Neutrophils in cancer: heterogeneous and multifaceted

Catherine C. Hedrick[1] ✉ *and Ilaria Malanchi*[2] ✉

Abstract | Neutrophils are the most abundant myeloid cells in human blood and are emerging as important regulators of cancer. However, their functional importance has often been overlooked on the basis that they are short-lived, terminally differentiated and non-proliferative. Recent studies of their prominent roles in cancer have led to a paradigm shift in our appreciation of neutrophil functional diversity. This Review describes how neutrophil diversification, which in some contexts can lead to opposing functions, is generated within the tumour microenvironment as well as systemically. We compare neutrophil heterogeneity in cancer and in other pathophysiological contexts to provide an updated overview of our current knowledge of the functions of neutrophils in cancer.

Neutrophils are the first line of defence against microbial infection. They are the most abundant cells circulating in human blood and are recruited rapidly to sites of tissue injury. It has long been assumed that circulating neutrophils, mainly owing to their inability to proliferate after maturation, become exhausted rapidly and thus have a short half-life in homeostatic conditions[1]. This led to the view that neutrophils are specialized cells that fulfil only a specific set of functions in immune defence. Thus, the functional diversity of neutrophils has not been investigated as deeply as it has for other myeloid cells.

In recent years, neutrophils have received growing attention, and old paradigms are being challenged. Indeed, even the short lifespan of human circulating neutrophils has been questioned: one study showed that neutrophils can persist in the blood for 19 hours, and another showed survival for up to 5.4 days[2,3]. The lifespan of neutrophils may also change following their activation. Again, moving away from the idea that neutrophils exist only to prevent infections, neutrophils were reported to undergo reverse transmigration from the site of injury and re-emerge in the circulation[4–6]. Why this reverse migration occurs is still unclear, but studies of recirculating neutrophils from the inflamed skin suggest that migration to lymphoid organs increases T cell proliferation and responses[7].

The recent reassessment of neutrophil biology is largely thanks to technical advances, such as in vivo imaging, high-dimensional transcriptomic and epigenomic approaches and studies performed at single-cell resolution. These new strategies have not only allowed us to dissect the modulation of neutrophil gene expression in immune-related diseases[8,9] but have also revealed the high level of neutrophil heterogeneity between different healthy tissues and over time[10]. Recent work has also revealed the unappreciated plasticity of neutrophils. Neutrophils contribute to collateral damage during postinjury inflammation, but they also play a key role in postinjury tissue regeneration[11,12]. These opposing functional states[13] are analogous to those of neutrophils in cancer, which range from being protumoural to being antitumoural. Neutrophils have recently gained increased attention in cancer[14]. Indeed, their therapeutic potential has been highlighted by the characterization of biological events underlying their antitumoural and protumoural functions[15]. Recent reviews have provided a comprehensive analysis of the function of neutrophils in cancer and tumour resistance, as well as their clinical relevance to patients with cancer[14,15]. Here we discuss our current knowledge of the relationship between neutrophils and cancer, with emphasis on the context-dependent activities of these cells. We begin by analysing the parameters influencing neutrophil heterogeneity and consider their engagement during cancer, from the onset of disease through to its progression. We review the multifaceted interactions of neutrophils with the surrounding tissue during tumour onset and within the tumour microenvironment. We also cover how cancer-derived systemic signals modify neutrophil production in the bone marrow, and describe how these concepts have important implications for metastatic outgrowth. Overall, we aim to highlight the parallels between the diverse neutrophil responses to cancer and other pathological conditions, as this area of research touches on a wide array of potential therapeutic interventions.

Neutrophil development

Heterogeneity among neutrophil progenitors. Neutrophils develop in the bone marrow from a series of haematopoietic progenitor stem cells. The current model

[1]*Center for Cancer Immunotherapy, La Jolla Institute for Immunology, La Jolla, CA, USA.*

[2]*Tumour–Host Interaction Laboratory, The Francis Crick Institute, London, UK.*

✉*e-mail: hedrick@lji.org; ilaria.malanchi@crick.ac.uk*

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of neutrophil differentiation in human bone marrow describes several developmental stages, each with distinct gene expression signatures[16]. First, multipotent granulocyte–monocyte progenitors (GMPs), which develop from common myeloid progenitors, give rise to neutrophil and monocyte precursors. The GMP is considered to be the earliest developmental cell in haematopoiesis that gives rise to all neutrophils and monocytes[17]. Promyelocytes then arise immediately downstream of GMPs and express the neutrophil lineage marker CD66b[18]. Subsequent neutrophil development occurs through stepwise lineage and morphological maturation stages[19]. Following upregulation of CD11b and CD16 expression, promyelocytes differentiate through myelocytes and metamyelocytes into banded and segmented neutrophils (FIG. 1). Over the past decade, work has redefined common myeloid progenitors and GMPs and their ability to generate downstream subsets[20–24]. Unipotent progenitors for other myeloid cells, including monocytes (common monocyte progenitors[25,26]), basophils[27,28] and eosinophils[20,29], have been discovered.

The concept of the GMP as a single homogeneous cell type has been somewhat challenged recently by high-dimensional technologies, including single-cell RNA sequencing and mass cytometry[10,30–36]. Importantly, and highly relevant for this Review, are the recent discoveries of novel unipotent progenitors for both mouse and human neutrophils[32–36]. These recent studies support the notion that the GMP is not a homogeneous cell type, and instead consists of several heterogeneous myeloid progenitor cells, including these newly identified neutrophil progenitors[30–36]. However, these studies do not exclude the possibility of an 'earlier' as-yet-undiscovered progenitor that gives rise solely to either neutrophils or monocytes (BOX 1). Also possible is the notion that additional heterogeneity within these neutrophil progenitors could give rise to specific subsets of neutrophils, particularly in the setting of cancer. It is also possible that the induction of transcription factors in neutrophil progenitors during disease can alter the phenotypic heterogeneity of neutrophil progeny, as suggested recently in mouse studies by Luo and colleagues[34].

A spectrum consisting of several maturation states of neutrophils is always present in healthy bone marrow. These cell maturation states range developmentally from the newly discovered early unipotent neutrophil progenitors to several states of immature neutrophil precursors to more-mature neutrophils[21,37–40] (FIG. 1). The existence of a spectrum of immature to mature cells in the bone marrow, even in the steady state, is somewhat unique to the neutrophil lineage among myeloid cells, and is more reminiscent of the spectrum of innate lymphocytes, such as natural killer (NK) cells and NK T cells[41,42]. This diversity of neutrophils is likely due to their important function as the first responder cell to acute infections or inflammation.

Neutrophil heterogeneity outside the bone marrow. Like neutrophil precursors in the bone marrow, mature neutrophils in the periphery are heterogeneous. To maintain their levels in the circulation, they require mobilization from the bone marrow, which depends, at least in part, on granulocyte colony-stimulating factor (G-CSF); deletion of *Csf3* (which encodes G-CSF) or its receptor *Csf3r* leads to neutropenia in mice[43,44]. Mature neutrophils are technically challenging to study owing to their low mRNA content and fragility. However, strong evidence of heterogeneity of circulating neutrophils in humans and mice with cancer was shown by the detection of so-called low-density and high-density neutrophils, an early neutrophil classification that reflected different functional properties[45]. With the recent emergence of high-dimensional phenotyping data, the concept of neutrophil heterogeneity has exploded.

Recent studies using cytometry by time-of-flight mass spectrometry (CyTOF) analysis suggest there are at least seven mature neutrophil subsets in individuals with cancer[46]. Moreover, single-cell RNA sequencing

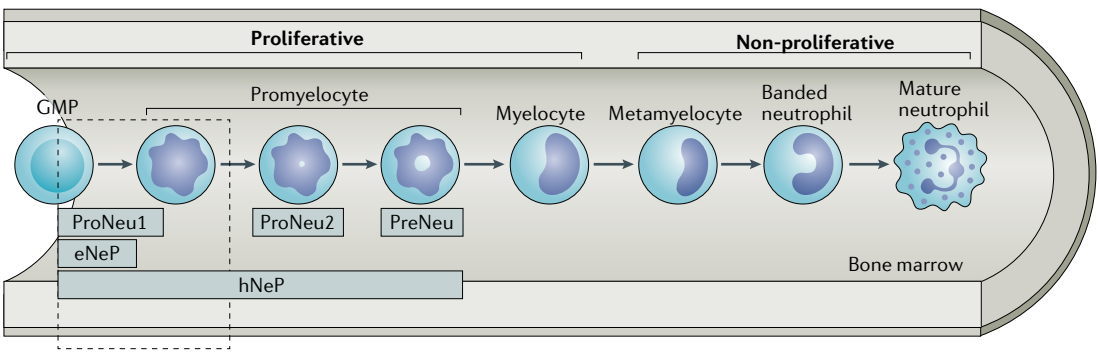


Fig. 1 | **Neutrophil development in the bone marrow.** Neutrophils develop in the bone marrow from a series of newly defined progenitors that originate as part of the granulocyte–monocyte progenitor (GMP) pool. Some of these neutrophil progenitors expand into the promyelocyte pool. The earliest unipotent neutrophil progenitors are termed 'early neutrophil progenitors' (eNePs) in humans and 'proneutrophils' (ProNeu1) in mice. In humans, eNePs are the earliest progenitors found within the human neutrophil progenitor (hNeP) pool. ProNeu1 and eNeP cells are proliferative and give rise to solely immature neutrophil precursors (also known as myelocytes and metamyelocytes). Shown here are the very early immature neutrophil precursors, ProNeu2 and PreNeu, found in mice. These precursor neutrophils continue to mature in the bone marrow and give rise to immature neutrophils (banded) and mature neutrophils. In homeostasis, mature neutrophils enter the circulation. In cancer, many immature neutrophils, including these earliest neutrophil progenitors, expand in the bone marrow and are released into the circulation.

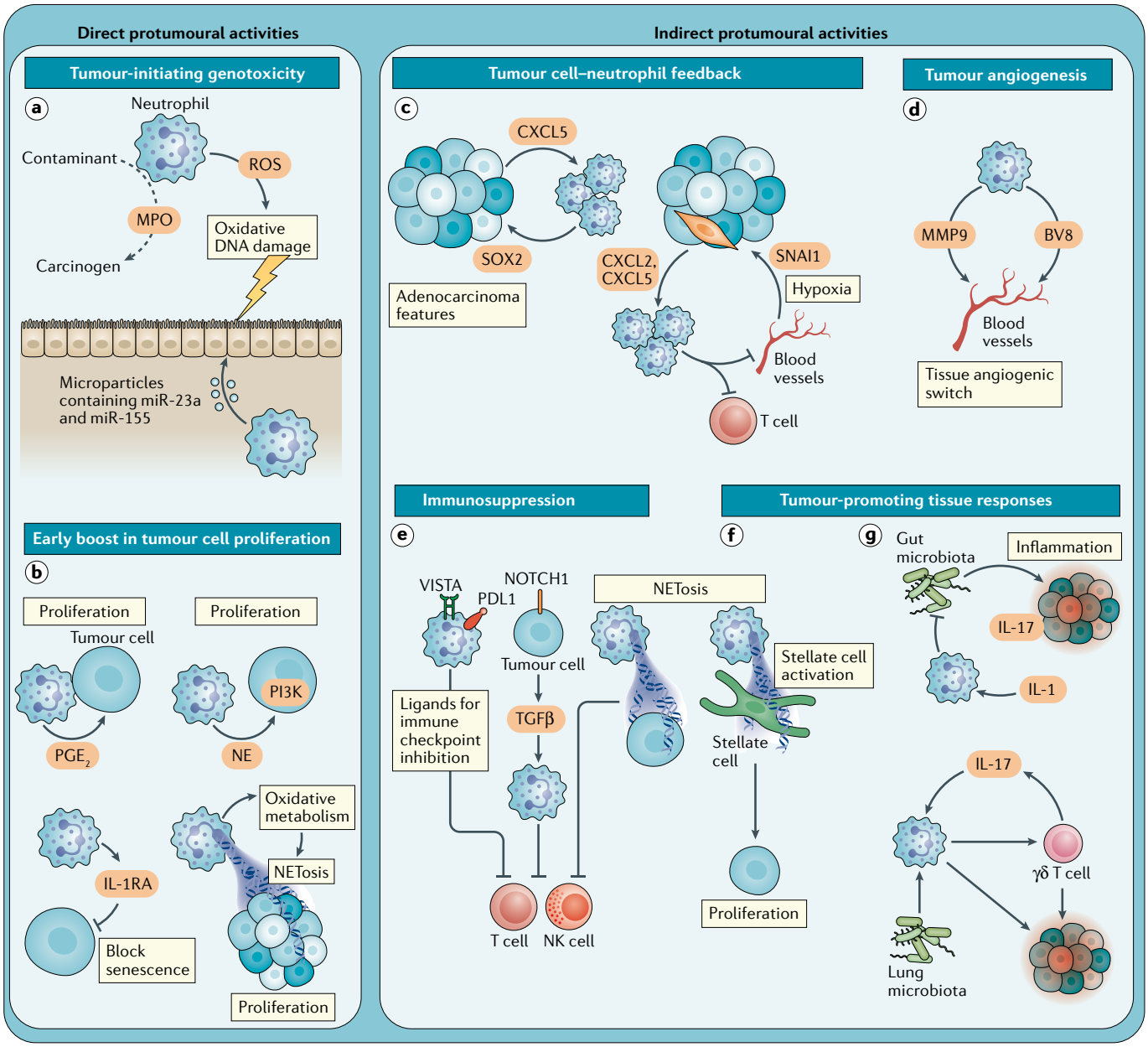
Box 1 | **Neutrophil progenitors in humans and mice**

In 2018, we identified a neutrophil progenitor population in human bone marrow, termed 'human neutrophil progenitors' (hNeP; $Lin^-CD66b^+CD117^+$)[33]. These cells constitute 1–3% of total bone marrow neutrophils and contain both $CD34^-$ and $CD34^+$ populations. When transferred into NSG-M3 mice that support re-engraftment of human myeloid cells, both $CD34^-$ hNeP and $CD34^+$ hNeP produce only neutrophils, confirming their status as unipotent neutrophil progenitors. In the same year, Ng and colleagues[32] identified early proliferative bone marrow neutrophil populations in both mice and humans, and termed these cells 'neutrophil precursors' (preNeu). These cells are $Lin^-CD66b^+CD34^-CD49d^+$ and constitute approximately 5% of total bone marrow neutrophils. PreNeu differentiate into non-proliferating, immature and mature neutrophils. At the same time, Kim et al.[38] identified in mice a late-stage precursor with neutrophil potency termed 'NeuP', and other reports of immature neutrophil precursors have also been published[21,39,40]. The mouse neutrophil progenitor NeuP identified by Kim et al.[38] appears to be further downstream in the neutrophil developmental tree[16,32,33]. Upon further mapping, the mouse neutrophil progenitors (PreNeu and NeuP) are likely to be the same cell identified by both groups. Very recently, Ng and colleagues and our own laboratory, both using high-dimensional mapping, identified ProNeu1 in mice[36] and eNeP in humans[35], respectively, as the earliest unipotent neutrophil progenitors present within the mouse and human neutrophil progenitor pools (FIG. 1). One thing that is clear in light of these many recent neutrophil progenitor discoveries is that a general consensus for nomenclature of these cell types is needed. Furthermore, whether different neutrophil progeny arise from different progenitors or immature precursors in response to infection or disease is unclear, but there is some very recent evidence for this in mice[34]. This study showed the development of distinct neutrophil progeny from progenitors in mice, and identified some of the transcription factors related to their development and how this is modified in disease states[34]. A recent study by Muench et al. shows the importance of the transcription factor zinc-finger protein GFI1 in mice in directing a transcriptional programme that is modulated through successive neutrophil differentiation states[183]. With the discovery of these earliest neutrophil progenitors, new therapies that selectively target early neutrophil development, rather than overall myeloid cell development or more-mature neutrophils, are now possible.

analysis has defined the molecular signatures of distinct neutrophil subsets in mice, with three of these clusters abundant in the circulation[34]. These subsets are present in the steady state and do not depend on the activation state or transendothelial migration. One of these subsets is enriched in expression of interferon-stimulated genes, conserved between humans and mice, and expanded during infection[34]. However, on the basis of high-throughput analyses and molecular signatures, it is hard to discriminate whether these subsets are true lineage-dependent subsets or simply different cellular phenotypic states based on their environmental cues. Understanding neutrophil phenotypic heterogeneity is relevant given that certain subsets could be predisposed to respond differently to subsequent environmental challenges that influence their polarization and activation. However, whether every phenotypic difference found in neutrophils reflects different functional properties and activation states remains to be determined.

Three parameters contribute to neutrophil heterogeneity in the steady state and in the absence of a response to specific challenges: these are space, time and disease context. The spatial parameter refers to the ability of neutrophils to respond to local signals in the tissue or circulation and to modify their properties accordingly. Neutrophils are influenced by their interactions with tissue-specific surface molecules, chemokines and distinct vascular properties, leading to organ-specific recruitment patterns[47]. Evidence of transcriptomic changes in mouse neutrophils as they migrate from the bone marrow to tissues is emerging[9] and indicates molecular adaptation of neutrophils to tissue-specific cues[48]. Using neutrophil-tracking models in mice, Ballesteros and colleagues[48] uncovered several neutrophil subsets that differ in their receptor expression, transcriptional activity, chromatin landscape and lifespan during the steady state. These subsets associate with distinct functions, such as tissue angiogenesis in the lungs and intestines and vascular repair upon genotoxic injury, viral infection or radiation in the lungs. Ballesteros and colleagues also showed that CXCL12–CXCR4 signalling is responsible for guiding the reprogramming of lung-resident neutrophils[48]. This study formally consolidates the idea of functional adaptation of neutrophils to different tissues, which was previously only hinted at by observations such as the presence of tissue-specific neutrophils displaying specialized B cell-stimulatory functions in the perimarginal zone of the spleen in mice[49].

The temporal parameter of neutrophil heterogeneity refers to the observations that, in some circumstances, neutrophils can be released from the bone marrow as immature cells and that mature neutrophils in the blood change their phenotype and function over time. After neutrophils are released from the bone marrow, they undergo changes over time via an ageing process, which alone generates neutrophils that elicit different responses in host defence and vascular protection[50]. Indeed, there are significant differences in properties, including production of reactive oxygen species (ROS) and formation of neutrophil extracellular traps (NETs), between young and aged neutrophils that suggest a progressively acquired spontaneous activated state. The regulation of daily production of neutrophils in the bone marrow and their release into the circulation relies on the expression of specific transcription factors, such as GATA1 and CCAAT/enhancer-binding proteins (C/EBPs); however, the factors that control their circadian expression are still unknown. Current knowledge of circadian regulation of neutrophils (reviewed in REF.[51]) involves the presence of modulatory signals orchestrated by bone marrow stromal, osteoblast and endothelial cells, and by the sympathetic nervous system, which together control the release or retention of neutrophil-regulatory chemokines (namely CXCL12). The ageing of neutrophils in the circulation seems to be regulated intrinsically by the molecular clock and extrinsically through CXCL12–CXCR4 signalling[51]. These life cycle changes in neutrophils are likely to be important for efficacy and control of their functions in host defence[52]. Indeed, a more recent study in mice and also in humans with respiratory infections demonstrated the presence of a neutrophil-intrinsic programme driven by regulators of the circadian cycle and CXCR2, causing progressive loss of granule contents and reduction of NET-forming capacity[53]. However, Zhang et al. showed that interactions with the microbiota can foster neutrophil ageing[54], indicating that this process can be influenced by external factors. Although an influence of the microbiota in regulating haematopoiesis in the bone marrow was already recognized[55], Zhang et al. showed that the aged


Direct protumoural activities
Indirect protumoural activities
Tumour-initiating genotoxicity
a
Neutrophil
Contaminant
ROS
MPO
Oxidative DNA damage
Carcinogen
Microparticles containing miR-23a and miR-155
Early boost in tumour cell proliferation
b
Proliferation
Tumour cell
PGE2
Proliferation
PI3K
NE
IL-1RA
Block senescence
Oxidative metabolism
NETosis
Proliferation
Tumour cell–neutrophil feedback
c
CXCL5
SOX2
Adenocarcinoma features
CXCL2, CXCL5
SNAI1
Hypoxia
Blood vessels
T cell
Tumour angiogenesis
d
MMP9
BV8
Blood vessels
Tissue angiogenic switch
Immunosuppression
e
VISTA
PDL1
NOTCH1
Tumour cell
Ligands for immune checkpoint inhibition
TGFβ
T cell
NK cell
Tumour-promoting tissue responses
f
NETosis
Stellate cell activation
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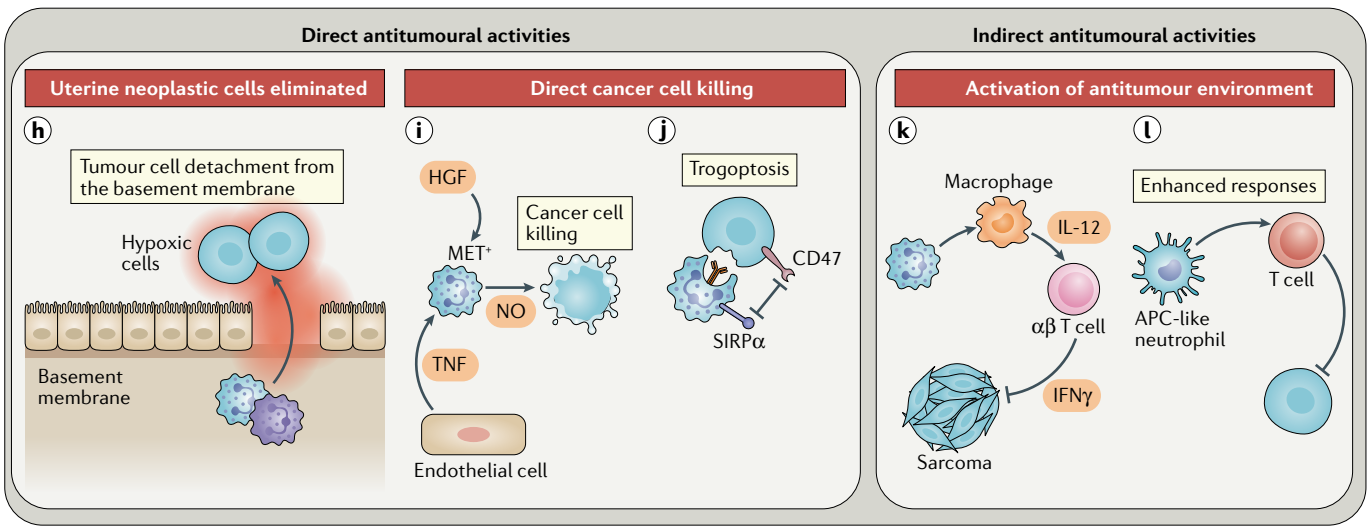
g
Gut microbiota
Inflammation
IL-17
IL-1
IL-17
γδ T cell
Lung microbiota


Direct antitumoural activities
Indirect antitumoural activities
Uterine neoplastic cells eliminated
h
Tumour cell detachment from the basement membrane
Hypoxic cells
Basement membrane
Direct cancer cell killing
i
HGF
MET+
Cancer cell killing
NO
TNF
Endothelial cell
j
Trogoptosis
CD47
SIRPα
Activation of antitumour environment
k
Macrophage
IL-12
αβ T cell
IFNγ
Sarcoma
l
Enhanced responses
T cell
APC-like neutrophil

◀ Fig. 2 | **Neutrophil effects on tumour initiation and growth.** Neutrophils have direct and indirect protumour and antitumour effects during early stages of tumour initiation and growth. **a** | Neutrophils can cause oxidative DNA damage and convert substances into carcinogens. **b** | Neutrophils enhance the proliferation of cancer cells directly or by antagonizing senescence; tumour-associated neutrophils in anaplastic thyroid cancer change their mitochondrial metabolism and release protumoural neutrophil extracellular traps (NETs). **c** | Expression of SOX2 in non-small-cell lung cancer triggers features of squamous cell carcinoma in a neutrophil-dependent manner. Neutrophils induce expression of zinc-finger protein SNAI1 and promote epithelial-to-mesenchymal transition in cancer cells via hypoxia. In turn, SNAI1 promotes neutrophil recruitment. **d** | A proangiogenic function of neutrophils involves activation of quiescent pancreatic vasculature via matrix metalloproteinase 9 (MMP9) and BV8. **e** | Immunosuppressive functions of neutrophils. NOTCH1 activation in cancer cells promotes transforming growth factor-β (TGFβ)-dependent immunosuppressive neutrophils that hinder antitumour immune responses, allowing subsequent tumour growth and progression. Expression of programmed cell death 1 ligand 1 (PDL1) by neutrophils impairs antitumour immunity. Myeloid cell expression of V-type immunoglobulin domain-containing suppressor of T cell activation (VISTA) mediates T cell-suppressive function. NETs released within the tumour microenvironment can shield cancer cells from interacting with cytotoxic immune cells. **f** | Tumour-promoting tissue responses induce indirect promotion of cancer cell proliferation by NETs via activation of stellate cells. **g** | Neutrophils also influence tumour-promoting inflammatory responses. In the context of cancer-induced dysbiosis of the microbiota, neutrophils limit protumoural inflammation and cancer growth. In the lungs, the microbiota activates neutrophils that foster protumoural inflammation and γδ T cell activation. **h** | In terms of antitumoural activities, neutrophils antagonize uterine carcinogenesis by inducing neoplastic cell detachment. **i** | Expression of the receptor tyrosine-protein kinase MET (also known as HGFR) by neutrophils is induced by inflammatory modulators, such as tumour necrosis factor (TNF), and induces neutrophil recruitment. Upon stimulation with hepatocyte growth factor (HGF), anticancer MET^+ neutrophils kill melanoma cells via release of nitric oxide. **j** | Neutrophils can kill cancer cells via Fc-mediated ingestion of their plasma membrane (trogoptosis). **k** | Neutrophils create an IFNγ-rich microenvironment to support antitumour activities of macrophages via unconventional αβ T cells in dermal sarcoma. **l** | Antigen-presenting cell (APC)–neutrophil hybrids in lung cancer trigger antitumour T cell responses. CXCL, CXC-chemokine ligand; IL-1RA, interleukin-1 receptor antagonist; MMP9, matrix metalloproteinase 9; NE, neutrophil elastase; NK cell, natural killer cell; PGE_2, prostaglandin E_2; PI3K, phosphoinositide 3-kinase; ROS, reactive oxygen species; SIRPα, signal regulatory protein-α.

phenotype of neutrophils is triggered by the microbiota via Toll-like receptors and myeloid differentiation factor 88-mediated signalling pathways[54]. Microbiota-driven neutrophil ageing correlated with a proinflammatory phenotype, with enhanced αMβ2 integrin activation and NET formation under inflammatory conditions, identifying a direct role for the microbiota in fostering a disease-promoting neutrophil subset.

As mentioned already, circadian features of neutrophils are relevant to understand their activity in the disease context[50], but aged neutrophils can also impact the status of the organ they infiltrate in the steady state. This was shown in an elegant study by Hidalgo and colleagues, in which the circadian clock regulated neutrophil granule content and responsiveness, orchestrated a diurnal state in the lungs and influenced the transcriptome of the tissue[56]. This study suggests that circadian oscillation in neutrophils in the tissue contributes to organ function in the steady state. Remarkably, this diurnal pattern in the lungs controlled by neutrophils was shown to better support the growth of seeded cancer cells[56]. This work highlights the spectrum of functional differences among neutrophils and the potential effects on a tissue[51].

The third parameter contributing to neutrophil heterogeneity — the disease context — reveals how neutrophil subsets are produced and phenotypically modified by different disease states, which generate more neutrophil heterogeneity compared with healthy states. Notably, the tissue adaptations of neutrophils described by Ballesteros et al.[48] in the steady state are reinforced by signals generated within diseased tissues. For instance, the pathogenic toxin Candidalysin induces IL-1β and CXCL1 production by microglia and triggers protective recruitment of neutrophils to the infected central nervous system[57]. At the gene expression level, bacterial infection in mice reprogrammes neutrophil populations to exhibit particular signatures and transition between subsets[34]. In particular, neutrophil subsets expressing interferon-stimulated genes expand upon infection[34], and interferons also modulate responses by tumour-infiltrating neutrophils[58]. Still, it remains unclear how different interferons generate divergent overall neutrophil signatures and functions[59].

Thus, spatial-, temporal- and disease-specific parameters combine to influence the phenotypic heterogeneity of neutrophils that we observe in tumours, at both early and late stages[46]. In the following sections, we review the literature concerning the plasticity of neutrophils in cancer and their contributions to the control and progression of the disease.

Neutrophils in tumour onset and growth

Tumour initiation involves several important events: oncogenic mutations in tissue progenitor cells, events that enhance their proliferation and function, such as tissue injury and regeneration[60,61], and the establishment of a protumour inflammatory environment[62]. Given that neutrophils are important immune components in inflammatory responses, it is easy to envisage their contribution to tumour onset (FIG. 2).

Protumoural neutrophil functions. The first evidence of a direct procarcinogenic effect of neutrophils was linked to their release of ROS, inducing oxidative DNA damage in the lungs[63], which was later also reported to increase mutational load in an inflammation-driven intestinal cancer model[64]. A more recent study using a lung chemical carcinogenesis model showed that neutrophil-derived ROS amplify DNA damage exclusively at the time of carcinogen exposure, thereby promoting tumorigenesis[65] (FIG. 2a). Activated neutrophils from inflamed colon promote double-stranded DNA breaks in epithelial cells via the release of microparticles containing proinflammatory microRNAs, in both humans and mice. In this inflammatory context, tissue healing is impaired due to the genomic instability of the epithelium[66].

Neutrophils also directly support tumour cell proliferation via various paracrine signalling pathways (FIG. 2b). For example, in a zebrafish model of RAS-driven neoplasia, neutrophils enhanced cancer cell growth by releasing prostaglandin E_2 (REF.[67]). Similarly, in RAS-driven lung cancer, neutrophil elastase was shown to degrade insulin receptor substrate 1 and trigger cancer cell proliferation[68]. And in pancreatic cancer, neutrophils release IL-1 receptor antagonist (IL-1RA) to counteract the programme of oncogene-induced senescence in tumour cells[69].

Within the tumour microenvironment, neutrophils are functionally perturbed and show a high level of plasticity in response to signals that drive their polarization and activation[70]. This drives both direct and indirect mechanisms of cancer promotion. For example, in anaplastic thyroid cancer, changes in oxidative mitochondrial metabolism in tumour-associated neutrophils (TANs) allows them to remain viable while releasing NETs, which promote cancer cell proliferation[71] (FIG. 2b). The contribution of NETs to non-infectious disease and cancer growth is currently the subject of intense investigation[72] and is discussed later in the context of metastatic progression.

Recent insights into the relationship between genetic alterations in cancer cells and their microenvironment[73] suggest a role for cancer–neutrophil crosstalk. Examples of this crosstalk have been reported in a model of non-small-cell lung cancer, in which neutrophils dominate the immune landscape[74]. Overexpression of the transcription factor SOX2 yields squamous tumours with CXCL5-dependent recruitment of TANs. Depletion of TANs reduced squamous tumour growth while promoting adenocarcinoma features, which suggests a TAN-dependent modulation of tumour characteristics[75] (FIG. 2c). In another study, neutrophils were responsible for T cell exclusion and hypoxia, which in turn induced epithelial-to-mesenchymal transition in a mouse model of lung cancer, promoting the invasion and further recruitment of neutrophils[76] (FIG. 2c). This phenomenon whereby infiltrated immune cells influence the intrinsic properties of cancer cells was observed in patients with other tumour types, such as glioma, in which the immune environment could shape cancer gene expression subtypes[77]. The effects of neutrophil–cancer crosstalk can be sex specific and thus context dependent[78]. In a RAS-driven lung adenocarcinoma model, deletion of *Stat3* resulted in neutrophil-dependent tumour growth in male but not female mice[79], whereas immunosuppressive neutrophils promoted glioblastoma growth via IL-1β only in female mice[80]. Finally, neutrophils also influence the composition of the tumour microenvironment, for example through neutrophil proangiogenesis functions, which are key during early phases of pancreatic tumour growth[81,82] (FIG. 2d).

Another key function of neutrophils is their ability to influence the behaviours of other immune cells. As we discuss in the context of metastasis, the capacity of neutrophils to suppress anticancer activities of other immune cells is one of their more frequently reported protumorigenic functions. Again the specific genetic make-up of tumour cells determines neutrophil behaviour; for example, immunosuppressive neutrophils are generated by transforming growth factor-β (TGFβ) released in an engineered colorectal cancer model and support tumour growth as well as liver metastasis[83] (FIG. 2e). Immunosuppression by neutrophils is achieved by mediators such as ROS, inducible nitric oxide synthase and arginase 1. However, neutrophils can also express ligands for immune checkpoint inhibitory receptors that induce T cell exhaustion (FIG. 2e). Neutrophils expressing the inhibitory receptor ligand programmed cell death 1 ligand 1 (PDL1) were reported in human and mouse hepatocellular carcinomas, as well as in patients with gastric cancer, to impair antitumour immunity[84,85]. Deletion of *Vista*, which encodes an inhibitory receptor ligand expressed on tumour-associated myeloid cells, including neutrophils, diminishes their ability to suppress T cells in a transplantation mouse model of melanoma[86].

The complexity of neutrophil-based immunosuppressive mechanisms was recently exemplified by evidence that NETs released in the tumour microenvironment shield cancer cells from cytotoxic immune cells both in transplantable tumour models and in a spontaneous pancreatic adenocarcinoma mouse model[87,88] (FIG. 2e). And in a genetic model of RAS-driven pancreatic cancer, NETs indirectly enhanced cancer cell proliferation, via activation of stellate cells and promotion of desmoplastic stromal cell activation[89] (FIG. 2f).

Immunosuppressive neutrophils generally have a more immature phenotype (although mature immunosuppressive neutrophils have been described in the blood)[45]. This led to the term 'myeloid-derived suppressor cells' (MDSCs) to describe neutrophils in the context of tumours[90]. However, given the complexity of the properties acquired by TANs, this terminology can generate confusion in defining cell identity versus cell functions, possibly undermining efforts to define mechanisms that regulate neutrophil plasticity (BOX 2). Therefore, in this Review, we do not use the term 'myeloid-derived suppressor cells' when discussing immunosuppressive neutrophils.

Neutrophils are also able to modulate inflammatory responses, and in models of spontaneous skin and intestinal tumorigenesis, which rely on tumour-promoting inflammation, the absence of neutrophil recruitment via deletion of *Cxcr2* decreased overall inflammation, leading to tumour inhibition[91]. In a subsequent study, the same reduction of intestinal tumorigenesis and neutrophil recruitment in mice lacking CXCR2 was linked to immunosuppressive activity of neutrophils on T cells[92]. However, the understanding of neutrophil functions in the context of intestinal cancers is complicated by the presence of the microbiota. For example, depletion of neutrophils by deletion of *Mcl1*, which encodes a neutrophil survival factor, led to enhanced intestinal tumour growth and IL-17-dependent inflammation owing to the coincident increase in intratumoural bacteria[93]. Similarly, ablation of *Il1r1* in neutrophils, which limits their antibacterial function, led to bacterial invasion of tumours, again resulting in increased inflammation and cancer progression[94] (FIG. 2g). Conversely, in a lung cancer model, the local microbiota activated neutrophil release of cytokines, inducing lung-resident γδ T cells that promoted inflammation and tumour growth[95] (FIG. 2g). Taken together, these studies highlight the context-dependent activities of neutrophils in carcinogenesis. Given the recent appreciation of distinct microbiotas in different tumour types[96], it is possible that intratumoural bacteria might alter the behaviour of neutrophils and other immune cells in the tumour microenvironment. Therefore, an increased understanding of the interaction between neutrophils and the microbiota[97] will be essential to better understand neutrophil behaviour in cancer and beyond.

Box 2 | **Neutrophils versus myeloid-derived suppressor cells: two sides of the same coin**

Neutrophils with immunosuppressive functions can be found in the blood following trauma and infection, in which they can limit damage to host tissues during inflammation, but their presence can also be detrimental for certain immune responses, such as sepsis[184,185]. Immunosuppressive neutrophils are also reported to limit autoimmunity in diseases such as systemic lupus erythematosus[186]. Conversely, neutrophils support adaptive immune responses to infection, as demonstrated in the context of influenza virus infection, in which early recruited neutrophils deposited long-lasting uropod trails enriched in chemokines that were crucial for virus-specific $CD8^+$ T cell recruitment[147].

'Myeloid-derived suppressor cells' (MDSCs) is a term assigned to a group of myeloid cells on the basis of their function in suppressing adaptive immune responses in cancer. In mice, MDSCs are defined by the expression of the markers CD11b and Gr-1. As Gr-1 comprises two antigens, Ly6C and Ly6G, this classification comprises both monocytes and neutrophils. Discrimination of the two components in mice is possible using Ly6G- and Ly6C-specific antibodies. Recently, the terminology has been refined as granulocytic MDSCs ($CD11b^+Ly6G^+Ly6C^{low}$ cells in mice and $CD66b^+CD14^-CD11b^+CD15^+$ cells in humans) and monocytic MDSCs ($CD11b^+Ly6G^-Ly6C^+$ cells in mice and $CD11b^+CD14^+HLA\text{-}DR^{low}CD15^-$ cells in humans)[187]. On the basis of these markers, the cells defined as granulocytic MDSCs are identical to neutrophils in terms of their cellular morphology.

In the context of cancer, both neutrophils and monocytes can suppress adaptive immune responses. However, this functional adaptation in leukocytes does not change their cell identity. Some of the phenotypic and functional changes in neutrophils responding to cancer can be extreme, such as the acquisition of hybrid phenotypes and dendritic cell functions[104,106]. To mechanistically understand this plasticity, we must keep cell identity in mind to find the molecular triggers guiding cellular changes. This notion is extremely important in the context of neutrophils in cancer, because not all neutrophils that are either locally or systemically perturbed by tumour cells are immunosuppressive. Therefore, using MDSCs as the general terminology to describe $CD11b^+Ly6G^+Ly6C^{low}$ neutrophils in cancer runs the risk of mislabelling some neutrophils as immunosuppressive, even though they might display other functions. Indeed, as we have discussed here, neutrophils have a broad range of functions in tumours, but maintain their lineage-defined cellular identity, despite functional plasticity. Moreover, there are important similarities between the function of neutrophils in cancer and other non-cancer responses, which are crucial to understand their behaviour. We suggest that the use of the MDSC terminology should be re-evaluated as it is not only restrictive, but the idea of those cells being an alternative myeloid cell type existing exclusively in cancer might limit efforts to understand how neutrophils can adopt such a multifaceted range of behaviours.

Antitumoural neutrophil functions. Although most studies support a protumoural role for neutrophils, experimental evidence exists for an early antitumorigenic role of neutrophils. One example of this is a study showing that human neutrophils express TNF-related apoptosis-inducing ligand (TRAIL) and mediate cancer cell killing in vitro, a pathway that is enhanced by IFNγ stimulation of neutrophils[98]. In a mouse model of uterine cancer, neutrophils had a direct anticancer role by inducing cancer cell detachment from the surrounding basement membrane, thereby blocking early-stage tumour growth[99] (FIG. 2h). Interestingly, the antitumoural activity of neutrophils was enhanced by limiting the extent of hypoxia[100], suggesting fine-tuning of the cytotoxic functions of neutrophils. Another direct anticancer activity was reported in a model of transplanted tumours, in which stimulation of a neutrophil subset expressing the receptor tyrosine-protein kinase MET (also known as HGFR) with hepatocyte growth factor (HGF) led to direct killing of cancer cells via nitric oxide release[101] (FIG. 2i). More recently, a novel neutrophil cytotoxic activity was described termed 'trogoptosis', whereby neutrophils ingest pieces of cancer cell plasma membrane[102]. This neutrophil-dependent cytotoxic effect against antibody-bound cancer cells was enhanced by interference of the phagocytosis inhibitory pathway involving signal regulatory protein-α (SIRPα)–CD47 interaction, suggesting a potential therapeutic application of this phenomenon (FIG. 2j).

Neutrophils can also oppose carcinogenesis indirectly via the creation of an antitumour microenvironment, as recently reported in studies using the 3-methylcholanthrene-induced carcinogenesis model of dermal sarcoma[103]. This study showed that antitumoural neutrophils drive IL-12 production by macrophages, which in turn promotes activation of the IFNγ pathway in a subset of unconventional αβ T cells. Consequently, the absence of neutrophil recruitment enhanced sarcoma growth owing to lack of neutrophil-mediated anticancer immunity (FIG. 2k). Another example of neutrophils fostering anticancer responses is represented by the identification of neutrophils with an antigen-presenting cell-like phenotype in human lung cancer that trigger antitumour T cell responses and support anticancer activity[104] (FIG. 2l). Interestingly, the existence of atypical neutrophils cross-presenting antigens to influence $CD8^+$ T cell responses was recognized earlier[105], and more recently, neutrophil–dendritic cell hybrids were shown to be potent effectors of antifungal immunity[106]. NET release was also shown to prime and increase T cell responses[107]. However, this phenomenon was reported with human immune cells tested ex vivo in response to inflammatory stimuli and was not described in the context of cancer, in which NETs are consistently reported to be protumoural.

When discussing the protumoural and antitumoural functions of neutrophils, we need to consider that similar functions are observed for tumour-associated macrophages (TAMs) and TANs and that there is cross-regulation during immune responses[108,109]. Both TANs and TAMs can suppress adaptive immune responses, but the mechanisms by which they might influence each other within the tumour are less clear. As exemplified by Ponzetta et al.[103], antitumoural neutrophils can influence macrophages to produce IL-12 and orchestrate anticancer immunity (FIG. 2k). Similar complex interactions could underlie many TAN and TAM activities in the tumour microenvironment and could be important at certain tumour stages. Therefore, further work is required to understand the modulatory cross-talk between neutrophils and macrophages in tumour responses, especially for the development of antitumour strategies targeting these cells, as we discuss later. Additional investigation is also needed to determine the molecular drivers and transcription factors guiding the different phenotypes and functions of neutrophils infiltrating different tumour types.

Systemic neutrophil perturbations

The neutrophil activities presented so far describe the different behaviours of tissue neutrophils in response to neoplastic transformation leading to tumour onset, as well as their involvement in tumour growth. However, once a primary tumour has formed, tumour-derived

signals engage in long-distance interactions with the bone marrow to perturb neutrophil production, which can change the TAN composition (FIG. 3). As discussed earlier, evidence is accumulating on cancer-dependent neutrophil perturbations involving neutrophil progenitors in the bone marrow[33] and perturbation of neutrophils in the circulation[45,46] (FIG. 1b). In a landmark study, Engblom et al.[110] identified systemic crosstalk between lung adenocarcinoma cells and osteoblasts in bone inducing the mobilization of cancer-promoting Siglec-F^{high} neutrophils (FIG. 3b), which accumulate in lung tumours and boost tumour growth. More recently, a systemic inflammatory loop involving neutrophils was reported in renal cell carcinoma[111]. Here, epigenetic remodelling drove cancer cell-intrinsic inflammation and systemically altered neutrophils (FIG. 3b) to promote angiogenesis and metastasis. This is a fundamental emerging concept in neutrophil biology: that cancer acts systemically to perturb neutrophil numbers, properties and heterogeneity. This phenomenon is also an important event during metastatic progression. As we discuss in the next section, not only do the numbers of neutrophils increase in the setting of cancer via tumour-induced granulopoiesis but neutrophils also undergo cancer-dependent priming, which modulates their properties in the circulation and in distant organs, where they can influence metastatic spreading (FIG. 3c).

Tumour-induced granulopoiesis. Early studies in mice showed that G-CSF secreted by tumour cells could feed back to the bone marrow to trigger the onset of massive neutrophil production[43,112]. This occurs by expanding the GMPs and related neutrophil progenitors[32,33] and early neutrophil precursors[112], and has been termed 'emergency granulopoiesis'[113]. Multiple studies[33,45,46,114–116] have shown a vast premature release of these neutrophils and their progenitors into the circulation in various cancer types in mice and humans (FIG. 3a). Such neutrophils often reside in distant organs, such as the spleen, where they rapidly respond to states of emergency with expanded granulopoiesis (termed 'extramedullary granulopoiesis')[117]. Extramedullary haematopoiesis in the lymph nodes, liver and kidneys is observed in patients with various malignant solid tumours[118]. Thus, cancer could also contribute to the heterogeneity of circulating neutrophil populations via this process.

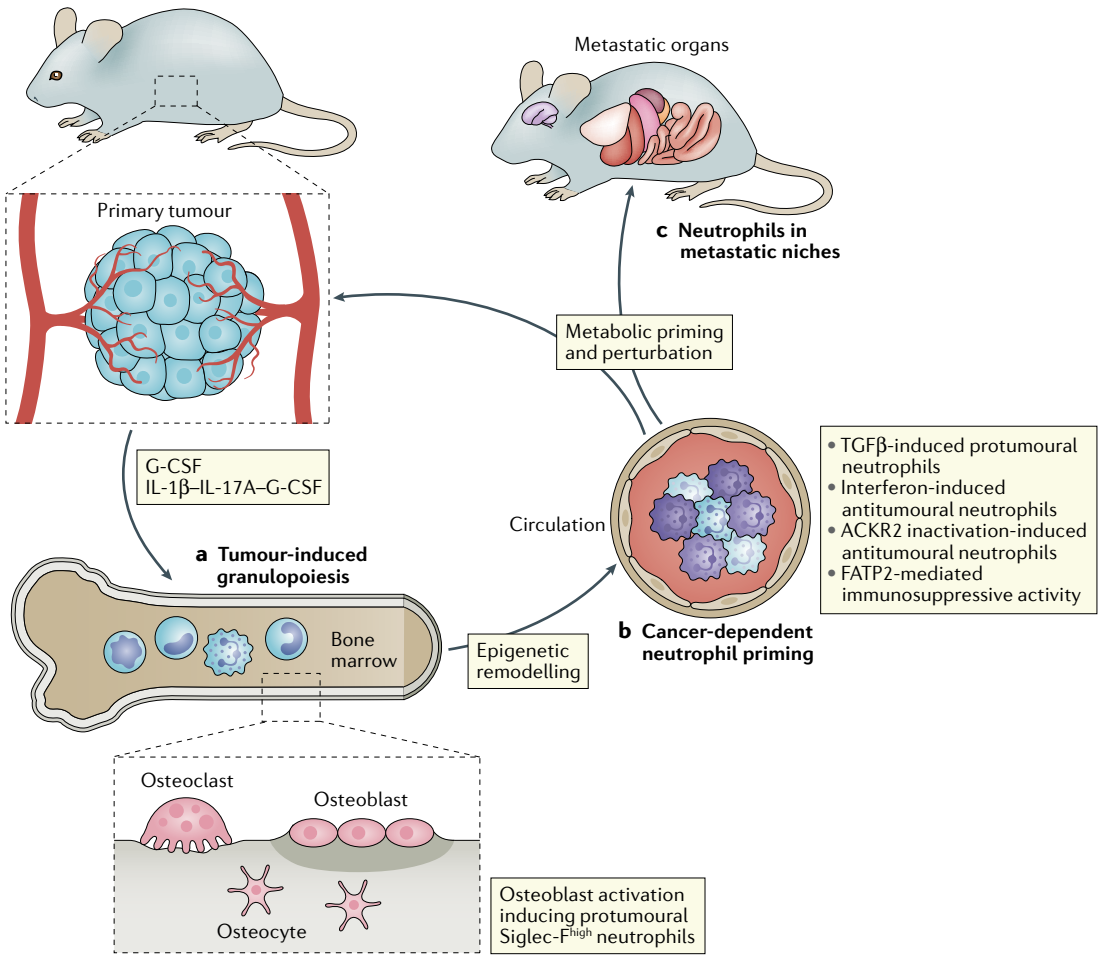


Fig. 3 | **Neutrophil perturbations during metastatic progression.** Systemic perturbation induced by primary tumour signals to produce and mobilize neutrophils (part **a**) and prime circulating neutrophils (part **b**) to foster cancer cell metastatic growth at distant organs (part **c**). ACKR2, atypical chemokine receptor 2; FATP2, fatty acid transporter protein 2; G-CSF, granulocyte colony-stimulating factor; TGFβ, transforming growth factor-β.

Modulators such as IL-17A released from the tumour microenvironment induce neutrophilia[119,120]. In mouse models of breast cancer with deletion of the gene encoding p53 (*Trp53*), release of WNT ligands induced IL-1β secretion from TAMs and caused systemic recruitment of KIT$^+$ neutrophils[121]. This finding supports a link between cell-intrinsic features of cancer and the immune landscape[73]. Other inflammatory circuits might drive tumour-dependent neutrophilia in other genetic contexts; p53-competent tumours induce neutrophilia, but the mobilized neutrophils do not express KIT[121].

Growth factors and cytokines, including G-CSF, granulocyte–macrophage colony-stimulating factor and IL-6, which can be released by cancers, are important in mediating emergency granulopoiesis[113,122,123] (FIG. 3a). Emergency granulopoiesis induced by infection is driven by a switch from the master regulator of steady-state granulopoiesis C/EBPα to C/EBPβ[39] in neutrophil precursors. In human and mouse cancers this switch is driven by retinoic acid-related orphan receptor (RORC)[124] and inhibits neutrophil differentiation, leading to a larger pool of more-immature neutrophils. However, as increased numbers of mature neutrophils are also detected in cancer[14], it is likely that many other extrinsic factors influence neutrophil maturation in this context. For instance, tumour-released factors, such as TGFβ, influence neutrophil maturation in the circulation, tissues and tumours[125]. Indeed, soluble mediators released systemically by cancers not only influence the number of neutrophils in the circulation and in the different tissues but also influence their behaviour by changing their cellular status, metabolism and activation (FIG. 3b). This cancer-mediated priming of neutrophils has important repercussions for disease progression.

Cancer-dependent neutrophil priming. Although there is growing appreciation of heterogeneity among circulating neutrophils in cancer, as determined by physical properties of neutrophils such as low or high density[45] or by CyTOF and flow cytometry[46], it is not clear how this heterogeneity influences tumour growth or metastasis. Comparison of mediators that influence neutrophil activities in cancer and in response to infections revealed interesting parallels between antitumoural functions and infection-mediated tissue damage. Type I interferons were one of the first signals reported to drive antitumoural activity in neutrophils in the context of breast cancer metastasis[126,127]. Interestingly, a type I interferon signature was observed in activated neutrophils that mediated liver tissue damage in malaria[128]. Moreover, genetic inactivation of ACKR2, a scavenger receptor for most proinflammatory CC chemokines and a negative regulator of inflammation, increased expression of proinflammatory chemokine receptors by neutrophils with antimetastatic activity[129]. However, ACKR2-deficient mice exhibit reduced survival against sepsis owing to tissue damage in the lungs and kidneys, suggesting that neutrophils in these animals may be beneficial against cancer spread but at the cost of greater tissue damage upon infectious challenges.

Cancer-mobilized neutrophils show perturbations in their metabolism, which affects their functions. Neutrophils are generally reliant on glycolysis for their survival and function, but different metabolic pathways are also important during neutrophil development in infectious and autoimmune diseases[130]. Evidence of this metabolic shift is emerging in various cancer contexts and is linked to immunosuppressive capacity. In a model of breast cancer, tumour-elicited neutrophils were able to maintain ROS production to suppress T cells by engaging oxidative mitochondrial metabolism. This cancer-dependent priming of systemic neutrophils allows these cells to maintain immunosuppressive functions when infiltrating the glucose-restricted tumour microenvironment[114]. More recently, lung neutrophils found locally within the metastatic environment displayed increased oxidative phosphorylation[131] compared with neutrophils located in lung sites that were distant from metastases, indicating that this metabolic shift can be induced both locally and systemically. Similarly, the subset of low-density neutrophils defined by a C/EBPε transcriptional signature found in the blood of patients with cancer was shown to be primed for NETosis via a metabolic shift occurring in the absence of glucose and could promote breast cancer liver metastasis[132]. Interestingly, a shift from glycolysis to fatty acid oxidation occurs during physiological neutrophil differentiation and is essential to provide free fatty acids to support mitochondrial respiration[133]. Notably, neutrophils in patients with cancer and in cancer models exclusively upregulate fatty acid transporter protein 2 (FATP2), which was essential for their immunosuppressive activity via arachidonic acid uptake and prostaglandin E_2 synthesis[134] (FIG. 3b). Intriguingly, other metabolites of arachidonic acid, namely leukotrienes, produced by cancer-mobilized neutrophils within the metastatic lungs, were shown to directly signal to invading breast cancer cells to support metastatic colonization[135].

Neutrophils at metastatic sites

In the previous section, we examined how systemic inflammatory loops triggered by cancer mobilize and prime neutrophils to promote phenotypic heterogeneity and cell production. In this section, we discuss how primed neutrophils impact the metastatic cascade (FIG. 4).

Neutrophils engage in the metastatic process during cancer cell dissemination. For example, in a model of ultraviolet radiation-induced inflammation, neutrophils supported interactions between melanoma cells and endothelial cells, promoting dissemination through the circulation[136]. More recently, neutrophils were found to support metastatic cancer cells while in the circulation by forming cell clusters (FIG. 4a). Cancer cells in neutrophil–cancer cell clusters acquire a proliferative advantage and are more efficient at metastasis than cancer cells not associated with neutrophils[137]. As primary tumour-derived signals generate neutrophils with specific phenotypic profiles, it would be interesting to determine whether neutrophils associated with circulating tumour cell clusters represent a unique subset.

Cancer-dependent granulopoiesis and neutrophil priming leads to neutrophil accumulation within distant organs well before cancer cell dissemination. This leads to the induction of premetastatic niches, wherein

primary tumours perturb distal organs to favour future metastatic seeding. This metastatic pioneering observed in preclinical models depends on cancer-dependent factors, inducing extracellular matrix remodelling[138], stromal cell activation[139] and, more relevant for this Review, cancer-primed neutrophil accumulation.

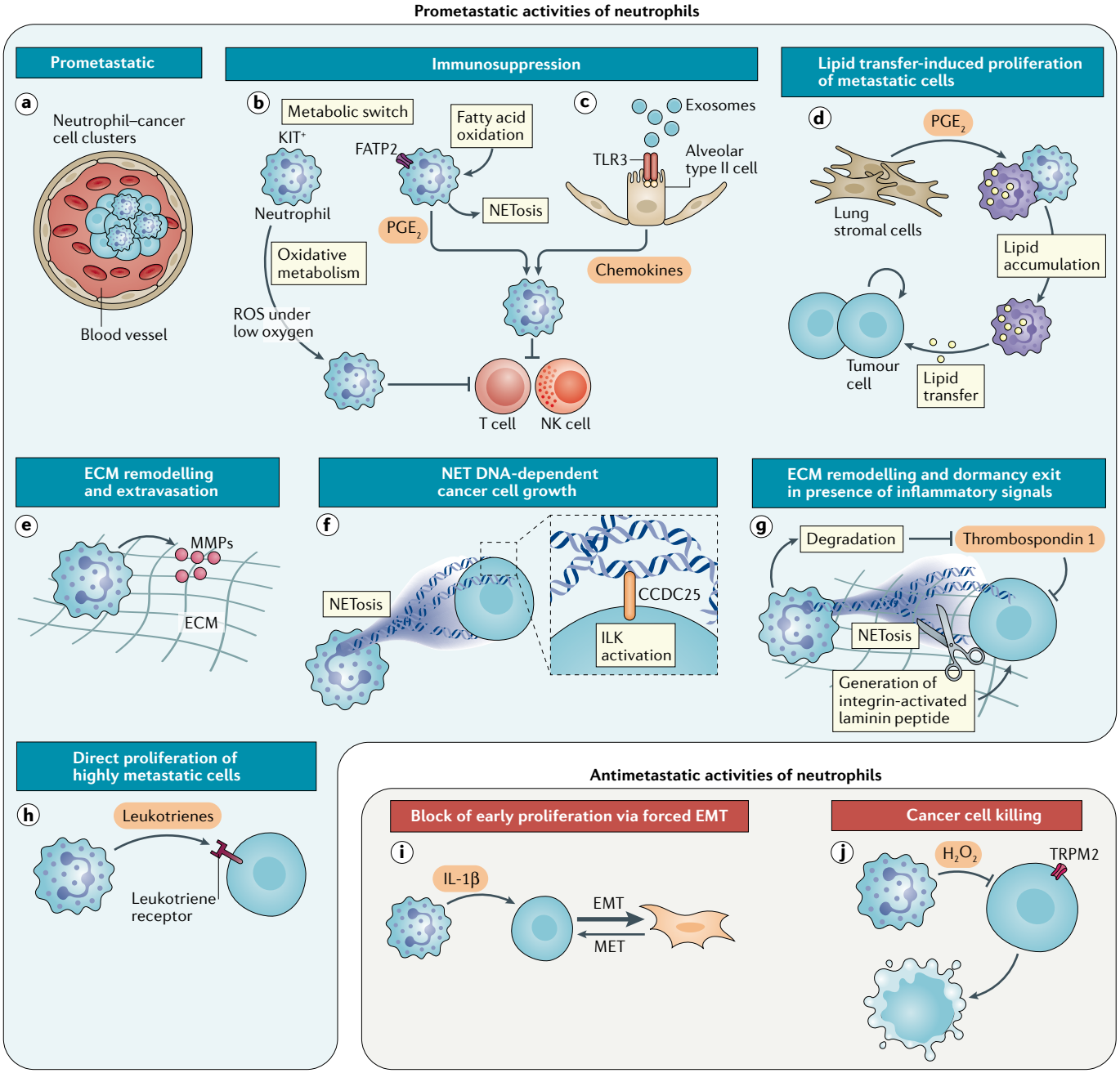


Fig. 4 | **Neutrophil engagements during metastatic progression.** **a** | Neutrophils associate with circulating tumour cells to form clusters, which support cancer cell cycle progression. **b** | Neutrophil mitochondrial oxidative phosphorylation and upregulated fatty acid transporter protein 2 (FATP2) promote immunosuppression and cancer cell growth. **c** | Primary tumour cell-derived exosomes activate Toll-like receptor 3 (TLR3) signalling in lung epithelial cells, promoting immunosuppressive neutrophil recruitment. **d** | Lung mesenchymal cells promote lipid accumulation in neutrophils, which transfer lipids to cancer cells, enhancing their proliferation. **e** | Prometastatic neutrophils induce extracellular matrix (ECM) remodelling. **f** | A specific sensor of neutrophil extracellular trap (NET)-associated DNA (known as CCDC25) on cancer cells promotes liver metastasis via activation of integrin-linked kinase (ILK) signalling. **g** | Exposure to tobacco smoke, which contains lipopolysaccharide, triggers NET-mediated remodelling of the ECM and awakens dormant cancer cells. The lipopolysaccharide-recruited neutrophils also degrade thrombospondin 1 via azurophilic granule release and allow metastatic growth. **h** | Prometastatic neutrophils directly boost proliferation of metastatic initiating cells. **i** | Neutrophils sustain epithelial-to-mesenchymal transition (EMT), inhibiting metastasis initiation. **j** | Neutrophils accumulating at the premetastatic site kill infiltrating disseminated breast cancer cells via H_2O_2 in a process dependent on expression of transient receptor potential cation channel M2 (TRPM2) on mesenchymal cancer cells. MET, mesenchymal-to-epithelial transition; MMP, matrix metalloproteinase; NK cell, natural killer cell; PGE_2, prostaglandin E_2; ROS, reactive oxygen species.

The first evidence for the existence of premetastatic niches emerged around 15 years ago when Kaplan et al. described the mobilization of bone marrow-derived cells at distant sites that enhanced future metastasis[140,141]. Over the following years, similar accumulations of neutrophils in mouse models were reported, in some cases inhibiting and in other cases supporting metastasis[112,119,135,142,143]. We consider some of the underlying mechanisms of the opposing behaviours of neutrophils during metastatic growth that have been clarified to date.

As previously mentioned, metabolic priming of circulating neutrophils leads to their accumulation in metastatic tissues and predisposes them to acquire immunosuppressive functions or to release NETs[114,131,132,134] (FIG. 4b). Besides secreted factors, tumour-derived exosomes have been reported to recruit premetastatic neutrophils to specific tissues and to support metastatic growth. This neutrophil priming by cancer-derived exosomes was first shown in melanoma[144], and later, exosomes from different types of metastatic tumours in experimental models were shown to specifically target the tissue in which they typically metastasize, and thus contribute to metastatic organotropism[145]. A subsequent study showed that tumour-derived exosomes within the lungs can activate Toll-like receptor 3 in alveolar cells and induce chemokine production and recruitment of immunosuppressive neutrophils[146] (FIG. 4c). Interestingly, although mimicking a viral infection, neutrophils recruited by tumour-derived exosomes opposed adaptive immune responses rather than enhanced $CD8^+$ T cell activity[147].

A more recent study reconfirmed the influence of lung-resident cells on premetastatic function in breast cancer models[148]. Here, lung mesenchymal cells suppressed adipose triglyceride lipase (ATGL) activity in neutrophils, leading to lipid accumulation. Subsequent transfer of lipids from neutrophils to cancer cells via a macropinocytosis–lysosome pathway enhanced cancer cell proliferation and metastatic activity (FIG. 4d). This work also highlights how tissue-specific changes can be induced in infiltrating neutrophils via interaction with tissue-resident cells. In turn, prometastatic neutrophils recruited to metastatic lungs can modify the lung environment via the release of matrix metalloproteinases to further enhance metastasis in breast cancer models[143] (FIG. 4e).

Another important function of neutrophils that promotes tumour growth is their ability to release NETs. NET release by cancer-primed neutrophils can occur during spontaneous metastasis, and the presence of NETs in the tissue around infiltrating cancer cells supports metastatic potential in various mouse models[149,150]. More recently, a mechanism by which NETs can directly support metastasis was revealed by the discovery of a specific receptor for NET-associated DNA on cancer cells, CCDC25 (REF.[151]), where engagement of CCDC25 by NET-associated DNA triggered the integrin-linked kinase (ILK) pathway and increased adhesion, motility and growth of metastatic cancer cells in the liver (FIG. 4f). Inflammatory signals triggered independently of cancer can predispose neutrophils to undergo NETosis[152,153] (FIG. 4g), which has important implications for tumour progression, as discussed in the next section.

Premetastatic neutrophils influence early metastatic seeding and growth in mouse cancer models both positively, by secreting inflammatory signals such as leukotrienes[135] (FIG. 4h), and negatively, by releasing IL-1β, which blocks the mesenchymal-to-epithelial transition needed for cancer cell growth[154] (FIG. 4i). Interestingly, because cancer cells switch their mesenchymal/epithelial status during different stages of the metastatic cascade[155], IL-1β-dependent epithelial-to-mesenchymal transition was conversely reported to promote tumour cell migration and invasion of primary gastric cancer[156]. This highlights that even the same neutrophil-secreted factor can have opposing effects during different phases of the metastatic cascade. Moreover, the immune cell composition of a metastatic lung guides neutrophils towards either prometastatic or antimetastatic functions[157]. By use of a combination of immunocompetent and immunodeficient animal models, it was shown that neutrophils could restrict metastatic cell growth in the lung in the absence of NK cells. Conversely, in the presence of NK cells, one of the protumorigenic functions of neutrophils was to limit NK cell activity[157]. NK cells can also directly influence neutrophil activities, for example by inhibiting tumour-promoting angiogenic functions of neutrophils via IFNγ[158], whereas in the context of haematopoietic cell transplantation, neutrophils support the survival and antitumoural activity of NK cells[159]. Thus, more studies are needed to define the relationship between neutrophils and NK cells, which appears to be multifaceted and highly context dependent.

Finally, depending on their phenotype, cancer cells interact with neutrophils in different ways. For example, the increase in cancer cell proliferation induced by neutrophil-derived leukotrienes in mouse breast cancer models affects only the subgroup of metastasis-initiating cells that express leukotriene receptors[135] (FIG. 4h). Similarly, only certain cancer cell phenotypes are susceptible to ROS-mediated killing by neutrophils[142]. This H_2O_2-dependent killing activity is mediated by transient receptor potential cation channel M2 (TRPM2), which is expressed on cancer cells particularly upon epithelial-to-mesenchymal transition[160,161] and drives expression of the neutrophil chemoattractant CXCL2 (REF.[162]). Thus, only cancer cells expressing high levels of TRPM2 are sensitive to this neutrophil-mediated inhibition (FIG. 4j).

Disease-modified neutrophils in cancer

Neutrophils are modified in other pathophysiological contexts, such as in infections, and this has implications for their responses to cancer. For example, circulating lipopolysaccharide induces complement C3a receptor expression on neutrophils and triggers complement activation, which boosted intestinal carcinogenesis by prompting NETosis by TANs[163]. Therefore, other conditions that increase blood coagulation could also alter neutrophil functions in cancer. Similarly, exposure of tumour-bearing mice to infection following caecal ligation and puncture led to deposition of NETs, which trap tumour cells and increase micrometastases of different

carcinoma cells to the liver[164]. Also, surgery triggers a systemic inflammatory wound healing response involving neutrophils and monocytes and promotes the growth of disseminated cancer cells that are otherwise restricted by antitumour T cells[165]. Indeed, patients who develop port-surgery infections following primary breast tumour resection are at higher risk of tumour recurrence[166]. Systemic or local lipopolysaccharide exposure enhances lung metastasis via the accumulation of neutrophils that induce degradation of the antitumorigenic factor thrombospondin 1 (REF.[152]) (FIG. 4g) or release cytokines that reactivate dormant cancer cells[167]. More recently, it was demonstrated that exposure to tobacco smoke containing lipopolysaccharide contaminants is a powerful trigger of NETosis by lung-resident neutrophils. The NET-associated proteases induced extracellular matrix remodelling involving laminin cleavage and the generation of a signalling peptide that awakened dormant cancer cells[153] (FIG. 4g).

Finally, chronic metabolic conditions influence neutrophil behaviour in cancer. In the context of obesity, increased adipose tissue in mice with breast cancer not only induced neutrophilia but also induced the release of ROS and NET production, facilitating the influx of tumour cells to the lungs and favouring metastasis[168,169]. Conversely, in the context of hyperglycaemia, a hallmark of diabetes, neutrophil mobilization from the bone marrow was reduced and led to increased breast cancer metastasis[170]. It remains to be clarified whether these systemic effects also reflect shifts in neutrophil phenotype and activation status. Although a high-fat diet and hyperglycaemia have more complex metabolic implications, these studies show that those conditions induce specific perturbations within the neutrophil compartment that have important implications for cancer.

Treatment resistance and neutrophils

The immune cell infiltrate of each tumour is a unique ecosystem that can provide an indication of disease prognosis and potential therapy responses[171]. An abundance of TANs is generally associated with poorer responses to chemotherapy and radiotherapy in various cancer types, except for ovarian and gastric cancers, in which higher TAN numbers predicted better chemotherapy responses (reviewed in REF.[15]). Moreover, the neutrophil-to-lymphocyte ratio in the blood has been used as a prognostic marker of survival in many tumour types (reviewed in REF.[14]). Different levels of heterogeneity among circulating neutrophils are observed between patients[14], but their association with clinical prognosis remains elusive.

As mentioned earlier, neutrophils express ligands for immune checkpoint receptors, and patients with lung cancer with high intratumoural neutrophil content showed poor responses to immune checkpoint blockade (ICB)[172]. Moreover, two studies that together analysed more than 2,500 individuals with advanced solid cancers (melanoma, non-small-cell lung cancer, urothelial carcinoma and renal cell carcinoma) found that higher levels of serum IL-8 were associated with increased TAN numbers, decreased survival and poorer response to ICB[173,174]. In a mouse model of lung cancer, anti-PD1 treatment efficacy was enhanced by treatment with a CXCR1 and CXCR2 inhibitor to antagonize neutrophils[172], showing the potential value of targeting neutrophils to improve ICB success.

A large-scale single-cell analysis of inflammatory compartments showed a broad remodelling of the immune cell infiltrate in patients undergoing therapy with ICB[175]. Immune cell profiling of multiple mouse models and clinical datasets led to the identification of three immune subtypes of triple-negative breast cancer: macrophage-enriched subtype (MES) tumours; neutrophil-enriched subtype (NES) tumours; and immunologically 'cold', ICB-insensitive tumours[176]. NES tumours were also resistant to ICB, whereas MES tumours showed variable levels of sensitivity, shifting to become NES-resistant tumours. Thus, the presence of myeloid cells determines the response to ICB and their composition in tumours is modified by treatment. The effectiveness of immunotherapy is also limited by the presence of neutrophils expressing the receptor tyrosine-protein kinase MET, which inhibit T cell expansion and effector functions following HGF stimulation[177].

When considering neutrophil-targeting therapies, one should consider the shared contributions of macrophages and neutrophils within the tumour microenvironment and their interchangeable functions. Indeed, the protumoural and antitumoural functions of neutrophils and macrophages are regulated by many common soluble factors, which could be used as potential targets[109]. For example, preclinical studies have shown that blocking CXCR2, which affects both neutrophils and macrophages[87,92,178–180], can reduce tumour growth and progression in various tumours, suggesting the potential benefit in targeting common regulators. Another factor common to both neutrophils and macrophages is the myeloid-specific phagocytosis checkpoint receptor SIRPα, which engages the 'do not eat me' signal CD47 on tumours and is proving to be a valuable target in preclinical and early clinical studies either alone or in combination with other cancer therapies[181].

The main issue with designing neutrophil-targeted approaches is the high plasticity of neutrophils in the tumour microenvironment and during standard anticancer therapy, which leads to a shift in their characteristics as well as their production and mobilization from splenic and bone marrow niches. Understanding the molecular drivers of neutrophil development and plasticity would open up possibilities of not only blocking specific protumoural functions of neutrophils but also harnessing their antitumoural activities[182].

Concluding remarks

Neutrophils are fascinating cells that respond rapidly to changes in homeostasis, with heterogeneous functions and an ability adapt to their environment. However, they are technically challenging to study owing to their fragility, short lifespan and low RNA content. Depending on the cancer type, they can represent a large population of cells within the tumour, and have crucial roles in cancer metastasis and overall patient outcomes[14]. Although there have been considerable advances in our

understanding of the biology, functions and heterogeneity of neutrophils, there are still many outstanding questions regarding their roles in cancer. For example, does the heterogeneity in neutrophil progenitors give rise to specific neutrophil progeny in the circulation? What are the functions of different neutrophil subsets in cancer, and would targeting all neutrophil subsets be a successful therapeutic approach? So far, research has focused on targeting the immunosuppressive or other protumoural functions of neutrophils, but will it be possible to control and exploit their cancer-killing functions? Future studies designed to answer these questions will certainly help to define new roles of neutrophils in cancer growth and metastasis, and may help in the development of new treatments that alter their behaviour in a context-dependent manner.

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