence of cell death [8, 9]. NETs have potent antimicrobial activity and have been shown to kill bacteria, fungi, viruses, and parasites [5, 10–12]. In agreement with this, patients whose neutrophils are defective in NET formation suffer from recurrent infections [13]. On the other hand, chronic exposure to NETs can cause inflammation. NET products can cause tissue injury or serve as autoantigens [14–16].

Importantly, the role of NETs in cancer has recently attracted significant interest. NETs have been shown to contribute to tumor progression and metastasis and is associated with adverse patient outcomes [17–20]. In this review, we will provide an overview of the role of neutrophils and NETs in cancer and discuss potential applications of nanoparticle-based targeting of neutrophils for cancer therapeutics.

Mechanisms of NET formation

Several physiological stimuli have been shown to induce NET formation, including microbes, microbial products like lipopolysaccharide (LPS), cytokines, antibodies, immune complexes, and reactive oxygen species (ROS) [21]. Several signaling pathways contribute to the formation of NETs [22–24]. For example, the production of ROS is critical in NET formation. NADPH oxidase complex facilitates the conversion of molecular oxygen to superoxide and hydrogen peroxide. Hydrogen peroxide reacts with MPO to generate hypohalous acids, including hypochlorous acid (HOCl), which has been implicated in NET formation [25]. The role of ROS and MPO in NET formation has been confirmed in several studies. Notably, mutations in the gene encoding the NADPH oxidase complex lead to a rare immune deficiency disorder known as chronic granulomatous disease (CGD). Neutrophils isolated from patients with CGD are defective in ROS production and NET formation [13]. Likewise, MPO-deficient neutrophils do not form NETs [26]. The granular protein neutrophil elastase (NE) is also essential for NET formation. ROS can initiate the translocation of NE from granules to the nucleus, where NE facilitates histone processing and chromatin decondensation [27]. Notably, we and others have shown that inhibition of NE activity prevents NET formation [27, 28]. Another crucial component of NET formation involves peptidylarginine deiminase 4 (PAD4) that catalyzes the conversion of arginine residues in histones to citrulline. The citrullination of histone has been identified as an indicator of NET formation [29]. Hence, PAD4 is essential in NET formation, and inhibition of PAD4 activity prevents NET formation [30]. Furthermore, phorbol 12-myristate 13-acetate (PMA) is one of the most potent inducers of NET formation in vitro. PMA activates the protein kinase C (PKC) pathway, leading to the activation of Raf-MEK-ERK pathway and ROS production [31, 32]. The molecular mechanisms involving NET formation have been previously discussed and can be referred to for further reading [22–24]. These critical pathways in NET formation are potential drug targets for the inhibition of NET formation and may be relevant for cancer therapeutics.

Role of neutrophils and NETs in cancer

There is significant interest in the role of neutrophils in cancer (Fig. 2). Prior studies have demonstrated both protumorigenic and anti-tumorigenic roles for neutrophils [33, 34]. These opposing roles of neutrophils may be attributed to the different animal models utilized by investigators or to the type of cancer being studied. However, most studies attribute a protumorigenic role for neutrophils, and neutrophil

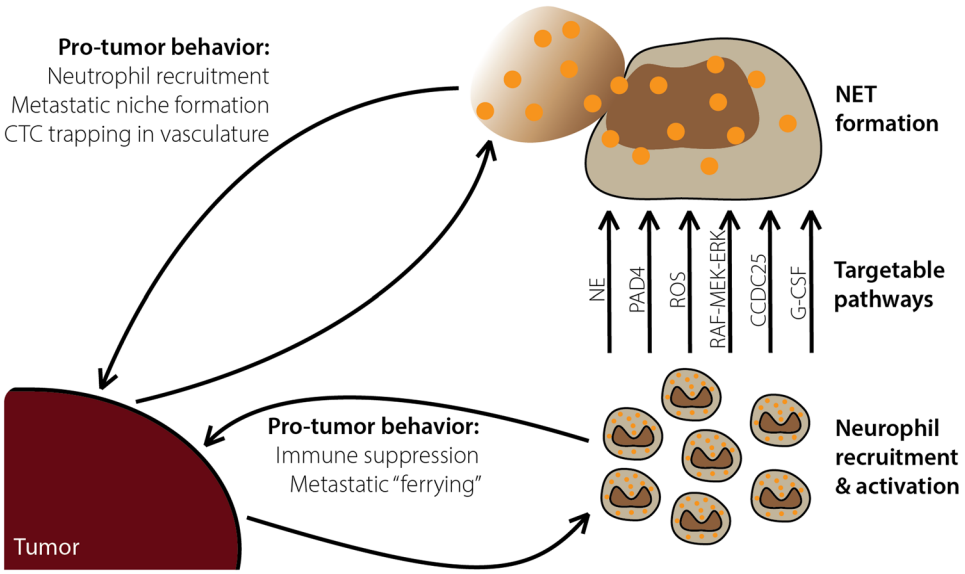

Fig. 2 Roles of neutrophils and NETs in cancer progression

depletion or cytokine-based phenotype modulation has been shown to inhibit tumor growth [35–37].

In humans, neutrophils are the most abundant leukocytes in circulation, consisting of up to 50–70% of all leukocytes. Notably, neutrophils have a very short half-life of about hours to days, and mature neutrophils are produced continuously in the bone marrow and released into the circulation in a process termed granulopoiesis. However, in homeostatic conditions, only about 2% of neutrophils produced in the bone marrow are released into the circulation [38]. Granulocyte-colony stimulating factor (G-CSF) is a major regulator of neutrophil generation and differentiation [39, 40] and plays a significant role in neutrophil development and release into the circulation. During infection, emergency granulopoiesis is initiated, and more neutrophils are rapidly mobilized to the infection site for the protection of the host. There is emerging evidence indicating that tumors induce emergency granulopoiesis. Cancer cells produce G-CSF [41, 42], leading to an increased number of neutrophils. Indeed, studies have shown that cancer patients have a higher number of circulating neutrophils, which is associated with adverse outcomes [43, 44].

The role of the tumor microenvironment (TME) in neutrophil activation and function is of significant interest. Depending on the cytokine cues they receive from the TME, neutrophils can be polarized to different phenotypes. In their seminal paper, Fridlender et al. identified two distinct subsets of neutrophils [37]. Tumor-bearing mice treated with TGF-β inhibitor resulted in a subset of tumor-associated neutrophils (TANs) termed N1 neutrophils with more significant tumor cytotoxicity. In contrast, the presence of TGF-β resulted in a subset of TANs called N2 neutrophils with protumorigenic function. Importantly, their study suggested that N2 neutrophils suppress the activation of CD8 + T cells, highlighting the role of neutrophils in adaptive immunity. However, the distinctive phenotype of N1 versus N2 neutrophils, including their lineage markers and transcription factors, is yet to be further delineated.

Another population of myeloid-derived cells with a granulocytic phenotype that is important in tumor immunology is myeloid-derived suppressor cells (MDSCs). A subset of MDSCs express neutrophil markers, have potent suppressive ability, and are called granulocytic MDSCs (G-MDSCs). Whether G-MDSCs are a distinct lineage of immune cells or are simply immature neutrophils that acquire a suppressive phenotype based on cytokine cues from the TME is a subject of ongoing debate [45]. The origins of G-MDSCs and their relation to neutrophils in the immature, mature, or tumor-associated stage remain unclear although several authors have encouraged a large-scale continuum view of myeloid cell involvement in cancer rather than specific phenotype assignment [45, 46]. One recent study compared neutrophils, tumor-associated neutrophils, and G-MDSCs at a transcriptomic level, allowing for discrimination over conventional cell surface marker analysis [47]. MDSCs are prevalent in human cancers [48–50], and MDSCs contribute to tumor progression by suppressing activities of T cells and NK cells, production of immunosuppressive cytokines, and induction of regulatory T cells [51]. Interestingly, tumors have been shown to produce IL-8, which attracts G-MDSCs to the tumor site and induce NET formation [52].

NETs contribute to tumor progression, and neutrophils are associated with adverse outcomes [18–20]. Cancer cells induce NET formation through G-CSF release [18], and neutrophil proteins released during NET formation promote the proliferation of cancer cells. Hence, neutrophil-tumor interaction can drive a feed-forward mechanism that propagates cancer progression via NET formation. But how does NET

formation promote tumor growth, metastases, and mortality? NETs contribute to cancer progression by promoting metastasis through interaction with cancer cells, by release of protumorigenic mediators, and promoting thrombus formation (Fig. 3). These will be discussed in detail in the following sections.

NETs interact with cancer cells and promote metastasis

The interactions of NETs with cancer cells contribute to metastasis. It is well established that NETs function as mechanical traps for pathogen capture [5]. NETs can also trap and sequester circulating tumor cells (CTCs), thereby promoting metastasis in distant sites. Cools-Lartigue et al. demonstrated the physical trapping of CTCs within NETs in vitro both during static and dynamic conditions [17]. This entrapment of CTCs by NETs led to increased metastasis in vivo. The same group showed that β-1 integrin expression on both cancer cells and NETs is important for the adhesion of CTCs to NETs [53]. In the context of inflammatory stimuli, TLR4 induced the activation of platelets and platelet-tumor cell aggregate formation in an ERK5-GPIIb/IIIa integrin-dependent manner [54]. NETs trap platelet-tumor cell aggregates, leading to metastasis at distant sites. Interestingly, inhibition of TLR4 activity or blocking platelet activation protected mice from inflammation-induced metastasis without CTC entrapment by NETs [54]. In addition to physical trapping of CTCs, NETs have also been shown to shield tumor cells from the host's immune responses. NETs shielded tumor cells from cytotoxicity by CD8 + T cells and NK cells, thereby promoting metastasis [55]. These studies shed new light on how the physical presence of NETs enables cancer progression.

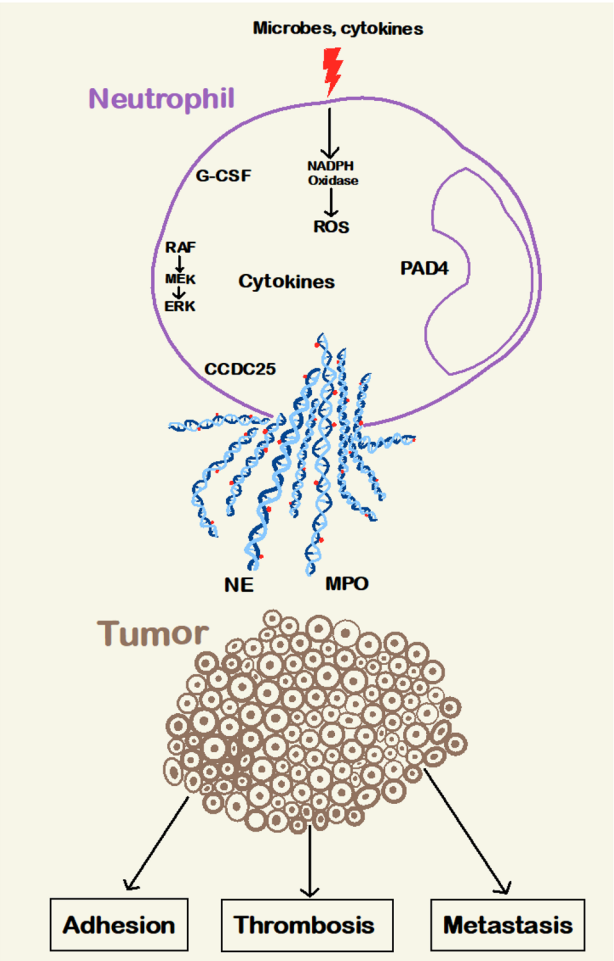

Fig. 3 Contributions of NETs in tumor cell adhesion, thrombus formulation, and cancer metastasis

The formation of NETs by neutrophils leads to the release of several proteins that activate the inflammatory cascade. One of the major consequences is the upregulation of adhesion molecules on the vascular endothelium and their receptors on leukocytes. It is conceivable that this increase in adhesion molecules also leads to the adhesion of CTCs, thereby seeding de novo metastatic niches. Indeed, Szczerba et al. used single-cell RNA sequencing to show that the majority of white blood cells associated with CTCs are neutrophils [56]. The interaction between neutrophils and CTCs was mediated by vascular cell adhesion molecule 1 (VCAM-1), and this neutrophil-CTC association led to cell cycle progression and increased the metastatic potential of CTCs. Neutrophils increased the adhesion of cancer cells to the liver in the context of systemic inflammation [57]. Cancer cells can bind to NETs through integrins α5 β1 and αvβ3 [58]. Interestingly, this increased adhesion of cancer cells was abrogated using DNase or integrin blocking antibodies. These studies support the notion that neutrophils and NETs promote tumor progression by increasing the adhesion of CTCs and capturing them.

NETs release protumorigenic mediators

Long before NETs were discovered, several proteins released by neutrophils during NET formation were implicated in tumor progression. For example, NE is a serine protease stored in the azurophilic granules of neutrophils whose name is derived from its ability to degrade the extracellular matrix protein elastin. NE is notable for its promiscuous activity and has also been shown to induce cell proliferation and cell migration [59–62]. NE plays an essential role in the formation of NETs, and inhibition of NE activity prevents NET formation [27]. NE is also a major product of NET formation. Elevated levels of NE have been reported in cancer patients, and NE has been proposed as a marker of severity in colorectal and breast cancer with higher levels of

NE associated with worse outcomes [63–65]. Recently, NE produced by NETs has been shown to re-awaken dormant cancer cells by remodeling laminin and activation of integrin α3 β [19]. Interestingly, neutrophils are not the sole source of NE in the TME as cancer cells can produce NE [66]. NE can also induce the proliferation and migration of cancer cells [67]. Pharmacologic and genetic inhibition of NE decreased tumor growth and improved survival in tumor-bearing animals [68–70]. In addition to NE, matrix metalloproteinase-9 and cathepsin G, which are produced during NET formation, have been shown to increase tumor progression [20].

Another major byproduct of NET formation is DNA, and NET-associated DNA has been shown to promote cancer progression. Yang et al. demonstrated that NETs do not merely act as a "trap" for CTCs but also that NET-DNA serves as a chemotactic factor to attract cancer cells [71]. They identified the transmembrane protein coiled-coil domain containing protein 25 (CCDC25) as a NET-DNA receptor on cancer cells that senses extracellular DNA and subsequently activates the integrin-linked kinase-β-parvin pathway to enhance cell motility. Moreover, the expression of CCDC25 on primary cancer cells was closely associated with a poor prognosis for patients, suggesting an active role of NETs in promoting cancer metastasis.

NETs induce thrombosis in cancer

Dysregulation of the coagulation pathway is a frequent occurrence in cancer patients and significantly increases mortality [72, 73]. Dysregulation of the coagulation pathway leads to blood vessel occlusion and thrombosis associated with pathologic events, including cardiovascular disease, deep vein thrombosis, and pulmonary embolism. It is conceivable that NET scaffold can occlude blood vessels and cause thrombosis. Indeed, NET scaffolds promote adhesion of platelets, leading to thrombus formation [74, 75], and NET formation has been associated with increased thrombus formation and blood vessel occlusion in several autoimmune diseases [76]. For example, depletion of neutrophils decreased thrombus formation [77], and inhibition of NET formation by infusion of DNase or genetic ablation of PAD4 protected mice from thrombosis [78, 79]. Interestingly, the etiology of thrombosis in cancer has been recently attributed to NET formation. Cancer cells can induce platelet activation and promote thrombosis [80, 81]. NET formation led to a prothrombotic state in tumor-bearing mice [42], and citrullinated histone H3, a biomarker of NET formation, is a predictor of the risk of venous thromboembolism in cancer patients [82]. Chloroquine, an anti-malarial drug, decreases NET formation [83] and reduces coagulation factors in cancer patients [84]. Tumor-derived microparticles adhere to NETs at the site of thrombus formation [85], and human pancreatic tumors grown in mice release tissue factor-positive microvesicles that increase venous thrombosis [86]. Moreover, inhibition of NET formation decreased thrombus formation associated with tumor occurrence [87, 88]. These studies highlight the significance of NET-associated thrombus formation in cancer.

Targeting NETs in cancer

Inhibition of NET formation is rapidly gaining traction as a therapeutic strategy, and several studies have shown that inhibition of NET formation can lead to tumor retardation and cancer regression. The use of DNAse to inhibit NETs has shown therapeutic benefit in several cancer models. For example, inhibition of NETs using DNAse prevented lung metastasis in a mouse model of breast cancer [18], and DNAse treatment also prevented adhesion and metastasis of CTCs [17, 53].

NETs have also been targeted in cancer via the inhibition of PAD4 activity. Cedervall et al. showed that NETosis was associated with cancer-induced renal dysfunction, and inhibition of NET formation using DNAse or PAD4 inhibition abrogated cancer-induced renal dysfunction [89]. PAD4 inhibition synergized with immune checkpoint blockade to prevent tumor progression [55]. Another molecular target of NET inhibition in cancer therapy is NE. Unpublished data from our lab showed that inhibition of NE decreased the growth of murine breast cancer cells. Other studies have shown that pharmacologic and genetic inhibition of NE decreased tumor growth and improved survival in tumor-bearing animals [68–70]. These studies have highlighted the prospect of targeting NET formation in cancer immunotherapy.

Engineering materials for targeting neutrophils in cancer

Nanoparticles (NPs) have emerged as a new platform for the delivery of therapeutics to cells with high specificity and reduced toxicity. Nanoencapsulation of drugs increases drug efficacy and circulation half-life [90]. In the case of cancer, NPs offer the advantage of precision targeted delivery of cytotoxic drugs to tumor cells while sparing healthy cells [90, 91]. Notably, tumor vasculature is characterized by weakened barrier integrity which enables the passive accumulation of NPs at the tumor site, a phenomenon known as the enhanced permeability and retention (EPR) effect [92]. In addition, NPs can be actively targeted to tumor-specific ligands [93, 94], and such NPs can reduce off-target toxicity of anti-cancer drugs. For example, recent work has shown that anti-tumor drugs, such as 5-fluorouracil (5FU), when

given in the free form induced NETs in the blood, but this effect was not observed if 5FU was delivered using polymeric NPs [95].

As the most abundant innate immune cell population, neutrophils inevitably take up injected NPs due to their phagocytic functions [96]. The development of NPs that can be efficiently taken up by neutrophils for therapeutic purposes has gained considerable interest in recent years [97]. Human neutrophils can internalize particles varying in both size (5 nm to 2 μm) and chemical composition (e.g., lipids, poly(styrene), poly(lactic-co-glycolic acid), and gold) [98]. Particle uptake by neutrophils is rapid, typically plateauing within 15 min. Importantly, uptake of nanoscale poly(styrene) and liposomal particles at concentrations of up to 5 μg/mL did not increase neutrophil apoptosis, activation, or cell death [98]. Additionally, ingested particles resided in intracellular compartments that were retained in neutrophils during activation and degranulation. Particle-laden neutrophils also retained the ability to degranulate normally in response to chemical stimulation [98]. Hence, neutrophils are promising targets for NP-mediated drug delivery.

The inherent ability of neutrophils to take up injected particles has been utilized for therapeutic purposes in recent studies. Notably, neutrophils possess several Fcγ receptors (FcγRs) that can mediate NP uptake via endocytosis [99]. Cationic liposomes loaded with paclitaxel (PTX-CL) were readily internalized by neutrophils and have been developed as a basis for neutrophil-based drug delivery (Fig. 4A) [100]. In a mouse model of glioblastoma, PTX-CL/NEs efficiently crossed the blood–brain barrier and showed greater accumulation in the brain, compared to untreated controls (Fig. 4B). PTX-CL/NEs administered after glioblastoma resection surgery significantly increased the animal survival up to 61 days, compared with 29 days for Taxol and 38 days for PTX-CL (Fig. 4C) [100]. These results indicate the efficacy of neutrophils as vehicles for drug delivery of nanomaterials.

Albumin-based NPs have been recently reported to target neutrophils in the setting of acute lung injury as well as cancer. Albumin NPs were synthesized by ethanol desolvation of bovine serum albumin (BSA) followed by glutaraldehyde-mediated cross-linking (Fig. 5A) [101]. Albumin NPs were preferentially taken up by activated neutrophils but not by activated monocytes (Fig. 5B). Albumin NP uptake was partly dependent on FcγRIII expressed on neutrophils, as the absence of FcγRIII significantly decreased NP uptake by 50%. Drug-loaded albumin NPs were more effective than free drug in limiting neutrophil infiltration and lung MPO production in an LPS-induced model of acute lung injury (Fig. 5C). The authors have further developed the albumin NP platform for targeted drug delivery to cancer (Fig. 5D) [102]. In the mouse model of B16 melanoma, TA99, a monoclonal antibody specific for gp75 antigen, is known to cause neutrophil recruitment to the tumor site. Hence, in B16 tumor-bearing mice, the authors administered TA99, followed by injection of albumin NPs carrying a photosensitizer Ppa, leading to significantly improved delivery of Ppa to the tumor site and increased efficacy of photodynamic therapy with an extended animal survival (Fig. 5E, F). Albumin NPs were taken up by a distinct subset of neutrophils with a pro-inflammatory phenotype [103]. These results highlight the potential of neutrophil-targeted drug delivery in the settings of cancer as well as inflammation.

Another strategy employed for therapeutic manipulation of neutrophils involves active targeting of neutrophil ligands. In this approach, the NPs engineered to bind to specific ligands on neutrophils are used for receptor-mediated endocytosis into neutrophils. For example, NPs have been coupled to the tri-amino acid sequence, arginine-glycine-aspartate (RGD) which promotes cellular adhesion to the extracellular matrix for targeted drug delivery to neutrophils. These RGD-coupled NPs have been shown to be effective in neutrophil-mediated drug delivery across the blood–brain barrier [104, 105]. In a mouse model of cerebral ischemia, RGD liposomes loaded with an antioxidant ER administered after 3 h resulted in decrease in infarct volume of 40–52% compared to animals treated with free drug alone, demonstrating the potential of RGD liposomes in drug delivery across the blood–brain barrier [104]. In addition, neutrophil membranes are rich with receptors that can be actively targeted with monoclonal antibodies. Thus, NP-antibody conjugates can be utilized for active targeting of neutrophils. Lymphocyte antigen 6 complex locus G6D (Ly-6G) is a component of the myeloid differentiation antigen Gr-1 that is predominantly expressed on neutrophils and widely utilized as a neutrophil marker. For example, superparamagnetic iron oxide nanoparticles conjugated with Ly-6G antibody have been developed for non-invasive, in situ labeling and tracking of neutrophils in vivo [106]. In another example, a NIMP-R14 antibody (which recognizes Ly-6G) was conjugated on the surfaces of PEGylated polylactide-coglycolide (PLGA)-NPs for the neutrophil-targeted drug delivery in the lungs [107]. PEGylation was employed to limit the clearance of the NPs by the airway defense mechanisms. This PEGylated immuno-conjugated PLGA-NP loaded with ibuprofen was shown to accumulate in the lungs, decrease the production of neutrophil MPO, and inhibit lung inflammation. Alternatively, peptides specific to neutrophil markers may be employed for targeting neutrophils. For instance, the phage display technology has been used to identify peptides that bind specifically to CD177 (also known as human neutrophil antigen NB1). These CD177-binding peptides were conjugated to lipid-based NPs for targeted drug delivery to neutrophils [108], providing an alternative approach to antibody-conjugated NPs. These approaches showcase the potential of NPs decorated with neutrophil-binding antibodies or other ligands for active targeting of neutrophils.

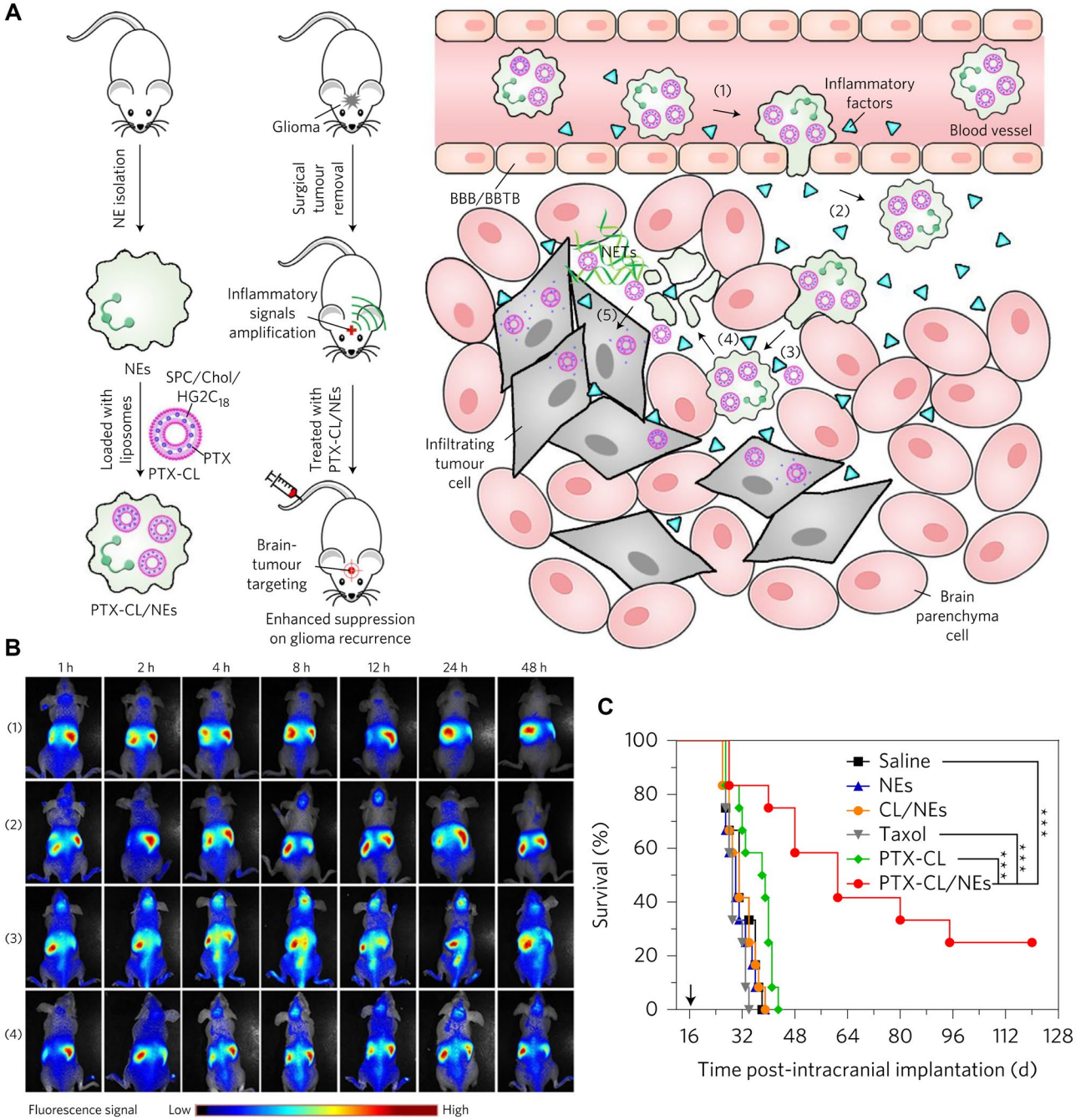

Fig. 4 Neutrophil-mediated anticancer drug delivery suppresses postoperative glioma recurrence. **A** Schematic illustration of PTX-CL/NE-mediated suppression of postoperative glioma recurrence in mice. PTX-CL/NEs transmigrate to the inflamed brain across the blood–brain barrier and delivers PTX into the infiltrating tumor cells to produce an anti-tumor effect. **B** Fluorescence imaging of the normal mice (1), G422-bearing mice (2), surgically treated G422-bearing mice (3), and the sham-operated mice (4) after intravenous administration of PTX-CL/DiR-NEs. **C** Survival curves of the surgically treated G422-bearing mice after intravenous administration of saline, the blank NEs, CL/NEs without PTX, Taxol, PTX-CL, and PTX-CL/NEs. Reprinted with permission from Xue et al. [100]

Moreover, drug-loaded NPs can be coated with neutrophil membranes rich in integrin ligands for tumor-targeted delivery of anti-cancer drugs [109]. These neutrophil-mimicking NPs (NM-NP) have found important applications for targeted drug delivery to cancer. For example, Zhou et al. demonstrated the efficacy of PEG-PLGA NPs coated with neutrophil membranes and loaded with an anti-inflammatory drug, celastrol, for the treatment of acute pancreatitis [110]. Compared to control NPs without membrane coating, NM-NP were shown to selectively accumulate in the pancreas of rats with acute

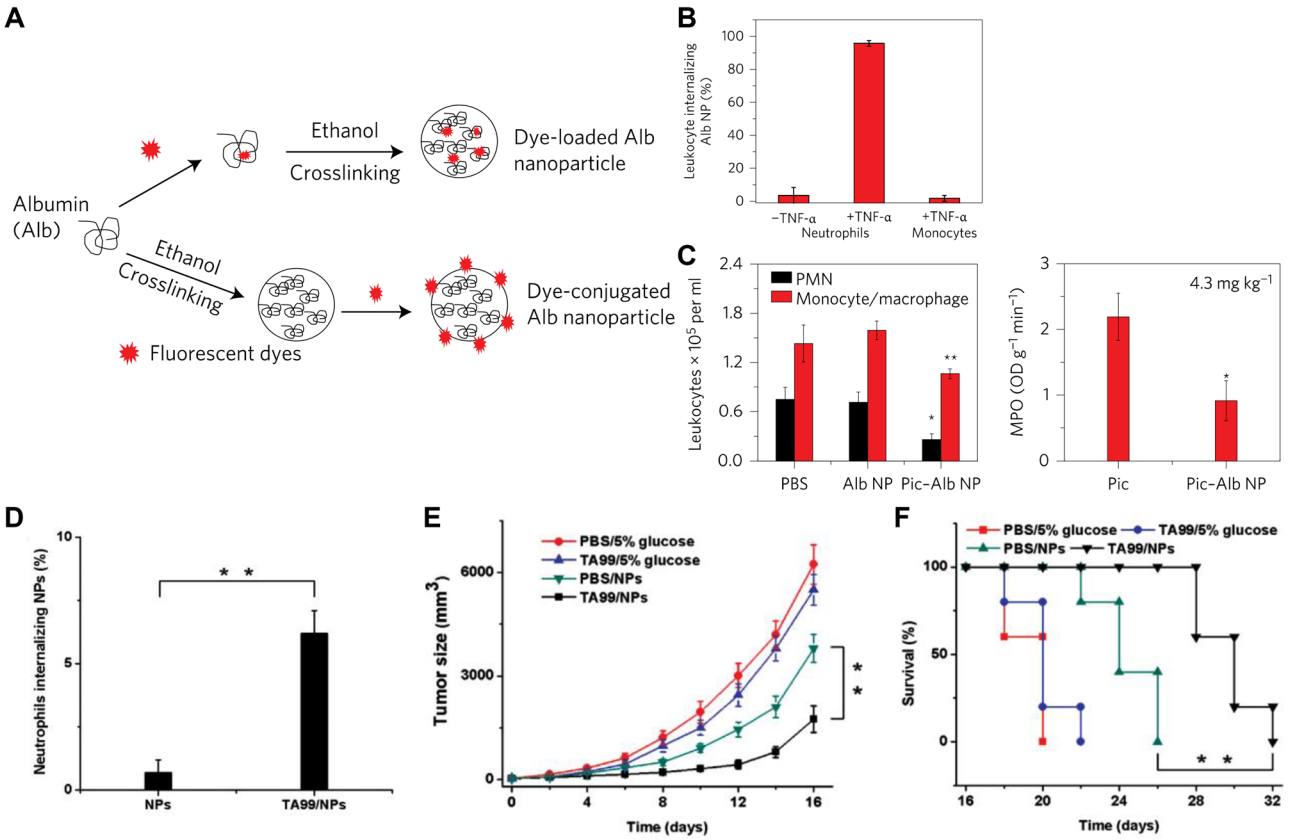

Fig. 5 Albumin nanoparticles for targeting neutrophils. **A** Schematic illustration for synthesis of albumin nanoparticles (Alb-Nano). **B** The percentage of neutrophils and monocytes internalizing Alb-Nano as assessed by intravital microscopy of mouse cremaster muscle. **C** Intravenous treatment with Alb-Nano carrying piceatannol decreased leukocyte infiltration and MPO activities in a mouse model of LPS-induced acute lung inflammation. **D** Administration of TA99 increased the internationalization of albumin nanoparticles by neutrophils in the blood. **E**, **F** Mice bearing B16 melanoma were treated with TA99 and albumin nanoparticles carrying Ppa, followed by photodynamic therapy. The animals were monitored for **E** tumor growth and **F** survival. Reprinted with permission from Wang et al. [101] for panels **A**–**C** and from Chu et al. [102] for panels **E**–**F**

pancreatitis. In a murine model of pancreatic cancer, NM-NP significantly inhibited tumor growth, prevented metastasis, and increased the animal median survival from 28 days (the saline control group) to 63 days (Fig. 6A, B) [111]. Furthermore, NM-NP loaded with an anti-tumor drug carfilzomib has been shown to deplete CTCs and prevent metastasis in a murine model of breast cancer (Fig. 6C, D) [112]. These studies have demonstrated the therapeutic potential of NM-NPs in preclinical models of cancer.

Notably, the approaches presented above for NP-neutrophil targeting can be utilized for the prevention of NET formation in cancer and other diseases. We have recently showed that lipid-based NPs, termed interbilayer-crosslinked multilamellar vesicles (ICMVs), can be rapidly taken up by neutrophils and used to increase the efficacy of sivelestat (a neutrophil elastase inhibitor) to prevent NET formation [28]. ICMVs loaded with sivelestat exhibited a greater efficacy than the free drug in preventing NET formation and reducing mortality in a murine model of endotoxic shock (Fig. 7). Moreover, other studies have also shown the utility of NPs in prevention of NET formation in the setting of cancer prevention. For example, NPs coated with DNase have been shown to inhibit NET formation and prevent metastasis in a murine model of breast cancer [18]. In addition, NPs modified with α2,8-linked sialic acid chains have been shown to inhibit NET formation [113]. These examples show the potential of NPs engineered to target and inhibit NET formation for cancer therapy.

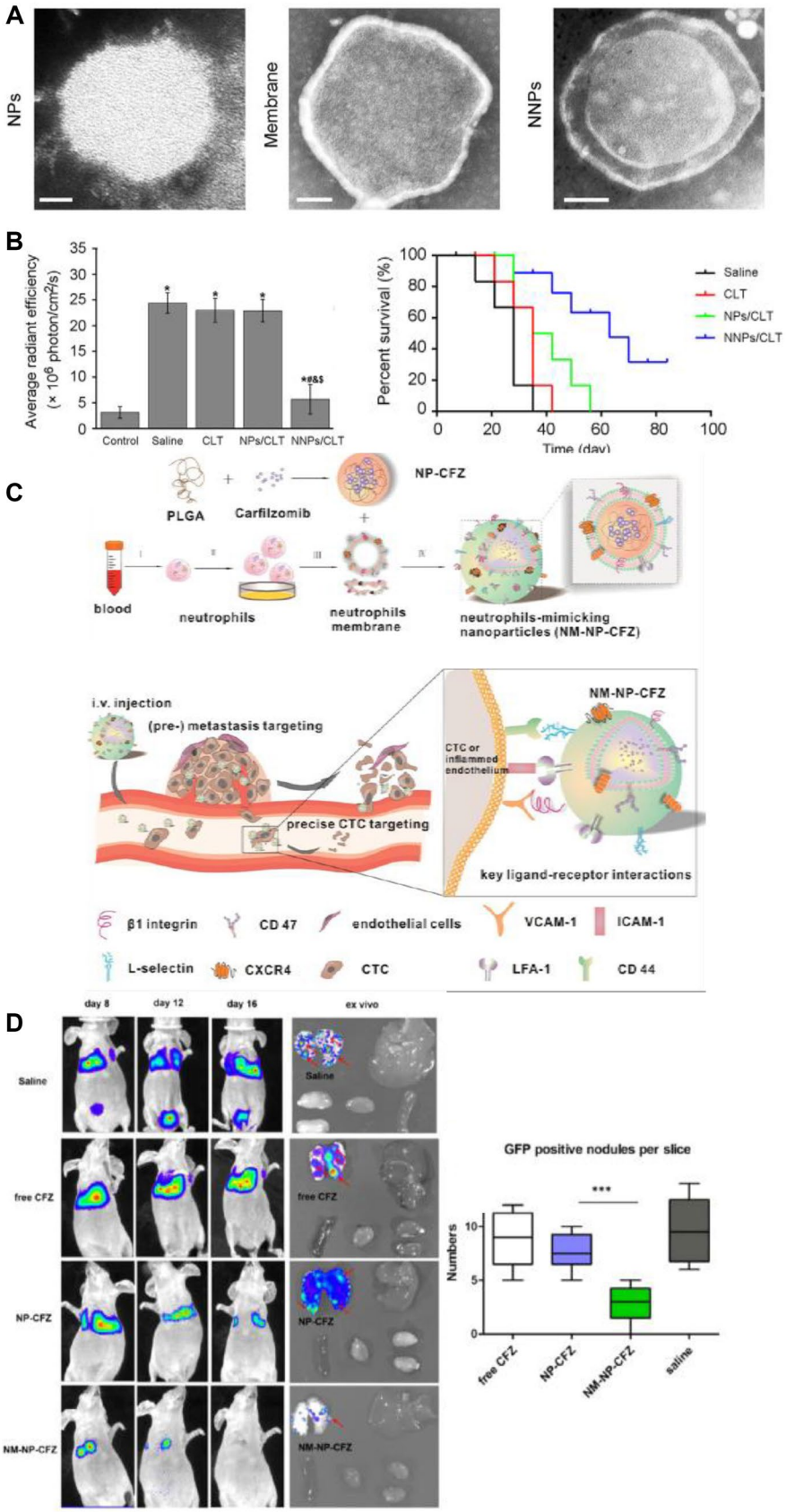

Fig. 6 Neutrophil membrane-coated nanoparticles for cancer treatment. **A** Transmission electron microscopy images of nanoparticles, neutrophil membrane, and neutrophil membrane-coated nanoparticles (NNPs). **B** Anti-tumor efficacy of NNPs carrying celastrol as quantified by GFP signal and animal survival in mice bearing orthotopic GFP-Panc02 pancreatic cancer cells. **C** Schematic illustration of synthesis of NM-NP carrying carfilzomib. The cocktail of neutrophil membrane-associated proteins enables the resulting NM-NP-CFZ to target circulating tumor cells (CTCs) in circulation and inflamed endothelial cells in the premetastatic lesion. **D** NM-NP-CFZ inhibited the development of already formed 4T1 lung metastasis. In vivo imaging of mice bearing GFP-4T1 lung metastasis, followed by treatments. The right panel shows quantification of GFP + lung nodules. Reprinted with permission from Cao et al. [111] for panels **A**–**B** and from Kang et al. [112] for panels **C**–**D**

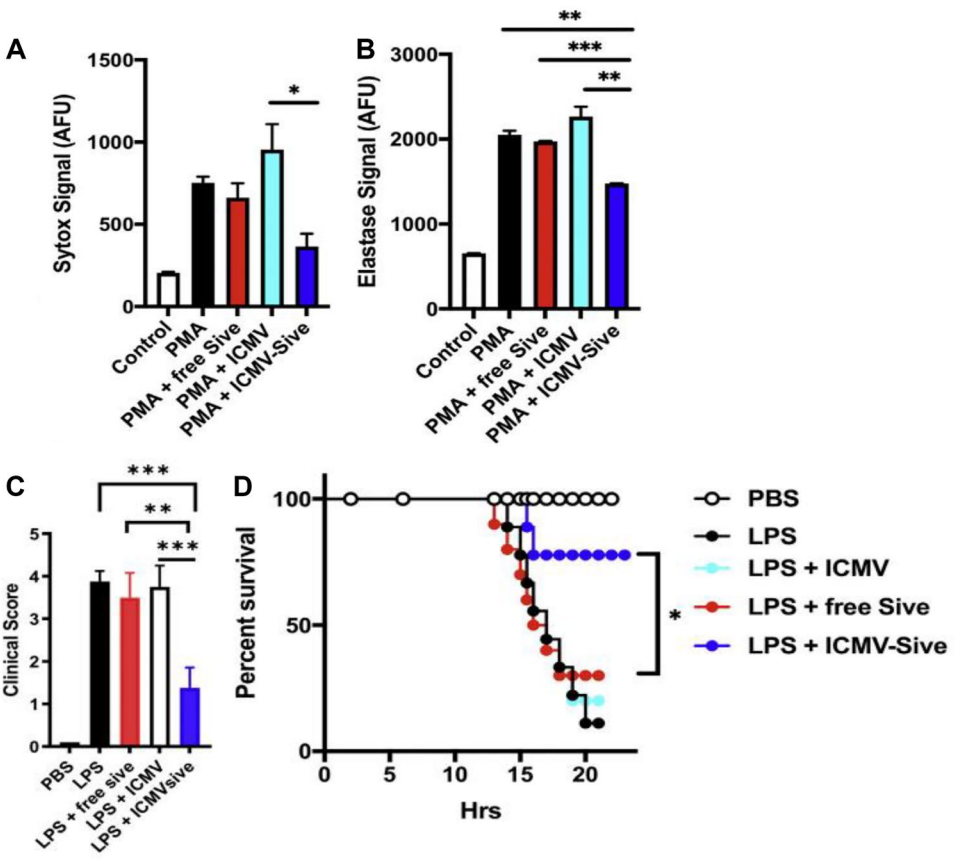

Fig. 7 ICMVs decrease NET formation and rescue mice from endotoxic shock. Drug-loaded ICMVs decrease NET formation as measured by release of extracellular DNA (**A**) or neutrophil elastase (**B**). Drug-loaded ICMVs significantly improve the clinical score (**C**) and survival of mice (**D**) in a LPS model of endotoxic shock compared to controls. Reprinted with permission from Okeke et al. [28]

Conclusions and perspectives

There is mounting experimental evidence showing that neutrophils and NETs contribute to tumor progression and metastases [20]. Recently, our understanding of tumor biology and immunology has greatly expanded the potential for cancer treatment through several immunological targets. Neutrophils have long been known as mediators of inflammation. In this context, neutrophil-mediated inflammation has been known to contribute to tumor progression, and therapies targeting the inflammation cascade can exert anti-tumor effects in humans. For example, clinical trials for cancer treatment with monoclonal antibodies blocking IL-6, an inflammatory cytokine, have shown promising results [114].

Strategies to inhibit NET formation have significant potential to advance cancer therapeutics. Experimental evidence has shown anti-tumor efficacy for inhibitors of NET formation, including DNase and inhibitors of PAD4 and neutrophil elastase. However, the anti-tumor efficacy of these inhibitors of NET formation has not been investigated in human studies. Such efforts are necessary to give a clearer picture on the effectiveness of NET inhibitors in cancer therapy. Indeed, there is potential for targeting the pathways that drive NET formation in cancer therapy (Fig. 2). For example, the PKC and MAPK pathways are involved in NET formation, and targeting the PKC and MAPK pathways may inhibit NET formation and exert an anti-tumorigenic effect.

Combining NET inhibition or neutrophil targeting with another anti-tumor therapy may be synergistic. For example, neutrophils have been shown to mediate tumor refractoriness to anti-VEGF treatment in mice [115]. Hence, anti-VEGF therapy combined with neutrophil depletion may be more effective than anti-VEGF treatment alone. Likewise, tumors resistant to immune checkpoint blockade were eliminated by inhibition of myeloid-derived cells [116], thus demonstrating the potential of neutrophil-targeting combination therapies.

Overall, more studies are needed to elucidate the role of neutrophils and NETs in cancer biology. Understanding the complex interplay between NET formation and tumor progression will further improve the options for targeting neutrophils in cancer therapy. Additionally, more work needs to be done in engineering NPs for anti-tumor drug delivery since there is tremendous opportunity in the rational design of NPs for targeting neutrophils and NET formation as potential therapeutics against cancer as well as other inflammatory diseases.

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References

1. Bodey GP, Buckley M, Sathe YS, Freireich EJ. Quantitative relationships between circulating leukocytes and infection in patients with acute leukemia. Ann Intern Med. 1966;64:328–40.
2. Donowitz GR, Maki DG, Crnich CJ, Pappas PG, Rolston KV. Infections in the neutropenic patient--new views of an old problem. Hematology Am Soc Hematol Educ Program. 2001;113–39.
3. Bouma G, Ancliff PJ, Thrasher AJ, Burns SO. Recent advances in the understanding of genetic defects of neutrophil number and function. Br J Haematol. 2010;151:312–26.
4. Nordenfelt P, Tapper H. Phagosome dynamics during phagocytosis by neutrophils. J Leukoc Biol Wiley. 2011;90:271–84.
5. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, et al. Neutrophil extracellular traps kill bacteria. Science. 2004;303:1532–5.
6. Branzk N, Lubojemska A, Hardison SE, Wang Q, Gutierrez MG, Brown GD, et al. Neutrophils sense microbe size and selectively release neutrophil extracellular traps in response to large pathogens. Nat Immunol Nature Publishing Group. 2014;15:1017–25.
7. Steinberg BE, Grinstein S. Unconventional roles of the NADPH oxidase: signaling, ion homeostasis, and cell death. Sci STKE. 2007.
8. Yipp BG, Petri B, Salina D, Jenne CN, Scott BNV, Zbytnuik LD, et al. Infection-induced NETosis is a dynamic process involving neutrophil multitasking in vivo. Nat Med. 2012;18:1386–93.
9. Pilsczek FH, Salina D, Poon KKH, Fahey C, Yipp BG, Sibley CD, et al. A novel mechanism of rapid nuclear neutrophil extracellular trap formation in response to Staphylococcus aureus. J Immunol. 2010;185:7413–25.
10. Urban CF, Reichard U, Brinkmann V, Zychlinsky A. Neutrophil extracellular traps capture and kill Candida albicans yeast and hyphal forms. Cell Microbiol. 2006;8:668–76.
11. Guimarães-Costa AB, Nascimento MTC, Froment GS, Soares RPP, Morgado FN, Conceição-Silva F, et al. Leishmania amazonensis promastigotes induce and are killed by neutrophil extracellular traps. Proc Natl Acad Sci USA. 2009;106:6748–53.
12. Saitoh T, Komano J, Saitoh Y, Misawa T, Takahama M, Kozaki T, et al. Neutrophil extracellular traps mediate a host defense response to human immunodeficiency virus-1. Cell Host Microbe. 2012;12:109–16.
13. Bianchi M, Hakkim A, Brinkmann V, Siler U, Seger RA, Zychlinsky A, et al. Restoration of NET formation by gene therapy in CGD controls aspergillosis. Blood. 2009;114:2619–22.
14. Villanueva E, Yalavarthi S, Berthier CC, Hodgin JB, Khandpur R, Lin AM, et al. Netting neutrophils induce endothelial damage, infiltrate tissues, and expose immunostimulatory molecules in systemic lupus erythematosus. J Immunol NIH Public Access. 2011;187:538–52.
15. Khandpur R, Carmona-Rivera C, Vivekanandan-Giri A, Gizinski A, Yalavarthi S, Knight JS, et al. NETs are a source of citrullinated autoantigens and stimulate inflammatory responses in rheumatoid arthritis. Sci Transl Med. NIH Public Access. 2013;5:178ra40.
16. Kessenbrock K, Krumbholz M, Schönermarck U, Back W, Gross WL, Werb Z, et al. Netting neutrophils in autoimmune small-vessel vasculitis. Nat Med. 2009;15:623–5.
17. Cools-Lartigue J, Spicer J, McDonald B, Gowing S, Chow S, Giannias B, et al. Neutrophil extracellular traps sequester circulating tumor cells and promote metastasis. J Clin Invest. 2013;123:3446–58.
18. Park J, Wysocki RW, Amoozgar Z, Maiorino L, Fein MR, Jorns J, et al. Cancer cells induce metastasis-supporting neutrophil extracellular DNA traps. Sci Transl Med. 2016;8:361ra138–361ra138.
19. Albrengues J, Shields MA, Ng D, Park CG, Ambrico A, Poindexter ME, et al. Neutrophil extracellular traps produced during inflammation awaken dormant cancer cells in mice. Science (80-). 2018;361:eaao4227.
20. Cools-Lartigue J, Spicer J, Najmeh S, Ferri L. Neutrophil extracellular traps in cancer progression. Cell Mol Life Sci Birkhauser Verlag AG. 2014; 4179–94.
21. Brinkmann V, Zychlinsky A. Neutrophil extracellular traps: is immunity the second function of chromatin? J Cell Biol. 2012;198:773–83.
22. Kenny EF, Herzig A, Krüger R, Muth A, Mondal S, Thompson PR, et al. Diverse stimuli engage different neutrophil extracellular trap pathways. Elife. 2017;6.
23. Kolaczkowska E, Jenne CN, Surewaard BGJ, Thanabalasuriar A, Lee W-Y, Sanz M-J, et al. Molecular mechanisms of NET formation and degradation revealed by intravital imaging in the liver vasculature. Nat Commun. 2015;6:6673.
24. Yang H, Biermann MH, Brauner JM, Liu Y, Zhao Y, Herrmann M. New insights into neutrophil extracellular traps: mechanisms of formation and role in inflammation. Front Immunol Frontiers Media S.A. 2016;1.
25. Palmer LJ, Cooper PR, Ling MR, Wright HJ, Huissoon A, Chapple ILC. Hypochlorous acid regulates neutrophil extracellular trap release in humans. Clin Exp Immunol John Wiley & Sons Ltd. 2012;167:261–8.
26. Metzler KD, Fuchs TA, Nauseef WM, Reumaux D, Roesler J, Schulze I, et al. Myeloperoxidase is required for neutrophil extracellular trap formation: implications for innate immunity. Blood. 2011;117:953–9.
27. Papayannopoulos V, Metzler KD, Hakkim A, Zychlinsky A. Neutrophil elastase and myeloperoxidase regulate the formation of neutrophil extracellular traps. J Cell Biol. 2010;191:677–91.

28. Okeke EB, Louttit C, Fry C, Najafabadi AH, Han K, Nemzek J, et al. Inhibition of neutrophil elastase prevents neutrophil extracellular trap formation and rescues mice from endotoxic shock. Biomaterials. Elsevier Ltd. 2020;238:119836.
29. Li P, Li M, Lindberg MR, Kennett MJ, Xiong N, Wang Y. PAD4 is essential for antibacterial innate immunity mediated by neutrophil extracellular traps. J Exp Med. The Rockefeller University Press; 2010;207:1853–62.
30. Knight JS, Zhao W, Luo W, Subramanian V, O'Dell AA, Yalavarthi S, et al. Peptidylarginine deiminase inhibition is immunomodulatory and vasculoprotective in murine lupus. J Clin Invest. 2013;123:2981–93.
31. Gray RD, Lucas CD, Mackellar A, Li F, Hiersemenzel K, Haslett C, et al. Activation of conventional protein kinase C (PKC) is critical in the generation of human neutrophil extracellular traps. J Inflamm (United Kingdom). BioMed Central. 2013;10:12.
32. Hakkim A, Fuchs TA, Martinez NE, Hess S, Prinz H, Zychlinsky A, et al. Activation of the Raf-MEK-ERK pathway is required for neutrophil extracellular trap formation. Nat Chem Biol. 2011;7:75–7.
33. Powell DR, Huttenlocher A. Neutrophils in the Tumor Microenvironment. Trends Immunol. Elsevier Ltd. 2016;41–52.
34. Giese MA, Hind LE, Huttenlocher A. Neutrophil plasticity in the tumor microenvironment. Blood. Am Soc Hema. 2019;2159–67.
35. Dumitru CA, Lang S, Brandau S. Modulation of neutrophil granulocytes in the tumor microenvironment: mechanisms and consequences for tumor progression. Semin Cancer Biol Semin Cancer Biol. 2013;141–8.
36. Tazawa H, Okada F, Kobayashi T, Tada M, Mori Y, Une Y, et al. Infiltration of neutrophils is required for acquisition of metastatic phenotype of benign murine fibrosarcoma cells: implication of inflammation-associated carcinogenesis and tumor progression. Am J Pathol Am Soc Investig Pathol Inc. 2003;163:2221–32.
37. Fridlender ZG, Sun J, Kim S, Kapoor V, Cheng G, Ling L, et al. Polarization of Tumor-Associated Neutrophil Phenotype by TGF-β: "N1" versus "N2" TAN. Cancer Cell. 2009;16:183–94.
38. Semerad CL, Liu F, Gregory AD, Stumpf K, Link DC. G-CSF is an essential regulator of neutrophil trafficking from the bone marrow to the blood. Immunity Cell Press. 2002;17:413–23.
39. Lieschke GJ, Grail D, Hodgson G, Metcalf D, Stanley E, Cheers C, et al. Mice lacking granulocyte colony-stimulating factor have chronic neutropenia, granulocyte and macrophage progenitor cell deficiency, and impaired neutrophil mobilization. Blood Am Soc Hema. 1994;84:1737–46.
40. Richards MK, Liu F, Iwasaki H, Akashi K, Link DC. Pivotal role of granulocyte colony-stimulating factor in the development of progenitors in the common myeloid pathway. Blood. 2003;102:3562–8.
41. Kowanetz M, Wu X, Lee J, Tan M, Hagenbeek T, Qu X, et al. Granulocyte-colony stimulating factor promotes lung metastasis through mobilization of Ly6G+Ly6C+ granulocytes. Proc Natl Acad Sci USA. 2010;107:21248–55.
42. Demers M, Krause DS, Schatzberg D, Martinod K, Voorhees JR, Fuchs TA, et al. Cancers predispose neutrophils to release extracellular DNA traps that contribute to cancer-associated thrombosis. Proc Natl Acad Sci USA. Nat Acad Sci. 2012;109:13076–81.
43. Ethier JL, Desautels D, Templeton A, Shah PS, Amir E. Prognostic role of neutrophil-to-lymphocyte ratio in breast cancer: a systematic review and meta-analysis. Breast Cancer Res. BioMed Central Ltd. 2017;19.
44. Bowen RC, Little NAB, Harmer JR, Ma J, Mirabelli LG, Roller KD, et al. Neutrophil-to-lymphocyte ratio as prognostic indicator in gastrointestinal cancers: a systematic review and meta-analysis. Oncotarget Impact J LLC. 2017;8:32171–89.
45. Coffelt SB, Wellenstein MD, De Visser KE. Neutrophils in cancer: neutral no more. Nat Rev Cancer Nat Publish Grp. 2016;431–46.
46. Leach J, Morton JP, Sansom OJ. Neutrophils: homing in on the myeloid mechanisms of metastasis. Mol Immunol Elsevier Ltd. 2019;110:69–76.
47. Fridlender ZG, Sun J, Mishalian I, Singhal S, Cheng G, Kapoor V, et al. Transcriptomic analysis comparing tumor-associated neutrophils with granulocytic myeloid-derived suppressor cells and normal neutrophils. PLoS One. 2012;7.
48. Wang L, Chang EWY, Wong SC, Ong S-M, Chong DQY, Ling KL. Increased myeloid-derived suppressor cells in gastric cancer correlate with cancer stage and plasma S100A8/A9 proinflammatory proteins. J Immunol Am Assoc Immunol. 2013;190:794–804.
49. Yang G, Shen W, Zhang Y, Liu M, Zhang L, Liu Q, et al. Accumulation of myeloid-derived suppressor cells (MDSCs) induced by low levels of IL-6 correlates with poor prognosis in bladder cancer. Oncotarget Impact J LLC. 2017;8:38378–88.
50. Dominguez C, McCampbell KK, David JM, Palena C. Neutralization of IL-8 decreases tumor PMN-MDSCs and reduces mesenchymalization of claudin-low triple-negative breast cancer. JCI Insight. Am Soc Clin Investig. 2017;2.
51. Kumar V, Patel S, Tcyganov E, Gabrilovich DI. The nature of myeloid-derived suppressor cells in the tumor microenvironment. Trends Immunol. Elsevier Ltd. 2016;208–20.
52. Alfaro C, Teijeira A, Oñate C, Perez G, Sanmamed MF, Andueza MP, et al. Tumor-Produced Interleukin-8 Attracts human myeloid-derived suppressor cells and elicits extrusion of Neutrophil Extracellular Traps (NETs). Clin Cancer Res Am Asso Canc Res Inc. 2016;22:3924–36.
53. Najmeh S, Cools-Lartigue J, Rayes RF, Gowing S, Vourtzoumis P, Bourdeau F, et al. Neutrophil extracellular traps sequester circulating tumor cells via β1-integrin mediated interactions. Int J cancer. 2017;140:2321–30.
54. Ren J, He J, Zhang H, Xia Y, Hu Z, Loughran P, et al. Platelet TLR4-ERK5 axis facilitates NET-mediated capturing of circulating tumor cells and distant metastasis after surgical stress. Cancer Res. 2021;81:2373–85.
55. Teijeira Á, Garasa S, Gato M, Alfaro C, Migueliz I, Cirella A, et al. CXCR1 and CXCR2 chemokine receptor agonists produced by tumors induce neutrophil extracellular traps that interfere with immune cytotoxicity. Immunity. 2020;52:856-871.e8.
56. Szczerba BM, Castro-Giner F, Vetter M, Krol I, Gkountela S, Landin J, et al. Neutrophils escort circulating tumour cells to enable cell cycle progression. Nat Publish Grp. 2019;566:553–7.
57. McDonald B, Spicer J, Giannais B, Fallavollita L, Brodt P, Ferri LE. Systemic inflammation increases cancer cell adhesion to hepatic sinusoids by neutrophil mediated mechanisms. Int J Cancer. 2009;125:1298–305.
58. Monti M, Iommelli F, De Rosa V, Carriero MV, Miceli R, Camerlingo R, et al. Integrin-dependent cell adhesion to neutrophil extracellular traps through engagement of fibronectin in neutrophil-like cells. PLoS One. Pub Library of Sci. 2017;12.
59. Pham CTN. Neutrophil serine proteases: specific regulators of inflammation. Nat Rev Immunol. 2006;6:541–50.
60. Rogalski C, Meyer-Hoffert U, Proksch E, Wiedow O. Human leukocyte elastase induces keratinocyte proliferation in vitro and in vivo. J Invest Dermatol. Blackwell Publish Inc. 2002;118:49–54.
61. Devaney JM, Greene CM, Taggart CC, Carroll TP, O'Neill SJ, McElvaney NG. Neutrophil elastase up-regulates interleukin-8 via toll-like receptor 4. FEBS Lett. 2003;544:129–32.
62. Zhao P, Lieu T, Barlow N, Sostegni S, Haerteis S, Korbmacher C, et al. Neutrophil elastase activates protease-activated receptor-2 (PAR2) and transient receptor potential vanilloid 4 (TRPV4) to cause inflammation and pain. J Biol Chem. 2015;290:13875–87.
63. Foekens JA, Ries C, Look MP, Gippner-Steppert C, Klijn JGM, Jochum M. The prognostic value of polymorphonuclear

leukocyte elastase in patients with primary breast cancer. Cancer Res. 2003;63:337–41.
64. Akizuki M, Fukutomi T, Takasugi M, Takahashi S, Sato T, Harao M, et al. Prognostic significance of immunoreactive neutrophil elastase in human breast cancer: long-term follow-up results in 313 patients. Neoplasia. 2007;9:260–4.
65. Ho AS, Chen CH, Cheng CC, Wang CC, Lin HC, Luo TY, et al. Neutrophil elastase as a diagnostic marker and therapeutic target in colorectal cancers. Oncotarget Impact J LLC. 2014;5:473–80.
66. Yamashita JI, Tashiro K, Yoneda S, Kawahara K, Shirakusa T. Local increase in polymorphonuclear leukocyte elastase is associated with tumor invasiveness in non-small cell lung cancer. Chest Am Coll Chest Physic.1996;109:1328–34.
67. Wada Y, Yoshida K, Tsutani Y, Shigematsu H, Oeda M, Sanada Y, et al. Neutrophil elastase induces cell proliferation and migration by the release of TGF-α, PDGF and VEGF in esophageal cell lines. Oncol Rep Spandidos Publications. 2007;17:161–7.
68. Wada Y, Yoshida K, Hihara J, Konishi K, Tanabe K, Ukon K, et al. Sivelestat, a specific neutrophil elastase inhibitor, suppresses the growth of gastric carcinoma cells by preventing the release of transforming growth factor-α. Cancer Sci. 2006;97:1037–43.
69. Nawa M, Osada S, Morimitsu K, Nonaka K, Futamura M, Kawaguchi Y, et al. Growth effect of neutrophil elastase on breast cancer: favorable action of sivelestat and application to anti-HER2 therapy. Anticancer Res. 2012;32:13–9.
70. Caruso JA, Akli S, Pageon L, Hunt KK, Keyomarsi K. The serine protease inhibitor elafin maintains normal growth control by opposing the mitogenic effects of neutrophil elastase. Oncogene Nature Publishing Group. 2015;34:3556–67.
71. Yang L, Liu Q, Zhang X, Liu X, Zhou B, Chen J, et al. DNA of neutrophil extracellular traps promotes cancer metastasis via CCDC25. Nat Res. 2020;583:133–8.
72. Rickles FR, Levine M, Edwards RL. Hemostatic alterations in cancer patients. Cancer Metastasis Rev Kluwer Academic Publishers. 1992;11:237–48.
73. Fernandes CJ, Morinaga LTK, Alves JL, Castro MA, Calderaro D, Jardim CVP, et al. Cancer-associated thrombosis: the when, how and why. Eur Respir Rev Eur Respir Soc. 2019;28.
74. Fuchs TA, Brill A, Duerschmied D, Schatzberg D, Monestier M, Myers DD, et al. Extracellular DNA traps promote thrombosis. Proc Natl Acad Sci U S A. 2010;107:15880–5.
75. Laridan E, Martinod K, De Meyer SF. Neutrophil extracellular traps in arterial and venous thrombosis. Semin Thromb Hemost. Thieme Med Publish Inc. 2019;45:86–93.
76. Rao AN. Do neutrophil extracellular traps contribute to the heightened risk of thrombosis in inflammatory diseases? World J Cardiol. Baishideng Publish Grp Inc. 2015;7:829.
77. von Brühl ML, Stark K, Steinhart A, Chandraratne S, Konrad I, Lorenz M, et al. Monocytes, neutrophils, and platelets cooperate to initiate and propagate venous thrombosis in mice in vivo. J Exp Med. 2012;209:819–35.
78. Brill A, Fuchs TA, Savchenko AS, Thomas GM, Martinod K, De Meyer SF, et al. Neutrophil extracellular traps promote deep vein thrombosis in mice. J Thromb Haemost NIH Public Access. 2012;10:136–44.
79. Martinod K, Demers M, Fuchs TA, Wong SL, Brill A, Gallant M, et al. Neutrophil histone modification by peptidylarginine deiminase 4 is critical for deep vein thrombosis in mice. Proc Natl Acad Sci USA. 2013;110:8674–9.
80. Heinmöller E, Schropp T, Kisker O, Simon B, Seitz R, Weinel RJ. Tumor cell-induced platelet aggregation in vitro by human pancreatic cancer cell lines. Taylor & Francis. 2009;30:1008–16.
81. Yan MJ, Jurasz P. The role of platelets in the tumor microenvironment: from solid tumors to leukemia. Biochim Biophys Acta - Mol Cell Res Elsevier. 2016;1863:392–400.
82. Mauracher LM, Posch F, Martinod K, Grilz E, Däullary T, Hell L, et al. Citrullinated histone H3, a biomarker of neutrophil extracellular trap formation, predicts the risk of venous thromboembolism in cancer patients. J Thromb Haemost Blackwell Publishing Ltd. 2018;16:508–18.
83. Boone BA, Orlichenko L, Schapiro NE, Loughran P, Gianfrate GC, Ellis JT, et al. The receptor for advanced glycation end products (RAGE) enhances autophagy and neutrophil extracellular traps in pancreatic cancer. Cancer Gene Ther Nature Publishing Group. 2015;22:326–34.
84. Boone BA, Murthy P, Miller-Ocuin J, Doerfler WR, Ellis JT, Liang X, et al. Chloroquine reduces hypercoagulability in pancreatic cancer through inhibition of neutrophil extracellular traps. BMC Cancer. BioMed Central Ltd. 2018;18.
85. Thomas GM, Brill A, Mezouar S, Crescence L, Gallant M, Dubois C, et al. Tissue factor expressed by circulating cancer cell-derived microparticles drastically increases the incidence of deep vein thrombosis in mice. J Thromb Haemost. 2015;13:1310–9.
86. Hisada Y, Ay C, Auriemma AC, Cooley BC, Mackman N. Human pancreatic tumors grown in mice release tissue factor-positive microvesicles that increase venous clot size. J Thromb Haemost. 2017;15:2208–17.
87. Hisada Y, Grover SP, Maqsood A, Houston R, Ay C, Noubouossie DF, et al. Neutrophils and neutrophil extracellular traps enhance venous thrombosis in mice bearing human pancreatic tumors. Haematologica Ferrata Storti Foundation. 2020;105:218–25.
88. Leal AC, Mizurini DM, Gomes T, Rochael NC, Saraiva EM, Dias MS, et al. Tumor-derived exosomes induce the formation of neutrophil extracellular traps: implications for the establishment of cancer-associated thrombosis. Sci Rep. Nature Publishing Group. 2017;7.
89. Cedervall J, Dragomir A, Saupe F, Zhang Y, Ärnlöv J, Larsson E, et al. Pharmacological targeting of peptidylarginine deiminase 4 prevents cancer-associated kidney injury in mice. Oncoimmunology. Taylor and Francis Inc. 2017;6.
90. Ferrari M. Cancer nanotechnology: opportunities and challenges. Nat Rev Cancer. 2005;5:161–71.
91. Mahmood M, Casciano D, Xu Y, Biris AS. Engineered nanostructural materials for application in cancer biology and medicine. J Appl Toxicol. 2012;32:10–9.
92. Maeda H. Polymer therapeutics and the EPR effect. J Drug Target. 2017;25:781–5.
93. Kumari P, Ghosh B, Biswas S. Nanocarriers for cancer-targeted drug delivery. J Drug Target. 2016;24:179–91.
94. Drago JZ, Modi S, Chandarlapaty S. Unlocking the potential of antibody-drug conjugates for cancer therapy. Nat Rev Clin Oncol. 2021;18:327–44.
95. Basyreva LY, Voinova EV, Gusev AA, Mikhalchik EV, Kuskov AN, Goryachaya AV, et al. Fluorouracil neutrophil extracellular traps formation inhibited by polymer nanoparticle shielding. Mater Sci Eng C Elsevier Ltd. 2020;108.
96. Boraschi D, Italiani P, Palomba R, Decuzzi P, Duschl A, Fadeel B, et al. Nanoparticles and innate immunity: new perspectives on host defence. Semin Immunol Acad Press. 2017;33–51.
97. Lin MH, Lin CF, Yang SC, Hung CF, Fang JY. The interplay between nanoparticles and neutrophils. J Biomed Nanotechnol Am Scientific Publish. 2018;66–85.
98. Bisso PW, Gaglione S, Guimarães PPG, Mitchell MJ, Langer R. Nanomaterial interactions with human neutrophils. ACS Biomater Sci Eng. Am Chem Soc. 2018;4:4255–65.
99. Lee WL, Harrison RE, Grinstein S. Phagocytosis by neutrophils. Microbes Infect Elsevier Masson. 2003;5:1299–306.
100. Xue J, Zhao Z, Zhang L, Xue L, Shen S, Wen Y, et al. Neutrophil-mediated anticancer drug delivery for suppression of postoperative malignant glioma recurrence. Nat Nanotechnol Nat Nanotechnol. 2017;12:692–700.

101. Wang Z, Li J, Cho J, Malik AB. Prevention of vascular inflammation by nanoparticle targeting of adherent neutrophils. Nat Nanotechnol. 2014;9:204–10.
102. Chu D, Zhao Q, Yu J, Zhang F, Zhang H, Wang Z. Nanoparticle targeting of neutrophils for improved cancer immunotherapy. Adv Healthc Mater Adv Healthc Mater. 2016;5:1088–93.
103. Bachmaier K, Stuart A, Singh A, Mukhopadhyay A, Chakraborty S, Hong Z, et al. Albumin nanoparticle endocytosing subset of neutrophils for precision therapeutic targeting of inflammatory tissue injury. ACS Nano ACS Nano. 2022;16:4084–101.
104. Hou J, Yang X, Li S, Cheng Z, Wang Y, Zhao J, et al. Accessing neuroinflammation sites: monocyte/neutrophil-mediated drug delivery for cerebral ischemia. Sci Adv. 2019;5.
105. Jain S, Mishra V, Singh P, Dubey PK, Saraf DK, Vyas SP. RGD-anchored magnetic liposomes for monocytes/neutrophils-mediated brain targeting. Int J Pharm Int J Pharm. 2003;261:43–55.
106. Chandrasekharan P, Fung KLB, Zhou XY, Cui W, Colson C, Mai D, et al. Non-radioactive and sensitive tracking of neutrophils towards inflammation using antibody functionalized magnetic particle imaging tracers. Nanotheranostics. 2021;5:240–55.
107. Vij N, Min T, Bodas M, Gorde A, Roy I. Neutrophil targeted nano-drug delivery system for chronic obstructive lung diseases. Nanomedicine. 2016;12:2415–27.
108. Miettinen HM, Gripentrog JM, Lord CI, Nagy JO. CD177-mediated nanoparticle targeting of human and mouse neutrophils. PLoS One. 2018;13.
109. Sionov RV, Fridlender ZG, Granot Z. The multifaceted roles neutrophils play in the tumor microenvironment. Cancer Microenviron. 2015;8:125–58.
110. Zhou X, Cao X, Tu H, Zhang ZR, Deng L. Inflammation-targeted delivery of celastrol via neutrophil membrane-coated nanoparticles in the management of acute pancreatitis. Mol Pharm. 2019;16:1397–405.
111. Cao X, Hu Y, Luo S, Wang Y, Gong T, Sun X, et al. Neutrophil-mimicking therapeutic nanoparticles for targeted chemotherapy of pancreatic carcinoma. Acta Pharm Sin B. Chinese Acad Med Sci. 2019;9:575–89.
112. Kang T, Zhu Q, Wei D, Feng J, Yao J, Jiang T, et al. Nanoparticles coated with neutrophil membranes can effectively treat cancer metastasis. ACS Nano Am Chem Soc. 2017;11:1397–411.
113. Bornhöfft KF, Viergutz T, Kühnle A, Galuska SP. nanoparticles Equipped with α2,8-linked sialic acid chains inhibit the release of neutrophil extracellular traps. Nanomater (Basel, Switzerland). Nanomaterials (Basel). 2019;9.
114. Trikha M, Corringham R, Klein B, Rossi J-F. Targeted anti-interleukin-6 monoclonal antibody therapy for cancer: a review of the rationale and clinical evidence. Clin Cancer Res Inserm. 2003;9:4653–65.
115. Shojaei F, Wu X, Malik AK, Zhong C, Baldwin ME, Schanz S, et al. Tumor refractoriness to anti-VEGF treatment is mediated by CD11b +Gr1+ myeloid cells. Nat Biotechnol. 2007;25:911–20.
116. Kim K, Skora AD, Li Z, Liu Q, Tam AJ, Blosser RL, et al. Eradication of metastatic mouse cancers resistant to immune checkpoint blockade by suppression of myeloid-derived cells. Proc Natl Acad Sci USA. Nat Acad Sci. 2014;111:11774–9.