Tumour-associated neutrophils in patients with cancer

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Abstract | The role and importance of neutrophils in cancer has become increasingly apparent over the past decade. Neutrophils accumulate in the peripheral blood of patients with cancer, especially in those with advanced-stage disease, and a high circulating neutrophil-to-lymphocyte ratio is a robust biomarker of poor clinical outcome in various cancers. To date, most studies investigating the role of neutrophils in cancer have involved animal models or investigated the function of circulating human neutrophils. Thus, only limited information is available on the roles of intratumoural neutrophils (also known as tumour-associated neutrophils (TANs)) in patients with cancer. In this Review, we initially describe the evidence correlating the neutrophil-to-lymphocyte ratio with prognosis, followed by a discussion on the predictive value of TANs, which remains debatable, with conflicting data from different cancer types, including variations based on neutrophil location within and/or around the tumour. We then explore available data on the implications of TAN phenotypes and functions for cancer development and progression, highlighting the reported effects of various treatments on TANs and how neutrophils might affect therapeutic efficacy. Finally, we examine the various compounds capable of modulating neutrophils and suggest future research directions that might ultimately enable the manipulation of TANs in patients with cancer.

A general consensus exists that multiple immune cell types, including neutrophils, macrophages, dendritic cells, natural killer (NK) cells, T cells and B cells, are present in the tumour microenvironment (TME) and that these cells have a major role in cancer biology[1–4]. Neutrophils make up a substantial proportion of the immune infiltrate in a wide variety of cancer types, including lung, breast and gastric cancers, melanoma, renal cell carcinoma (RCC) and others[5–8]. However, the role of neutrophils in cancer has long been a matter of controversy. The results of various studies suggest that tumour-associated neutrophils (TANs) have various antitumour properties, including direct cytotoxicity towards tumour cells and inhibition of metastasis[9–12]. Conversely, the findings of numerous other studies suggest that TANs are capable of supporting tumour progression by promoting the angiogenic switch, stimulating tumour cell motility, migration and invasion, and modulating other immune cells as part of the 'immunosuppressive switch' (described in detail elsewhere[3,13–15]). Only since 2015 have researchers recognized that cancer-related neutrophils (both circulating and tumour-associated) are able to retain some functional plasticity and can undergo 'alternative activation' when exposed to various cues found in the TME[16,17]. For example, the presence of transforming growth factor-β (TGFβ) has been demonstrated to promote a protumour phenotype (referred to as N2 TANs), whereas the presence of interferon-β (IFNβ) or the inhibition of TGFβ signalling results in TANs of an antitumour (or N1) phenotype[18,19]. Furthermore, multiple heterogeneous neutrophil subsets have been observed in the circulation of both tumour-bearing mouse models and that of patients with cancer. Thus far, at least three distinct neutrophil populations have been identified in the circulation of both patients with cancer and mouse models. These can be roughly divided into mature high-density neutrophils (HDNs), mature low-density neutrophils (LDNs) and immature LDNs[20–24]. Neutrophils of the mature HDN subtype have been shown to have an N1-like phenotype and to kill tumour cells; however, mature LDNs are not cytotoxic and typically have impaired functionality and immunosuppressive properties[21–23].

Various different terminologies have been used across various studies to describe neutrophils or polymorphonuclear (PMN) cells in the context of cancer. The terms myeloid-derived suppressor cells (MDSCs) or PMN-MDSCs were originally used to refer to cells with immunosuppressive properties that differentiated and expanded from the immature granulocytes that are often observed in both mouse models and patients with cancer[25]; however, the extent of phenotypic and functional

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Key points

- The traditionally held belief that neutrophils are merely a bystander in the tumour microenvironment has been revolutionized over the past decade, and research has now established that neutrophils have an important contribution in the initiation, development and progression of cancer.
- Tumour-associated neutrophils (TANs) predict poor overall survival in many types of cancer, with their location in the tumour and specific markers being important deferential determinants.
- Data on the phenotype and function of TANs in patients with cancer remain limited and are mostly from those with early stage disease.
- Both antitumour and protumour functions of TANs have been described in patients with cancer, with both direct and indirect effects on tumour cells as well as indirect effects on the tumour microenvironment and its immune content.
- The importance of neutrophils in mediating the effects of existing and established cancer therapies is an emerging and exciting area of research.
- The ability to target TANs clinically, either by suppression or phenotypic manipulation, might be an important outcome of research into the role of TANs in cancer and could enable the development of a new generation of immunotherapies.

overlap between neutrophils and PMN-MDSCs has resulted in some confusion regarding the classification of these cells[13,26,27] (BOX 1).

Neutrophils are short-lived cells with a circulating half-life of ~7–10 hours in both humans and mice[13], although cytokines secreted by tumour cells, such as granulocyte colony-stimulating factor (G-CSF), IL-1β, IL-6 or tumour necrosis factor (TNF), have been suggested to extend their lifespan[28,29]. Owing to the short lifespan of neutrophils and the technical difficulties encountered in handling these cells, most knowledge on the role of neutrophils in cancer is derived from animal models. To date, our understanding of the functions of neutrophils in patients with cancer remains limited. The majority of clinical data on the roles of neutrophils in cancer have been obtained from the isolation of peripheral blood neutrophils. Nonetheless, many patients with advanced-stage cancers have high counts of blood neutrophils[30], and the neutrophil-to-lymphocyte ratio (NLR) has been introduced as a prognostic factor for survival (and, in some scenarios, to indicate a response to treatment) in many tumour types. However, the phenotypes and possible functions of TANs in patients with cancer have only begun to be investigated over the past decade.

In this Review, we initially discuss the prognostic relevance of circulating neutrophils and TANs in patients with cancer and expand on the correlation between prognostic markers and the balance between their tumour-promoting versus antitumour functions. Multiple factors that are suggested to affect the specific prognostic importance of TANs, including the type of tumour, specific histological features, stage of disease and localization within the tumour, are discussed. We then examine the available data obtained from patients with cancer that shed light on the various phenotypes and roles of TANs in tumour biology. We further explore the mutual effects of various anticancer therapies on neutrophils in the TME, and outline current insights into the effects of neutrophils on the efficacy of treatments. Finally, we summarize data from current clinical studies using compounds that are capable of modulating neutrophils as part of the treatment of patients with cancer, and evaluate possible future directions for the manipulation of TANs.

Neutrophils as a prognostic factor

Multiple immune hallmarks have been evaluated in cancer in the search for strong prognostic factors that might help to inform treatment strategies and elucidate the mechanisms through which tumours evade immune recognition. In this context, many researchers have assessed the prognostic value of circulating neutrophils or the NLR in various cancers. Furthermore, with the acknowledgment that neutrophils constitute a substantial proportion of the immune infiltrate in a wide variety of cancer types, an increasing number of studies have examined the prognostic value of tumour-infiltrating neutrophils[7].

Circulating neutrophils and NLR

The NLR has been introduced as a prognostic factor for survival in many tumour types, including non-small-cell lung cancer (NSCLC)[31], breast cancer[32], metastatic melanoma[30], prostate cancer[33], colorectal cancer (CRC)[34], hepatocellular carcinoma (HCC)[35], intrahepatic cholangiocarcinoma (ICC)[36] and others[37]. In a meta-analysis from 2014, investigators explored the correlation between the NLR and overall survival (OS) in data from 100 studies involving patients with solid tumours[38]. The prognostic effect of a high NLR (defined as ≥4) was the strongest in patients with mesothelioma, pancreatic cancer, RCC or CRC. High counts of circulating neutrophils were also shown to correlate with an increased risk of cancer-related thrombosis[39,40].

Although direct comparisons between circulating and tumour-infiltrating neutrophils from the same patient are difficult to perform, a high NLR and high numbers of circulating neutrophils might be associated with a higher frequency of tumour-infiltrating neutrophils in patients with pancreatic cancer[41]. In this study, the authors[41] found a NLR of >5 to be a robust and significant prognostic factor for decreased disease-free survival (DFS) and OS. When examining the density of intratumoural $CD66b^+$ neutrophils in the high and low NLR groups, a trend for higher $CD66b^+$ density was observed in the high NLR group, although this association was not statistically significant, mostly owing to a high level of interpatient variability. Clearly, a general association between the NLR and the extent of neutrophil infiltration cannot currently be made or extrapolated to all cancer types.

TANs

In a meta-analysis published in 2015, gene signatures obtained from >18,000 cancer biopsy samples were analysed and neutrophils were found within the tumour in the vast majority of the solid tumour samples examined[42]. Strikingly, a high ratio of infiltrating TANs to plasma cells emerged as the strongest prognostic predictor of inferior OS among all cellular populations and across all cancer types. Interestingly, the authors reported no relationship between the estimated neutrophil portion of the immune cell infiltrate and necrotic

Box 1 | **Neutrophils versus PMN-MDSCs: two faces of a similar entity**

When describing the roles and importance of neutrophils in cancer, the widespread use of the term 'polymorphonuclear myeloid-derived suppressor cells' (PMN-MDSCs) cannot be ignored. MDSC is a name assigned to a heterogeneous group of myeloid cells that suppress immune responses and, in mice, express the cell surface proteins CD11b and Gr1 (REFS[237,238]). A nomenclature and characterization for cells of this phenotype has been suggested[239], although no full consensus on their definition in humans has been reached[3,13,240]. These cells are thought to have inhibitory effects on lymphocytes, specifically, inhibition of lymphocyte proliferation[241–243]. MDSCs have been shown to accumulate in the circulation when tumours are present[244,245], and MDSC numbers generally correlate with an inferior prognosis[246–248]. The exact definition of an MDSC is debated, although the importance of their description is widely accepted and the definition of this cell type enabled the development of an understanding of the role of innate immunity in inhibiting the adaptive response to cancer and thus markedly advanced the understanding of the roles of myeloid regulatory cells in cancer[249,250]. However, PMN-MDSCs are closely related to neutrophils and are even considered by us and other investigators to be neutrophils of a certain phenotype[22,251,252]. Interestingly, the immunosuppressive capabilities of neutrophils were originally attributed to immature neutrophils only and, in some initial studies, these cells were even referred to as immature myeloid cells[241,244,253–255]. However, data from 2018 show that mature neutrophils (referred to as PMN-MDSCs) are capable of substantial suppression of T cell proliferation and cytokine release and, in some scenarios, are even more immunosuppressive than immature neutrophils[23].

Mature neutrophils can be defined by a $CD14^{-}CD15^{+}CD66b^{+}CD16^{+}$ pattern of cell-surface protein expression[256]. A more complex panel of at least six markers is typically used to identify human MDSCs (CD11b, CD14, CD15, CD66b, HLA-DR and CD33), and PMN-MDSCs are mostly referred to as $CD14^{-}CD15^{+}CD66b^{+}CD16^{+}CD11b^{+}CD33^{+}HLA\text{-}DR^{-}$ (REFS[257–259]), which is, in fact, a definition of a subset of neutrophils as all of these markers have been shown to also be expressed by neutrophils[13,84,260,261]. In 2018, Lang et al.[23] suggested that PMN-MDSCs can be divided into three different subsets, based on differences in CD11b and CD16 expression, while limiting separation only to the suppressive activity of these neutrophils isolated from the low-density fraction.

In our view, and as previously suggested[13], assigning a specific name to a cell or group of cells based entirely on one function, such as immunosuppression, implies that they exist predominantly for a single purpose or are unable to mediate any other activity. In fact, neutrophils are extremely dynamic and adaptable cells that are able to carry out many different and occasionally opposing functions simultaneously[19,22,84,262,263]. This reality is often overlooked — individual studies often tend to focus on one particular functional aspect of a cellular population (such as immunosuppression), whereas other functions remain largely untested. Therefore, we believe that the PMN-MDSCs described in most studies are, in fact, a subset of neutrophils, possibly with a different level of cellular activation, and not a separate cellular entity.

tissue content of the tumours, suggesting that intratumoural neutrophils are not simply a consequence of tissue necrosis.

Several other studies have revealed a correlation between the presence of TANs and a poor prognosis. These studies involved patients with early-stage melanoma[43], head and neck cancer[44] and HCC[45], and demonstrated that the presence of TANs was independently associated with poor OS, recurrence-free survival (RFS) and disease-specific survival outcomes. In addition to poor prognosis, high densities of tumour-infiltrating neutrophils were correlated with more advanced-stage disease in patients with gliomas[46] and in those with gastric cancer[47,48] and are more likely to be detected in more aggressive pancreatic tumours, such as those of the micropapillary and undifferentiated subtypes[49,50]. Similarly, in patients with non-metastatic clear cell RCC, the presence of $CD66^{+}$ neutrophils in the tumour was correlated with inferior OS and lower RFS following nephrectomy[51,52]. However, in some types of cancer, such as CRC and lung cancer, the prognostic relevance of TANs remains a matter of debate. Several studies of tissue samples from patients with CRC revealed no prognostic relevance of TANs, although the use of nonspecific staining methods, such as haematoxylin and eosin[53,54] or elastase staining[55], might have compromised the specificity of the analysis. In a more recent study from 2012, Rao et al.[5] reported that TANs are an adverse prognostic factor in patients with CRC and that a correlation exists between high tumour $CD66b^{+}$ neutrophil count and size, degree of spread to lymph nodes and clinical stage. By contrast, data from other studies suggest that infiltration by myeloperoxidase $(MPO)^{+}$ neutrophils is associated with a favourable prognosis in patients with CRC[56–59].

Lung cancer is another example in which the role of intratumoural neutrophils remains controversial[60,61]. Investigations of the clinical implications of neutrophil density in the tumour have described differences in the prognostic relevance of neutrophils depending on the specific type of lung cancer, including small-cell carcinoma, adenocarcinoma or squamous cell carcinoma (SCC). In a study involving patients with early-stage (stage I–III) NSCLC[60], high $CD66b^{+}$ neutrophil density had a minimal effect on OS but was correlated with a greater incidence of relapse following surgical resection. However, a high TAN-to-$CD8^{+}$ T cell ratio was found to be a poor prognostic indicator of both RFS and OS. More surprisingly, Rakaee et al.[8] described opposing subtype-specific prognostic implications of TANs in patients with early-stage NSCLC: the presence of $CD66b^{+}$ TANs was described as a positive prognostic factor in patients with SCC but an adverse prognostic factor in those with adenocarcinoma. Conversely, in another study, a high neutrophil count was associated with a poor prognosis in patients with SCC, although no such association was found in those with adenocarcinoma[62].

From our own experience of studying tissue samples from patients with lung cancer, we have observed a surprising level of heterogeneity in the presence of neutrophils (defined as MPO^{+} cells): some tumours are heavily infiltrated, whereas others have only moderate or even

no neutrophil infiltration. The prognostic implications of neutrophil infiltration in these patients clearly require further investigation.

Implications of TAN location

Differences in neutrophil localization within the tumour provide a possible explanation for the controversial and sometimes confusing findings on the prognostic implications of TANs. Indeed, evidence from multiple studies suggests that not only the presence of neutrophils but also their specific location within the tumour has prognostic relevance[59–61,63,64]. Data from a few studies comparing the presence and preponderance of intratumoural, peritumoural or stromal neutrophils in various cancer types suggest that neutrophils in different locations can have different prognostic implications (FIG. 1). In most studies, intratumoural neutrophils, as opposed to peritumoural or stromal neutrophils, were shown to have the strongest association with detrimental prognosis. Nevertheless, in certain types of cancer, associations between peritumoural neutrophils and inferior OS have been detected. In patients with HCC, multiple studies indicate that neutrophils, if present, are predominantly located in the peritumoural stroma rather than within the tumour and are correlated with inferior OS[63–65]. He et al.[64] confirmed that high intratumoural or peritumoural CD66b$^+$-to-CD3$^+$ immune cell ratios in liver sections from patients with HCC are predictive of inferior OS. Similarly, in patients with HCC with high counts of peritumoural CD15$^+$ neutrophils, MET expression in malignant cells was inversely correlated with both OS and DFS[65]. In women with cervical cancer, high densities of CD66b$^+$ neutrophils in the peritumoural and stromal regions have been shown to correlate with inferior RFS, whereas neutrophils within the tumour nest have not[6]. In the same study, the whole-tumour (but not region-specific) neutrophil-to-CD8$^+$ ratio provided a high level of discriminatory power for RFS. High counts of intratumoural neutrophils were strongly associated with metastasis, advanced-stage disease, and inferior OS and DFS in an analysis of surgical specimens from patients with oesophageal SCCs[66], although high ratios of peritumoural neutrophil-to-CD8$^+$ lymphocytes were also associated with more advanced-stage disease and lymph node metastasis.

In an analysis of surgical specimens from patients with CRC, CD11b$^+$CD15$^+$CD10$^+$ TANs were found predominantly at the invasive front[67]. This subset strongly correlated with TGFβ expression in CRC cells and with the presence of tumour buds, which were described as small groups of cancer cells believed to have gone through the epithelial–mesenchymal transition and to be associated with inferior prognosis in patients with cancer[68,69].

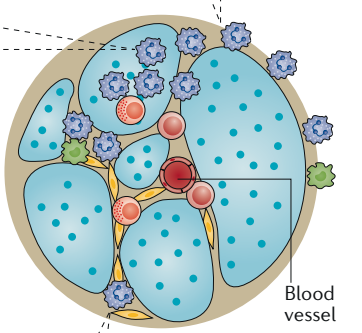
The hypothesis that TAN phenotypes are modulated by local cues encountered in different regions of the tumour might explain the differences in prognosis related to neutrophil location. For example, He et al.[64] reported that high levels of granulocyte–macrophage colony-stimulating factor (GM-CSF) and TNF are expressed in the peritumoural area, but also to a lesser extent in the intratumoural area in HCC specimens, and that these cytokines drive neutrophils to develop an immunosuppressive phenotype. The authors attributed this change to stronger suppression of CD3$^+$ T cells in the peritumoural regions and differences in the CD66b$^+$-to-CD3$^+$ ratio between the peritumoural and intratumoural regions. We have also previously suggested that, at the early stages of tumour development (and in a mouse model of mesothelioma), neutrophils remain predominantly located at the edges of the tumour and have an N1 phenotype[70]. However, on tumour progression, neutrophils are often found deeper within the tumour and have an N2 phenotype, enabling tumour growth to be supported. The exact cues that dictate differences in TAN phenotypes during tumour progression require further clarification.

Intratumoural

Cancer types	OS	Refs
RCC	↓↓↓	51,52
HCC	↓↓	45,63,73,74
HNSCC	↓↓	44,76
NSCLC	~	60,61
CRC	↑↓	5,57–59
Melanoma	↓↓	43
Gastric	↑↓	47,48
ICC	↓	75
PDAC	~	50
Lymphoma	↓↓↓	285
ESCC	↓	66

Peritumoural

Cancer types	OS	Refs
HCC	↓	63
NSCLC	–	61
ESCC	–	66



Stromal

Cancer types	OS	Refs
HNSCC	~	63
NSCLC	–	61

Fig. 1 | **Prognostic implications of neutrophils and their location in patients with cancer.** The prognostic implications of neutrophils, in terms of overall survival (OS), can vary dramatically by primary tumour histology and whether the neutrophils are predominantly localized in the tumour (intratumoural), around the tumour (peritumoural) or in the tumour-associated stroma. ↓↓↓ hazard ratio (HR; for OS) >2.5; ↓↓, HR 2.0–2.5; ↓, HR 1.5–1.9; ~, HR 1.0–1.5; ↓↑, mixed conclusions. CRC, colorectal cancer; ESCC, oesophageal squamous cell carcinoma; HCC, hepatocellular carcinoma; HNSCC, head and neck squamous cell carcinoma; ICC, intrahepatic cholangiocarcinoma; NSCLC, non-small-cell lung cancer; PDAC, pancreatic ductal adenocarcinoma; RCC, renal cell carcinoma.

Specific markers as prognostic factors

As will be discussed later, the exact phenotypes of TANs are a matter of controversy. However, a general consensus exists that neutrophils can express a range of different cell-surface markers and receptors and that these are likely to be clinically relevant, affecting tumour growth and eventual prognosis. To improve prognostication,

multiple studies have suggested combining the quantification of other immune markers in addition to a general neutrophil marker (such as CD66b). Thus far, most studies combining neutrophil-specific markers with other immune biomarkers have involved patients with HCC. Zhou et al.[71] demonstrated that intratumoural neutrophils express high levels of CC-chemokine ligand 2 (CCL2) and CCL17, and that these are correlated with disease progression and prognosis. The number of $CCL2^+$ and $CCL17^+$ TANs was found to positively correlate with tumour size, microvascular invasion, level of tumour differentiation and staging. Tissue microarray-based immunohistochemical quantifications of CCL2 and CCL17 levels showed that these chemokines are preferably expressed by neutrophils throughout the tumour stroma but not in the adjacent nonmalignant tissues. Patients with low $CCL2^+$ or $CCL17^+$ TAN counts had substantially better outcomes than those with higher numbers of these cells. Importantly, these markers were previously demonstrated, in mice, to be part of the N2 signature[70,72]. Outcomes of another study involving samples from patients with HCC who underwent curative resection suggest that the combination of CD66b and CXC-chemokine receptor 6 (CXCR6) levels is a better predictor of both disease recurrence and dismal survival than CXCR6 levels or neutrophil counts alone[73]. The authors demonstrated that sections with high levels of the chemokine receptor CXCR6 also contained an abundance of $CD66b^+$ neutrophils and blood vessels. Although intratumoural neutrophil density alone appeared to be an independent prognostic indicator of short RFS, but not OS, the combination of intratumoural $CD66b^+$ neutrophil density and CXCR6 expression was independently associated with both short RFS and OS durations. In an additional study, Zhou et al.[74] suggested that CXCL5 expression, either alone or in combination with the presence of intratumoural neutrophils, was an independent prognostic factor for shorter OS durations and cumulative risk of recurrence in patients with HCC. Gu et al.[75] proposed the combination of $IL\text{-}17^+$ cell density with that of $CD66b^+$ neutrophil density as a stronger negative prognostic factor for OS, although both intratumoural $IL\text{-}17^+$ cell density and intratumoural neutrophil density alone were described as independent prognostic factors for OS in patients with ICC. Furthermore, increased intratumoural CD66b and IL-17 densities were correlated with more aggressive forms of ICC, and intratumoural neutrophil levels alone were correlated with vascular density, as indicated by CD34 immunohistochemistry.

Specific neutrophil phenotypes have also been shown to have prognostic implications in tumours of other primary histologies. Dumitru et al.[76] suggested that strong staining intensities and high percentages of $CD66b^+$ and MIF^+ or $CD66b^+$ and $AHNAK^+$ cells in skin biopsies are associated with poor survival outcomes in patients with laryngeal carcinoma. Interestingly, AHNAK expression was demonstrated to be essential for rearrangement of the cytoskeleton and to promote tumour cell migration and invasion in cell lines derived from patients with metastatic solid tumours[77]. CXCR2 signalling has also been shown to be upregulated in biopsy samples from patients with pancreatic cancer, predominantly in neutrophils and/or MDSCs, whereas very few isolated pancreatic cancer cells express CXCR2. The authors found a significant correlation between expression of MPO and CXCR2 in cells of the stroma located at the edge of the tumours, adjacent to non-malignant regions[78]. CXCR2 signalling and myeloid cell recruitment at the tumour border were also linked with inferior outcomes in patients[78]. Patients with NSCLCs characterized by a high density of $CD15^+$ neutrophils also have an increased proportion of tumours expressing the epithelial–mesenchymal transition-promoting transcription factor Snail, reflecting a population of patients with NSCLC with a poor prognosis[79]. In this study, infiltration with $CD15^+$ neutrophils and CD31 staining were negatively correlated.

Characterization of TANs in patients

TAN phenotype in patients

Most studies related to the phenotype and function of neutrophils in the TME thus far have been conducted in animal models. To date, data on the phenotype and function of intratumoural neutrophils in patients with cancer remain limited and are mostly from patients with early-stage disease, from whom tumour material is more widely available. Furthermore, the majority of data available from patients are focused on the bilateral effects of neutrophils and T cells (FIG. 2).

In a study looking at the effects of T cells on neutrophil function, innate IL-17-producing γδT (γδT17) cells were found to promote the accumulation and survival of immunosuppressive neutrophils (described as PMN-MDSCs in this study) in the tumour, mainly in an IL-8, TNF and GM-CSF-dependent manner[80]. TANs in CRC were defined by cell-surface protein expression ($CD45^+Lin^-HLA\text{-}DR^-CD33^+CD66b^+CD11b^+$) and had a typical PMN morphology, but produced much more arginase 1 (ARG1) and reactive oxygen species (ROS) than autologous neutrophils. These TANs were able to inhibit the proliferation of activated autologous T cells and their ability to produce IFNγ, in contrast to circulating PMN cells isolated from the same patients, which did not inhibit T cell proliferation. The authors also reported that, in the CRC tissues analysed, TANs were ~80-fold more abundant than monocytic MDSCs[80].

As previously mentioned, high numbers of $CCL17^+$ and $CCL2^+$ TANs are correlated with greater tumour size, level of differentiation and more advanced vascularization of the tumour in patients with HCC[71]. Using a humanized mouse model of HCC, the authors suggested that TANs support these processes by promoting the infiltration of macrophages ($F4/80^+$) and regulatory T (T_{reg}) cells ($FoxP3^+$) from the TME. Because TANs, but not circulating neutrophils, had higher levels of CCL2 and CCL17 expression, the authors suggested that HCC cells are able to 'educate' circulating neutrophils towards a TAN phenotype by activating the MAPK and PI3K signalling pathways.

Research by Cui et al.[81] suggests that immature myeloid cells identified as $Lin^-CD45^+CD33^+$ (referred to as MDSCs) support the progression and metastatic dissemination of ovarian cancer. These cells, which comprised 37% of the non-neoplastic cells in patients with high-grade ovarian cancer, suppressed $CD4^+$ and $CD8^+$

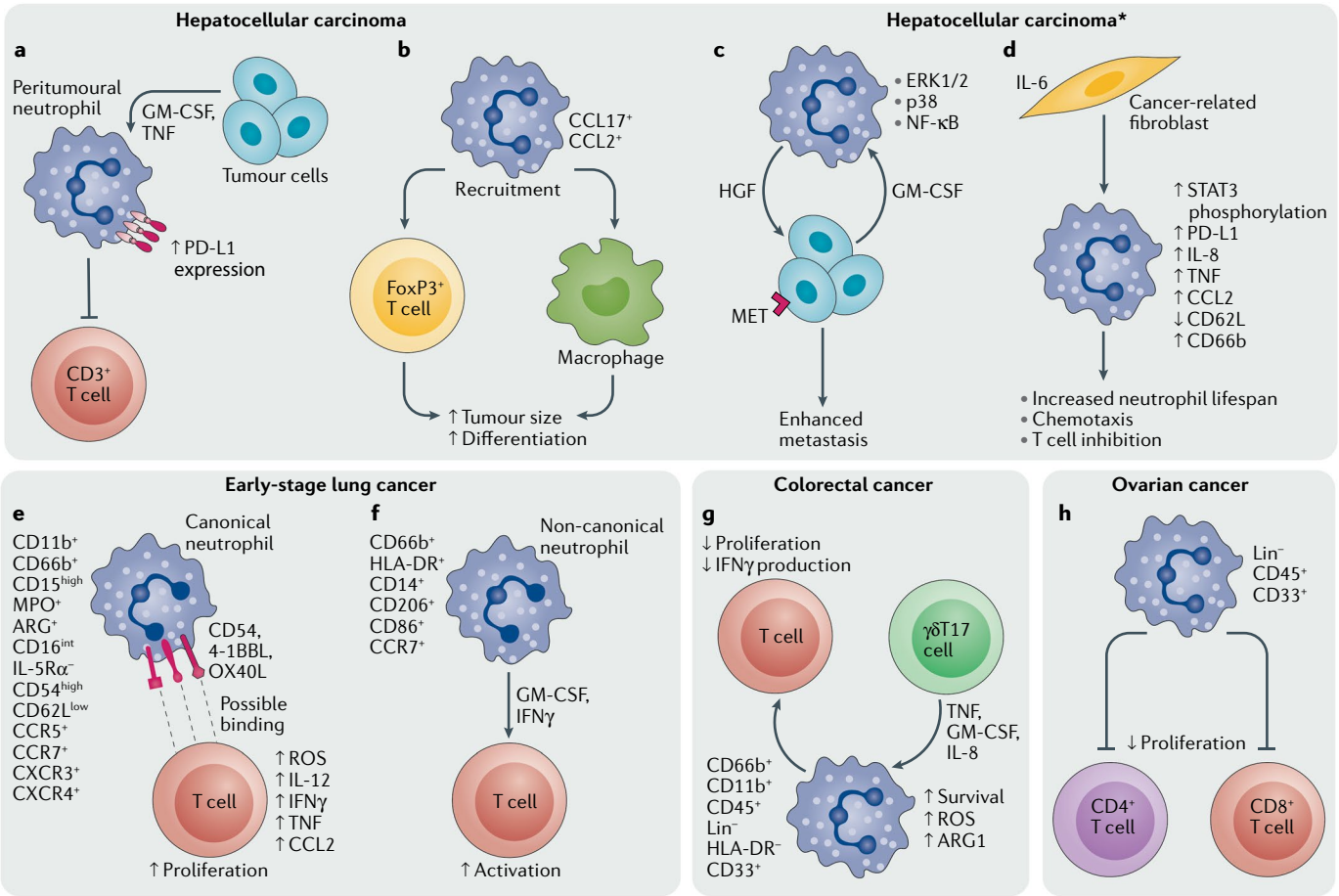


Fig. 2 | **Proposed phenotypes, markers and functions of tumour-associated neutrophils in a range of human cancers.** The available data suggest, thus far, that tumour-associated neutrophils (TANs) can have different locations and, therefore, different functional roles depending on the histology of the primary tumour. **a**–**d** | Role of neutrophils in hepatocellular carcinoma (HCC). Granulocyte–macrophage colony-stimulating factor (GM-CSF) and tumour necrosis factor (TNF) are highly expressed in the peritumoural area of HCCs and modulate neutrophils towards a stronger immunosuppressive profile, increasing their levels of programmed cell death 1 ligand 1 (PD-L1) expression and enhancing their ability to suppress T cells[64] (part **a**). CC-chemokine ligand 17-positive ($CCL17^+$) and $CCL2^+$ TANs support the growth and vascularization of HCCs by promoting the infiltration of macrophages ($F4/80^+$) and regulatory T cells ($FoxP3^+$) to the tumour microenvironment[71] (part **b**). GM-CSF expressed by HCC tissue promotes the activation of TANs and their production of hepatocyte growth factor (HGF) in an extracellular signal-regulated kinase 1/2 (ERK1/2)-, p38- and nuclear factor-κB (NF-κB)-dependent manner. The binding of HGF to MET on malignant cells enhances the migration and invasiveness of these cells[65] (part **c**). Cancer-associated fibroblasts infiltrating HCCs promote the survival and activation of TANs, reflected by increased expression of CD66b, PD-L1, IL-8, TNF and CCL2, but decreased expression of CD62L, through an IL-6–signal transducer and activator of transcription 3 (STAT3)–PD-L1 signalling cascade[264] (part **d**). **e**,**f** | Role of neutrophils in early-stage non-small-cell lung cancer. TANs isolated from early-stage lung cancer tissues display an activated phenotype with a distinct repertoire of chemokine receptors, pro-inflammatory factors and co-stimulatory molecules. TANs enhance the activation and proliferation of T cells in an OX40L-, 4-1BBL- and CD54-dependent manner[84] (part **e**). Early-stage lung cancer 'non-canonical' TANs express markers typically found on antigen-presenting cells (part **f**). This unique subset of neutrophils stimulates and supports antitumour T cell responses in a GM-CSF- and interferon-γ (IFNγ)-dependent manner[85]. **g** | Role of neutrophils in colorectal cancer. In colorectal cancer, IL-17-producing γδT (γδT17) cells promote the accumulation and survival of immunosuppressive TANs, in an IL-8-, TNF- and GM-CSF-dependent manner[80]. **h** | Role of neutrophils in ovarian cancer. In ovarian cancer, $Lin^-CD45^+CD33^+$ cells support ovarian cancer progression and metastasis by weakening T cell proliferation and antitumour immunity[81]. ARG1, arginase 1; MPO, myeloperoxidase; ROS, reactive oxygen species.

T cell proliferation. Using an immunocompromised (NOD/Shi-*scid*/*Il2R*^{null}) mouse xenograft model created by injection of a primary human ovarian cancer cell line, the authors showed that T cells 'educated' via in vitro incubation with tumour-associated $Lin^-CD45^+CD33^+$ cells had weakened antitumour immunity when subsequently injected into tumour-bearing mice, resulting in enhanced tumour volume compared with mice injected with tumour cells alone or with tumour cells and 'non-educated' T cells.

The expression of LOX-1 has also been proposed as a method of identifying a subset of circulating and intratumoural PMN-MDSCs with T cell-suppressive properties[82]. $CD15^+LOX\text{-}1^+$ cells were found to comprise ~5%

of the circulating PMN cells and ~20% of intratumoural PMN cells (TANs) in samples from patients with head and neck squamous cell carcinoma or NSCLC[82]. Endoplasmic reticulum stress was further shown to induce LOX-1 expression and to promote the suppressive functions of $CD15^+$ circulating neutrophils isolated from donors without cancer[83].

In one of the largest studies on the functions and phenotypes of TANs, Eruslanov et al.[84] investigated the phenotypes of intratumoural neutrophils in patients with early-stage lung cancer. TANs from these patients were broadly characterized as $CD11b^+CD15^{hi}CD66b^+MPO^+ARG1^+CD16^{int}IL\text{-}5R\alpha^-$. Intratumoural neutrophils were found to have a more activated phenotype than that of circulating neutrophils with high levels of phagocytic activity, ROS production and CD54 expression, but low CD62L levels, an altered cytokine profile and a unique chemokine receptor expression profile including CC-chemokine receptor 5 (CCR5), CCR7, CXCR3 and CXCR4 (REF.[84]). These 'early' TANs were shown to secrete a large variety of cytokines and chemokines, mostly with pro-inflammatory effects, such as IFNγ, IL-12 and TNF, with limited expression of anti-inflammatory cytokines (IL-4 and IL-10). The TANs isolated in this study also produced larger quantities of the pro-inflammatory factors CCL2, CCL3 (also known as MIP1α), IL-8 and IL-6 than blood neutrophils. Many co-stimulatory molecules, such as OX40L, 4-1BBL and CD54, became upregulated upon contact with activated T cells, which might explain the ability of TANs to stimulate the proliferation of both $CD4^+$ and $CD8^+$ T cells[84]. Furthermore, the authors identified a small group of 'non-canonical' TANs expressing additional markers typically expressed on antigen-presenting cells, but not on circulating neutrophils, such as HLA-DR, CD14, CD206, CD86 and CCR7. The frequency of this hybrid population varied from 0.5% to ~25% of all TANs and was substantially higher in patients with adenocarcinoma than in those with SCC. This unique subset of neutrophils was demonstrated to stimulate and support antitumour T cell responses in a GM-CSF-dependent and IFNγ-dependent manner, and were named 'antigen-presenting cell-like' hybrid TANs[85]. In 2017, Governa et al.[86] showed that, although neutrophil infiltrates from patients with CRC are often devoid of direct cytotoxicity towards cancer cells, co-culture of $CD8^+$ T cells with tumour-associated TANs or peripheral blood neutrophils promotes $CD8^+$ T cell activation, proliferation and cytokine release. In an analysis of the presence and phenotype of neutrophils in patients with melanoma, investigators found a high frequency of $CCR5^+$ cells (described as $HLA\text{-}DR^{-/low}CD11b^+CD14^-CD15^+$ PMN-MDSCs) in both the circulation and tumour samples[87]. The authors also found high levels of CCL3, CCL4, CCL5, GM-CSF and IFNγ in tumour lysates, which might explain the migration of $CCR5^+$ MDSCs from the circulation into melanoma lesions. Patients with stage III or stage IV disease also had a higher frequency of circulating $ARG1^+$ and programmed cell death 1 ligand 1 $(PD\text{-}L1)^+CCR5^+$ PMN-MDSCs than those with stage I or stage II disease, suggesting a stronger immunosuppressive phenotype than that of the $CCR5^-$ PMN-MDSC fraction. In 2018, circulating neutrophils or PMN-MDSCs of a similar phenotype were described in patients with NSCLC[88].

The ability of cancer-related neutrophils to release neutrophil extracellular traps (NETs) is attracting considerable research interest. NETs are released from neutrophils in response to extracellular pathogens and typically consist of fibrous decondensed chromatin with associated histones, MPO and various cytoplasmic proteins, such as neutrophil elastase, cathepsin G and lactoferrin. NETs have been reported to be released in the context of cancer, although the exact importance of this effect has yet to be clarified. NETs are hypothesized to act within the primary tumour to promote disease progression and dissemination; however, thus far, most studies have only described this phenomenon in circulating neutrophils[89–91]. Compared to neutrophils from people without cancer, circulating neutrophils isolated from patients with CRC release substantially more NETs following stimulation with IL-8 and/or lipopolysaccharide, and are associated with relapsed disease[91]. The authors also described spontaneous NET production in samples from patients with cancer. An increase in NET-derived MPO–DNA complex levels in patient serum samples following liver resection was associated with a more than fourfold reduction in DFS, suggesting that NETs promote metastatic dissemination following surgical stress[90]. Limited evidence for the formation of NETs in tumour tissues has been reported thus far. NET release, also referred to as 'NETosis', was identified in biopsy samples from two out of eight paediatric patients with Ewing sarcoma[92].

An increase in the ability of neutrophils to release NETs has been broadly suggested to promote tumour progression and metastatic dissemination. As mentioned previously, the accompanied release of ROS together with the trapping of cancer cells could theoretically promote a cytotoxic effect and inhibit the dissemination of cancer cells[93,94]. However, the protumour versus antitumour functions of this phenomenon require further clarification. Furthermore, direct evidence of NETosis occurring under physiological conditions (as opposed to when stimulated with phorbol 12-myristate 13-acetate, lipopolysaccharide or *N*-formylmethionine-leucyl-phenylalanine), together with a better description of this effect in human tumour tissues, remains elusive.

Protumour versus antitumour functions

In vivo manipulations in mouse models have demonstrated that TANs are able to acquire different phenotypes based on specific features of the TME. In a TGFβ-rich environment, neutrophils typically have an N2 profile, which is associated with protumour properties, whereas in the presence of IFNβ or inhibition of TGFβ signalling, neutrophils switch to an N1 phenotype, which is associated with antitumour properties. This change in phenotype is accompanied by a change in the expression of genes encoding various cytokines, chemokines, adhesion molecules, granule-associated proteins and others. Whether the N1/N2 profile described in mouse models is applicable to human TANs remains largely unknown. Interestingly, however, some of the genes and markers associated with the tumour-promoting effects of N2 TANs have also been reported in humans.

The gene encoding the chemokine CCL17 was found to be one of the most strongly upregulated genes among TANs of an N2 phenotype in a mouse model of mesothelioma (AB12) and expression of this chemokine was found to increase with tumour progression[70,72]. CCL17 released from TANs was also shown to support tumour growth by promoting the recruitment of $CD4^+$ T_{reg} cells to the tumour[70]. In an assessment of the expression of various chemokines in human HCC[71], CCL17 was also shown to be predominantly expressed by neutrophils and high levels of CCL17 observed in histological sections were found to correlate with disease progression and an inferior prognosis in these patients. As previously mentioned, $CCL17^+CCL2^+$ TANs are clearly associated with a poor prognosis in patients with HCC.

Upregulation of ARG1 is also associated with a tumour-supportive, T cell inhibitory phenotype[95] and has been described in several studies as a phenotypic marker of MDSCs[87,96,97]. In 2017, higher frequencies of $ARG1^+$ circulating neutrophils were reported in patients with stage III–IV CRC than those with stage I–II disease, suggesting that a more immunosuppressive neutrophil phenotype emerges during tumour progression[98]. Research by Lang et al.[23] also showed that mature $CD16^+CD11b^+CD66b^+$ circulating neutrophils expressing high levels of ARG1 are superior in their ability to suppress T cell proliferation and cytokine production than other MDSC subtypes. By contrast, in an analysis of specimens from patients with CRC, investigators observed a significant and positive correlation between the number of TANs and the level of ARG staining, in which higher TAN counts were associated with a better prognosis[58]. This observation adds to the conclusions of other studies, which have questioned the sufficiency of ARG1 activity alone in the impairment of T cell function in patients with cancer[99].

Matrix metalloproteinase 9 is another marker of N2 TANs in mouse models. This enzyme is also reported to be secreted from human neutrophils and to have a pivotal role in the activation of angiogenesis by counteracting the effects of anti-angiogenic molecules and possibly also by promoting the release of VEGF[63,100].

In contrast to the multiple tumour-promoting effects of neutrophils, several studies involving tumour-bearing mouse models have highlighted the antitumour and antimetastatic potential of neutrophils[12,19,101–104]. However, evidence supporting an antitumour role of TANs in patients with cancer is scant, and most studies involving patients are reliant on data from isolated circulating neutrophils only. Research by Dissemond et al.[105], for example, demonstrated that circulating PMN cells isolated from adults without cancer that were 'primed' with TNF and GM-CSF are highly cytotoxic to melanoma cells in vitro owing to the release of ROS. The antitumour potential of neutrophils from donors without cancer has been confirmed elsewhere by the demonstration that circulating PMN cells express and release TNF-related apoptosis-inducing ligand (TRAIL), thus driving apoptosis in Jurkat cells (a human T cell leukaemia cell line)[106]. Furthermore, Eruslanov et al.[84] showed that TANs isolated from patients with early stage NSCLC can stimulate T cell proliferation and promote IFNγ production.

Assigning the expression of single specific genes as being restricted only to TANs of either an N1 subtype or an N2 subtype is an inaccurate assumption. The N2 versus N1 phenotypes rather appear to result from more complex changes in gene expression patterns that tilt the overall phenotypic balance towards a more protumour or antitumour profile, respectively. For example, inducible nitric oxide synthase and TNF, both of which are pro-inflammatory proteins classically associated with the antimicrobial role of neutrophils, were shown to be overexpressed in N1 TANs. However, these same proteins were also shown to be crucial to the ability of N2 TANs to induce $CD8^+$ T cell apoptosis. In the same manner, the expression of inflammatory mediators, such as IL-6 (REF.[107]), prostaglandin E_2 (REF.[108]), matrix metalloproteinases[109] and adhesion molecules[110], has been reported in both protumour and antitumour contexts, and the role of these proteins in human neutrophils has yet to be established in the context of cancer.

The N1/N2 phenotype does not relate directly to polarization towards a T helper cell 1 or 2 phenotype, or to a group 1 or 2 innate lymphoid cell-like phenotype, which drives the polarization of M1/M2 macrophages via secretion of IFNγ or IL-4, respectively[111]. The M1/M2 nomenclature, which was originally defined in the context of infection, has been used to describe the phenotypic modulation of macrophages in the TME, although the polarization of these cells in the context of cancer seems to be much more complex[112].

Whether TANs can be manipulated in patients with cancer to acquire either a protumour or antitumour phenotype, as described in mouse models, also remains largely unknown. Most studies exploring the possibility of such effects in patients have done so only in circulating neutrophils. For example, He et al.[64] reported that neutrophils isolated from patients with HCC have a substantial increase in PD-L1 expression following exposure to GM-CSF and TNF. The interaction of PD-L1 with programmed cell death 1 (PD-1) is part of a critical T cell negative regulatory mechanism; therefore, these results support the theory that the TME, by promoting the upregulation of specific chemokines and cytokines, can modulate neutrophil phenotypes. Nevertheless, whether neutrophils infiltrating the tumour are able to acquire a definite phenotype in the circulation, or whether neutrophil phenotypes continue to evolve in response to signals from the TME itself after their infiltration, remains unknown.

We believe that neutrophils in the TME tend to have either a predominantly protumour or predominantly antitumour phenotype. However, these phenotypes or activation states probably coexist within the tumour, and the findings reported thus far are more likely based on the analysis of cell populations rather than on changes occurring at the single-cell level.

Correlations with other immune cell types

The findings of numerous studies demonstrate that neutrophils are able to influence tumour development by modulating the recruitment, profile and phenotype of other immune cells that infiltrate the TME. Most of our knowledge of this aspect is, again, based on data

from animal models of cancer and has been extensively reviewed elsewhere[14,113,114]. Here, we focus on the available data on the correlation between TAN infiltration and other immune cells in the TME in patients with cancer, and the associated prognostic implications. Tumour-associated macrophages (TAMs) and tumour-infiltrating lymphocytes are major components of the immune TME, and have attracted considerable research interest; both TAMs and tumour-infiltrating lymphocytes have also been demonstrated to undergo phenotypic modulation or selective exclusion upon entering the TME, thus creating a tumour-promoting microenvironment.

Similar to neutrophils, tumour infiltration with $CD3^+$ lymphocytes or $CD68^+$ macrophages has been associated, in different reports, with both an inferior and a more favourable prognosis. The reasons for the discrepancies between studies appear to be dependent on the cancer type as well as on the activation status of and/or specific immune cell types present in the tumour. High densities of TAMs have been correlated with an inferior prognosis in patients with certain cancer types[51,115,116], whereas with a better prognosis in others[117,118]. The infiltration of $CD8^+$ T lymphocytes into solid tumours has generally been associated with a better prognosis in various types of cancer[119–121], whereas the prognostic relevance of intratumoural T_{reg} ($CD4^+FoxP3^+$) cells has been more controversial[119,122,123]. The prognostic implications of tumour-infiltrating B cells[124] and NK cells[125] have also been examined in patients with solid tumours.

Nevertheless, limited data are available on the possible associations between TANs and the intratumoural density of other leukocytes in patients with cancer. TANs isolated from patients with HCC were shown to release large amounts of CCL2 and CCL17, which promoted the in vitro activation and migration of macrophages and T_{reg} cells from patients with HCC[71,124]. Research by Wu et al.[80] showed that intratumoural γδT17 cells from patients with CRC promote the migration and survival of intratumoural PMNs via a mechanism involving IL-17, IL-8, TNF and GM-CSF.

Research published in 2017 suggests the existence of crosstalk between tumour cells and cancer-associated fibroblasts (CAFs) that serves to limit the recruitment of PMN-MDSCs to tumours[126]. By producing CSF1, tumour cells are able to impair the production of neutrophil-specific chemokines by CAFs, which thus limits the migration of these cells to tumours. CAFs isolated from resected lung tumour specimens from patients with NSCLC were shown to express high levels of the CSF1 receptor, and the proportion of intratumoural $CD11b^+CD14^-CD15^+$ PMN-MDSCs among $CD45^+$ cells was found to inversely correlate with the amount of CSF1 secreted by the tumour tissue.

Therapeutic implications

The importance of neutrophils in mediating the effects of cancer therapies and the changes that these therapies induce in neutrophils within the TME is an emerging area of research (FIG. 3). Several chemotherapies are known to 'deplete' circulating neutrophil levels (and other immune cells), thus causing neutropenia and eliminating neutrophils from the TME. This effect is known to expose the patient to regular opportunistic infections[127,128], although the implications for phenotypic modulation and the subsequent consequences for the efficacy of the treatment itself are largely unknown. Furthermore, in patients receiving the new anticancer modality immune-checkpoint inhibition, neutrophils should be, as far as we understand, a key mediator of the efficacy, clinical value and toxicities of these therapies[129].

Only a few studies thus far have specifically aimed to understand the effects of drugs on cancer-related neutrophil phenotypes. The therapeutic potential of neutrophils is exemplified by the reported effects that the drugs mentioned above have on them as part of the mechanism of action of these treatments. Several drugs that could potentially recruit, activate, inhibit or otherwise modulate the phenotypes of neutrophils in the TME are currently being investigated in patients with cancer (TABLE 1).

One of the few clinical trials designed to understand the effects of drug therapy on neutrophil phenotypes involves the use of the dietary supplement β-glucan in patients with NSCLC (NCT00682032). The investigators in this study are looking more specifically into the ability of β-glucan to prime circulating neutrophils towards stronger antitumour activity following treatment. To the best of our knowledge, however, no clinical trials explicitly designed to test agents that modulate the recruitment or phenotype of intratumoural neutrophils are currently ongoing in patients with solid tumours.

Chemotherapy

Using a combination of data from patients with cancer and from animal models, Zhou et al.[71] proposed that the tyrosine-kinase inhibitor sorafenib promotes infiltration of the tumour by neutrophils in patients with HCC. Histological sections obtained from patients with HCC who received sorafenib before surgery were found to contain greater $CD66b^+$ TAN densities than those from patients who did not. Sorafenib, as an anti-angiogenic agent, is expected to induce hypoxia, and the authors demonstrated in vitro that this agent induces the expression of CXCL5 in HCC cells via the hypoxia-inducible factor 1α–nuclear factor-κB pathway, resulting in an increase in TAN recruitment. In addition, the authors showed in mice that the combination of sorafenib and TAN depletion (by injection of anti-Ly6G antibodies) had an additive effect and inhibited tumour growth and neovascularization to a greater extent than sorafenib alone.

As mentioned above, neutropenia is a common occurrence in patients receiving chemotherapy, representing the main dose-limiting constraint[130]. Recombinant G-CSF or GM-CSF are, therefore, commonly prescribed in combination with chemotherapy to increase neutrophil counts and reduce the risk of infection[131,132]. Besides promoting the release of neutrophils from the bone marrow, the effects of G-CSF or GM-CSF on human neutrophil phenotypes are unclear, and controversy surrounds the question as to whether these recruited neutrophils have a protumour or antitumour effect. In vitro assessments of peripheral blood neutrophils isolated from patients who

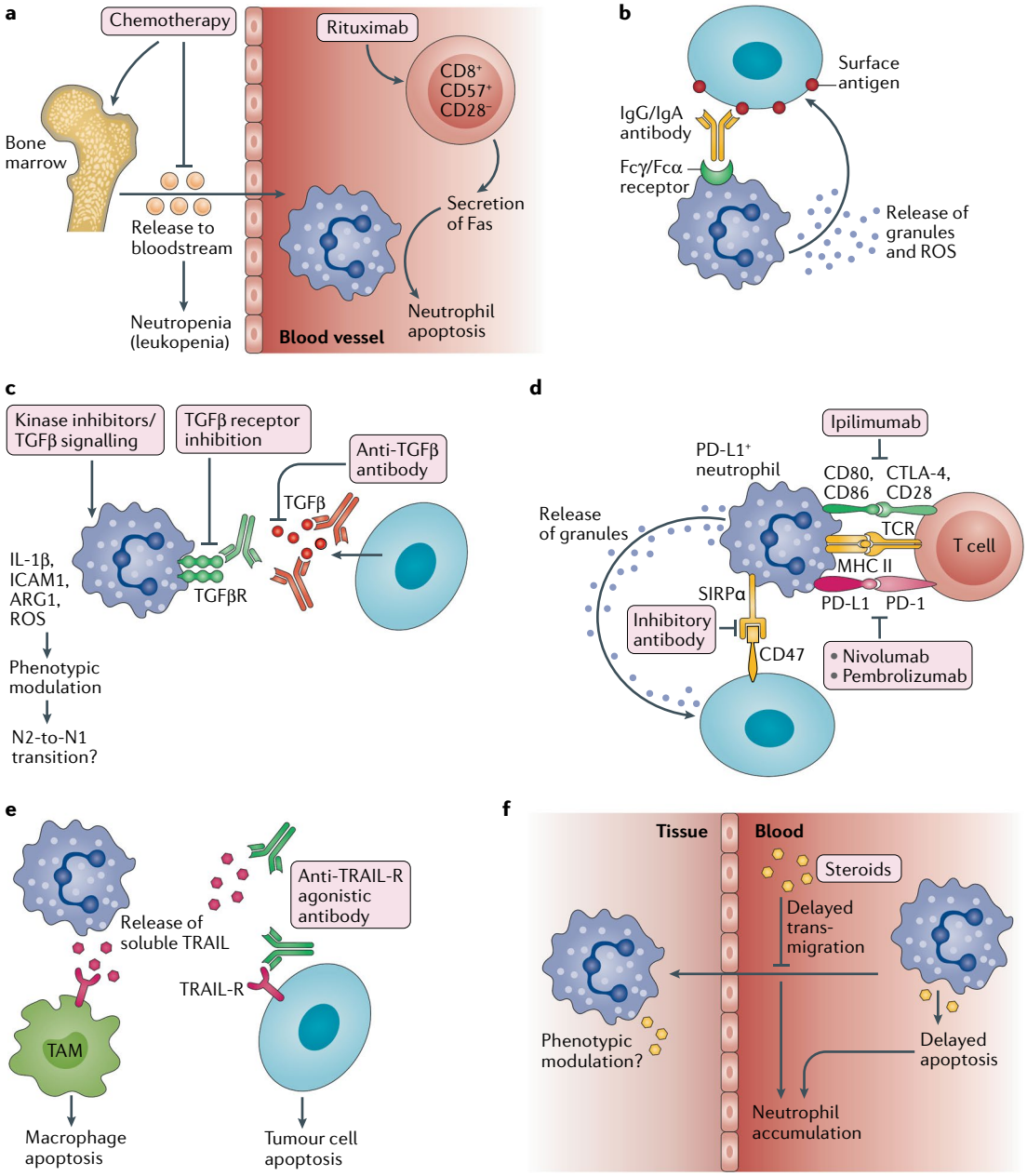


Fig. 3 | **Mechanisms by which routinely used treatments of cancer might modulate the phenotype and/or functions of cancer-related neutrophils. a** | The cytotoxic effects of chemotherapy on white blood cell precursors include a decreased release of leukocytes into the blood, resulting in neutropenia[130]. Neutrophil apoptosis could also be mediated via the release of Fas from T cells following chemotherapy (rituximab)[265–267]. **b** | Antibody-dependent cellular cytotoxicity is triggered by the binding of monoclonal antibodies with an intact Fc domain to tumour cells. Following the binding of the antibody through Fc receptors (Fcγ/Fcα receptors), activated neutrophils (similar to other phagocytes) release their granule contents and reactive oxygen species (ROS), resulting in tumour cell death[180]. **c** | Inhibition of transforming growth factor-β (TGFβ) signalling, obtained via neutralization of the cytokine itself, inhibiting its receptor (TGFβR), or inhibition of the intracellular kinases involved in downstream signalling induces the phenotypic modulation of neutrophils towards an antitumour phenotype in mice[18]. **d** | Immune-checkpoint inhibitors target key regulators of the immune system, which are mainly located on the tumour and/or immune cell membranes. Activation of the immune checkpoints promotes tumour immune evasion by suppressing cell-mediated cytotoxicity. These checkpoints include, among others, cytotoxic T lymphocyte-associated protein 4 (CTLA-4), programmed cell death 1 (PD-1) and CD47. **e** | Tumour necrosis factor-related apoptosis-inducing ligand (TRAIL), a TNF homologue, can induce apoptosis in tumour cells by binding to TRAIL receptors (TRAIL-Rs) expressed on the tumour cell surface. Neutrophils can release soluble TRAIL upon exposure to interferon-α, which can then induce tumour cell death, but is also cytotoxic to macrophages[176]. **f** | Steroids induce the accumulation of neutrophils from the circulation by blocking their recruitment to tissues and by delaying apoptosis[213]. Whether specific steroids are able to specifically modulate neutrophil phenotypes remains to be clarified[214,217]. ARG1, arginase 1; ICAM1, intercellular adhesion molecule 1; PD-L1, programmed cell death 1 ligand 1; SIRPα, signal regulatory protein-α; TAM, tumour-associated macrophage; TCR, T cell receptor.

Table 1 | **Ongoing clinical trials of agents with putative effects on neutrophils in patients with cancer**

Class of agent	Putative effects on neutrophils	Agents	Ongoing trials
G-CSF mimetics	Induce the release of immature neutrophils/PMN-MDSCs with tumour-promoting and metastasis-promoting properties[268,269]	Pegfilgrastim	NCT00035594
		YPEG-rhG-CSF (a long-acting form of pegfilgrastim)	NCT02005458
TGFβ pathway inhibitors	Promote the development of neutrophils with an antitumour phenotype[197,270]	Galunisertib (a TGFβR1 kinase inhibitor)	NCT02734160, NCT01582269, NCT01682187 and NCT02452008
		Fresolimumab (an anti-TGFβ monoclonal antibody)	NCT02581787
Angiogenesis inhibitors	Ameliorate the effects of chemotherapy-driven neutropenia; extends the lifespan of neutrophils through induction of cytokines (IL-1β, IL-6 and IL-12), which promote neutrophil survival[271–273]	Plinabulin (an inhibitor of tubulin polymerization, as required for the formation of new blood vessels)	NCT00630110, NCT02504489 and NCT03102606
Neutrophil elastase inhibitors	Block elastase activity in neutrophils, which is upregulated in numerous cancer types and correlates with poor prognosis; inhibit the tumour-promoting and metastasis-promoting effects of neutrophils[235,274]	Sivelestat	NCT01170845
CXCR2 inhibitors	Inhibit neutrophil recruitment to the tumour[78,198]; attenuate granulocytosis, neutrophil recruitment and vascular permeability by inhibiting the CXCR2 chemotactic axis	Reparixin	NCT02370238 and NCT02001974
PI3K inhibitors	Ameliorate neutrophil/PMN-MDSC-mediated inhibition of T cell function[275]	Buparlisib (a PI3Kδγ inhibitor)	NCT02194049 and NCT01629615
Anti-CD40 monoclonal antibody	Neutrophil depletion (possibly neutropenia); activates ADCC[276]	CP-870,893	NCT01103635 and NCT00607048
PDE5 inhibitors	Reduce ARG1, NOS2 and IL-4Rα expression in tumour-associated neutrophils of an immunosuppressive phenotype, thus impairing tumour-promoting effects[277,278]	Sildenafil	NCT02544880 and NCT00752115
		Tadalafil	NCT01697800
NSAIDs	Inhibit COX2 (the activation of which correlates with increased tumour cell proliferation) and impair prostaglandin-mediated immunosuppression, thus causing neutrophil inhibition[279]	Aspirin and ibuprofen (COX1 and COX2 inhibitors)	NCT01786200
		Celecoxib (a COX2 inhibitor)	NCT02429427
CCR5 antagonists	Inhibit both the release of immature neutrophils from bone marrow and their recruitment to the tumour[280,281]	Maraviroc	NCT03274804 and NCT01736813
β-Glucans	Promote neutrophil-mediated cytotoxicity via complement receptor 3-dependent priming[282]	ImuCell WGP	NCT00682032
TRAIL-R agonist	Triggers neutrophil apoptosis and clearance from tissues by targeting TRAIL-Rs expressed on neutrophils[178,179,283]	Mapatumumab	NCT01088347
		AMG 951	NCT00508625
		TRM-1	NCT00092924
CD47–SIRPα inhibitors	Delay the transmigration of neutrophils to tumour tissues, thus inducing macrophage-mediated phagocytosis of tumour cells[284,285]	Hu5F9-G4	NCT02216409
		IBI188	NCT03717103
		CC-90002	NCT02367196

ADCC, antibody-dependent cellular cytotoxicity; ARG1, arginase 1; CCR5, CC-chemokine receptor type 5; COX, cyclo-oxygenase; CXCR2, CXC-chemokine receptor 2; G-CSF, granulocyte colony-stimulating factor; NOS2, nitric oxide synthase 2; PDE5, phosphodiesterase type 5; PMN-MDSC, polymorphonuclear myeloid-derived suppressor cell; SIRPα, signal regulatory protein-α; TGFβ, transforming growth factor-β; TRAIL-R, tumour necrosis factor-related apoptosis-inducing ligand receptor.

received G-CSF have generated many conflicting reports regarding the effects of these chemokines on neutrophil function, including phagocytosis, oxidative burst, bacterial killing and chemotaxis[133]. One study comparing the effects of filgrastim (non-glycosylated G-CSF) versus those of lenograstim (glycosylated G-CSF) revealed different effects of these two compounds on the chemotaxis and morphology of circulating neutrophils isolated from patients with non-Hodgkin lymphoma[134]. Whereas neutrophils from patients who received lenograstim had impaired chemotaxis, those from patients who received filgrastim had a morphology suggestive of higher levels of activation with increased expression of integrin β2. G-CSF and GM-CSF have been reported to have both protumour and antitumour effects and can affect both tumour and immune cells[135–139]. G-CSF has been reported to induce the phagocytic and antibacterial activity of neutrophils[140] together with enhanced ROS production following stimulation[141]; however, the phenotypic modulation of TANs following treatment with either glycoprotein is still under investigation.

Radiotherapy

Radiotherapy remains one of the most important treatment modalities in cancer therapy. Data from many studies involving animal models have shown that radiotherapy activates both the adaptive and the innate immune responses through the release of antigens, Toll-like receptor ligands and pro-inflammatory cytokines from tumour cells, thereby promoting the recruitment of myeloid cells, such as macrophages, dendritic cells and neutrophils, and inducing T cell-mediated immunogenic cell death[142–144]. In preclinical models, radiotherapy induces sterile inflammation with rapid and transient infiltration of neutrophils into the tumours[145]. These newly recruited neutrophils produce increased amounts of ROS and induce apoptosis in tumour cells. Recent studies have identified a correlation between baseline blood neutrophil count and survival following radiotherapy across different cancer types[146–149]. Yet, very little information is available on the effects of radiation on the phenotypes of immune cells in general, and of neutrophils in particular, in patients with cancer. Data from clinical studies demonstrate that radiotherapy can initiate a response outside the local radiation field known as the abscopal effect, which might be linked to enhanced recruitment of immune cells[150–153]. On the basis of these observations, the combination of radiotherapy with immunotherapy or GM-CSF might improve the outcomes of patients[154–156]. Nevertheless, the effects of radiotherapy on human neutrophils remain elusive[146] and are yet to be determined.

Immune-checkpoint inhibitors

Antibodies that inhibit the cytotoxic T lymphocyte-associated protein 4 (CTLA-4) and PD-1 immune checkpoints have been successful in many patients with advanced-stage cancers, especially in patients with melanoma whose lesions contain high counts of $CD8^+$ T cells. Although not all patients benefit from these agents, immune-checkpoint inhibitors are being used more frequently as first-line therapies in patients with other forms of cancer[157,158], based on the expression of PD-1, PD-L1 and/or CTLA-4 in biopsy samples. Several studies have described the effects of immune-checkpoint inhibitors on the TME in mouse models[159] and in patients with cancer[160–162]. Nevertheless, our knowledge of the specific effects of immune-checkpoints inhibitors on intratumoural neutrophils in patients with cancer remains limited.

In patients with melanoma, a substantial reduction in granulocytic cells (defined as $Lin^-HLA\text{-}DR^{-/low}CD15^+CD33^+CD11b^+$ cells) was noted following treatment with the anti-CTLA-4 antibody ipilimumab[163]. This decrease was accompanied by downregulated ARG1 expression.

In a study published in 2017, changes in the intratumoural immune cell subpopulations were investigated in patients with melanoma following treatment with the anti-PD-1 antibody nivolumab[160]. The investigators found no difference in the number of intratumoural neutrophils between patients who benefited from nivolumab and those who did not, although substantial levels of variability in intratumoural neutrophil counts were observed in both groups. Finally, data from several studies have suggested a correlation between PD-L1 expression on neutrophils and an immunosuppressive phenotype. For example, $PD\text{-}L1^+$ neutrophils were shown to suppress T cell function and to promote disease progression in patients with gastric cancer[164], and this suppressive effect might be reversed by inhibition of PD-L1. Similarly, intratumoural and peritumoural neutrophils have higher levels of PD-L1 expression than circulating neutrophils in patients with HCC[64], suggesting that TANs have strong immunosuppressive effects in these patients and highlighting the possible role of $PD\text{-}L1^+$ neutrophils as targets of anti-PD-1 and/or anti-PD-L1 antibodies.

Agents in clinical development

CD47–SIRPα. CD47 is a glycoprotein broadly expressed on the membranes of virtually all cell types. By contrast, signal regulatory protein-α (SIRPα) is expressed predominantly on myeloid cells, including monocytes, macrophages, granulocytes and $CD4^+$ dendritic cells, and to some extent on neuronal cells. Different types of cancer cells have been reported to overexpress CD47, and multiple studies have described a negative association between extent of CD47 expression on cancer cells and response to therapy, including to both chemotherapies and targeted therapies, in patients with solid tumours and in those with haematological cancers[165–167]. The CD47–SIRPα signal has since been referred to as a 'don't eat me' signal that inhibits the phagocytosis of cancer cells and thus promotes tumour cell survival[167,168]. Several different agents, including those targeting either CD47 or SIRPα, have been used either as single agents or in combination with other therapies to investigate the effects of impairing this interaction on cancer progression (as reviewed elsewhere[169]).

Most studies have focused on the effects of CD47–SIRPα inhibition on the ability of macrophages to phagocytose cancer cells; however, it should be emphasized that neutrophils also express high levels of SIRPα, and are therefore also likely to be affected by agents targeting the CD47–SIRPα interaction. The use of an intact anti-CD47 antibody has also been proposed as a method of stimulating antibody-dependent cellular cytotoxicity (ADCC) by IgG Fc receptor (FcγR)-expressing cells. Neutrophils are known to express FcγRs and could therefore be expected to have a strong ADCC response towards tumour cells in patients receiving this type of therapy. In accordance with this hypothesis, Ring et al.[170] demonstrated that circulating neutrophils mediate cancer cell phagocytosis, which, together with antibody-driven opsonization, promotes tumour cell death following treatment with an anti-human SIRPα antibody in mouse models expressing human SIRPα.

CD47–SIRPα signalling has also been suggested to have a role in neutrophil transmigration. Research by Liu et al.[171] demonstrated that stimulation of circulating neutrophils with *N*-formylmethionine-leucyl-phenylalanine resulted in increased cell-surface expression of CD47, and both anti-SIRPα and anti-CD47 monoclonal antibodies delayed the transmigration of neutrophils through epithelial layers. Tumour infiltration by neutrophils has been broadly regarded as being

correlated with a poor prognosis, although the clinical implications of impairing neutrophil recruitment in specific forms of cancers require further clarification, as previously discussed.

ACKR2. Atypical chemokine receptor 2 (ACKR2) has also been suggested to be a novel immune checkpoint that regulates neutrophil differentiation, mobilization to tumour tissues and anti-metastatic activity[172]. This effect has currently only been demonstrated in animal models, although it is interesting to note that genetic inactivation of ACKR2 drives an increase in primary tumour growth as well as a decrease in metastatic burden, thus supporting the hypothesis that neutrophils have opposing (protumour versus antitumour) effects in primary versus metastatic lesions.

TRAIL. TRAIL was first identified, based on its high level of homology with TNF and CD95L, as a signal that induces apoptosis in tumour cells while sparing non-tumour cells. In contrast to TNF, it can be injected systemically without causing toxicities. Two TRAIL receptors, TRAIL-R1 (also known as DR4) and TRAIL-R2 (also known as DR5), are capable of inducing apoptosis. These discoveries led to extensive attempts to develop TRAIL-R agonists as cancer therapeutics. The 'first generation' of these agonists failed owing to limited antitumour efficacy and because most patients rapidly developed resistance to apoptosis induction following treatment with these agonists[173]. New strategies designed to improve the efficacy of TRAIL receptor agonists are currently being investigated[174] (TABLE 1). Selective targeting of MDSCs using an agonistic TRAIL-R2 antibody (DS-8273a) was investigated in a phase I trial involving patients with stage III head and neck squamous cell carcinoma[175]. This trial showed that, specifically in patients with elevated numbers of what they described as circulating PMN-MDSCs ($CD11b^+CD14^-CD33^+CD15^+$ cells from the low-density fraction), treatment with DS8273a caused a substantial reduction in their number, with a decrease in the number of TANs (defined as elastase-positive cells) observed in one of six biopsy samples following treatment. Nevertheless, the safety and tolerability of TRAIL receptor agonists require further investigation.

The release of a soluble form of TRAIL by human neutrophils (and monocytes) can be stimulated upon exposure to IFNα[176]. This interferon, among other type I interferons, is administered to patients in combination with other therapies for the treatment of a diverse range of cancers, including breast cancer, advanced-stage melanoma and chronic myeloid leukaemia. Neutrophils might therefore be an important source of soluble TRAIL and have a role in the therapeutic effects of IFNα[177]. TRAIL receptors are also expressed on the surface of circulating neutrophils and TANs, where they might have a role in apoptosis[178] and clearance from sites of inflammation[179].

ADCC

Another approach to stimulating the cytotoxic capacities of immune cells involves the use of therapeutic antibodies characterized by an intact Fc domain triggering an immune response and tumour cell death via ADCC[180–182]. Following binding to the Fc domain of the antibody, activated neutrophils can produce several cytotoxic agents, including proteases, oxidative metabolites and defensins, and induce apoptosis through the release of granzymes and perforins. Similar to macrophages and NK cells, neutrophils also express a wide variety of Fc receptors and could therefore be important effector cells for therapies designed to engage targeted ADCC[183].

Evaluations of markers of neutrophil activation have shown that activation of neutrophils with GM-CSF is associated with improved outcomes following immunotherapy with monoclonal antibodies directed against the tumour-associated disialoganglioside GD2 in patients with neuroblastoma[184,185]. Research involving the human breast carcinoma cell line SK-BR-3 suggests that neutrophils isolated from individuals without cancer are able to facilitate autophagy and thus induce tumour cell death[186,187]. Otten et al.[188] demonstrated that the crosslinking of a monoclonal IgA antibody recognizing a tumour-associated antigen with an IgA Fc receptor (FcαRI) induces the migration and degranulation of neutrophils, leading, in turn, to a reduction in tumour volume in vitro as well as the release of IL-1β and TNF by the neutrophils themselves. Interestingly, the authors observed that the production of CXCL8 (also known as IL-8), which induces the migration of neutrophils in vivo, was sustained by IL-1β and TNF, thus supporting a cytokine-mediated, indirect antitumour effect activated by neutrophils.

FcγRs have been demonstrated to have an important role in the therapeutic success of antitumour monoclonal antibodies. However, the identity of the FcγR-bearing cells that provide cytotoxic activity in patients and the extent to which neutrophils can be manipulated by monoclonal antibodies remains unknown[183].

TGFβ signalling

Another potential treatment, which could both affect and be partially mediated by neutrophils, involves inhibition of TGFβ signalling. Multiple trials of agents designed to impair tumour development using inhibitors of TGFβ signalling were conducted before TGFβ was known to have potent immunomodulatory effects on neutrophils[189]. In patients with cancer, treatments targeting TGFβ (directed towards the cytokine itself, its receptors or associated signalling pathways) have encountered difficulties owing to the involvement of TGFβ in multiple signalling pathways, resulting in many off-target effects. In particular, given the well-documented dual role of TGFβ in cancer, the risk of tumour-promoting off-target or indirect effects is a major concern. Currently, new strategies including molecules either targeting TGFβ directly or its receptors are being clinically tested (NCT02160106, NCT03620201 and NCT01058785)[190]. TGFβ can act as a tumour suppressor in non-malignant cells and in early-stage cancers, while it promotes invasiveness, dissemination, metastatic colonization and maintenance of cancer stem-like cells in patients with advanced-stage cancers (as reviewed elsewhere[191]). Activation of different TGFβ receptors might also have opposing effects on the development of primary versus metastatic lesions, as demonstrated in animal

models[192]. Upregulation of TGFβ receptor 2 on cancer cells following chemotherapy has been described as a common cause of acquired drug resistance in patients with cancer[193]. This effect was demonstrated following exposure to agents such as the EGFR inhibitors erlotinib and gefitinib, sorafenib, the BRAF inhibitor vemurafenib, and the MET and ALK inhibitor crizotinib in cell line models and in tumour material from patients. This mechanism of TGFβ receptor-driven resistance has been observed in various different tumour types[193–196]. In addition to direct effects on tumour cells, treatments targeting TGFβ could have a major effect on the TME and on neutrophils in particular. Inhibition of TGFβ signalling in tumour-bearing animal models has been demonstrated to modify the polarization of neutrophils and switch their phenotypes from protumour to antitumour[18]. Nonetheless, the effects of such treatments on the phenotypes of neutrophils (circulating or intratumoural) in patients with cancer remain unknown.

TGFβ antagonists were proposed as a method of improving the efficacy of immune-checkpoint inhibitors[197]. For example, galunisertib is a novel TGFβ receptor 1 kinase inhibitor that is currently being investigated in a phase I trial in combination with the anti-PD-L1 antibody durvalumab in patients with recurrent and/or refractory metastatic pancreatic cancer (NCT02734160). Galunisertib is also being investigated as a monotherapy or in combination with lomustine chemotherapy in two clinical trials involving patients with recurrent glioblastoma (NCT01582269 and NCT01682187) and in combination with temozolomide-based chemoradiotherapy (NCT01220271).

TGFβ has been described as a strong neutrophil chemoattractant; therefore, inhibiting the effects of this chemokine could potentially result in impaired neutrophil recruitment to the tumour. Further studies are needed to understand how manipulating TGFβ in patients with cancer might affect the various different neutrophil subtypes.

Chemokine signalling

Multiple agents, including those targeting CXCR1/CXCR2 (SX-682), CXCR4 (ulocuplumab), CCR2 (MLN1202) and CCR5 (maraviroc), have been developed in an attempt to inhibit the effects of a diverse range of chemokines, cytokines and/or their receptors, and several of these agents are currently in clinical trials. Agents targeting chemokine signalling could, theoretically, interfere with neutrophil migration to the tumour and/or modulate their phenotype. A notable example of this effect is provided by attempts to target CXCR2 signalling. CXCR2 was shown to be upregulated in patients with pancreatic cancer, predominantly in neutrophils or MDSCs, with a strong correlation between the expression of MPO and CXCR2 in the tumour-adjacent stroma[78]. Furthermore, CXCR2 signalling and myeloid cell recruitment at the tumour border were linked with poor outcomes in patients. Importantly, very few tumour cells expressed CXCR2 (REF.[78]), suggesting that the effects of agents targeting CXCR2 are specifically mediated by immune cells. In mouse models of pancreatic ductal adenocarcinoma, genetic ablation or inhibition of *Cxcr2* abrogated metastases, improved the extent of T cell infiltration, and the combination of inhibition of CXCR2 and PD-1 substantially extended the survival of mice with established disease[78].

Paediatric patients with sarcomas have high serum levels of CXCL1 and CXCL8, two major ligands of CXCR2. Disruption of *Cxcr2* in a mouse model of rhabdomyosarcoma was also shown to improve the efficacy of anti-PD-1 antibodies by inhibiting the trafficking and homing of $CD11b^{+}Ly6G^{high}$ cells into the tumour[198].

Reparixin, a non-competitive allosteric CXCR1/CXCR2 inhibitor, has been shown to attenuate granulocytosis, neutrophil recruitment and vascular permeability following lung injury in mice or reperfusion injury in patients undergoing coronary artery bypass graft surgery[199–201]. CXCR2 is the main chemokine receptor expressed on neutrophils and regulates the recruitment of neutrophils to tissues; therefore, inhibition of this receptor might interfere with neutrophil recruitment to the tumour. Reparixin is currently being tested in combination with paclitaxel chemotherapy in a phase II study as a first-line therapy in patients with metastatic triple-negative breast cancer (NCT02370238).

Implications for treatment efficacy

The prognostic value of intratumoural neutrophils in indicating a response to therapy has also been examined over the past few years. In a study involving patients with cervical cancer[202], investigators indirectly linked pretreatment levels of intratumoural neutrophils with prognosis following radiotherapy. The authors reported the presence of TANs in 44% of pretreatment biopsy samples and found the level of TAN infiltration to be correlated with a high interstitial fluid pressure (IFP) inside the tumour, with TAN infiltration identified in 61% of high IFP tumours. IFP has been described elsewhere as causing inefficient uptake of therapeutic agents by the tumour[203] and has been correlated with a high risk of recurrence and a poor prognosis in patients with cervical cancer who receive radiotherapy[204–206].

High intratumoural (but not peritumoural) TAN infiltration is associated with improved DFS following fluorouracil chemotherapy in patients with stage III CRC[57]. By contrast, Rakaee et al.[8] reported no significant interaction between receiving adjuvant chemotherapy, TAN numbers and OS, DFS and disease-specific survival in patients with NSCLC receiving adjuvant chemotherapy, neither in the entire cohort, nor in the adenocarcinoma and SCC subgroups separately. In the same study, the authors mentioned a tendency towards greater differences in survival outcomes between patients with a high versus those with a low CD66b density who received adjuvant radiotherapy, although they did not explicitly explain this claim.

When assessing biomarkers that predict a response to immunotherapy, the vast majority of studies have focused on tumour markers and characteristics, or absolute lymphocyte count. Nevertheless, the predictive value of neutrophils in determining response to immune-checkpoint inhibition has been evaluated more recently. In a study in which patients with metastatic melanoma received ipilimumab, elevated absolute neutrophil

counts were associated with a substantial reduction in both OS and PFS[207]. Another study involving patients with metastatic melanoma demonstrated that a pretreatment NLR of >4 is associated with significantly reduced OS in patients receiving ipilimumab[208].

TANs as a therapeutic target?

The traditionally held belief that neutrophils are merely a bystander in the TME has been completely revolutionized over the past decade[14,209,210]. Research has now established that neutrophils have a major role in cancer, with an important contribution to initiation, development and disease progression. Furthermore, emerging data suggest that the presence and phenotype of neutrophils in the TME is an important determinant of therapeutic success, in response to both traditional and newer therapies such as immune-checkpoint inhibitors[64,164]. The majority of published data indicate that neutrophils have tumour-promoting effects, although many reports show that, with the proper environmental cues, neutrophils can have antitumour and/or antimetastatic effects[17,211]. The appealing concept of targeting TANs, either by suppression or phenotypic manipulation, has been raised by several researchers. Addressing this clinically will be an important outcome of research into the role of TANs in cancer and might enable the development of the next generation of immunotherapies[212].

Besides the different approaches described here, treatments designed to specifically target neutrophils can be administered to patients with cancer, usually as additional supportive measures, such as G-CSF. These treatments have been hypothesized to modulate the contribution of neutrophils to cancer progression or regression and/or response to therapy.

Steroids during chemotherapy

Glucocorticoids (corticosteroids) are included in treatment regimens for patients with various cancers, such as leukaemia, lymphoma and multiple myeloma, as well as in the treatment of chemotherapy-induced nausea and vomiting and to stimulate appetite. Glucocorticoids have inhibitory effects on a broad range of immune responses, although impairment of migration to sites of inflammation or infection, rather than effects on function, is their main neutrophil-specific effect[213]. Endogenous glucocorticoids promote neutrophil maturation in the bone marrow and their release into the circulation. In addition, neutrophil migration through the vasculature to sites of inflammation is impaired in patients receiving corticosteroids. These effects, combined with inhibition of neutrophil-mediated apoptosis[214,215], result in increased numbers of circulating neutrophils[216]. By contrast, neutrophil functions, such as phagocytosis or bactericidal effects, do not seem to be impaired at low-to-moderate doses of glucocorticoids[217,218]. Whether corticosteroids have a beneficial or detrimental overall effect on tumour progression and what role modulation of cancer-related neutrophils might have remain unknown. Interestingly, in a study published in March 2019, Obradovic et al.[219] showed that activation of the glucocorticoid receptor by glucocorticoids promotes tumour heterogeneity and metastasis in a patient-derived xenograft model of breast cancer in immunocompromised (NOD-*scid-Il2rg*^{null}) mice. A few studies have specifically evaluated the response to glucocorticoids using changes in NLR and have revealed a possible correlation with response to treatment and survival in patients with castration-resistant prostate cancer receiving chemotherapy and low-dose corticosteroids. These studies demonstrated that the use of corticosteroids at baseline or during treatment did not affect the association between NLR and prognosis[220,221].

Granulocyte transfusion

Granulocyte transfusion is used in patients with severe, prolonged forms of neutropenia following chemotherapy owing to concerns of vulnerability to life-threatening infections. Compatible granulocyte donors are typically pretreated with G-CSF (or corticosteroids) to increase granulocyte numbers before isolation and transfusion[222]. Nevertheless, the clinical utility of this technique remains controversial and this is not widely used, in part owing to the inability to retain neutrophils in a functional state for long periods of time, to the large number of neutrophils required (>30×10^9 cells) to achieve benefit[223] and to the risk of serious adverse effects[224]. Naturally, most clinical studies have focused their efforts on determining host response in terms of changes in absolute neutrophil count (which is typically maintained for 1–1.5 days) and improvements in clinical symptoms versus the associated risks[224–226]. When considering the possible outcomes of granulocyte transfusion in terms of tumour progression and/or regression, multiple aspects require consideration. The phenotypic effects of G-CSF or steroids on the donor's granulocytes in terms of protumour versus antitumour function are still not clear. First, the administration of G-CSF to individuals without cancer has been shown to promote the release of both HDNs and LDNs, which have opposite effects on T lymphocyte proliferation, including immature LDNs[227]. Therefore, the effects of transfusion of the entire granulocyte fraction, which contains both HDNs and LDNs, remain unclear. Second, multiple transfusions would seem mandatory in order for a patient to benefit from the long-term effects of neutrophils on tumour development and/or immune composition. This approach, in addition to requiring a high level of donor availability and dedication, would require close follow-up monitoring for known adverse events (such as chills, fever, pulmonary adverse events, transfusion-associated graft-versus-host disease and others). The effects of specific transfusion with HDNs versus LDNs and the feasibility of repetitive transfusions in patients with cancer have yet to be investigated.

Early studies involving mouse models demonstrated the feasibility of neutrophil depletion as a method of inhibiting tumour growth and metastasis in specific circumstances[102,228,229]. However, deliberately inducing continuous neutropenia in patients with cancer is unrealistic given the obvious risks of severe and even fatal infections. Considering the contradictory potential of TANs to have either a protumour or antitumour phenotype, a major question remains regarding whether neutrophils can be polarized towards a cytotoxic antitumour phenotype. Importantly, data from several studies have shown that direct activation of neutrophils, for

example, by high-dose G-CSF, might induce antitumour functions in TANs[145,230]. About a decade ago, we established, for the first time, that TANs are able to polarize in mouse models, influencing the TME and affecting tumour growth[18]. These neutrophils of the antitumour or 'N1' phenotype emerged following blockade of TGFβ signalling, had a hypersegmented appearance with a pro-inflammatory phenotype and cytotoxic effects on tumour cells. Although some researchers argue that this is merely a heightened state of activation[212], the authors demonstrated that neutrophils within tumours can be modified in vivo, thus altering their behaviour and responses to tumour and immune cells and enabling them to react to specific cues from the microenvironment. Data from another study showed that IFNβ can drive TANs to an antitumour, less pro-angiogenic phenotype[231]. Importantly, TANs generally have a higher level of activation than circulating neutrophils, but can have a protumour or antitumour phenotype[232], thus complicating our understanding of their manipulation.

These data from mouse models[18,19,231,232] could provide the theoretical basis for attempts to manipulate TANs or circulating neutrophils towards an antitumour and/or more active immune phenotype, although whether cancer-related neutrophils can be manipulated in this way in patients remains unclear. Furthermore, no robust data exist that answer the questions regarding the potential risks associated with neutrophil manipulation in patients with cancer, such as inducing acute lung injury, as can be seen following transfusion of neutrophils[224].

As mentioned above, most studies suggest that neutrophils have a harmful role in cancer. However, generalized approaches targeting all TANs or cancer-related circulating neutrophils in patients might lead to serious adverse events, most likely relating to severe immunosuppression, which would complicate their use. Such approaches could include depletion of all neutrophils, general manipulation of neutrophils as described above (such as by TGFβ blockade or using IFNβ), and even the more subtle possibility of extracting neutrophils, manipulating them and then returning them to the patient. This last approach is even more complex when considering the short-lived nature of neutrophils.

Future directions

In light of these various challenges, we believe that more delicate, restrained and probably more complex approaches are needed to successfully and safely target neutrophils therapeutically in patients with cancer. Some possible approaches could include direct targeting of neutrophils using novel approaches that are currently unknown, targeting specific neutrophil subpopulations (such as immature cells or LDNs), targeting the recruitment of neutrophils into the tumour or even specific, as-yet-undefined signalling axes that regulate the recruitment of tumour-promoting neutrophil subpopulations (for example, the blockade of CXCR2 signalling, as mentioned above[78,198]), or targeting specific substances and/or enzymes secreted by neutrophils (either exclusively or as part of the tumour stroma) to ameliorate their detrimental effects (for example, the direct inhibition of neutrophil elastases[233–236]).

Conclusions

In our opinion, the specific targeting of neutrophils will become a viable therapeutic approach in the treatment of patients with cancer. Various methods designed to target TANs and/or circulating neutrophils, perhaps in addition to myeloid regulatory cells, may very well become part of the next generation of immunotherapy. However, further investigation of the exact roles, recruitment pathways, subpopulations and mechanisms of action of TANs is still needed to eventually develop better and more specific therapeutic approaches, with a maximal level of therapeutic potential and a minimal level of harm.

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