Neutrophil diversity and plasticity in tumour progression and therapy

Sebastien Jaillon[1,2], Andrea Ponzetta [1,2], Diletta Di Mitri[2], Angela Santoni[3,4], Raffaella Bonecchi[1,2], Alberto Mantovani[1,2,5]

[1]Department of Biomedical Sciences, Humanitas University, 20090 Pieve Emanuele, Italy.
[2]Humanitas Clinical and Research Center, 20089 Rozzano, Italy.
[3]Dipartimento di Medicina Molecolare Istituto Pasteur-Fondazione Cenci Bolognetti, Università di Roma "La Sapienza", 00161 Rome, Italy.
[4] IRCCS Neuromed, 86077 Pozzilli (IS), Italy.
[5]The William Harvey Research Institute, Queen Mary University of London, London EC1M 6BQ, UK.

Corresponding authors:

Sebastien Jaillon (sebastien.jaillon@humanitasresearch.it) and Alberto Mantovani (alberto.mantovani@humanitasresearch.it)

Abstract

Neutrophils play a key role in defence against infection and in the activation and regulation of innate and adaptive immunity. In cancer, tumour-associated neutrophils (TANs) have emerged as an important component of the tumour microenvironment. Here they can exert dual functions. TANs can be part of tumour-promoting inflammation by driving angiogenesis, extracellular matrix remodelling, metastasis and immunosuppression. On the other hand, neutrophils can mediate anti-tumour responses by direct killing of tumour cells and by participating in cellular networks that mediate anti-tumour resistance. Neutrophil diversity and plasticity underlie the dual potential of TANs in the tumour microenvironment. Myeloid checkpoints as well as the tumour and tissue contexture shape neutrophil function in response to conventional therapies and immunotherapy. We surmise that neutrophils can provide tools to tailor current immunotherapy strategies and pave the way to myeloid cell-centred therapeutic strategies complementary to current approaches.

Introduction

Neutrophils have long been known to serve as an essential line of resistance against infectious agents in innate immunity and downstream of polarized T_H17-driven adaptive immune responses [1-3]. Moreover, in addition to representing a hallmark of acute inflammation, neutrophils are an important component of circuits which orchestrate the activation, orientation and regulation of adaptive immune responses and chronic inflammation. By expressing a wide repertoire of cytokines, immunosuppressive and stimulatory molecules, neutrophils engage in complex bidirectional interactions with lymphoid cells and macrophages [4,5].

The tumour microenvironment (TME) has emerged as an essential component of neoplasia [6]. Inflammatory cells and components of the humoral arm of innate immunity are key players of cancer-related inflammation [6-9]. Inflammatory cells and mediators contribute to tumour progression, from initiation to seeding at distant anatomical sites [6-9].

While attention has long been focused on macrophages as a paradigm of cancer-related inflammation [10], several lines of evidence in preclinical and clinical conditions point to a role of neutrophils [11,12]. Neutrophils infiltrate solid tumours to a variable extent, as assessed by conventional immunohistochemistry staining for neutrophil markers (e.g. CD66b in human and Ly6G in mice) and expression of a neutrophil transcriptional signature [11-17]. In most but not all human tumours high infiltration with tumour-associated neutrophils (TANs) has been associated with poor prognosis [12]. Accordingly, in transplanted tumours and models of carcinogenesis TANs have been reported to be a component of tumour-promoting inflammation [3,11]. However, neutrophils can engage in pathways of anti-tumour resistance by killing tumour cells and/or by interacting with other components of immunity [3,11,12]. Thus, neutrophils have the potential to be both pro-tumorigenic and anti-tumorigenic within the TME and this dual function is likely a reflection of their unexpected plasticity in response to environmental cues.

Here we will review current evidence on the role of neutrophils in tumour progression and metastasis in the light of their diversity and plasticity. Emphasis will be on diversity and on prognostic

and therapeutic implications. Previous reviews on the immunobiology of neutrophils and TANs [1-4,11,12,18,19] will provide a framework for the present essay.

Homeostasis and recruitment

Development and mobilization

Neutrophils represent 50 to 70% and 10 to 25% of circulating leukocytes in humans and mice, respectively [20,21]. In peripheral blood, neutrophils are short lived cells and require a constant replenishment from bone marrow precursors dependent on signalling by the granulocyte-colony stimulating factor receptor (G-CSFR) (Box1) [2,22]. Therefore, deficiency in G-CSF or G-CSFR leads to severe neutropenia [23,24]. In addition to the essential role played by G-CSF, mediators such as granulocyte-macrophage colony-stimulating factor (GM-CSF) and the proinflammatory cytokine IL-6 are involved in the regulation of the development of neutrophils, in particular during an inflammatory response [2,22,25,26].

The trafficking of neutrophils from bone marrow into peripheral blood is tightly regulated, in particular through signalling by the chemokine receptors CXCR2 and CXCR4. Expression of CXCL12 by bone marrow stromal cells mediates the retention of $CXCR4^+$ immature neutrophils (N_I; as an operational laboratory nomenclature, we use N_I, N_M, N_A and N_{ISG} to refer to immature, mature, aged and interferon gene signature neutrophils, respectively; see below and see Box2). Decreased expression of CXCR4 in bone marrow mature neutrophils coupled with activation of CXCR2 signalling triggers the entry of N_M into the circulation [27]. Ageing neutrophils upregulate the expression of CXCR4, driving their homing back to the bone marrow and their elimination by macrophages [27,28]. The cellular composition and molecular signature of the hematopoietic niche, including the number of reticular cells expressing CXCL12, is regulated by the rhythmic clearance of neutrophils by macrophages [28].

The process of neutrophil ageing, which can take place in the circulation, is regulated by gut microbiota and is controlled by neutrophils themselves through a cell-autonomous transcriptional

program [28-30]. Indeed, circadian expression of the transcription factor Bmal1 controls the production of CXCL2 in a cell-intrinsic manner. In turn, CXCL2 signals through CXCR2 to induce neutrophil ageing [30]. Elimination of apoptotic neutrophils and formation of fresh cells are interconnected as a homeostatic rheostat essential to prevent exacerbated inflammation and tissue damage [30-32].

Recruitment in cancer

In established neoplasia in mice and humans, altered haematopoiesis is usually observed, a reflection of the production of growth factors (G-CSF and GM-CSF) and inflammatory cytokines (e.g. IL-6, IL-1β, IL-17) by tumour cells, stromal cells and tumour-infiltrating leukocytes, including T cells, macrophages and neutrophils [33-35]. Neutrophilia and appearance in the circulation of immature myeloid cells occur and these cells are potent mediators of immunosuppression as discussed below (Figure 1a) [11,12,36].

Neutrophils express high levels of the chemokine receptors CXCR1 and CXCR2, which play a major role in their recruitment in the TME where CXC chemokines (e.g. CXCL1, CXCL2, CXCL5, CXCL6, CXCL8/IL-8) are expressed by tumour cells, tumour-infiltrating leukocytes, endothelial cells and fibroblasts [37,38]. In addition to chemokines, inflammatory cytokines (e.g. IL-17, IL-1β and TNFα) have been implicated in neutrophil mobilization and recruitment in cancer. In particular, these cytokines are part of an inflammatory circuit that leads to the production of G-CSF and the subsequent formation and mobilization of neutrophils [11,33,39]. Moreover, IL-1β and G-CSF dramatically prolong the survival of neutrophils [40]. In addition to these molecules, tumour-derived oxysterols and the complement component anaphylatoxin C5a have been shown to contribute to neutrophil recruitment in mouse tumours [41-43].

The dynamics and lifespan of neutrophils in tumours remain to be fully elucidated. In a transplantable model of head and neck cancer and in primary sarcomagenesis induced by 3-methycholanthrene (3MCA) in mice, neutrophils were already present at the site at early time points (hours/days) [8,15,44]. Intravital multiphoton imaging revealed that neutrophils infiltrated the tumour

within 3h after tumour cell injection and persisted for up to 3 days in the TME [44]. Interestingly, the motility of intra-tumour neutrophils was reduced compared to peri-tumour neutrophils, suggesting different states of activation or polarization [44]. Accordingly, early in cancer development, neutrophils from tumour-bearing mice and cancer patients showed increased spontaneous and chemokine-induced migration mediated by autocrine ATP signalling through the purinergic receptors $P2Y_1$ and $P2Y_2$, compared to neutrophils from late stage cancer [45]. Therefore, neutrophils undergo dynamic changes during cancer development and progression as also discussed below [45].

Neutrophils have also been reported to accumulate in the premetastatic niche where the expression of G-CSF, CXCL1 and CXCL2 by cancer cells and stromal cells promoted their recruitment [33,46-50] (Figure 1b). In an orthotopic model of breast cancer and oncogene-driven mammary carcinogenesis, the mobilisation of neutrophils into the metastatic lung was regulated by the atypical chemokine receptor 2 (ACKR2), a decoy and scavenger receptor for inflammatory CC chemokines expressed in early hematopoietic precursors [47]. Genetic deficiency of this molecule accelerated the maturation rate, mobilization and activation of neutrophils which restrained metastasis [47]. In patients with breast cancer, expression of ACKR2 was found to be inversely correlated with the stage of the disease [51]. However, ACKR2 was also expressed by mammary epithelial cells and the relative importance of hematopoietic versus tumour cell expression in this neoplasm remains to be assessed.

Thus, results obtained in preclinical mouse models and in humans suggest that neutrophil recruitment and survival in neoplastic tissues involves upstream regulation of myelopoiesis and a complex network of chemokines, cytokines, G-CSF, and Complement components.

Neutrophil diversity

Neutrophil diversity in health

Under homeostatic conditions, circulating and tissue neutrophils exhibit considerable diversity, with phenotypic and functional heterogeneity driven by maturation and ageing as well as

tissue microenvironmental cues [18]. Circadian oscillation and ageing affect the neutrophil proteome, including the repertoire of chemokine receptors, pattern recognition receptors and molecules involved in adhesion, inflammasome and vesicular transport as well as the production of neutrophil extracellular traps (NETs) and the capacity to migrate [28-30,52].

In the circulation, N_M freshly released from the bone marrow are characterized by a high expression of CD62L and CXCR2 and low expression of CXCR4 (Box 2). N_M are released during the night and the early morning and predominate at zeitgeber time (ZT) 13 (i.e. 13h after light on) [28]. The process of neutrophil ageing is a bone fide circadian process. Over a period of 6 to 8 hours, expression of CD62L is dramatically reduced and N_A, characterized by a high expression of CXCR4 and CD11b and a hypersegmented nucleus, predominate at ZT5 [28-30]. These phenotypic variations favour neutrophil clearance and suggest that neutrophil-dependent immune and inflammatory responses are not stable over time and may respond to environmental changes during the circadian cycle. In agreement with this hypothesis, N_A displayed reduced migration into inflamed tissues, compared to N_M [30].

Divergent results concerning the capacity to produce NETs by ageing neutrophils have been reported [29,52]. This apparent divergency may reflect different methodological approaches for enriching N_A (i.e. injection of antibodies to block P- and E- selectins or isolation of neutrophils at ZT5 in untreated mice). In steady state conditions, circulating neutrophils were shown to undergo homeostatic degranulation and to lose their capacity to form NETs before they penetrate tissues, limiting their tissue damaging potential [52]. This process is driven by a cell-intrinsic mechanism controlling the circadian expression of CXCL2 induced by Bmal1, as observed for neutrophil ageing (see above) [52].

In addition to N_M and N_A, results available in preprint form and obtained by single-cell RNA sequencing (scRNAseq) analysis of circulating neutrophils identified N_{ISG}, which are characterized by the expression of a set of interferon-stimulated genes [53]. This neutrophil subset is present in mice

and humans and could represent a population of neutrophils primed to fight infections. Interestingly, a similar population has been observed in tumours (see below and Box2) [54].

In tissues, neutrophils can accomplish important homeostatic functions and acquire specific immunomodulatory properties, as it occurs in the lymph nodes and spleen [3,32,55]. In particular, under homeostatic conditions neutrophils expressing the major histocompatibility complex (MHC) class II molecules are present in lymph nodes in proximity to T cells, suggesting a role as antigen presenting cell (APC) [56]. Neutrophil present in the marginal zone of the spleen promote immunoglobulin class switching and production of antibodies by activating B cells through the expression of B cell-activation factor of the TNF family (BAFF), a proliferation-inducing ligand (APRIL) and IL-21 [55].

Thus, in homeostasis neutrophils exhibit a previously unanticipated heterogeneity and are integrated in regulatory circuits of immunity [5,30,32]. Among mononuclear phagocytes, cells originating from embryonic precursors subserve mainly homeostatic functions, whereas in postnatal life the main function of bone marrow-derived macrophages is response to damage and inflammation, with plasticity of these cells as a major driver of diversity [57]. There is no evidence for ontogenetically distinct neutrophil or sensu stricto defined subsets. We therefore surmise that neutrophil diversity is a reflection of plasticity in response to differentiation and environmental signals.

Neutrophil diversity in cancer

Cancer has served as a paradigm for the plasticity and diversity of neutrophils, reflecting maturation stage, response to tissue cues and cancer progression (Box2). Neutrophil differentiation and maturation trajectories are profoundly altered in tumour-bearing mice [58,59]. In advanced neoplasia, immature myeloid cells endowed with immunosuppressive properties appear in the circulation, primary tumours and metastases (Box2) [36,60,61]. In the same vein, early unipotent neutrophil progenitors (NePs) (Box1) accumulated in a melanoma mouse model and a similar cell subset ($CD66b^{+}$, $CD117^{+}$, $CD34^{+/-}$) was identified in the blood of melanoma patients [61]. Despite that

these progenitors do not correspond to a subset of N_M, they are part of the diversity of myeloid cells found in patients and tumour-bearing mice.

Transcriptomic analysis of myeloid differentiation in mice bearing mammary carcinomas, revealed a profound alteration of the transcriptional trajectory leading to an immunosuppressive phenotype, characterized by reactive oxygen species (ROS), nitric oxide (NO) and Arginase-1 (Arg1) production, with potential to inhibit T cell proliferation *ex vivo* [58,59] (Figure 1c). In human and mouse lung cancers, scRNAseq analysis of tumour-infiltrating myeloid cells revealed that TANs formed a continuum of phenotypic states, which can be resolved in five and six cell clusters in human and mouse, respectively [54]. Three modules of cell subsets are conserved in human and mouse, including a module expressing canonical neutrophil markers (e.g. *MMP9, S100A8, S100A9*), a module expressing molecules involved in tumour inflammation and growth (e.g. *CCL3*, *CSF1*) and a module with a limited number of cells displaying strong expression of type I interferon-response genes (e.g. *IRF7, IFIT1*) [54].

Analysis of TANs has revealed how signals present in the TME shape their function. In primary carcinogenesis and transplantable lung tumours, TGFβ was found to polarize neutrophil function in a pro-tumour direction characterized by a high expression of Arg1, CCL17 and CXCL14 and a low expression of CXCL10, CXCL13, CCL6, TNFα and ICAM1 [62,63]. Mirroring the M1-M2 nomenclature used for polarized macrophages (for a discussion see [10,57]), N1 and N2 have been used to refer to anti-tumour and pro-tumour neutrophils, respectively (see Glossary and Box2) [62-64]. In 3MCA-induced primary sarcomagenesis, TANs presented a hybrid phenotype between N1 and N2 [15].

In contrast to TGFβ, IFNβ and combined IFNγ plus GM-CSF set neutrophils in an anti-tumour mode [65-67] (Figure 2a). In early, but not late, non–small-cell lung cancer (NSCLC), IFNγ and GM-CSF have been shown to drive the differentiation of APC-like $MHCII^+$ neutrophils expressing the co-stimulatory molecules OX40 ligand (OX40L), CD86 and 4.1BBL (Figure 2a) [66,67]. A similar HLA-

212 DR^+ neutrophil population was observed in human head and neck cancer, spatially associated with
213 activated T cells [68].

214 In general, early in carcinogenesis TANs are part of networks mediating anti-tumour
215 resistance, whereas progression is associated with a functional switch setting these cells in an
216 immunosuppressive pro-tumour mode (see also below) [12,45,64,66,67,69].

217

218 **Neutrophils in tumour-promoting inflammation**

219 Evidence in mouse models and patients, the latter discussed below, strongly suggests that
220 neutrophils are an important component of tumour-promoting inflammation in many types of cancer
221 (Figure 1d) [11,12]. Antibody-mediated neutrophil depletion (see Box3 for discussion of neutrophil
222 depletion strategies and their limitations) resulted in protection against primary carcinogenesis and
223 transplanted tumours [33,62].

224 Within the TME, TANs have been shown to affect genetic instability, tumour cell
225 proliferation, angiogenesis, tissue remodelling and suppression of innate and adaptive lymphoid cell-
226 mediated immunity (immunosuppression will be discussed in detail in the next section). Production
227 of high quantity of ROS is a fundamental property of neutrophils [3]. In cancer, neutrophil-derived
228 ROS have been associated with DNA damage and genetic instability in epithelial cells [70-72]. ROS-
229 independent mechanisms include neutrophil-derived microparticles which deliver specific
230 proinflammatory microRNAs (i.e miR-23A and miR-155) into intestinal epithelial cells, promoting
231 the accumulation of DNA double-strand breaks via downregulation of the nuclear envelope protein
232 lamin B1 (LB1) and of RAD51, a regulator of the homologous recombination pathway [70].

233 Neutrophils can express a host of cytokines and growth factors relevant to tumour growth and
234 progression including epidermal growth factor (EGF), hepatocyte growth factor (HGF) and platelet-
235 derived growth factor (PDGF) (reviewed in [73,74]). In an *in vivo* mouse model of lung adenocarcinoma
236 induced by oncogenic *Kras*, the tumour burden was dramatically reduced in neutrophil elastase (NE)-
237 deficient mice and this was associated with a reduction in tumour cell proliferation [75]. *In vitro*, NE

238 activated the proliferation of human and mouse lung cancer cells by entering into an endosomal
239 compartment and degrading the insulin receptor substrate 1 (IRS-1), which interacted with the
240 phosphoinositide 3-kinase (PI3K) and limited its interaction with the PDGF receptor (PDGFR) [75].
241 The *in vitro* mitogenic activity of NE was also observed with cells with different origin, including
242 human oesophageal cell lines and mammary epithelial cells, through the transactivation of the EGF
243 receptor (EGFR) and TLR4, and human prostate cancer cells through activation of the mitogen-
244 activated protein kinase (MAPK) signalling pathway [76-78].

245 Neutrophils play an important role in promoting tumour angiogenesis through the production
246 of pro-angiogenic factors, including Bv8, MMP-9 and VEGF-A (Figure 1d) [49,79-82]. In the
247 extracellular matrix, TAN-derived MMP-9 induced the liberation and activation of the VEGF and the
248 consequent angiogenesis whereas Bv8 induced myeloid cell mobilization and acted as a mitogen for
249 endothelial cells [79,81]. Neutrophil-derived Bv8 has been implicated in resistance to anti-VEGF therapy
250 and inhibition of G-CSF or IL-17 increased the therapeutic efficacy of anti-VEGF [83-85]. These *in vivo*
251 studies performed in animal models support a role for neutrophils in the initial angiogenic switch
252 during tumorigenesis [79,81,83-85].

253 NETs have been observed in different tumours, (i.e. liver, breast, intestinal and gastric) and
254 have been shown to be driven by hypoxia, complement, or fatty acids [86-90]. NET-associated molecules
255 such as high mobility group box 1 (HMGB1), neutrophil elastase (NE) and matrix metalloproteinase-
256 9 (MMP9) can induce the proliferation of cancer cells (Figure 1d) [86,88]. In a mouse model of lung
257 metastasis, sustained lung inflammation promoted the formation of NETs, which in turn induced the
258 proliferation of dormant cancer cells [88]. Indeed, the proteolytic remodelling of the extracellular matrix
259 component laminin-111 by NE and MMP9, contained in NETs, induced the generation of a new
260 epitope that triggered the proliferation of dormant cancer cells through α3β1 integrin activation [88]. In
261 addition, entrapment of circulating tumour cells (CTCs) into NETs promoted the formation of
262 metastases, and *in vivo* administration of DNAse reduced the formation of metastasis [86,89,91].

Therefore, NETs participate in tumour-promoting inflammation by driving angiogenesis, extracellular matrix remodelling and proliferation of tumour cells.

The tumour-promoting function of neutrophils covers the multistep process of dissemination and implantation at distant anatomical sites. Neutrophils have been reported to prepare the metastatic niche at organs as diverse as lung and liver [48,92,93]. Moreover, neutrophils have been reported to engage with CTCs in the bloodstream and to favour implantation and subsequent growth (Figure 1b) [94-97].

Primary oncogene-driven cancer models in gene-modified mice, including mammary tumours induced in *K14^{cre};Cdh1$^{fl/fl}$;Trp53$^{fl/fl}$* (KEP) mice and MMTV-polyoma middle T antigen (PyMT) mice and colorectal cancer (CRC) induced in *villinCre^{ER}; Kras$^{G12D/+}$; Trp53$^{fl/fl}$;Rosa26$^{N1icd/+}$* (KPN) mice, have provided insights into molecular mechanisms underlying neutrophil-mediated promotion of metastasis [33,34,48,93]. In a mouse model of breast carcinogenesis (i.e. KEP mice), systemic accumulation of neutrophils with immunosuppressive activity was associated with the formation of lung metastases [33]. Mechanistically, the loss of p53 in cancer cells promoted the secretion of WNT ligands that stimulated the production of IL-1β by macrophages [34]. In turn, IL-1β activated the production of IL-17 by γδ T cells that drives neutrophil accumulation in the circulation and in the lung and promoted metastasis [34]. IL-17 was upstream of G-CSF, which increased the formation of neutrophils and their polarization into cells with immunosuppressive activity [11,33,35]. Therefore, IL-17-derived from T_H17 cells or γδ T cells participates in the neutrophilia observed in tumour-bearing individuals and drives the neutrophil-derived pro-tumour activities [11,33]. In the pre-metastatic lung, neutrophils produced factors facilitating the extravasation and growth of metastasis-initiating cells, including the proangiogenic molecules Bv8 and matrix metallopeptidase 9 (MMP-9) (observed in MMTV-PyMT mice), the chemoattractants S100A8 and S100A9 (observed in KEP mice), the proteases neutrophil elastase (NE) and cathepsin G that mediate the degradation of thrombospondin-1 (Tsp-1) (observed in MMTV-PyMT mice), the proinflammatory cytokine IL-1β and the leukotriene B4 (LTB4) (observed in MMTV-PyMT mice) (Figure 1d) [33,48,49,98].

Immunosuppression

Immature and mature neutrophils can express a host of mediators capable of suppressing innate and adaptive lymphoid cell function. These include ROS, reactive nitrogen intermediates (RNI), Arg1, prostaglandins and ligands of immune checkpoints.

Neutrophil-derived ROS have long been associated with suppression of T cells activation in cancer (Figure 1c) in particular in advanced tumours [11,45,62,99,100]. In a murine model of transplantable mammary tumour, glucose deprivation in the TME triggered a metabolic switch in neutrophils that resulted in enhanced mitochondrial fatty acid oxidation, increased ROS production and consequent T cell suppression [101]. In addition to ROS, neutrophils can inhibit T cell activation through the inducible NO synthase (iNOS)–dependent production of NO, as observed in neutrophils from tumour-bearing KEP mice (Figure 1c) [33,36].

The production of Arg1 by TANs reduced the availability of L-arginine in the TME, resulting in T cell dysfunction and alteration of T cell-mediated anti-tumour immunity [62,64,102]. The expression of ARG1 by TANs can be driven by TGFβ (Figure 1c) [62,64]. Importantly, production of Arg1 by neutrophils has been shown to hamper the T cell response in human cancer, including in renal cell carcinoma and advanced-stage of NSCLC [103,104].

Endoplasmic reticulum (ER) stress has been associated with altered lipid metabolism, pathological activation and immunosuppressive activity of myeloid cells in cancer [105-107], including neutrophils in patients with NSCLC and head and neck cancer [108-110]. Here, immunosuppressive neutrophils were characterized by their low-density and increased expression of genes associated with ER stress response (e.g. *Chop*, *Xbp1*, *Bip* and *Atf4*) [110]. Induction of ER stress in neutrophils upregulated the expression of LOX-1, a scavenger receptor involved in lipid metabolism, together with the onset of potent immunosuppressive activity [108]. In patients with NSCLC and head and neck cancer, LOX-1^{+} neutrophils showed higher expression of ROS and ARG1 compared to LOX-1^{-} neutrophils and defined the neutrophil population with immunosuppressive activity [108]. In addition to

LOX-1, immunosuppressive neutrophils present in tumour-bearing mice and patients with head and neck, breast and lung tumours presented an upregulation of other proteins involved in trafficking of lipids, such as CD36 and fatty acid transport protein (FATP) 2 [109]. While the role of LOX-1 in the immunosuppressive activity of neutrophils remains to be defined, increased uptake of arachidonic acid by FATP2-expressing neutrophils drove the biosynthesis of the prostaglandin E2 (PGE2) and subsequent immunosuppression (Figure 1c) [109]. Therefore, administration of a FATP2 inhibitor in tumour bearing mice reduced the immunosuppressive activity of neutrophils and tumour growth [109]. These results may pave the way to new strategies targeting neutrophil lipid metabolism.

Neutrophils can express ligands which activate immune checkpoints in T cells (Figure 3a). PD-L1 was shown to be induced by the hypoxia-inducible factor-1α (HIF-1α) pathway in the mouse and by inflammatory cytokines (e.g. IL-6, IFNγ and GM-CSF) in humans [111-114]. PD-L1 expressing neutrophils have been identified in hepatocellular carcinoma and gastric carcinoma and to have prognostic significance [114,115]. Therefore, neutrophils are part of the myeloid stromal cell network expressing PD-L1 driving checkpoint engagement and T cell exhaustion. Further studies are needed to evaluate the expression and function of PD-L1 in neutrophils in different cancer types.

In addition to PD-L1, V-domain immunoglobulin suppressor of T-cell activation (VISTA) is expressed on tumour-associated myeloid cells, including monocytes, macrophages, dendritic cells and neutrophils [116,117]. In a murine model of transplantable melanoma, blockade of VISTA generated a MyD88-mediated pro-inflammatory response that resulted in the development of anti-tumour immunity [116]. VISTA inhibition enhanced the production of IL-12 by tumour-associated dendritic cells and monocytes and reversed their immunosuppressive activity on T cells [116]. In contrast, the immunosuppressive activity of neutrophils was not altered during VISTA inhibition, indicating that further investigations are needed to determine the role of VISTA in neutrophils and its impact in anti-tumour immunity [116]. These data suggest that tumours with elevated levels of immunosuppressive TANs may be resistant to treatment with antibodies targeting VISTA and approaches combining VISTA inhibitors and neutrophil depletion or reprogramming should be considered in these tumours.

Although the existence of an important cross-talk between neutrophils and innate lymphoid cells (ILCs), in particular natural killer (NK) cells in an inflammatory context is well established [118,119], only limited findings have been reported on the bidirectional interaction between these two innate cells in the TME. Neutrophils have been shown to promote metastatic dissemination by preventing NK cell–mediated clearance of tumour cells from initial sites of dissemination [120]. In humans, G-CSF-mobilized neutrophils inhibited the activation of NK cells [121]. On the other hand, NK cells can control the tumour-promoting and angiogenic function of neutrophils in an IFNγ-dependent manner by inhibiting VEGF-A expression [122]. However, significant anti-tumour NK cell-mediated activity attributable to enhanced NK cell activation and survival, has been also reported following NK cell interaction with neutrophils in hematopoietic stem cell transplantation recipients [123]. Thus, the interaction of neutrophils with NK cells in the TME can be context-dependent.

Neutrophils in anti-tumour resistance

The results discussed above and clinical correlative evidence suggest that neutrophils are an important component of tumour-promoting inflammation and immunosuppression in a number of murine and human tumours. In apparent contrast with these observations, neutrophils have been shown to mediate anti-tumour resistance *in vitro* and *in vivo*, suggesting a dual potential of these cells.

It has long been known that massive recruitment and activation of neutrophils can result in anti-tumour activity [124]. Neutrophils kill tumour cells through direct contact and via the generation of ROS (Figure 2b) [62,125,126]. ROS-mediated killing involved the transient receptor potential cation channel, subfamily M, member 2 (TRPM2), an H_2O_2-dependent channel which induces a lethal influx of Ca^+ in target cells (Figure 2b) [127]. Expression of TRPM2 was upregulated in cancer cells undergoing an epithelial-to-mesenchymal transition (EMT) and this was also associated with an increase in the secretion of CXCL2, suggesting that, in addition to triggering an apoptotic cascade, TRPM2 sustains the recruitment of neutrophils [127,128].

366 The neutrophil killing armamentarium includes NO, TNF-related apoptosis inducing ligand
367 (TRAIL) and TNFα [129,130]. The latter induced the expression of the hepatocyte growth factor receptor
368 (HGFR, also known as Met) on neutrophils [129]. Investigations performed in different transplanted
369 murine tumour models (e.g. Lewis lung carcinoma and a fibrosarcoma) showed that HGF present in
370 the TME induced neutrophil recruitment and production of NO, which resulted in killing of tumour
371 cells [129]. However, in a murine transplantable melanoma, HGF-Met signalling in neutrophils lead to
372 an immunosuppressive phenotype associated with a limited expansion of anti-tumour T cells and a
373 reduced response to adoptive T cell transfer and checkpoint blockade therapies [131]. Thus, the impact
374 of the expression of Met on neutrophils remains to be fully elucidated in different tumour contexts
375 and therapeutic conditions [129,131].

376 Accumulation and activation of neutrophils in the metastatic niche can reduce the formation
377 of metastases through the elimination of cancer cells [46,47,132]. In mouse models of breast cancer,
378 expression of G-CSF and CCL2 by the primary tumour induced the mobilization and activation of
379 neutrophils in the pre-metastatic lung, and consequent ROS-dependent killing of tumour cells [46]. This
380 result suggests an interplay between primary tumour and neutrophils to activate their anti-tumour
381 activity and control metastatic progression. In non-obese diabetic (NOD)/severe combined
382 immunodeficient (SCID) mice, the injection of human breast cancer cells with low spontaneous
383 metastatic potential resulted in the reprogramming of neutrophils in the pre-metastatic lung, with high
384 cytotoxic activity associated with expression of the transmembrane protein 173 (TMEM173, also
385 called STING) [132]. Here, breast cancer cells with low spontaneous metastatic efficiency showed
386 increased expression of CCL2 compared to cancer cells with high metastatic potential. Tumour-
387 derived CCL2 induced the recruitment of IFNγ-producing $CCR2^+$ monocytes. In turn, IFNγ up-
388 regulated TMEM173 and enhanced the cytotoxic activity of neutrophils [132]. These observations
389 highlight the capacity of neutrophils to act as effector cells.

390 In addition to mediating direct killing, neutrophils engage in circuits of T cell-dependent anti-
391 tumour immunity. TANs have been shown to produce chemokines including CXCL10, CCL2, CCL3,

CXCL1 and CXCL2, which recruit T cells as well as other leukocytes [3,15,133]. Neutrophils can acquire an antigen presenting cell phenotype and in early stage human lung cancer, a population of immature $CD11b^{+}$, $CD15^{hi}$, $CD10^{-}$, $CD16^{int/low}$ neutrophils stimulated the proliferation of both $CD4^{+}$ and $CD8^{+}$ T cells [66,67]. In response to GM-CSF and IFNγ present in the TME, neutrophils acquired APC features, characterized by the expression of HLA-DR and CD86 and the capacity to amplify the anti-tumour T cell response (Figure 2a) [66]. In CRC, neutrophils isolated from cancer specimens amplified the activation of $CD8^{+}$ T cells in response to T cell receptor (TCR) triggering [134].

Intestinal microbiota play a role in inflammation and colorectal carcinogenesis [135,136]. Neutrophils were reported to have a tumour-suppressive effect in CRC via the response to IL-1, which enhanced the expression of antimicrobial peptides by neutrophils and their subsequent anti-bacterial activities (Figure 2c) [137,138]. In addition to CRC, the process of lung carcinogenesis induced by *Kras* mutation coupled with *p53* loss has also been associated with a dysregulation of the airway microbiota, which stimulates IL-17 production by resident γδ T cells resulting in neutrophilia and tumour growth [35]. Therefore, neutrophils can play a role in the control of the microbiota-induced tumour-promoting inflammation [137,138].

In 3MCA-induced primary sarcomagenesis, a tripartite interaction between neutrophils, macrophages and a subset of $CD4^{-}$, $CD8^{-}$, $TCR\beta^{+}$ unconventional double negative T cells ($UTC_{\alpha\beta}$) was found to be essential for the establishment of an effective anti-tumour immunity (Figure 2d) [15]. During the early phase of sarcoma development, neutrophils amplified the production of IL-12 by macrophages, which in turn promoted type 1 polarization and IFNγ production of $UTC_{\alpha\beta}$ (Figure 2d) [15]. Further investigations are needed to determine the mechanism(s) by which a subset of $UTC_{\alpha\beta}$ act as an anti-tumour effector cells, as well as their presence, significance and role in human tumours. Interestingly, *in silico* analyses suggest that this neutrophil dependent anti-tumour axis is relevant in select human tumours [15].

Thus, neutrophils can exert dual, seemingly opposite, functions in tumour immunity. The disease stage as well as the tumour and tissue context are key determinants of actual role of these

cells in promoting or restraining cancer. The levels and nature of inflammatory mediators found in different tumour contexts and at different tumour stages may dictate the phenotype of neutrophils. [45,64,69]. The complexity of the regulatory pathways involved is underlined by the fact that the same growth factor, G-CSF, can drive the differentiation and activation of both anti- and pro-tumour neutrophils, by stimulating their cytotoxic activity or the acquisition of immunosuppressive activity, depending on the conditions [59,124,139]. In the perspective of crystallizing available information on the dual role of neutrophils in tumours, we surmise that at early stages of tumour development, myeloid cells are set in an anti-tumour mode [66,140,141], while progression to invasion and metastasis is associated with and driven by the acquisition of a pro-tumour, immunosuppressive phenotype [45,64,69].

Neutrophils in human cancer

Occurrence and significance

As discussed above increased myelopoiesis is a common feature of advanced neoplasia and neutrophil diversity has been also observed in human cancer patients, including lung cancer, head and neck cancer and melanoma [11,12,36,54,61,108,142]. By using mass cytometry by time-of-flight (CyTOF), a study available in preprint form identified distinct phenotypes of $CD66b^+$ neutrophils at different melanoma stages [142]. Notably, the abundance of the terminally differentiated N_M subset, characterized as $CD66b^+$, $CD10^+$, $CD101^+$ and $CD16^+$, was gradually decreased during tumour progression, while N_I, characterized as $CD66b^+$, $CD117^+$, $CD49d^+$ and $CD79b^+$, were increased [142]. It is important to note that the association between neutrophil immaturity and immunosuppressive activity remains a matter of discussion. Indeed, immature human $CD10^-$, $CD66b^+$ neutrophils have been described to promote T cell activation while an opposite effect has been reported for the mature $CD10^+$ N_M [143]. As observed in the mouse and discussed above, the immunosuppressive activity of neutrophils has been reported in patients with cancer and associated with the induction of ER stress and the expression of ROS and ARG1 (see above) [108-110].

In peripheral blood, high neutrophil counts and high neutrophil-to-lymphocyte ration (NLR) are associated with bad prognosis in a wide spectrum of solid tumours (e.g. CRC, melanoma, breast, prostate and lung cancer) [12]. The prognostic significance of NLR was validated in a metanalysis involving one hundred studies with 40,559 patients and 22 solid tumours [144]. However, the actual relevance of NLR in the clinic remains to be proven [145,146]. For instance, in patients with metastatic breast cancer, NLR was found associated with the stage of the disease, the involvement of the central nervous system and the presence of visceral metastasis but its prognostic significance was lost in multivariate analysis [146].

Neutrophils are present in variable numbers in human solid tumours, as assessed by conventional immunohistochemistry (e.g. CD66b) and transcriptional signatures [13-17,147]. In general, high TAN infiltration is associated with worse prognosis [12]. For instance, in a large study using the CIBERSORT (Cell type Identification By Estimating Relative Subsets Of known RNA Transcripts) method to quantify 22 leukocyte populations in ≈18,000 patients with 39 tumours, a neutrophil signature emerged as the most significant negative prognostic factor [14]. In early NSCLC, TANs were the most represented leukocyte and were negatively correlated with T cell infiltration [16]. Thus, suppression of T cell mediated immunity is likely one of the mechanisms underlying their adverse clinical significance. In addition, correlative analysis on tumour specimens from hepatocellular carcinomas revealed an association between the occurrence of neutrophils and angiogenesis [148].

In apparent contrast with the above results, in selected human tumours high levels of TANs assessed by immunohistochemistry or neutrophil transcriptional signatures were associated with better prognosis. These included CRC [13,134,149-151], endometrial cancer [125], invasive ductal breast carcinoma [125], low grade glioma [125] and undifferentiated pleomorphic sarcoma (UPS) [15]. In CRC, TANs colocalized with $CD8^+$ T cells and combined infiltration of TANs and $CD8^+$ T cells was associated with better prognostic value compared to $CD8^+$ T cell alone [134]. In UPS, but not in other sarcomas, neutrophil signatures were associated with a type I immune response and better clinical outcome [15]. Since UPS is likely the human counterpart of 3MCA-induced primary sarcomagenesis in

mice, here neutrophils may engage in anti-tumour resistance mediated by $UTC_{\alpha\beta}$ (see above and Figure 2d) [15]. Thus, in selected human tumours TANs can mediate anti-tumour resistance by direct killing of tumours cells [125,126] or by engaging in cooperative networks with innate and adaptive lymphoid cells [15,134]. Collectively, available information suggests that the significance of neutrophils and their functions, in the circulation and in the neoplastic context, may be strongly influenced by the tissue and tumor contexture.

Chemotherapy and immunotherapy

TAN infiltration affects response to different anticancer treatment modalities (Table 1). High neutrophil infiltration was generally reported to be associated with worse response to chemotherapy and radiotherapy (Table 1). Notable exceptions were CRC, gastric cancer and high-grade ovarian cancer where higher TANs were associated with better response to chemotherapy [13,152,153]. These discordant observations regarding the predictive significance of TANs for the response to cytoreductive regimens is likely a reflection of fundamental differences in the immunobiology of these cancers.

Peripheral blood neutrophilia and high NLR have been associated with poor response to immune checkpoint immunotherapy (ICI) (Table 1). As mentioned above, neutrophils express ligands of immune checkpoints including PD-L1 and VISTA [114-116]. High levels of PD-L1-expressing neutrophils in the TME have been associated with poor survival in patients with hepatocellular carcinoma and gastric cancer [114,115]. Therefore, neutrophils represent both a target and a mechanism of resistance in ICI.

In summary, neutrophils are an important determinant of the anti-tumour efficacy of established treatment modalities, ranging from chemotherapy, ICI and anti-tumour monoclonal antibodies (mAbs) mediating antibody-dependent cellular cytotoxicity (ADCC) (see below). Moreover, experimental therapies including myeloid checkpoint targeting strategies, new checkpoint blockade immunotherapies and TGFβ inhibitors have neutrophils as one of their therapeutic targets.

Neutrophil targeting and reprogramming

A better understanding of the complexity and mechanism of neutrophils' role in tumour progression provides a basis to design therapeutic approaches. Chemokine receptors CXCR2 and CXCR1 expressed by neutrophils are important for their recruitment, modulation of their activation state and circadian oscillation (see above) [30,37,52]. Based on results in preclinical models, inhibition of neutrophil recruitment by blocking CXCR1 and/or CXCR2 [37,38,50,154] has now entered clinical evaluation (Figure 3b). CXCR2 inhibitors (i.e. AZD5069, Reparixin[155], SX-682 and Navarixin) are undergoing clinical evaluation in patients with metastatic castration-resistant prostate cancer (NCT03177187 [156]: AZD5069 in combination with Enzalutamide), early breast cancer (NCT01861054[157]: Reparixin), metastatic breast cancer (NCT02001974[158,159] and NCT02370238[160]: Reparixin in combination with Paclitaxel), metastatic melanoma (NCT03161431[161]: SX-682 in combination with Pembrolizumab) and patients with NSCLC and CRC (NCT03473925[162]: Navarixin in combination with Pembrolizumab). It will be important to assess whether these agents indeed affect TAN infiltration and\or activation state given the disappointing results so far obtained with chemokine inhibitors in inflammatory conditions [37,163].

Reprogramming neutrophil function in the TME represents a challenge for which different approaches have been proposed including blocking TGFβ (Figure 3c and discussed above [62]). Along the same line, in mouse models targeting the angiotensin converting enzyme inhibitors (ACEis) and the angiotensin II type 1 receptor (AGTR1), the nicotinamide phosphoribosyltransferase (NAMPT) or CXCR4 has been reported to set neutrophils in an anti-tumour mode [164-166]. Consistently with the role of HIF-1α in setting neutrophils in a pro-tumour mode, in a uterine cancer model, hyperoxygenation and reverting hypoxia activated the anti-tumour potential of neutrophils [126]. Here we will focus on other approaches, namely ADCC and myeloid checkpoints.

Antibody-dependent cellular cytotoxicity

Neutrophils share with monocytes, macrophages and NK cells the expression of Fcγ receptors (FcγRs) and mediate tumour cell elimination via ADCC (Figure 3d) [167]. Depletion of neutrophils reduced the efficacy of treatment with mAbs directed against CD52 (anti-CD52, alemtuzumab) and CD20 (anti-CD20, rituximab) in mouse lymphomas, highlighting the requirement for neutrophils in this process [167].

Neutrophils express the high affinity receptor for IgAs, FcαRI (CD89), a potent inducer of ADCC, leading to increased killing of cancer cells compared to FcγR-mediated ADCC [167,168]. Human IgA anti-CD20 mAb was more efficient than IgG at inhibiting lymphoma development through a mechanism involving neutrophil recruitment [169]. Interestingly, IgA-elicited neutrophil mediated ADCC was enhanced by concomitant blocking of the CD47-signal regulatory protein-α (SIRPα) myeloid checkpoint (see below) [170]. Triggering of neutrophil FcαRI promoted the release of LTB4, a potent chemoattractant factor for neutrophils, IL-1β and TNFα, which in turn amplified the recruitment of neutrophils via the production of CXCL8 by endothelial cells [171]. Anti-tumour IgA treatment sustained the activation of neutrophils and created an amplification loop for neutrophil recruitment [171]. Thus, FcαRI-mediated ADCC may represent a valuable neutrophil-centred therapeutic strategy.

Myeloid checkpoints

The function of myeloid cells is under control by a number of negative regulators (checkpoints), which are expressed by neutrophils, monocytes and macrophages. These include SIRPα, CD200 receptor (CD200R), leukocyte immunoglobulin-like receptor B2 (LILRB2), paired immunoglobulin-like type 2 receptor alpha (PILRα), and, at the level of neutrophil precursors, PD-1 and ACKR2 (Figure 3a). Here we will focus on molecules for which there is evidence that neutrophils play a significant role in their anti-tumour activity.

SIRPα is highly expressed by neutrophils, monocytes and macrophages and acts as phagocytosis checkpoint via its interaction with the “don’t eat me” signal CD47 presented on target

cells [172]. CD47 is overexpressed on cancer cells, rendering them resistant to myeloid cells [172,173]. Interestingly, CD47-SIRPα checkpoint blockade increased the elimination of cancer cells during an antibody-based treatment, including non-Hodgkin lymphoma cells, melanoma cells and breast cancer cells, and potentiated the cytotoxic activity of neutrophils against breast cancer cells opsonized by trastuzumab, an anti-HER2 monoclonal antibody, through a process of trogoptosis (see Glossary) [174,175]. In transgenic mice expressing human SIRPα, the administration of an anti-SIRPα mAb increased the elimination of tumour cell by macrophages and neutrophils when combined with anti-tumour mAbs (e.g. anti-CD20, anti-HER2, or anti-EGFR mAbs) [176]. Importantly, full antitumor activity was neutrophil-dependent [176]. Anti-CD47, combined with anti-CD20, was reported to have remarkable anti-tumour activity in patients with non-Hodgkin's lymphoma [177]. The significance of neutrophils in this context and more in general in the activation and orientation of adaptive immunity downstream of blocking CD47-SIRPα remains to be defined. LILRB2 is expressed by myeloid cells, including neutrophils and acts as a negative regulator of cell activation [178]. LILRB2 binds to classical and non-classical HLA class I and contains immunoreceptor tyrosine-based inhibitory receptor motifs (ITIMs) in its cytoplasmic tails. Activation of LILRB2 on neutrophils by its ligand HLA-G inhibited their phagocytic activity and production of ROS [178]. In a model of lung cancer, LILRB2 blockade suppressed infiltration of immunosuppressive neutrophils and significantly promoted anti-tumour immunity when combined with anti-PD-L1 [179].

ACKR2 is expressed on hematopoietic precursors and is virtually absent in mature neutrophils [47]. Genetic deletion of ACKR2 resulted in an increase in the mobilization of neutrophils endowed with anti-tumour properties, characterized by their ROS-mediated cytotoxic activity against cancer cells [47]. Thus, targeting ACKR2 may on the one hand unleash the CC chemokine-mediated lymphocyte and monocyte recruitment in the periphery and on the other the neutrophil effector function.

The results discussed above suggest that myeloid cell function is under control of negative regulators (checkpoints) which restrain their effector function. Evidence obtained dissecting the

function of CD47/SIRPα suggests that blocking myeloid checkpoints unleashes adaptive immune responses [180-183]. The myeloid checkpoints presented in Figure 3a are expressed by macrophages and neutrophils, while other negative regulators (e.g. Clever-1) are present only in macrophages [184-186]. Preclinical evidence suggests that neutrophils contribute to the anti-tumour activity of agents which block the CD47-SIRPα axis and LILRB2, whereas for other molecules presented in Figure 3a the significance of neutrophil expression is unknown. Targeting neutrophil checkpoints may represent a new frontier in cancer immunotherapy. As these molecules enter the clinical arena, it will be important to assess neutrophil numbers, diversity and function as candidate correlates of anti-tumour activity

Conclusions and perspectives

The occurrence and significance of neutrophils in cancer has long been overlooked. More rigorous approaches to quantify their presence in the TME and dissection of their diversity and plasticity have opened new vistas on TAN immunobiology. TANs have therefore emerged as an important component of the ecological niche of many murine and human tumours. Current views on the yin-yang role of neutrophils in cancer are based on depletion in the mouse or correlative analysis at a whole population level. A more systematic effort using gene targeting approaches for neutrophil depletion and abrogation of selected functions in primary carcinogenesis rather than in transplanted tumour models is needed to actually dissect the yin-yang role of neutrophils in different neoplasias. Deconvoluting the diversity of TANs at single cell level and relating this complex information to function, prognosis and response to therapy represent important challenges in the field.

The current nomenclature of the diversity of neutrophils and related myeloid-derived suppressor cell (MDSC) populations can be a matter of disorientation for students outside and within the field (Box2). Even imperfect nomenclatures can have a “value of use” as communication tools and hence have euristic value [187]. Therefore, we call for a consensus effort to develop a provisional

596 nomenclature for neutrophils plasticity and diversity, on the track of previous exercises conducted
597 for ILCs, macrophages, IL-1, and more.

598 Myeloid cells at different stages of differentiation and activation represent a major pathway
599 of immune suppression at a systemic as well as at the local TME level. Dissecting the relative
600 importance and diversity of the monocytic versus the neutrophil differentiation pathway in different
601 tumour contexts and integrating it with the general immunological landscape may pave the way to
602 tailored personalized therapeutic approaches.

603 TANs can be part of anti-tumour resistance pathways. Neutrophils express myeloid
604 checkpoints and there is now clinical proof of principle that targeting the negative regulator CD47,
605 and unleashing myeloid cell function can result in clinical therapeutic benefit [177]. We surmise that
606 harnessing the anti-tumour potential of neutrophils in the tumours in which there is evidence for their
607 protective role (e.g. sarcomas, colorectal cancer) and in current immunotherapy-resistant patients may
608 represent a strategy worth to be pursued to complement the T-cell centred therapeutic
609 armamentarium.

610

Items

621

	Tumour type	Parameter assessed	Prognostic parameter	Therapy	Predictive value of neutrophils	References
Peripheral blood neutrophils	CRC (Stage IV)	NLR	OS	Chemotherapy	-	188
	CRC (Stage III)	NLR	OS	Chemotherapy	No correlation	189
	CRC (Stage IV)	NLR	OS, PFS	Chemotherapy + Bevacizumab	-	190
	Breast Cancer (Stage II-III)	NLR	DFS	Chemotherapy	-	191
	Breast Cancer	NLR	Complete/Partial response	Chemotherapy	-	192
	Breast Cancer (Stage I-III)	NLR	Complete response	Chemotherapy	-	193
	Melanoma (unresectable Stage III, IV)	NLR	OS, PFS, Clinical response	Immunotherapy (Ipilimumab)	-	194
	Melanoma (unresectable Stage III, IV)	NLR	OS, PFS, Clinical response	BRAF inhibitor	No Correlation	194
	Melanoma (Stage IV)	NLR	OS	Immunotherapies (Nivolumab, Ipilimumab)	-	195,196
	Melanoma (Stage IV)	ANC	OS	Chemotherapy ± IL-2	-	197
	Melanoma (Stage III- IV)	NLR	OS	Immunotherapies (Ipilimumab Ipilimumab+Nivolumab)	-	198-201
	Ovarian cancer (Stage I-IV)	NLR	OS, DFS	Chemotherapy	-	202
	Cervical cancer	ANC	OS, MFS	Chemoradiotherapy	-	203
	mRCC	ANC	OS, PFS	MVA-5T4 vaccination	-	204
	mRCC	NLR	OS, PFS	Immunotherapy (Nivolumab)	-	205
	Esophageal Cancer (Stage I-IV)	NLR	OS, DFS	Chemotherapy	-	206
	NSCLC (Stage III, IV)	NLR	OS, PFS	Immunotherapy (Nivolumab)	-	207-212
	NSCLC (Stage IIIB-IV)	ANC	OS, PFS	Immunotherapy (Nivolumab)	-	213
	NSCLC (Stage IIIB-IV)	Peripheral $CD15^+$ $CD33^+$ cells (FC)	Clinical response	Bevacizumab	-	214
	NSCLC (Stage IV)	NLR	OS, PFS	Chemotherapy	-	215
	Cervix, Anal, Esophagus, Lung, Glioma, HNC	ANC	OS	Radiotherapy	-	216
	Meta-analysis (Melanoma, NSCLC, mRCC)	NLR	OS, PFS	Immunotherapies (Ipilimumab, Nivolumab, Pembrolizumab)	-	217,218
Tumour-associated neutrophils	CRC (Stage III)	Density of $CD66b^+$ TANs (IHC)	DFS	Chemotherapy	+	13
	CRC (Stage IV)	Density of $CD177^+$ TANs (IHC)	OS	Bevacizumab	-	219
	Gastric Cancer (Stage I-IV)	Density of $CD66b^+$ TANs (IHC)	OS	Chemotherapy	+	152
	Ovarian cancer (high grade)	Density of $CD66b^+$ TANs (IHC)	PFS	Chemotherapy	+	153
	Biliary cancer (Stage I-IV)	Density of $CD66b^+$ TANs (IHC)	OS	Chemotherapy	-	220
	NSCLC	Ratio CD8/$CD66b^+$ TANs (IHC)	PFS	Immunotherapy (Nivolumab, Pembralizumab)	-	221

	DLBCL	*ELANE* mRNA expression (in silico)	OS	Immunotherapy (Rituximab)+Chemotherapy	-	[222]
	HCC	Density of $CD66b^+$ TAN (IHC)	OS	Sorafenib	-	[223]
	Cervical Cancer (Stage IB-IVA)	Density of $CD66b^+$ TANs (IHC)	PFS	Radiotherapy	-	[224]

Title: Predictive value of neutrophils in response to therapy

Abbreviations: ANC, Absolute Neutrophil Count; **CRC**, Colorectal cancer; **DLBCL**, Diffuse Large B cell Lymphoma; **DFS**, Disease-Free Survival; **HCC**, Hepatocellular Carcinoma; **HNC**, Head and Neck Cancer; **IHC**, Immunohistochemistry; **MFS**, Metastasis-Free Survival; **mRCC**, metastatic Renal Cell Carcinoma; **NSCLC**, Non-Small Cell Lung Cancer; **NLR**, Neutrophil-to-Lymphocyte Ratio; **OS**, Overall Survival; **PFS**, Progression-Free Survival; **TANs**, Tumour-Associated Neutrophils, **-**, Adverse prognosis; **+**, Favourable prognosis.

Figure legends

Figure 1: Neutrophils in tumour promotion

Neutrophils can sustain tumour growth via different mechanisms, including the suppression of T cell activation, the promotion of genetic instability, tumour cell proliferation, angiogenesis and metastasis. **a.** The production of growth factors, IL-17, complement component C5a, oxysterols and CXC chemokines drives the production, recruitment and life survival of neutrophils [11,33,41,42]. **b.** Chemokines induce the mobilization of neutrophils in the premetastatic niche which sustain the arrival of the metastatic cells via the production of several mediators. Circulating neutrophils escort circulating tumour cells and promote their survival and extravasation by direct interaction mediated by integrins [89,225]. **c.** Molecules present in the tumour microenvironment (TME), including granulocyte-colony stimulating factor (G-CSF) and transforming growth factor β (TGFβ), induce the expression of arginase-1 (Arg1), reactive oxygen species (ROS) and nitric oxide (NO) by neutrophils, which inhibit the activation of T cells [33,36,62]. Granulocyte-macrophage colony-stimulating factor (GM-CSF) induces the expression of fatty acid transport protein 2 (FATP2) in neutrophils. FATP2 promotes the uptake of arachidonic acid and the synthesis of prostaglandin E2 (PGE2) [109]. Neutrophils express the immune checkpoint ligands programmed death-ligand 1 (PD-L1) [111-114] and V-domain immunoglobulin suppressor of T-cell activation (VISTA) [116]. **d.** Neutrophils induce genetic instability via the production of ROS and the release of microparticles containing microRNA-23A (miR-23A) and miR-155, which downregulates the expression of molecules involved in nuclear integrity maintenance [70-72]. Neutrophils sustain tumour proliferation via the production of epidermal growth factor (EGF), hepatocyte growth factor (HGF) and platelet-derived growth factor (PDGF) [74,129], the release of neutrophil extracellular traps (NETs) containing HGMB1 that activates a TLR9-dependent pathway on cancer cells and neutrophil elastase (NE) and matrix metalloproteinase-9 (MMP-9) that cleaves laminin-111 [86,88]. Cleaved laminin-111 triggers the proliferation of cancer cells through integrin signaling activation [88]. Neutrophils sustain tumor angiogenesis through the release of the pro-angiogenic factors Bv8 and S100A8/9 and MMP-9 that activates the vascular endothelial growth factor-A (VEGF-A) in the extracellular matrix [49,79-81].

Figure 2: Anti-tumour potential of neutrophils.

Neutrophils are involved in different mechanisms of anti-tumour resistance, including the activation of T cell-dependent anti-tumour immunity (a,d), direct cytotoxic activity against tumour cells (b), or their antimicrobial activity (c). **a.** IFNγ and granulocyte-macrophage colony-stimulating factor (GM-CSF) present in the tumour microenvironment promote the maturation of immature neutrophils into

antigen-presenting cells (APCs) expressing the major histocompatibility complex class I (MHC-I) and MHC-II molecules and the costimulatory molecules CD86, 4-1BB ligand (4-1BBL) and OX40 ligand (OX40L) [66,67]. **b**. Different stimuli, such as granulocyte-colony stimulating factor (G-CSF), chemokines CXCL8 CXCL5 and CCL2, lipopolysaccharide (LPS) and interferonβ (IFNβ) promote an oxidative burst and the production of hydrogen peroxide (H_2O_2) in neutrophils. Blockade of the transforming growth factor β receptor (TGFβR) signaling enhances the production of H_2O_2 [62]. At the tumor cell level, H_2O_2 triggers an intracellular signaling leading to the activation and opening of the transient receptor potential cation channel, subfamily M, member 2 (TRPM2), a non-selective cation channel, which induces a lethal influx of Ca^{2+} in cancer cells [127]. **c.** In colorectal cancer (CRC), signaling of the IL-1 receptor type 1 (IL-1R1) in neutrophils enhances their antimicrobial activities, which limits bacteria-driven inflammation and CRC development [137,138]. **d.** Neutrophils engage in a tripartite interaction with macrophages and $CD4^-$, $CD8^-$ unconventional double negative T cells ($UTC_{\alpha\beta}$) [15]. Neutrophils amplify the production of IL-12 by macrophages, which in turn promotes type 1 polarization and IFNγ production of $UTC_{\alpha\beta}$. These cells are characterized by the expression of T cell receptor αβ chains (TCRαβ), IL-12 receptor (IL-12R), IL-18 receptor (IL-18R), molecules related to their innate-like phenotype (e.g Ly49, CD94/NKG2) and IFNγ [15].

Figure 3: Therapeutic targeting of neutrophils.

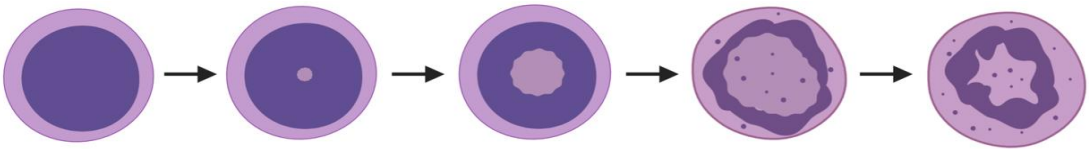
a. Neutrophils express a set of myeloid checkpoints, including signal regulatory protein-α (SIRPα), CD200 receptor (CD200R), leukocyte immunoglobulin-like receptor B2 (LILRB2), paired immunoglobulin-like type 2 receptor alpha (PILRα), and, at the level of precursors, programmed cell death 1 (PD-1) and atypical chemokine receptor 2 (ACKR2) [47,172,176,178,179,226,227]. The significance of neutrophils in targeting SIRPα, LILRB2 and ACKR2 has been established (see main text). Neutrophils or their progenitors also express CD200R [228,229], PILRα [226] and PD-1 [227], but their role in the antitumor activity of agents which target these molecules has not been demonstrated. Neutrophils also express a set of ligands of lymphocyte checkpoints (i.e. V-domain immunoglobulin suppressor of T-cell activation (VISTA), programmed death-ligand 1 (PD-L1), CD86, 4-1BB ligand (4-1BBL), OX40 ligand (OX40L)), representing a potential target to limit the process of neutrophil-mediated immunosuppression in cancer [66,67,114-116]. The interaction with their cognate receptors expressed by T cells (P-selectin glycoprotein ligand 1 (PSGL-1), PD-1, cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), 4-1BB (OX40)), delivers a positive (+) of negative (-) signals to T cells. **b.** Inhibition of CXCR1/2 dampens the recruitment of immunosuppressive neutrophils in cancer [37,38]. **c.** Transforming growth factor β receptor (TGFβR) blockade, interferonβ (IFNβ) or angiotensin II type 1 receptor (AGTR1) antagonist can increase the cytotoxic activity of neutrophils against

cancer cells**.** CXCR4 blockade increased the production of IL-18 by neutrophils and the activation of natural killer (NK) cells [62,164-166]. The immunosuppressive effect of neutrophils can be impaired by blocking the fatty acid transport protein 2 (FATP2) which, in response to arachidonic acid, induces the synthesis of prostaglandin E2 (PGE2) [109]. **d.** Neutrophils express the IgG Fc receptors (FcγR) and IgA Fc receptor (FcαRI) and are involved in the elimination of antibody-opsonized cancer cells [167,168].

Boxes

Box1: Neutrophil differentiation

The differentiation of neutrophils occurs in the bone marrow through a stepwise maturation process. In steady state, 1 to $2x10^{11}$ neutrophils are generated per day in one individual [2]. Hematopoietic stem cells differentiate into common myeloid progenitors (CMPs), which give rise to the granulocyte-monocyte progenitors (GMPs). The differentiation of neutrophils is orchestrated through the expression of specific transcription factors, including IRF8, Gfi1, C/EBPs, GATA-1 and PU.1 [11,22,230]. Neutropenia was observed in C/EBPα, C/EBPε and Gfi1 deficient mice, while IRF8 regulates cell fate choice promoting monocyte differentiation and reducing neutrophil differentiation [22,230,231]. Differentiation of GMPs into neutrophils is regulated by mediators expressed by bone marrow stromal cells (i.e G-CSF, GM-CSF) and begins with the formation of myeloblasts. Then, myeloblasts differentiate into promyelocytes, which subsequently give rise to myelocytes, metamyelocyte, band neutrophils and finally mature neutrophils [2]. Historically, this classification was based on histological staining and electron microscopy analysis [2]. Recent studies have characterized neutrophil development and identified multiple discrete steps along their differentiation process using single-cell RNA sequencing (scRNAseq) and mass cytometry by time-of-flight (CyTOF) [61,142,232,233]. In particular, two subsets of proliferative neutrophil-committed bone marrow-residing cells, named early unipotent neutrophil progenitors (NePs) and preNeutrophils (preNeus) have been identified [61,234]. NePs might represent a precursor of preNeus. Importantly, human NePs produce only neutrophils after adoptive transfer in NSG-M3 mice [61]. In cancer, distinct subsets of neutrophils composed by immature and mature cells and potentially endowed with immunosuppressive and pro-tumour activities appear in the circulation and tumour sites (Box2).



	GMP	Early unipotent neutrophil progenitors (NePs)	preNeutrophils (preNeus)	Band neutrophils	Mature neutrophils
Human	Lin^-, $CD34^+$, $CD38^+$, $CD45RA^+$, $CXCR4^+$, $CXCR2^-$,	Lin-, $CD117^+$, $CD66b^+$, $CD38^+$, $CXCR4^+$, $CXCR2^-$,	Lin^-, $CD117^-$, $Siglec8^-$, $CD15^+$, $CD34^-$, $CD66b^+$, $CD49d^+$, $CD101^-$ $CXCR4^+$, $CXCR2^-$,	$CD66b^+$, $CD15^+$, $CD33^{mid}$, $CD49d^-$, $CD101^{mid}$, $CD10^-$, $CD16^{low}$, $CXCR4^+$ $CXCR2^+$	$CD66b^+$, CD15+, $CD33^{mid}$, $CD49d^-$, $CD101^{mid}$, $CD10^+$, CD16+, $CXCR4^-$ $CXCR2^+$
Mouse	Lin^-, $IL7R^-$, $CD117^+$, $Sca1^-$, $CD34^+$, $CD16/32^+$, $CXCR4^+$, $CXCR2^-$,	Lin^-, $CD117^+$, $Sca1^-$, Siglec F^-, $FcεRIα^-$, $CD16/32^+$, $Ly6B^+$, $CD11a^+$, $CD162^{low}$, $CD48^{low}$, $Ly6C^{low}$, $CD115^-$, $Ly6G^-$, $CXCR4^+$, $CXCR2^-$	Lin^-, $CD117^{mid}$, $CD115^-$, Siglec-F^-, $Gr1^+$, $CD11b^+$, $Ly6G^{low}$, $CXCR4^{high}$, $CXCR2^-$,	$CD117^-$, CD115-, SiglecF-, $Gr1^+$, $CD11b^+$, $CD101^-$, $Ly6G^{low/mid}$, $CXCR4^+$,$CXCR2^-$,	$CD115^-$, $CD11b^+$, $SiglecF^-$, $Gr1^+$, $CD101^+$, $Ly6G^{high}$, $CXCR4^-$,$CXCR2^+$,

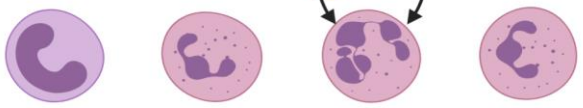
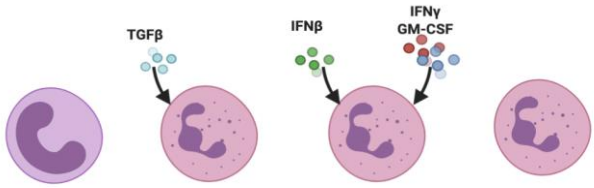
Box 2: Nomenclature of neutrophil diversity in cancer: a guiding map

There is no consensus nomenclature of the emerging complexity of neutrophil differentiation and activation states, including TANs and G-MDSCs. Here, we provide readers with a toolkit to navigate this complex, at times confusing, continent. Mirroring M1 and M2, N1 and N2 have been introduced to define neutrophils with anti-tumour and pro-tumour functions, respectively [62-64]. In contrast to other widely used type 1 and 2 nomenclatures, the definition of N1 and N2 does not mirror other dichotomous oversimplifications of polarized immune responses (e.g. Th1/Th2; type 1/2 immunity; M1/M2; ILC1/2) because IFNγ and IL-4 are not key drivers of functional polarization of these cells.

The expression on SSC^{hi} $CD45^{+}$ leukocytes of CD11b and CD66b in human and CD11b, $Ly6C^{low}$ and $Ly6G^{high}$ in mice represents the minimal set of surface molecules that unequivocally identifies neutrophils. This broad phenotypic definition encompasses distinct subsets, including immature neutrophils, mature neutrophils, aged neutrophil and neutrophils expressing a set of interferon-stimulated genes. As an operational laboratory nomenclature, we use N_I, N_M, N_A and N_{ISG} to refer to immature, mature, aged and interferon gene signature-neutrophils, respectively. MDSCs are operationally defined as a heterogeneous population of mostly immature myeloid cells with immunosuppressive activity related to the neutrophil (G-MDSC) or monocyte (M-MDSC) differentiation pathway [102]. MDSC are defined operationally based on function and therefore we feel that G-MDSC should refer to a neutrophil population with proven immunosuppressive activity.

Attempts have been made to determine specific markers of neutrophil subsets but an unequivocal strategy to detect immunosuppressive neutrophils and other neutrophil subsets by flow cytometry remains to be defined [102]. In addition to molecular markers, circulating low-density neutrophils (LDNs) consisting of immature and mature neutrophils have been identified [64]. LDNs accumulate in cancer and are generally endowed with immunosuppressive capacity [64,108,235]. However, immunosuppressive neutrophils were observed also in the normal density neutrophil (NDN) fraction [143], highlighting the need for a more robust system to define the neutrophil immunosuppressive subsets.

Here are reported selected molecules proposed to identify neutrophil subsets in cancer, including CD101 [234] and CD177 [151], which have been associated with tumour regression and CD117 [61,101,234], PD-L1[61,111-114], CD170[236], LOX-1[68,108], CD84 and junctional adhesion molecule-like (JAML)[58], which have been associated with T cell immunosuppression and disease progression.

	Circulating neutrophils				Tumour-associated neutrophils			
								
	Immature neutrophils **(N_I)**	Mature neutrophils **(N_M)**	Aged neutrophils **(N_A)**	Neutrophil with interferon-stimulated genes signature **(N_{ISG})**	Tumour-associated immature neutrophils	Tumour-associated pro-tumour neutrophils	Tumour-associated anti-tumour neutrophils	Tumour-associated neutrophil with interferon-stimulated genes signature
Human	$CD66b^+$, $CD11b^+$, $CD101^{mid}$, $CD10^-$,$CD16^{low}$, $CD62L^{high}$, $CD117^+$, CD49d+, CD79b+, $CXCR4^+$, $CXCR2^+$	$CD66b^+$, $CD11b^+$ $CD101^+$, $CD10^+$, $CD16^+$, $CD62L^{high}$, $CXCR4^-$ $CXCR2^+$	$CD66b^+$, $CD11b^+$ $CD101^+$, $CD10^+$, $CD16^+$, $CD62L^{low}$, $CXCR4^+$, CXCR2-	$CD66b^+$, $CD11b^+$ IFIT1 IRF7, RSAD2	CD66b+, $CD11b^+$, $CD117^+$, $CD10^-$, $CD16^{int/low}$, $LOX\text{-}1^+$ $CD84^+$ $JAML^+$	$CD66b^+$, $CD11b^+$, $CD170^{high}$, $PD\text{-}L1^+$	$CD66b^+$, $CD11b^+$ $CD101^+$, $CD177^+$ (in CRC), $CD170^{low}$, CD54+, HLA-DR^+, $CD86^+$, $CD15^{high}$	$CD66b^+$, $CD11b^+$ IFIT1, IRF7, RSAD2
Mouse	$Ly6G^{low/mid}$, $CD11b^+$ $CD101^{mid}$, $CD62L^{high}$ $CD117^+$, $CXCR4^+$, $CXCR2^{low}$	$Ly6G^{high}$, $CD11b^+$ $CD101^+$, $CD62L^{high}$, $CXCR4^-$, $CXCR2^+$	$Ly6G^{high}$, $CD11b^+$ $CD101^+$ $CD62L^{low}$, $CXCR4^+$, CXCR2-	$Ly6G^+$, $CD11b^+$, IFIT1 IRF7, RSAD2	$Ly6G^+$, $CD11b^+$, $CD117^+$, $CD170^{low}$, $CD101^-$ $CD84^+$ $JAML^+$	$Ly6G^+$, $CD11b^+$, PD-L1+, $CD170^{high}$,	$Ly6G^+$, $CD11b^+$, $CD170^{low}$, $CD177^+$ (in CRC), $CD54^+$, $CD16^+$	$Ly6G^+$, $CD11b^+$, $CD11b^+$, IFIT1, IRF7, RSAD2

Box 3: Strategies to deplete neutrophils.

Two monoclonal antibodies (mAbs) RB6-8C5 and 1A8 have been extensively used to deplete neutrophils in mice. The mAb RB6-8C5, characterized by its interaction with the granulocyte-differentiation antigen (Gr-1), can interact with both the Ly6G and Ly6C molecules, promoting the depletion of neutrophils and Ly6C expressing cells, including monocytes [237]. In contrast, the mAb 1A8 drives a specific depletion of neutrophils [237,238]. It is important to note that monitoring the efficacy of depletion using the same mAbs is not reliable as target antigens are "masked" by the depleting antibody. Thus, depletion efficacy should be assessed by using alternative antibodies (e.g. Ly6B). Investigations, including in a preprint article [239], have reported that sustained administration of the anti-Ly6G antibody leads to effective depletion in naïve FVB/N and BALB/c but not in C57BL/6J mice [46,239,240]. Data suggest that the combined use of 1A8 and a secondary anti-rat antibody results in increased efficacy and duration of neutrophil depletion *in vivo* [239].

Genetic strategies represent a valuable tool to overcome the limitations of mAbs-induced neutrophil depletion. Models of genetic neutropenia leverage the importance of molecules involved in neutrophil development (e.g. *Csf3*$^{-/-}$, *Csf3r*$^{-/-}$ and *Gfi*$^{-/-}$ mice) [15,22]. These models have also limitations because these genetic deficiencies can affect the development of other cell types. (i.e. monocytes). Conditional gene deficiency can represent a more sophisticated tools to achieve specific and durable depletion of neutrophils. For instance, ablation of the anti-apoptotic protein Mcl1 in myeloid compartment (*LysM*^{Cre} mice) determines neutrophil deficiency [241]. It is important to note that profound depletion of circulating and tissue neutrophils (>98%) using the *LysM*^{Cre} Mcl1$^{flox/flox}$ mice requires a high level of Cre-mediated deletion of Mcl1 for which a bi-allelic expression of the Cre-recombinase can be required [241-243]. The high specificity of Ly6G expression in neutrophils makes targeting this locus a promising strategy [244]. However, results available in preprint form showed that the combined used of Ly6G^{Cre} with Cre-inducible diphteria toxin receptor (iDTR) was inefficient to deplete neutrophils because these cells were resistant to DT [239]. In contrast, Mrp8^{DTR} mice display complete neutrophil depletion, suggesting that MRP8 expression during neutrophil development occurs when the progenitors are still sensitive to DT [239].

A conceptually different strategy to assess neutrophil functions in tumours is represented by genetic ablation of the chemokine receptor CXCR2 (*Cxcr2*$^{-/-}$). However, this approach presents significant limitations due to the expression of CXCR2 in other myeloid cells and non-immune cells, such as cancer cells [37,154,245-249].

To unequivocally demonstrate the involvement of neutrophils in tumour, the extent of neutrophil depletion in peripheral blood, distant organs and tumours should be assessed and different approaches of neutrophil depletion should be used [15].

Glossary

Anaphylatoxins. Protein fragments (C3a and C5a) produced by the cleavage of the complement components C3 and C5. They bind G-protein-coupled receptors expressed on myeloid cells and are potent chemotactic agents.

Antibody-dependent cellular cytotoxicity (ADCC). Lysis of an antibody-coated target cell triggered via the interaction of target-bound antibodies with Fc receptors (FcRs) expressed by effector cells. ADCC can occur through the release of cytotoxic molecules, the expression of cell death-inducing molecules and trogoptosis.

Atypical chemokine receptors (ACKRs). Seven transmembrane receptors belonging to the chemokine receptor family that lack a DRY motif and do not mediate chemotaxis (directional cell migration) but regulate chemokine bioavailability by scavenging, transcytosis or presentation of the ligand.

Circulating tumour cells (CTCs). Cancer cells stripped from a primary tumour and found in the bloodstream.

Fc receptors (FcRs). Surface receptors expressed by innate immune cells that recognize Fc fragment of immunoglobulins (IgG). Neutrophils express the IgG Fc receptors (FcγRs) and IgA Fc receptor (FcαRI).

Innate lymphoid cells (ILCs). A group of cells of the innate immune response that belong to the lymphoid lineage and are characterized by the lack of antigen-specific receptors.

Mass cytometry by time-of-flight (CyTOF). Flow cytometry platform which utilizes elemental mass spectrometry to detect metal-conjugated antibodies that are bound intracellularly or extracellularly to antigens of interest in single cells.

M1-M2. Terms referring to the opposite end of a continuum of macrophage polarization states. Classically activated M1 macrophages in response to signals such as IFNγ and lipopolysaccharide mediate resistance to intracellular pathogens and tumours, whereas in response to IL-4 and IL-13 elicit an alternative form of macrophage activation (M2) which mediates resistance to parasites, tissue

repair and tumour promotion. M1 and M2 are loose operational definitions of extremes of polarization in a universe of activation states driven by the integration of environmental signals

Neutrophil extracellular traps (NETs). Extracellular neutrophil-derived networks composed by DNA and proteins such as neutrophil elastase and histones.

N1-N2: These terms have been used to discriminate anti-tumour neutrophils (N1) and pro-tumour neutrophils (N2). IFNβ and TGFβ signalling pathways can trigger the formation of N1 and N2 neutrophils, respectively. N1 neutrophils are characterized by a normal density, a hypersegmented nucleus and a cytotoxic activity towards cancer cells, whereas N2 are neutrophils with immunosuppressive activity. N1 and N2 classification may represent an oversimplification of neutrophils polarization, activation or maturation states.

Oxysterols. Cholesterol metabolites involved in cholesterol homeostasis and immune and inflammatory responses. Oxysterols interact with the transcription factor liver X receptor (LXR) and the G-protein-coupled receptors (GPCRs) CXCR2 and Epstein–Barr virus-induced gene 2 (EBI2).

Pattern recognition receptors. Germline-encoded host sensors which recognize essential molecules expressed by microorganisms. The signalling through these receptors drives the initiation of the inflammation, innate and adaptive responses.

Premetastatic niche. Environment in a secondary organ that provides favourable conditions for the seeding of metastatic cells.

Reactive oxygen species (ROS). Chemically reactive species containing oxygen. ROS produced by the activation of the NADPH oxidase enzymatic system. They have important antimicrobial activity and induce genetic instability.

Sarcomagenesis. The process of initiation and development of a sarcoma, a tumour of mesenchymal origin.

T_H17. A subset of $CD4^+$ T helper cells characterized by production of IL-17. T_H17 cells are involved in neutrophil-dependent defence against extracellular pathogens and implicated in inflammatory disorders.

Trogoptosis. A process of cytotoxicity mediated by an active mechanism of plasma membrane transfer, called trogocytosis, between interacting cells.

Unconventional T cells (UTCs). A group of T lymphocytes that express T cell receptor (TCR) αβ or γδ chains and characterized by the lack of recognition of classical peptide antigens, such as the mucosal associated invariant T (MAIT) cells and invariant natural killer T (iNKT) cells.

Undifferentiated pleomorphic sarcoma (UPS). An aggressive sarcoma of soft tissues or bone that occurs in any part of the body. UPS is characterized by the presence of pleomorphic spindle cells with large atypical cells exhibiting numerous irregular mitotic figures

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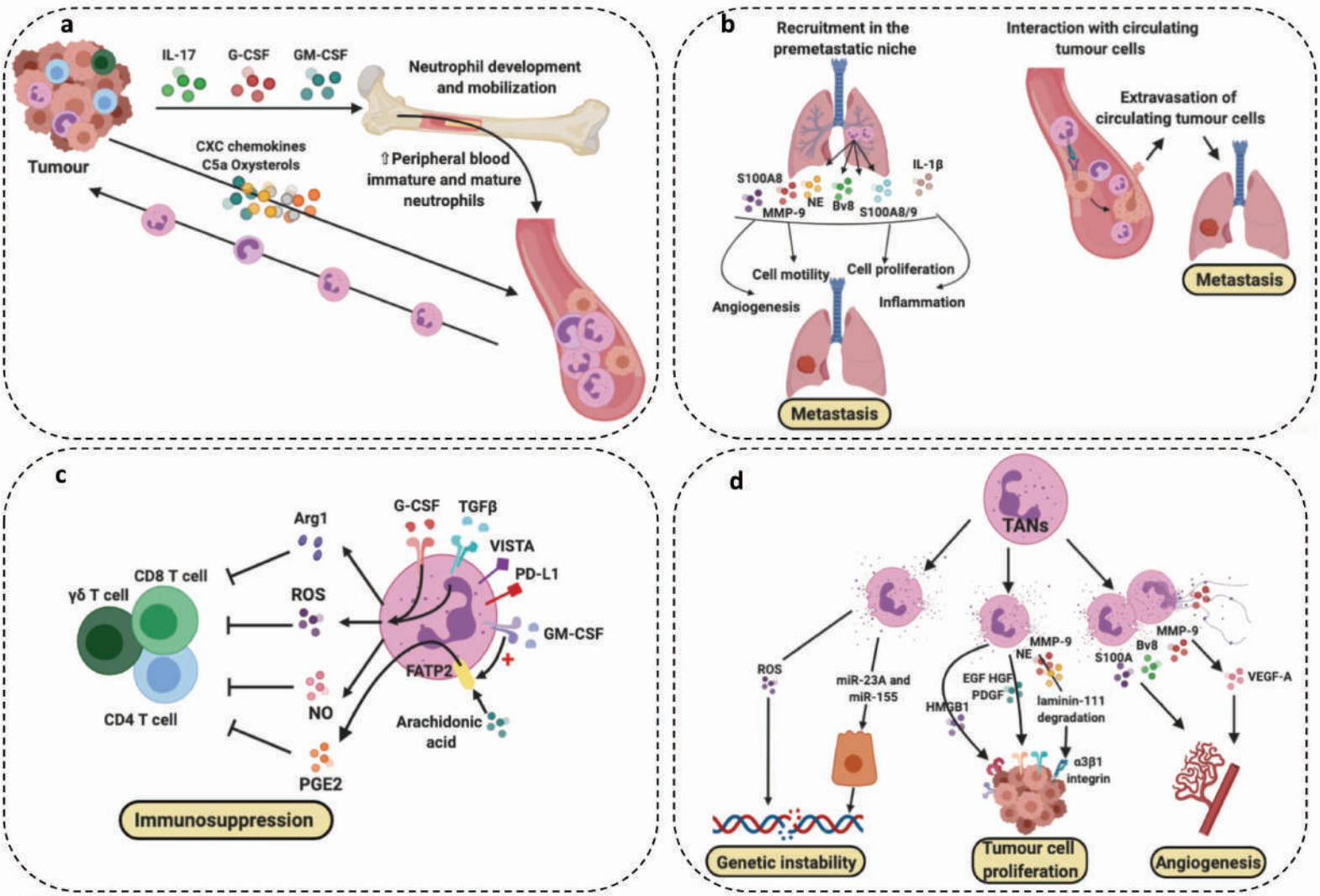

a
IL-17
G-CSF
GM-CSF
Neutrophil development and mobilization
Tumour
CXC chemokines
C5a Oxysterols
⇧Peripheral blood immature and mature neutrophils
b
Recruitment in the premetastatic niche
S100A8
MMP-9
NE
Bv8
S100A8/9
IL-1β
Cell motility
Cell proliferation
Angiogenesis
Inflammation
Metastasis
Interaction with circulating tumour cells
Extravasation of circulating tumour cells
Metastasis
c
Arg1
G-CSF
TGFβ
VISTA
PD-L1
GM-CSF
CD8 T cell
γδ T cell
ROS
NO
FATP2
Arachidonic acid
CD4 T cell
PGE2
Immunosuppression
d
TANs
ROS
miR-23A and miR-155
MMP-9
NE
EGF HGF PDGF
HMGB1
laminin-111 degradation
α3β1 integrin
S100A
Bv8
MMP-9
VEGF-A
Genetic instability
Tumour cell proliferation
Angiogenesis

Figure 1

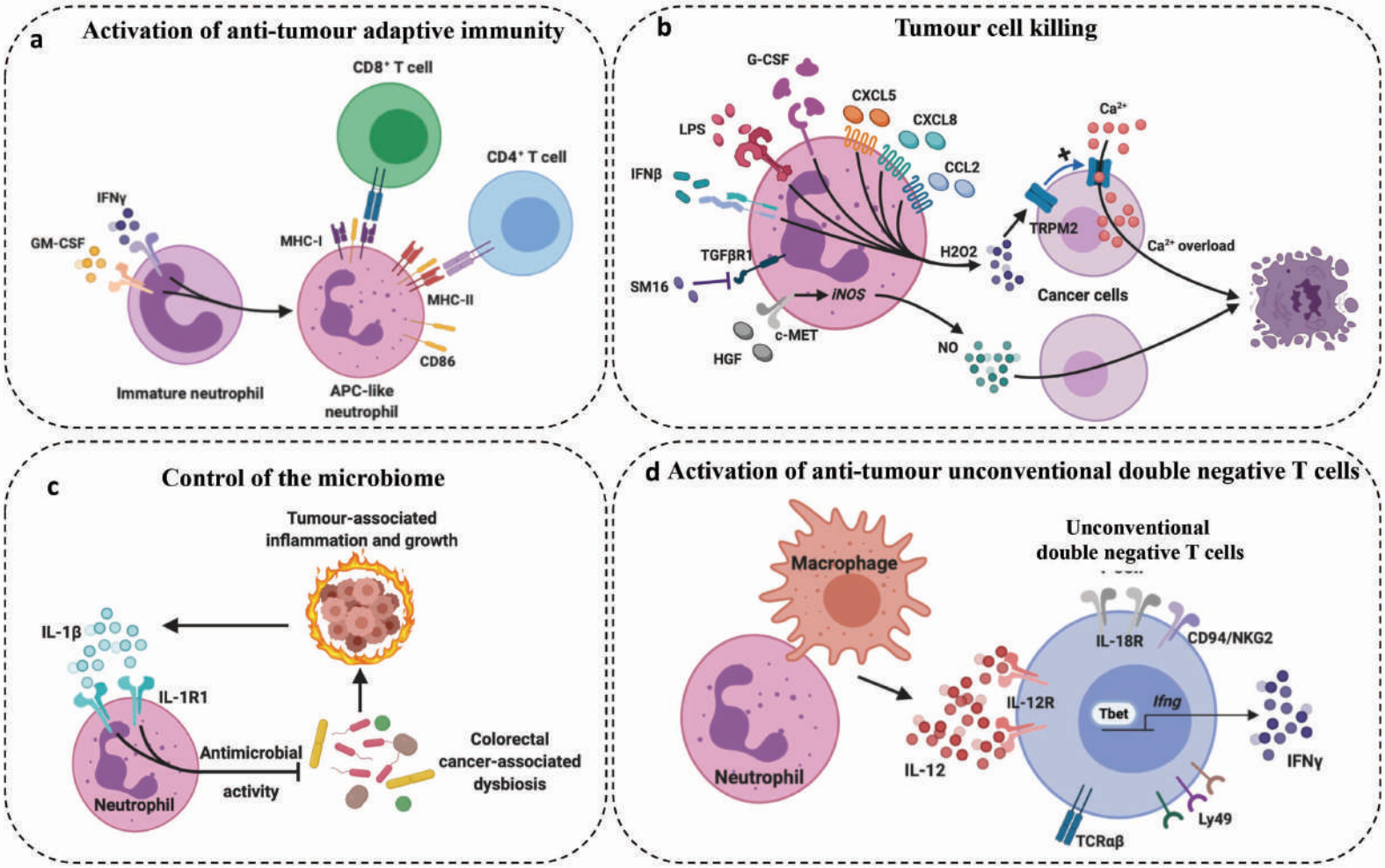
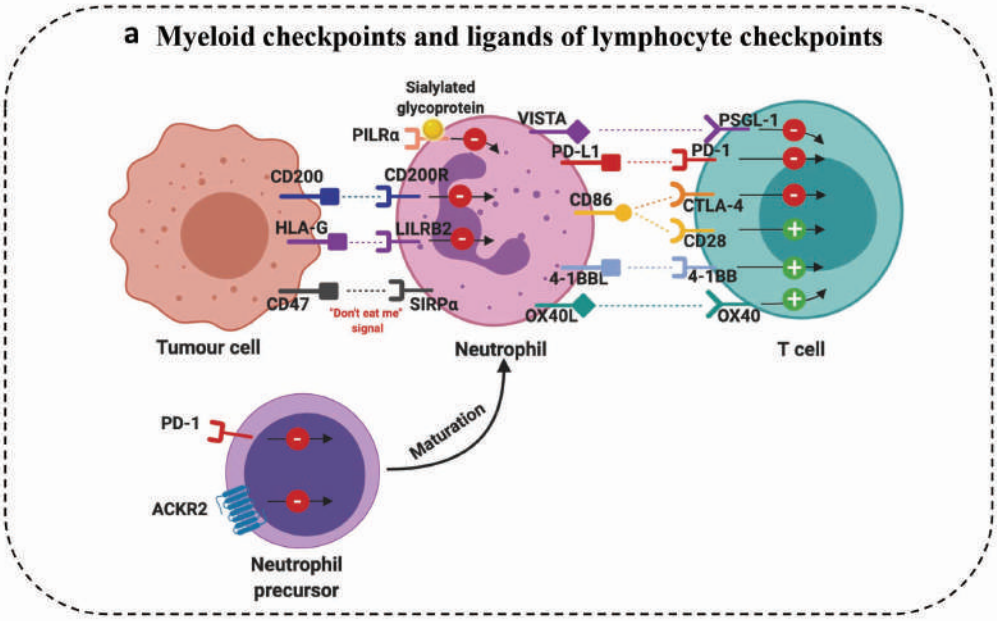
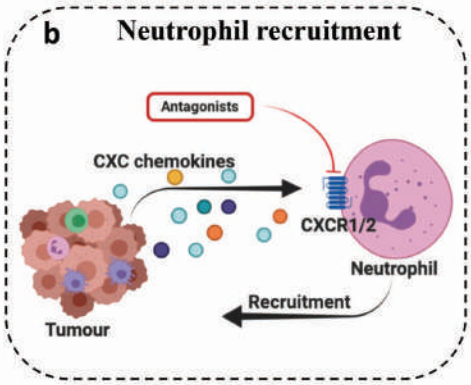
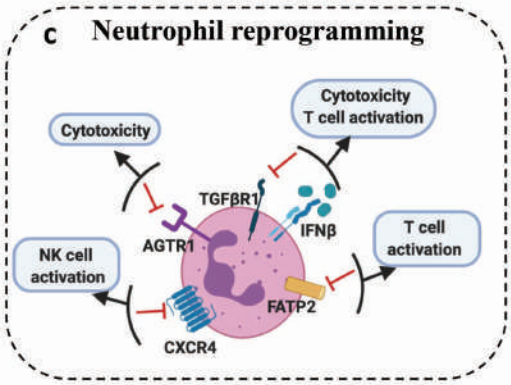

a
Activation of anti-tumour adaptive immunity
CD8⁺ T cell
CD4⁺ T cell
IFNγ
GM-CSF
MHC-I
MHC-II
CD86
Immature neutrophil
APC-like neutrophil
b
Tumour cell killing
G-CSF
CXCL5
CXCL8
LPS
CCL2
IFNβ
TGFβR1
SM16
iNOS
c-MET
HGF
H2O2
NO
Ca²⁺
TRPM2
Ca²⁺ overload
Cancer cells
c
Control of the microbiome
Tumour-associated inflammation and growth
IL-1β
IL-1R1
Antimicrobial activity
Neutrophil
Colorectal cancer-associated dysbiosis
d
Activation of anti-tumour unconventional double negative T cells
Macrophage
Neutrophil
Unconventional double negative T cells
IL-18R
CD94/NKG2
IL-12R
IL-12
Tbet
Ifng
IFNγ
Ly49
TCRαβ

Figure 2


a Myeloid checkpoints and ligands of lymphocyte checkpoints
Sialylated glycoprotein
PILRα
VISTA
PD-L1
PSGL-1
PD-1
CD200
CD200R
CD86
CTLA-4
HLA-G
LILRB2
CD28
4-1BBL
4-1BB
CD47
"Don't eat me" signal
SIRPα
OX40L
OX40
Tumour cell
Neutrophil
T cell
PD-1
Maturation
ACKR2
Neutrophil precursor


b Neutrophil recruitment
Antagonists
CXC chemokines
CXCR1/2
Neutrophil
Recruitment
Tumour


c Neutrophil reprogramming
Cytotoxicity
Cytotoxicity T cell activation
TGFβR1
IFNβ
AGTR1
T cell activation
NK cell activation
FATP2
CXCR4

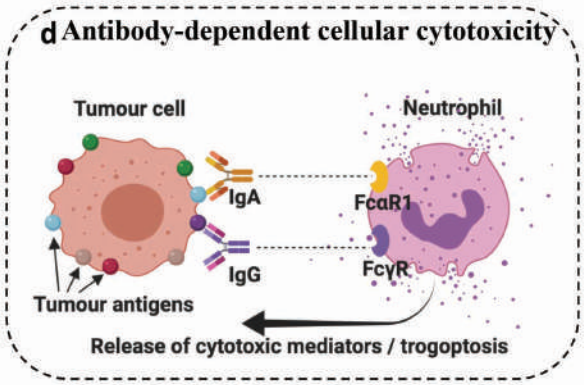

d Antibody-dependent cellular cytotoxicity
Tumour cell
Neutrophil
IgA
FcαR1
IgG
FcγR
Tumour antigens
Release of cytotoxic mediators / trogoptosis

Figure 3