A new dawn for eosinophils in the tumour microenvironment

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Abstract | Eosinophils are evolutionarily conserved, pleotropic cells that display key effector functions in allergic diseases, such as asthma. Nonetheless, eosinophils infiltrate multiple tumours and are equipped to regulate tumour progression either directly by interacting with tumour cells or indirectly by shaping the tumour microenvironment (TME). Eosinophils can readily respond to diverse stimuli and are capable of synthesizing and secreting a large range of molecules, including unique granule proteins that can potentially kill tumour cells. Alternatively, they can secrete pro-angiogenic and matrix-remodelling soluble mediators that could promote tumour growth. Herein, we aim to comprehensively outline basic eosinophil biology that is directly related to their activity in the TME. We discuss the mechanisms of eosinophil homing to the TME and examine their diverse pro-tumorigenic and antitumorigenic functions. Finally, we present emerging data regarding eosinophils as predictive biomarkers and effector cells in immunotherapy, especially in response to immune checkpoint blockade therapy, and highlight outstanding questions for future basic and clinical cancer research.

A.M. dedicates this Review to the memory of James J. Lee, who inspired and encouraged his work on defining the role of eosinophils in the tumour microenvironment.

Eosinophils are primitive cells of the innate immune system that are found in all vertebrate species. Although early studies of eosinophils focused primarily on their roles in helminth infections and allergic diseases, the presence of peripheral eosinophilia in patients with cancer was reported more than 120 years ago[1]. Since then, the generation of unique biochemical and genetic tools for the study of eosinophils, such as antibodies against eosinophil peroxidase (EPX), eosinophil major basic protein (MBP) and epidermal growth factor (EGF) module-containing mucin-like receptor 1 (EMR1) in humans as well as genetically engineered mice (for example, hypereosinophilic and eosinophil-deficient mice)[2], has helped define new cardinal roles for these cells in metabolism, tissue regeneration, development, innate and adaptive immunity and cancer[3]. Eosinophils have been shown to infiltrate multiple tumours, either as an integral part of the tumour microenvironment (TME) or in response to various therapeutic strategies. By reliably recognizing and responding to diverse stimuli and being able to synthesize and secrete a large range of molecules[4], eosinophils are equipped and have been shown to influence tumour progression. Better understanding of the activities of these cells will not only help define the cellular components of the TME but may lead to new therapeutic strategies.

In this Review we provide an overview of current knowledge regarding the roles of eosinophils in the TME, with an emphasis on solid tumours. We discuss mechanisms of eosinophil homing and survival in the TME, examine their diverse pro-tumorigenic and antitumorigenic functions, present compelling data regarding the use of eosinophils as potential predictive biomarkers in immune checkpoint blockade (ICB) therapy and highlight outstanding questions for future research. Although eosinophilia is also present in various haematological malignancies[5], the potential involvement of eosinophils in the malignant clone and/or their proliferation in response to interleukin-5 (IL-5) production by neoplastic lymphocytes creates a level of complexity that is beyond the scope of this Review.

Eosinophil biology

Eosinophils arise from multipotent $CD34^+$ progenitor cells in the bone marrow, where, under homeostatic conditions, they develop into eosinophil-lineage committed, IL-5 receptor-α (IL-5Rα)-expressing eosinophil progenitors[6]. This process involves a complex hierarchically expressed network of transcription factors, including PU.1, FOG1 (also known as ZFPM1), C/EBP family members, GATA1 and GATA2 (FIG. 1), as well as various microRNAs, long non-coding RNAs and other regulatory factors. Although eosinophil progenitors can be found in low numbers in peripheral blood, most differentiate into mature eosinophils in the bone marrow in response to cytokine and chemokine signals, of which the most

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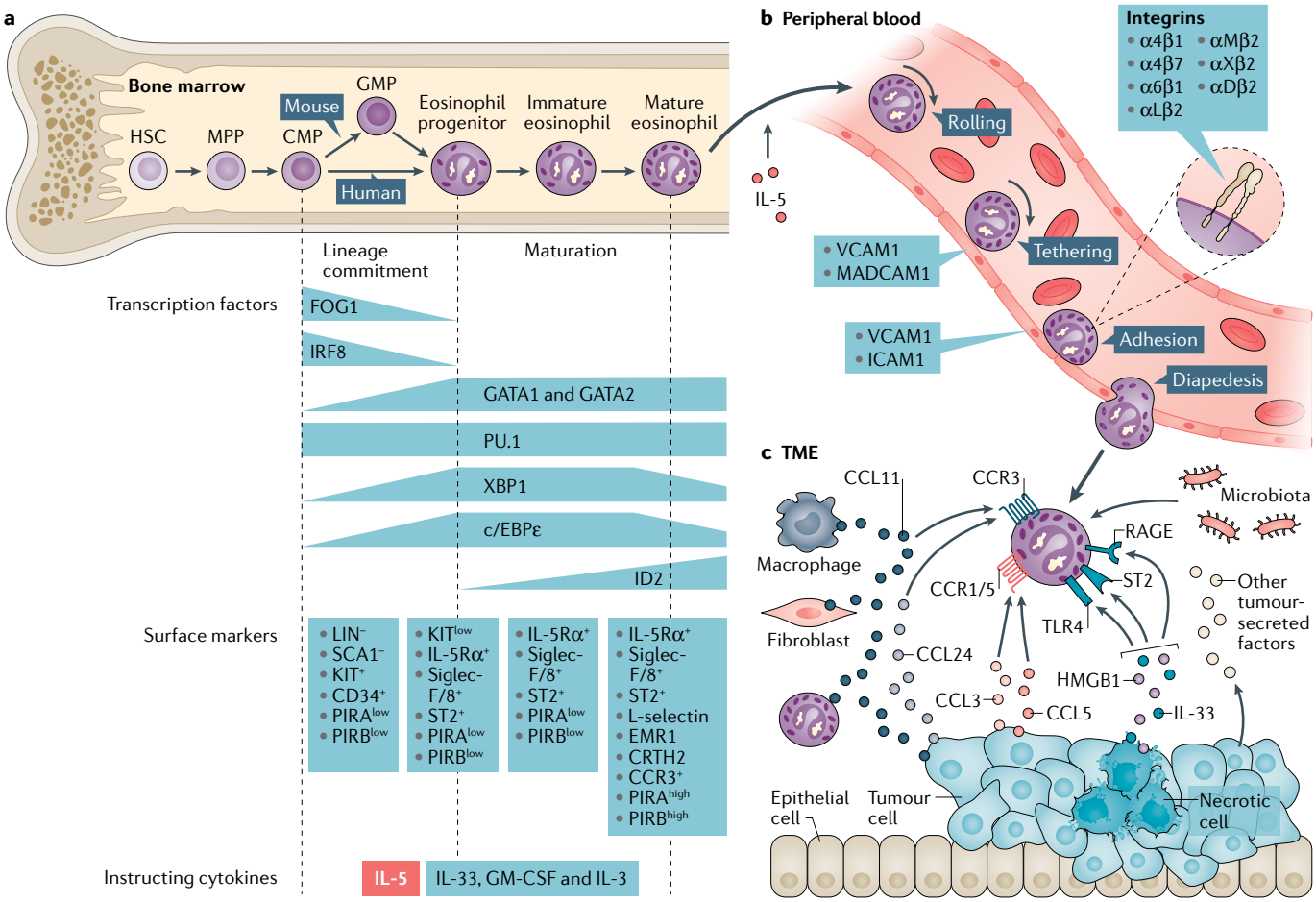

Fig. 1 | **Eosinophil maturation and homing into the tumour microenvironment. a** | Eosinophils differentiate in the bone marrow from a common myeloid progenitor (CMP) cell in a tightly regulated process directed by differential expression of several transcription factors. FOG1 enables the increased expression and activity of GATA1 and GATA2, which are necessary for production of eosinophil progenitors. Lineage commitment is primarily instructed by interleukin-5 (IL-5; highlighted in the red box) with a contribution from IL-33, granulocyte–macrophage colony-stimulating factor (GM-CSF) and IL-3. Eosinophil granule proteins are increased by coordinated and collaborative expression of c/EBPε, PU.1, IRF8, GATA1 and GATA2, and their maturation is enhanced by XBP1 and ID2. **b** | Mature eosinophils express various receptors, adhesion molecules and integrins that facilitate their transition from the bone marrow to the blood in an IL-5-dependent fashion. **c** | Following interactions with endothelial cell-expressed adhesion molecules, such as intracellular adhesion molecule 1 (ICAM1) and vascular cell adhesion molecule 1 (VCAM1), eosinophils transmigrate into the tumour microenvironment (TME) in response to chemotactic cues delivered by different cell types. One of the prominent pathways for eosinophil migration is dictated by eotaxins (CC-chemokine ligand 11 (CCL11) and CCL24) signalling through CC-chemokine receptor 3 (CCR3). Tumour-derived CCL24 and CCL11 as well as macrophage-derived, fibroblast-derived and even eosinophil-derived CCL11 can promote the homing of eosinophils to the TME. In addition, tumour-derived CCL3, CCL5 or as yet unidentified secreted factors can support the migration of eosinophils. Eosinophils express ST2, the receptor for advanced glycation end products (RAGE) and Toll-like receptor 4 (TLR4), which enables them to migrate in response to tumour necrotic cell alarmins, such as IL-33 and high mobility group box 1 (HMGB1). Finally, alterations in microbiota can either directly or indirectly influence the accumulation of eosinophils in the TME. EMR1, epidermal growth factor module-containing mucin-like receptor 1; GMP, granulocyte–macrophage progenitor; HSC, haematopoietic stem cell; IL-5Rα, IL-5 receptor-α; MADCAM1, mucosal vascular addressin cell adhesion molecule 1; MPP, multi-potent progenitor; Siglec-F/8, sialic acid-binding immunoglobulin-like lectin F (in mice) or 8 (in humans).

important and lineage-specific is IL-5 (REF.[6]). IL-5 signalling via IL-5Rα is not only crucial for eosinophil differentiation and maturation but plays a key role in eosinophil expansion[7], release from the bone marrow[8] and survival[9].

Mature eosinophils display a wide variety of surface receptors, including receptors for growth factors, cytokines, chemokines, adhesion molecules, phosphatidylserine, pathogen-associated molecular patterns and even co-inhibitory receptors (reviewed in detail elsewhere[4,10]). Of these, IL-5Rα, CC-chemokine receptor 3 (CCR3) and sialic acid-binding immunoglobulin-like lectin 8 (Siglec-8) in humans (Siglec-F in mice) are considered eosinophil-selective in their expression and are commonly used to detect eosinophils by flow cytometry in combination with additional myeloid cell markers such as CD11b (also known as integrin αM)[10]. Although differences exist between human and mouse eosinophils (thoroughly reviewed elsewhere[11]), basic eosinophil biology is generally conserved between the species.

A unique feature of mature eosinophils is the presence of acidophilic secondary granules. Eosinophil granule proteins are highly cationic proteins that have been implicated as important effector molecules in the anti-tumorigenic activities of eosinophils in cancer. In humans,

Pathogen-associated molecular patterns
Small molecular motifs derived from microorganisms that can be recognized by specialized pattern recognition receptors.

Extracellular DNA traps
A network of mitochondrial DNA fibres and eosinophil granule proteins, such as major basic protein (MBP) and eosinophil cationic protein (ECP), which facilitate bacterial clearance.

Lipid bodies
(Also known as lipid droplets.) Functionally active organelles that are actively formed within cells from the immune system in response to different inflammatory conditions and are sites for synthesis and storage of inflammatory mediators.

these granules comprise a crystalloid core containing MBP type I and II encoded by proteoglycan 2 (*PRG2*) and *PRG3*, respectively[12], and a matrix consisting of eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN; also known as ribonuclease A family member 2), EPX[13] and a wide array of preformed cytokines, chemokines and other soluble mediators, including interferon-γ (IFNγ), IL-4, IL-6, IL-10, IL-12, IL-13 and tumour necrosis factor (TNF)[14]. MBP can disrupt the integrity of lipid bilayers and is cytotoxic to helminths, tumour cells and respiratory epithelial cells[15–18]. EPX induces oxidative stress and subsequent cell death by catalysing the oxidation of halides (Cl^-, Br^- and I^-), pseudohalides (SCN^-)[19] and nitric oxide metabolites to form reactive oxygen species and reactive nitrogen metabolites[20]. ECP and EDN are ribonucleases with antiviral activity[21].

Activated eosinophils can undergo a process called piecemeal degranulation, whereby the cell remains viable despite selectively releasing granule contents, including specific cytokines and granule proteins. Secretion-competent eosinophil secondary granules can also be released through cytolysis in the context of extracellular DNA traps[22]. Another characteristic feature of activated eosinophils is the formation of lipid bodies. These serve as repositories for inflammatory mediators, including eicosanoids and TNF, which can be secreted in the context of inflammatory responses[23]. Clearly, eosinophils are well equipped to engage and interact with tumour cells and with surrounding haematopoietic and non-haematopoietic cells in the TME. Nonetheless, numerous caveats may affect the interpretation of current data as they relate to the role of eosinophils in the TME (see BOX 1).

Box 1 | **Caveats affecting the interpretation of data on the roles of eosinophils in the TME**

No standardized method for eosinophil quantitation
Tissue eosinophils are usually detected by haematoxylin and eosin (H&E) staining (BOX 2). Nonetheless, in the tumour microenvironment (TME), eosinophils may degranulate and therefore stain weakly. To accurately identify and quantify eosinophils, it is imperative to use standardized immunohistochemical staining techniques for eosinophil-specific granule proteins (for example, eosinophil peroxidase (EPX) or major basic protein (MBP)), which can also be used to assess eosinophil activation.

Anatomical relevance of experimental models
Eosinophils are tissue-resident cells that are likely educated by the specific microenvironment in which they reside[172]. Interpretation of data from subcutaneous tumour models may be difficult as they overlook the activities of the resident eosinophil population.

Use of genetically engineered tumour cells that promote a type 2 environment
Transformed tumour cell lines that can promote a type 2 T helper (T_H2) cell environment by secretion of cytokines or antigens that promote a T_H2 cell response (for example, interleukin-4 (IL-4)[123], IL-5 (REF.[70]), CC-chemokine ligand 11 (CCL11) (REF.[70]) and ovalbumin (OVA)[78]) have been used to study eosinophils. Conclusions regarding eosinophils from such experiments should be limited to the specific tumour-secreted factor and not extrapolated to other factors and/or settings.

Non-specific effects of depleted cytokines, chemokines and/or cells
Eosinophils are studied in settings where specific cytokines or chemokines (for example, IL-5 and CCL11 (REFS[37,78])) were depleted, or in the absence of regulatory cells[81]. Given the possible effects of these cytokines or chemokines on additional cells (for example, CCL11 (REFS[173,174]) on tumour cells, and IL-5 (in mice) on B cells[175] and myeloid-derived suppressor cells[84]), conclusions should be drawn cautiously.

Lack of transcriptome signature for eosinophils
Isolation of high-quality and high-quantity RNA from eosinophils is difficult owing to the presence of potent ribonucleases in the granules. Although some attempts have been made[176,177], lack of an eosinophil-associated signature renders them absent from the majority of transcriptome profiling databases (for example, The Cancer Genome Atlas) and from single-cell sequencing studies of the TME before and/or after treatment.

Recruitment: many roads to the TME

Multiple chemotactic factors, which are regulated by the dynamic changes that occur within a given TME, can induce the migration of eosinophils. These are discussed in the sub-sections below.

IL-5, eotaxins and CCR3

Under baseline conditions and in allergic inflammation, eosinophil trafficking to the gastrointestinal tract or the lungs is predominantly dependent on the eotaxin–CCR3 axis. CC-chemokine ligand 11 (CCL11), CCL24 and CCL26 (members of the eotaxin family) are C-C motif chemokines with eosinophil-selective chemoattractant activity[24–26]. Although the mouse genome contains only *Ccl11* and *Ccl24*, the specificity and activity of mouse and human eotaxin members are remarkably conserved[27]. CCR3, the eotaxin-family receptor, is a seven-transmembrane-spanning G protein-coupled receptor, primarily expressed by eosinophils[28–30], and although CCR3 interacts with multiple non-eotaxin ligands, including CCL8, CCL7, CCL13, CCL5 and CCL15, only the eotaxins signal exclusively through CCR3 (REF.[31]). Furthermore, IL-5 primes eosinophils to respond to eotaxins, promoting eosinophil migration in vivo[32,33].

Accumulation of eosinophils in lung cancer, colorectal cancer (CRC) and melanoma is at least partially dependent on the IL-5Rα–CCR3 signalling axis[34–36] (FIG. 1). In an experimental model of metastatic colonization of the lungs, eosinophil numbers were dramatically reduced in the lungs of *Il5*$^{-/-}$ mice compared with wild-type controls[37]. Furthermore, IL-25 and IL-33 regulate the production of IL-5 from innate lymphoid cells (ILCs) and have been shown to affect eosinophil accumulation and migration into the lungs that bear metastases[38]. Although these experiments clearly demonstrate a role for IL-5 in eosinophil accumulation in the lung TME, they should be interpreted cautiously as IL-5 likely regulates eosinophil accumulation indirectly by governing eosinophil expansion in the bone marrow, survival and/or responses to eotaxins.

A significant correlation between tumour-infiltrating eosinophil numbers and immunohistochemical staining of CCL11 and/or CCL24 has been shown in CRC[39], Hodgkin lymphoma[40] and oral squamous cell carcinoma[41], and increased CCL11 (but not CCL24) was observed in experimental models of CRC[42]. Increased concentrations of tumour cell-derived CCL24 have also been detected in culture supernatants of biopsy samples from hepatic metastases of primary CRCs[43]. Moreover, carcinoembryonic antigen-positive tumour cells stained strongly for CCL24, implicating these cells as the predominant source of CCL24 expression[43]. In oral squamous cell carcinoma, CCL11 was identified within eosinophils, which may represent an autocrine and/or paracrine pathway of eosinophil-induced eosinophil accumulation[41]. An additional source of CCL11 in the

TME could be fibroblasts, which have been implicated in eosinophil recruitment in Hodgkin lymphoma[44].

Although CCL11 has been shown to regulate eosinophil migration into the TME in several settings, it is clear that additional pathways exist for eosinophil recruitment. For example, induction of fibrosarcoma using methylcholanthrene in *Ccl11*$^{-/-}$ mice led to a minimal change in tumour-infiltrating eosinophil numbers[35], consistent with the involvement of CCL24 or other, as yet undefined, chemoattractants.

Danger signals

Damage-associated molecular patterns, such as high mobility group box 1 (HMGB1) and IL-33, also induce eosinophil recruitment to regions of dying cells and hypoxia within tumours[45,46] (FIG. 1). HMGB1 may mediate eosinophil migration indirectly, as physiological concentrations of HMGB1 have no direct impact on human eosinophil migration in vitro[47]. Nonetheless, HMGB1 can induce the expression of CCL13 and CCL4 (REF.[48]), which can directly lead to eosinophil chemotaxis. Similarly, IL-33 is a potent inducer of type 2 T helper (T_H2) cell immune responses[49], which are characterized by increased expression of CCL11 (REF.[50]). Additionally, eosinophils have the capacity to respond to HMGB1 and IL-33 as they express ST2 (also known as IL-1 receptor-like 1), the IL-1R accessory protein (IL-1RAcP) and the receptor for advanced glycation end products (RAGE), and may express Toll-like receptor 4 (TLR4)[51–53]. Thus, a direct role for HMGB1 and/or IL-33 in eosinophil effector functions in the TME cannot be excluded.

Eosinophil migration into solid tumours was induced in a mouse model of metastatic melanoma using subcutaneous injection of B16-F10 cells. In this model, eosinophils were shown to reside in necrotic areas of the primary tumour as well as its fibrotic capsule[54]. Notably, eosinophil recruitment was an early and active process that was independent of eosinophil production in the bone marrow, acquired immunity and eotaxins[54]. Moreover, conditioned media from post-confluent B16-F10 cell cultures induced eosinophil chemotaxis. The magnitude of eosinophil chemotaxis in this Transwell cell migration system was proportional to the level of cell death observed in the B16-F10 culture[54]. Similarly, eosinophils were recruited to and activated (degranulated) in the acellular necrotic and capsule regions of spontaneous mammary gland tumours arising in mouse mammary tumour virus (MMTV) promoter-driven polyoma middle T oncogene (MMTV-PyMT) transgenic mice[55].

Microbiota

The microbiota may also affect eosinophil migration to the TME. *Apc*^{A468} mice, which spontaneously develop colorectal adenomas, displayed increased intestinal eosinophilia that was further increased in the absence of IL-10 derived from CD4$^+$ T cells[56]. Broad-spectrum antibiotic treatment markedly decreased microbial diversity and eosinophilic infiltration[56], suggesting that eosinophils can respond to microbial intrusion by direct and/or indirect mechanisms. In support of direct eosinophil–bacteria interactions, gastrointestinal eosinophils express various receptors that can interact with pathogens, and in vitro studies have shown that different bacterial species, both Gram-positive and Gram-negative, can induce significant human eosinophil migration[57]. For example, *N*-formyl oligopeptides (such as *N*-formylmethionyl-leucyl-phenylalanine (fMLP)) present in Gram-negative bacteria can induce eosinophil migration[58]. Interestingly, eosinophils express the fMLP receptor formyl peptide receptor 1 (FPR1)[59], and intratumoural eosinophils in CRC upregulate FPR2, another receptor for fMLP[42].

CCL3, CCL5 and CCR1 signalling

An additional pathway for eosinophil recruitment in the TME could be dictated by the chemokines CCL3 and CCL5, which are CCR1 ligands. 4-Nitroquinoline-1-oxide (4NQO)-induced tongue tumours in *Ccl3*$^{-/-}$ and *Ccl5*$^{-/-}$ mice had reduced eosinophil infiltration and a concomitant decreased incidence of tumours in comparison with wild-type and *Ccr1*$^{-/-}$ mice[60] (FIG. 1). Interestingly, CCR1 negatively regulated eosinophil infiltration to the liver in experimental models of liver metastasis of colon cancer cells[61].

Controversial or pleiotropic?

Infiltration of eosinophils into the TME (often termed tumour-associated tissue eosinophils) has been reported in multiple tumours, including various lymphomas, breast, ovarian, uterine, bladder, lung, pancreatic, gastric, oesophageal and colorectal tumours, as well as head and neck squamous cell carcinomas (TABLE 1). Whereas the extent of eosinophil infiltration can vary widely in different tumour types and even within a given tumour, tumour-infiltrating eosinophils often comprise a substantial proportion of the immune infiltrate of the TME[2]. Of note, the methodology for identification of eosinophils within a given TME should be considered when assessing eosinophil involvement in the tissue (BOX 2). The presence of tumour-infiltrating eosinophils and/or peripheral blood eosinophilia has been associated with both good and poor prognosis[42,45] (TABLE 1), resulting in controversy regarding the perceived role of eosinophils in the TME. Whereas some of this controversy can be explained by variability in the staining technique (for example, haematoxylin and eosin staining versus immunohistochemical staining of eosinophil granule proteins) and/or other technical differences between studies[62,63], there is no physiologic reason to assume that eosinophils within a given microenvironment will be instructed towards a single and rigid end-stage effector function. In fact, gene expression profiling of intratumoural eosinophils obtained from adenomas of mice with colitis-associated cancer versus normal colonic eosinophils revealed a heterogeneous transcriptome signature between eosinophils in tumours from different mice[42], which indicates functional heterogeneity and plasticity in the eosinophil lineage[2,3]. This concept is now widely accepted for other myeloid immune cells, such as macrophages and neutrophils[64–66], which can display opposing functions as a consequence of their activation status.

In further support of this notion in the context of eosinophils, two distinct mouse eosinophil populations — namely, tissue-resident eosinophils (CD125^{int}SiglecF^{int}CD62L$^+$CD101^{low}, where int means intermediate levels of marker expression) and recruited eosinophils

Damage-associated molecular patterns
Host cell-derived biomolecules that can be recognized by pattern recognition receptors to initiate inflammatory responses.

Type 2 T helper (T_H2) cell immune responses
T_H2 cell responses involve production of cytokines, such as interleukin-4 (IL-4), which stimulate antibody production. T_H2 cytokines promote secretory immune responses of mucosal surfaces to extracellular pathogens and allergic reactions.

($CD125^{int}Siglec\text{-}F^{hi}CD62L^{-}CD101^{hi}$) — have been described in experimental asthma[67]. Transcriptome profiling and depletion experiments revealed that resident eosinophils displayed a regulatory phenotype and were able to inhibit the maturation of allergen-loaded dendritic cells (DCs), whereas recruited eosinophils promoted a T_H2 cell immune response. The same authors described differing eosinophil phenotypes in normal human lung

Table 1 | **Prognostic value associated with eosinophilia in various tumours**

Cancer type	Tissue	Clinical correlation	Prognosis
Bladder	Blood	Pro-tumorigenic	Risk of bladder cancer was increased with mild eosinophilia[142]
	Tumour	Pro-tumorigenic	Higher numbers of tumour-infiltrating eosinophils were observed in recurring urothelial carcinomas[143]
	Tumour	Antitumorigenic	Patients with eosinophilia in transitional (but not squamous) cell carcinomas displayed better prognosis than those without[144]
Breast	Blood	Antitumorigenic	Improved breast cancer-specific survival in patients with high relative eosinophil count[145]
	Blood	Antitumorigenic	Patients with low eosinophil counts displayed increased risk of recurrent disease compared with patients with normal or high eosinophil counts[146]
Cervical	Tumour	Pro-tumorigenic	Eosinophils were associated with tumour invasion[147]
	Tumour	Pro-tumorigenic	Tumour-infiltrating eosinophils served as an independent parameter, predicting worse overall survival in patients with tumour-negative lymph nodes and a tumour-negative resection margin[148]
Colorectal	Tumour	Antitumorigenic	High tumour stromal eosinophil score was inversely correlated with a decreased risk for all-cause and colorectal cancer death[149]
	Tumour	Antitumorigenic	High eosinophil counts were associated with a significantly better prognosis that is independent of staging, vascularization, p53 expression and histologic grade[150]
	Tumour	Antitumorigenic	Carcinomas with more than 30 eosinophils/mm^2 were associated with fewer metastases and an increased survival rate (independent of metastasis)[151]
Oesophageal	Tumour	Antitumorigenic	Number of tumour-associated eosinophils was higher in cases without venous invasion, lymph node metastasis or clinical recurrence[152]
	Tumour	Antitumorigenic	Tumour-infiltrating eosinophils were associated with increased survival[153]
Gastric	Tumour	Antitumorigenic	Amount of eosinophilic infiltration was associated with metastatic or unresectable tumours. Eosinophilic infiltration had a favourable prognostic value for overall survival[154]
	Tumour	Antitumorigenic	Eosinophilic infiltration was recognized more frequently in poorly differentiated than in well-differentiated adenocarcinomas. Higher levels of eosinophil infiltration were associated with better survival rates[155]
Hodgkin lymphoma	Tumour	Pro-tumorigenic	Increased tissue eosinophilia was the strongest prognostic factor for poor overall survival and shorter time to treatment failure[156]
	Tumour	Pro-tumorigenic	Patients with marked eosinophil infiltration had worse disease-free survival[157]
Larynx	Tumour	Pro-tumorigenic	Elevated eosinophil count was associated with tumour invasion[158]
	Tumour	Antitumorigenic	Patients with tumour eosinophilia displayed improved survival compared with patients with little to no tumour eosinophils[159]
Melanoma	Blood	Antitumorigenic	Melanoma patients with eosinophilia at any point in their course of disease showed a trend towards longer survival independent of their therapy[105]
Oral	Tumour	Antitumorigenic	Patients with higher numbers of tumour-associated eosinophils had a better prognosis than patients with intermediate or low counts[160]
	Tumour	Antitumorigenic	Increased numbers of eosinophils are found in non-metastatic carcinoma patients in comparison with metastatic carcinoma patients[161]
	Tumour	Pro-tumorigenic	Recurrence was significantly associated with an intense degree of tumour eosinophilia[162]
Ovarian	Blood	Pro-tumorigenic	Higher eosinophil count was predictive of both recurrence and mortality[163]
Liver	Blood	Antitumorigenic	Peripheral blood eosinophils were associated with increased survival[164]
	Blood	Antitumorigenic	Postoperative maximum number of eosinophils after hepatectomy was significantly associated with overall survival in patients with cholangiocarcinoma[165]

Box 2 | **Methods of eosinophil detection in pathological samples**

Cytological staining
Sirius Red, Congo Red and modified haematoxylin and eosin (H&E) are all practical options for detection of eosinophils, staining their nuclei blue and their granules bright red[166]. Alternatively, Fast Green and Neutral Red staining will mark the nuclei of eosinophils red and their granules bright green[167]. However, these methods only allow the detection of intact eosinophils and, as such, might lead to under-recognition of degranulated eosinophils, which may play an essential role in the tumour microenvironment (TME)[42].

Immunostaining
Eosinophil-specific granule proteins, such as major basic protein (MBP)[168] and eosinophil peroxidase (EPX)[169], can be detected by immunohistochemistry and/or immunofluorescence. This strategy provides two main advantages. First, it is more sensitive than cytologic staining methods. Second, it enables the detection and quantitative assessment of eosinophil degranulation, which is observable even in the absence of intact infiltrating eosinophils[169].

Electron microscopy
Although immunohistochemical staining may provide valuable insight into eosinophil degranulation in vivo, the only technique that can clearly identify and distinguish between different modes of eosinophil degranulation is ultrastructural analysis by transmission electron microscopy[170].

Reporter mice
Insertion of Cre recombinase in the open reading frame of *Epx* allows the development of a knock-in strain of mice (termed eoCRE) in which knockout or expression of a gene of interest is restricted to eosinophils. This mouse line is capable of mediating recombination of 'floxed' reporter cassettes in 95% of peripheral blood eosinophils[171].

Flow cytometry
Using recognized eosinophil cell surface markers[4,10], flow cytometry can be used to detect intact eosinophils that are recovered after tissue processing. However, this method does not permit cell localization within the organ or tumour, which is highly relevant in the TME.

parenchyma compared with asthmatic sputum (defined as Siglec-8$^+$CD62L$^+$IL-3R^{low} and Siglec-8$^+$CD62L^{low}IL-3R^{hi}, respectively), suggesting that regulatory and inflammatory eosinophils may also exist in humans[67]. Deciphering the extent of eosinophil heterogeneity in a given TME and linking eosinophil phenotypes to the diverse molecular pathways governing their recruitment, activation status and role in specific tumours is one of the challenges of eosinophil research in cancer.

Antitumorigenic roles of eosinophils

An antitumorigenic role for eosinophils has been demonstrated in various in vitro studies. Activated eosinophils, via secretion of IL-10 and IL-12, inhibited the growth of prostate cancer cells in vitro and increased the expression of the adhesion molecule E-cadherin, which may suppress metastatic seeding[68]. Furthermore, direct eosinophil-mediated cytotoxicity has been observed in co-culture studies of mouse or human eosinophils with mastocytoma cells (P815 cells)[69], Epstein–Barr virus-infected B cell lines (721.221 cells)[69], hepatocellular carcinoma cells (MH134 cells)[70], fibrosarcoma (MCA cells)[35], melanoma (HBL and B16-F10 cells)[71,72] and CRC cells (MC38, CT26, SW480, HBL and Colo-205 cells), but not L428 Hodgkin lymphoma cells[42,72,73]. Importantly, several mediators could augment eosinophil-mediated killing, including IFNγ, IL-5, IL-33 and CCL11 (REFS[35,42,70,71]), consistent with a role for eosinophil-derived secreted factors in eosinophil tumour cytotoxicity. The fact that eosinophil-mediated killing of Colo-205 cells was accompanied by the release of TNF, ECP, EDN and granzyme A (FIG. 2) further supports this hypothesis[72,73].

The ability of eosinophils to recognize and bind tumour cells appears to be important for their cytotoxic effects. Cytotoxicity towards Colo-205 cells was regulated by IL-18, which promoted adhesion of eosinophils to tumour cells by upregulating the integrin lymphocyte function-associated antigen 1 (LFA1) and intracellular adhesion molecule 1 (ICAM1)[73]. Interestingly, eosinophils from allergic donors were more cytotoxic towards Colo-205 cells than eosinophils from normal donors or patients with hypereosinophilic syndrome. This functional heterogeneity may be related to increased LFA1 expression and binding capabilities or differences in eosinophil activation[72]. Stimulation of eosinophils with IL-33 increased expression of CD11b and ICAM1, which were required for eosinophil adhesion to MC38 and B16-F10 cells, eosinophil degranulation and tumour cell cytotoxicity[71,74,75]. Eosinophil cytotoxicity following stimulation with IL-33 was associated with polarization of ECP, EPX and granzyme B to the surface membrane[74], suggesting the formation of an active degranulating synapse. This may occur through Fc receptor expression[76] and Fc receptor-bound antibodies targeting tumour antigens as well as natural killer (NK) cell-associated receptors such as 2B4, NKG2D and, perhaps, members of killer cell lectin-like receptor subfamily A (LY49 family), all of which can mediate eosinophil cytotoxic effects in vitro[69,70] (FIG. 2). Finally, eosinophils were able to infiltrate MCF-7 breast cancer cell spheroids and tightly bind tumour and endothelial cells in a tri-cell tumour spheroid model[77].

In vivo studies assessing eosinophil activities in cancer can be broadly divided into two types: studies assessing the physiological activities of eosinophils; and studies examining eosinophil responses to tumour cells engineered to secrete cytokines and/or chemokines (for example, IL-4 and CCL11). Although data from the latter need to be interpreted cautiously (BOX 1), these have demonstrated that, under specific settings, eosinophils can prevent tumour formation.

Experimental models using intravenous injections of B16-F10 melanoma cells, engineered to express chicken ovalbumin (OVA) as a surrogate tumour antigen, demonstrated clearance of lung and visceral metastases that was dependent on CD4$^+$ T_H2 cell secretion of IL-5 (REF.[78]). Metastatic clearance was also dependent on CCL11 expression and the presence of degranulating eosinophils[78]. Importantly, no tumour cell lysis was observed in eosinophil and tumour cell co-culture experiments using naive eosinophils or eosinophils stimulated with supernatants of OVA-stimulated T_H1 or T_H2 cells, with or without CCL11. Thus, the factors in the TME that instruct eosinophil degranulation and subsequent tumour cell lysis remain unclear in this model. In addition to CD4$^+$ T cell-derived IL-5, ILC production of IL-5 has been implicated in the antitumorigenic activities of eosinophils[38]. Intravenous injection of B16-F10 cells resulted in colonization of the lungs, which was accompanied by increased levels of IL-5-producing ILCs. Neutralization of IL-5 or injection of B16-F10 cells into *Il5*$^{-/-}$ mice decreased eosinophilic infiltration

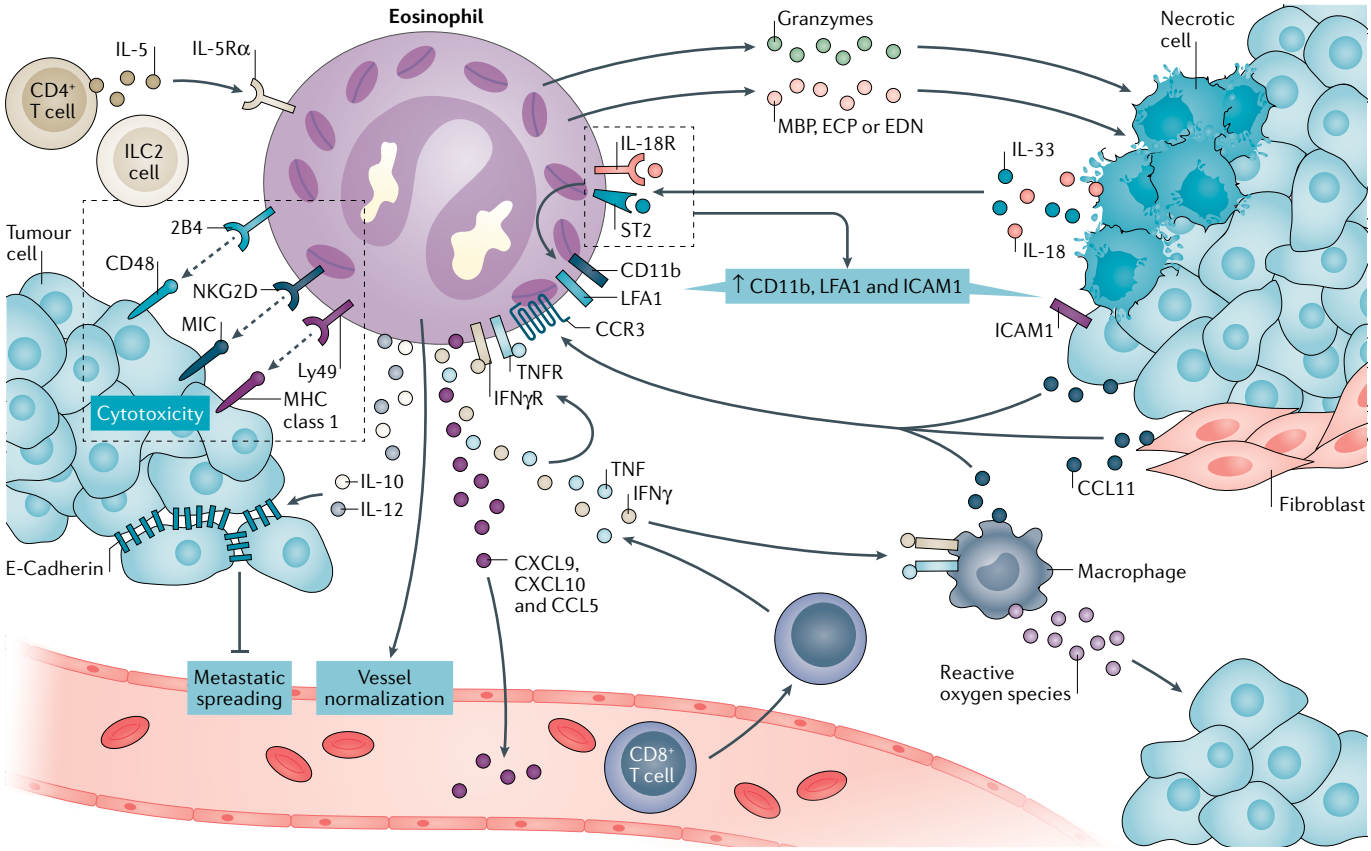

Fig. 2 | **Antitumorigenic activities of eosinophils.** Eosinophils mediate antitumour responses via direct and indirect mechanisms. CD4$^+$ T cell-derived and type 2 innate lymphoid cell (ILC2)-derived interleukin-5 (IL-5) enhances eosinophil survival and activation via IL-5 receptor-α (IL-5Rα). Furthermore, IL-18 and IL-33 enhance the expression of lymphocyte function-associated antigen 1 (LFA1) and CD11b on eosinophils and intracellular adhesion molecule 1 (ICAM1) on tumour cells to facilitate cell–cell recognition and adhesion. In response to IL-5, IL-33, CC-chemokine ligand 11 (CCL11), interferon-γ (IFNγ) and tumour necrosis factor (TNF), eosinophils are capable of secreting cytotoxic proteins, such as major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN) and granzymes, which can induce tumour cell death. Moreover, eosinophil-derived IL-12 and IL-10 can decrease metastatic migration of tumour cells by enhancing E-cadherin expression on tumour cells and strengthening their adhesion. Eosinophils also express natural killer (NK) cell-associated activation receptors, such as 2B4, NKG2D and LY49, which can bind several ligands on tumour cells, including major histocompatibility (MHC) class I, MHC class I chain-related proteins A and B (MIC) and CD48, that can induce cytotoxicity towards tumour cells. Indirectly, eosinophils promote antitumour immunity by releasing IFNγ, which acts in an autocrine fashion or in combination with CD8$^+$ T cell-derived IFNγ to secrete CXC-chemokine ligand 9 (CXCL9) and CXCL10 that induce CD8$^+$ T cell migration and subsequent cytotoxicity. In addition, IFNγ-activated and TNF-activated eosinophils support the polarization of macrophages towards an antitumorigenic phenotype. Finally, eosinophils support antitumour immunity by normalization of the vasculature. CCR3, CC-chemokine receptor 3; IFNγR, interferon-γ receptor; TNFR, TNF receptor.

and enhanced tumour growth[38]. IL-5 involvement in the antitumorigenic activities of eosinophils has also been demonstrated in fibrosarcoma and hepatocellular carcinoma mouse models. The incidence and growth of methylcholanthrene-induced fibrosarcomas were attenuated in hypereosinophilic *Il5*-overexpressing (*Il5*^{Tg}) mice[35]. Conversely, tumour growth was markedly increased in *Ccl11*$^{-/-}$ mice, *Il5*$^{-/-}$;*Ccl11*$^{-/-}$ mice and eosinophil-deficient ΔdblGATA mice[35]. Similarly, *Il5*^{Tg} mice, but not wild-type mice, implanted with MH134 hepatocellular carcinoma cells overexpressing CCL11 displayed decreased tumour cell growth[70]. Administration of anti-IL-5Rα antibodies resulted in decreased eosinophil levels and increased tumour cell growth[70]. Finally, CCL11-secreting mouse sarcoma cells (MS-4 cells) were capable of recruiting eosinophils, limiting angiogenesis and promoting tumour destruction in vivo[79]. Importantly, in mice, IL-5 may affect additional cells, especially B cells[80]. Thus, data that are based on modulation of this pathway in mice should be interpreted with caution.

Eosinophil recruitment, prolonged survival and degranulation have been demonstrated in both human and mouse models of CRC[42]. Unbiased RNA sequencing and proteomic analyses of sorted intratumoural mouse eosinophils revealed a signalling signature involving interferon-dependent transcripts. Consistently, activation of human and mouse eosinophils with IFNγ (but not TLR ligands) in vitro potentiated eosinophil-mediated killing of CRC cells. Finally, cytokine neutralization and cell depletion experiments demonstrated that eosinophil survival and antitumorigenic activities were independent of IL-5 and CD8$^+$ T cells, respectively[42].

Although the physiologic antitumorigenic role of eosinophils in CRC was independent of CD8$^+$ T cells, increasing data suggest that important crosstalk exists between these two cell types. Depletion of regulatory

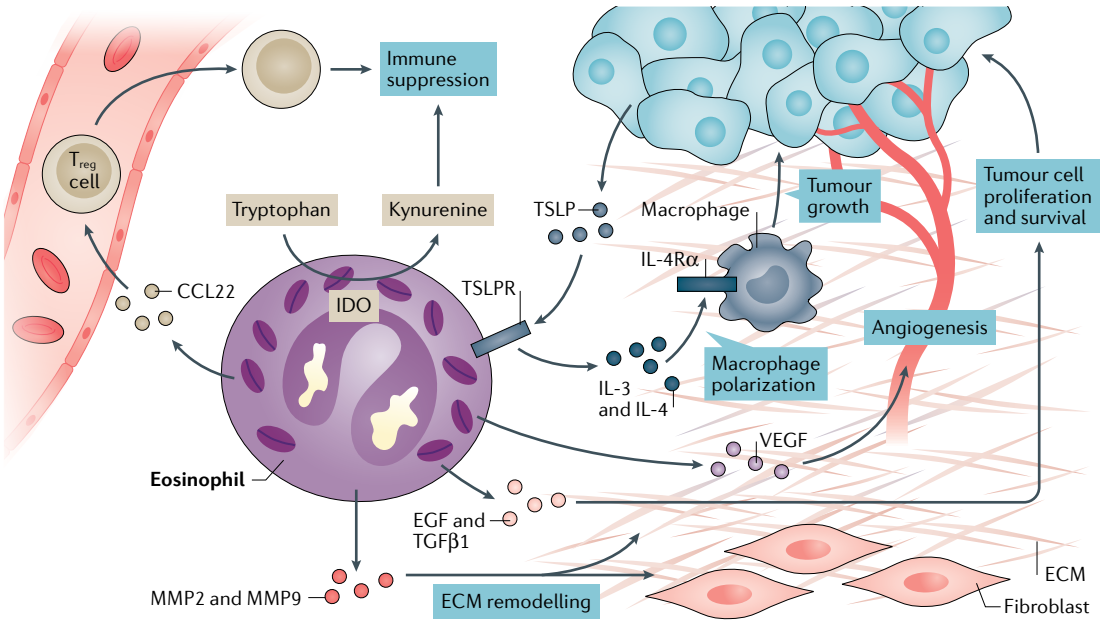

Fig. 3 | **Pro-tumorigenic activities of eosinophils.** Eosinophils can promote tumour growth via various mechanisms. Eosinophil-derived CC-chemokine ligand 22 (CCL22) can facilitate the migration of immune-suppressive regulatory T (T_{reg}) cells to the tumour microenvironment. In addition, eosinophils express indoleamine 2,3-dioxygenase (IDO), which catalyses the oxidative degradation of L-tryptophan through the kynurenine pathway, resulting in inhibition of effector T cell responses and induction of suppressive immunity. Furthermore, tumour cell-derived thymic stromal lymphopoietin (TSLP) increases the expression of interleukin-4 (IL-4) and IL-13 in eosinophils, and IL-13 (likely IL-4 as well) can polarize macrophages into a tumour-promoting activation state characterized by an immunosuppressive phenotype. Tumour cell-derived TSLP also increases the expression of vascular endothelial growth factor A (VEGFA), which induces angiogenesis. Eosinophils may synthesize and release a plethora of other growth factors, such as epidermal growth factor (EGF) and transforming growth factor-β1 (TGFβ1), which can induce tumour cell growth and epithelial mesenchymal transition, respectively. In addition, eosinophils induce matrix remodelling by secretion of matrix metalloproteinases, including MMP2 and MMP9, which could also facilitate metastatic seeding. ECM, extracellular matrix; IL-4Rα, IL-4 receptor-α; TLSPR, TLSP receptor.

T (T_{reg}) cells resulted in eosinophil-dependent tumour rejection owing to the ability of eosinophils to attract $CD8^+$ T cells via expression of CCL5, CXC-chemokine ligand 9 (CXCL9) and CXCL10[81]. Adoptive transfer of $CD8^+$ T cells alone failed to induce tumour rejection, whereas co-transfer of T cells with TNF-activated and IFNγ-activated eosinophils led to substantial infiltration of T cells and antitumour immunity[81]. Eosinophils also normalized the vasculature and promoted a TME characterized by increased IFNγ and TNF, which stimulated the activation of pro-inflammatory macrophages. A collective view of these data[42,81] suggests that IFNγ and IFNγ-induced pathways act as key regulators of antitumorigenic activities of eosinophils, which can promote both direct and indirect tumour rejection (FIG. 2).

Pro-tumorigenic roles of eosinophils

Several lines of evidence suggest that eosinophils have indirect pro-tumorigenic activities in the TME through regulation of immune cell composition and/or activity, secretion of growth factors and matrix metalloproteinases, and promotion of angiogenesis (FIG. 3). Tumour-derived thymic stromal lymphopoietin (TSLP) can activate eosinophils and increase their expression of IL-4, IL-5, IL-10 and IL-13, promoting the proliferation of cervical cancer cells[82]. Additionally, co-culture of cervical cancer cells and eosinophils resulted in increased eosinophil IL-8 and vascular endothelial growth factor A (VEGFA) production that stimulated angiogenesis of human umbilical vein endothelial cells in vitro[83].

Eosinophils and IL-5 also play a role in human and mouse models of malignant pleural effusions (MPEs)[84]. In an experimental model of MPEs in immunocompetent mice, MPE formation was markedly decreased in *Il5*$^{-/-}$ mice injected intrapleurally with Lewis lung carcinoma (LLC) or MC38 colon carcinoma cells[84]. This was associated with decreased levels of eosinophils and myeloid-derived suppressor cells (defined as $Gr\text{-}1^+CD11b^+$) in the blood and MPEs[84]. These findings were substantiated by neutralization of IL-5, which limited MPE formation, and exogenous delivery of IL-5 in wild-type mice, which promoted the formation of MPEs[84]. In a different model, intravenous injection of tumour cells resulted in an early and transient induction of IL-5 levels in the lung (but not peripheral blood)[37]. Neutralization of IL-5 decreased metastatic lung colonization of various tumour cells, including lung cancer, melanoma and CRC cells, whereas exogenous delivery of IL-5 and adoptive transfer of eosinophils into *Il5*$^{-/-}$ mice promoted lung metastasis[37]. Mechanistically, *Il5*$^{-/-}$ mice displayed decreased levels of CCL22 and T_{reg} cells[37] (FIG. 3). Interestingly, in the aforementioned

mouse model, eosinophils facilitated the colonization of lung metastases, but did not impact the formation and growth of primary tumours[37], implying that the activities of eosinophils are dependent, at least in part, on the specific anatomical site in which they reside and/or are recruited. In humans, epidemiological studies show a link between increased expression of IL-5 in breast carcinomas and higher rates of distant metastasis and poor prognosis[85]. Whether expression of IL-5 is associated with poor prognosis in additional solid tumours remains to be defined as IL-5 may have opposing effects on eosinophils in the TME.

Although clinical data suggest that eosinophils are associated with both good and poor prognosis in oral squamous cell carcinoma[86], experimental models have confirmed a primarily pro-tumorigenic role. Depletion of eosinophils in hamsters using anti-IL-5 antibodies reduced the tumour burden and incidence following treatment with the carcinogen 7,12-dimethylbenz(a) anthracene (DMBA)[87]. Similarly, administration of the carcinogen 4NQO in the absence of eosinophils was associated with a decreased occurrence of oral squamous cell carcinoma with lower cytological atypia[87]. Moreover, 4NQO-treated *Ccl3*$^{-/-}$ and *Ccl5*$^{-/-}$ mice displayed decreased eosinophilia and decreased incidence of tongue tumours compared with wild-type mice. The attenuated cytomorphological atypia and reduced tumour cell proliferation were associated with reduced transcript expression of growth factors (for example, those encoding EGF, transforming growth factor-β1 (TGFβ1) and VEGFA) and matrix metalloproteinases (for example, those encoding MMP2 and MMP9)[60]. Indeed, MMP9 and vimentin-positive eosinophils have been demonstrated in experimental models of colon cancer metastasis to the liver[61]. In this model, eosinophils expressed MMP9 and cooperated with monocytes, neutrophils and fibrocytes to promote colonization of metastatic cells (FIG. 3).

Multiple studies have shown that eosinophils can store and release a plethora of growth factors, including EGF, fibroblast growth factors (FGFs), nerve growth factor (NGF), TGFβ1, platelet-derived growth factor (PDGF) and VEGFA, as well as S100 family members, such as S100A8 and S100A9 (REF.[4]). Eosinophils infiltrate hypoxic tumour regions[54] and can potentially promote angiogenesis. Freshly isolated human eosinophils and eosinophil culture supernatants can directly induce endothelial cell proliferation and promote angiogenesis in the chick chorioallantoic membrane and rat aortic ring assays[88]. Additionally, EPX stimulated the growth, survival and metastasis of mammary tumours in mice, which was associated with increased collagen deposition and neovascularization within the primary tumour[89]. In fact, HER2 (also known as ERBB2) was shown to serve as a receptor for EPX, and EPX induced HER2-dependent upregulation of cell proliferation, as indicated by upregulation of the proliferation marker Ki67, and activation of focal adhesion kinase (FAK), ERK1, ERK2 and β1 integrin[90,91].

Eosinophils can promote glioblastoma cell growth in vitro[92]. Furthermore, eosinophil-derived TGFβ1 has been linked with epithelial growth, fibrosis and tissue remodelling[2,93]. Despite these data, it is important to note that causal tumour-promoting effects have not been demonstrated for eosinophil-derived growth or angiogenic factors in in vivo cancer models.

One way eosinophils can promote tumour growth is by secretion of IL-4 and IL-13 that may shape macrophage polarization in the TME. Orthotopic transplantation of various tumour cell lines, including LLC, B16 and mouse thymoma EG7 cells, into mice lacking the TNF receptor (*Tnfr*$^{-/-}$; also known as *Tnfrsf1a*$^{-/-}$ mice) caused increased expression of IL-13, accumulation of eosinophils and induction of hallmark M2 macrophage signature transcripts[94]. Intracellular flow cytometric analysis revealed that IL-13 expression was relatively restricted to the infiltrating eosinophils. Furthermore, neutralization of IL-13 in tumour-bearing *Tnfr*$^{-/-}$ mice partially reduced expression of an M2 macrophage transcript signature. These data demonstrated that eosinophil-derived IL-13 could serve as a factor provoking M2 macrophage polarization in the TME. An additional mechanism by which eosinophils can foster a suppressive TME is through production of indoleamine 2,3-dioxygenase (IDO), a central factor in tumour-induced tolerance. Eosinophil-derived IDO has been proposed to maintain the imbalance between T_H1 cells and T_H2 cells in allergic asthma[95]. Recently, IDO-expressing eosinophils were identified in human non-small-cell lung carcinoma; their presence inversely correlated with overall survival, suggesting a pro-tumorigenic role in this context[96].

Eosinophils in cancer therapy

Recently, eosinophils have been highlighted as potential cellular biomarkers and even end-stage effector cells in cancer therapy[97–100]. The following sub-sections will focus on eosinophil activities in response to ICB and cytokine immunotherapy. We will then discuss potential therapies that may specifically target eosinophils.

Eosinophils in ICB

Pharmacological blockade of immune checkpoints has provided one of the most promising avenues in cancer immunotherapy. These molecules mainly govern T cell activities and include cytotoxic T lymphocyte-associated protein 4 (CTLA4, inhibited by ipilimumab), PD1 (inhibited by pembrolizumab, nivolumab and cemiplimab-rwlc) and PDL1 (inhibited by atezolizumab, avelumab and dervalumab)[101,102]. However, not all patients with cancer respond to ICB. Consequently, there is an urgent need to identify predictive biomarkers and/or accessory mechanisms that forecast clinical response and enable development of better therapeutic agents.

The absolute eosinophil count (AEC) was positively correlated with overall survival in 209 patients with stage IV melanoma treated with at least one dose of ipilimumab[103]. Moreover, accumulating data suggest that an early increase in the eosinophil count following ICB is also associated with improved survival. In one study, AECs after the first infusion of ipilimumab were increased in responding patients with stage IV melanoma compared with non-responders[100]. In a second study, increased AECs during ipilimumab treatment for metastatic melanoma were correlated with longer survival rates[104]. A positive association between eosinophilia and disease outcome has also been reported with

Absolute eosinophil count
A clinical and pathological measurement that is used to define eosinophil numbers by calculating the percentage of peripheral blood eosinophils multiplied by the total white blood cell count.

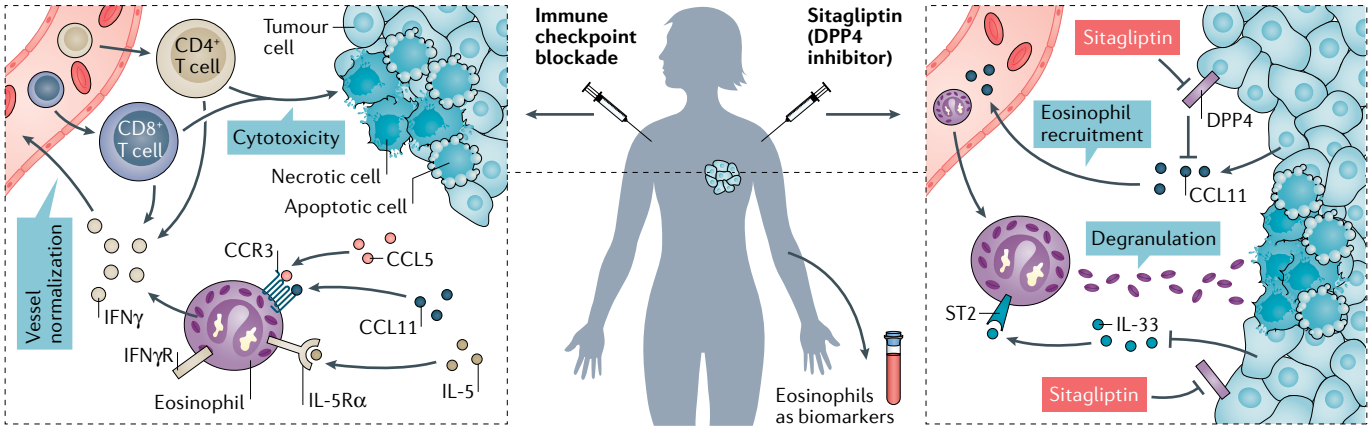

Fig. 4 | **Eosinophils as biomarkers or therapeutic targets of emerging therapies.** Eosinophils have been implicated as potential cellular biomarkers and/or end-stage effector cells in various forms of cancer therapies. In response to immune checkpoint blockade therapy, increased relative eosinophil and absolute eosinophil counts in the blood have been associated with better prognosis, including response to therapy and long-term survival. Mechanistically, anti-cytotoxic T lymphocyte-associated protein 4 (anti-CTLA4) treatment increased the infiltration of eosinophils into tumours. Infiltration of eosinophils was dependent on $CD4^+$ T cells and $CD8^+$ T cells, which both expressed intereukin-5 (IL-5), CC-chemokine ligand 11 (CCL11) and CCL5. Moreover, eosinophil-derived and T cell-derived interferon-γ (IFNγ) promotes vessel normalization, which is associated with response to anti-CTLA4 treatment. Dipeptidyl peptidase 4 (DPP4) is a type II transmembrane protein capable of cleaving various chemokines, including CCL11. Therapeutic blockade of DPP4 using sitagliptin results in increased expression of CCL11 and subsequent accumulation and degranulation of eosinophils in the tumour microenvironment leading to decreased tumour burden. In addition, sitagliptin can induce the expression of tumour-derived IL-33, which is required for increased expression of CCL11 and induction of eosinophil-dependent antitumour immunity. CCR3, CC-chemokine receptor 3; IFNγR, interferon-γ receptor; IL-5Rα, IL-5 receptor-α.

pembrolizumab treatment in metastatic melanoma[105], nivolumab treatment of classical Hodgkin lymphoma[106] and pembrolizumab treatment of patients with stage IV melanoma[107]. Likewise, all patients with stage IV or unresectable stage III melanoma who responded to a combination of intratumoural IL-2 treatment and PD1 inhibition displayed a profound increase in AEC[108].

Whereas the eosinophilia observed following ICB could reflect a generalized allergic response promoted by ICB, such as anti-CTLA4 (REF.[109]), recent experimental data suggest that eosinophils play an active role in the antitumour response following ICB treatment. In experimental models of breast cancer, anti-CTLA4 therapy led to intratumoural eosinophil accumulation that was dependent on $CD4^+$ and $CD8^+$ T lymphocytes expressing increased levels of IL-5, CCL5 (in $CD8^+$ T cells) and CCL11 (in $CD4^+$ T cells), and on IFNγ[110] (FIG. 4). Furthermore, IFNγ production by eosinophils was essential for anti-CTLA4-induced vessel normalization, and the pharmacological depletion of eosinophils attenuated the inhibition of tumour growth by anti-CTLA4 therapy[110]. Interestingly, the macrophage-derived, IFNγ-dependent chemokines CXCL9 and CXCL10 were critical for the antitumour immune response following dual blockade of PD1 and CTLA4, consistent with a requirement for accessory myeloid cells in this process[111]. Finally, implantation of a decellularized biologic scaffold with syngeneic B16-F10 melanoma cells in mice created an immune microenvironment that suppressed tumour formation in a $CD4^+$ T cell-dependent and macrophage-dependent fashion[112]. Tumour growth inhibition by PD1 and PDL1 blockade was potentiated in the scaffold immune microenvironment and was associated with eosinophilic inflammation that was dependent on lymphoid cells.

These data have uncovered exciting new roles for eosinophils as essential accessory cells in cancer immunotherapy and highlight eosinophils as potential cellular targets for future ICB therapy. Furthermore, they suggest an innovative new paradigm involving a positive feedback loop between eosinophils and T cells that results in enhanced recruitment of both cell types into the TME.

Eosinophils in cytokine treatment

Interleukin-2. The IL-2–IL-2R signalling axis plays critical roles in T cell expansion[113]. Therefore, IL-2 immunotherapy has been used to stimulate effector T cell proliferation with improved outcomes in various cancers[114]. The use of high-dose IL-2 is limited by adverse effects, including capillary leak, oedema and weight gain. Eosinophilia, driven primarily by IL-5-producing type 2 innate lymphoid cells (ILC2s), is also frequently observed[115], and eosinophil degranulation in the TME has been described following IL-2 treatment[116]. Although data supporting a role for eosinophils in the aforementioned adverse effects are lacking, several lines of evidence link eosinophil activation with the antitumour response generated by IL-2 in some settings. Expression of IL-2 in melanoma cells prevented tumour formation in nude mice, and the immune infiltrate at the site of injection was composed of macrophages, eosinophils and mast cells[117]. Furthermore, combination treatment with an antibody directed against a melanoma antigen (TA99) and an IL-2 fusion protein decreased the tumour burden in mice injected subcutaneously with B16-F10 cells[117]. Depletion of eosinophils using anti-IL-5 antibodies during the course of the TA99 and IL-2 treatment diminished therapeutic activity[117]. Moreover, the cytotoxic activity of eosinophils isolated from patients with small-cell lung cancer (SCLC) towards allogeneic

Type 2 innate lymphoid cells
Tissue-resident innate immune cells that are derived from a common lymphoid progenitor and are involved in the reaction to parasites and allergic diseases.

tumour cell lines (derived from SCLC, chronic myeloid leukaemia and melanoma) was markedly increased during IL-2 therapy in an IL-5-dependent fashion[118]. Finally, IL-2-induced eosinophilia may be mediated, at least in part, by CCL11, as suggested by a phase II trial of intraperitoneal IL-2 administration to patients with platinum-resistant or platinum-refractory ovarian cancer, in which IL-2 therapy was associated with induction of CCL11 and increased peripheral blood and peritoneal eosinophilia[119].

T_H2 cytokines. Whereas T_H2 cell immune responses in cancer have generally been associated with worse prognosis due to attenuated antitumour immunity[120], evidence suggests that promoting a T_H2 cell immune response and the subsequent recruitment of eosinophils to the TME can result in beneficial antitumour immune responses[121].

Interleukin-4. IL-4 is a 'hallmark' T_H2 cytokine that can stimulate major histocompatibility class (MHC)-restricted and MHC-unrestricted cytotoxic T cell proliferation[122]. The ability of IL-4 to promote antitumour immunity was assessed using tumour cell lines engineered to express IL-4. Injection of these cells into mice resulted in CCL11 production by non-haematopoietic cells, such as endothelial cells[123], and was associated with infiltration of eosinophils and macrophages and inhibition of tumour growth[124–126]. The antitumorigenic activities of IL-4 in immune-deficient mice lacking T cells, B cells, NK cells or mast cells were comparable to that observed in wild-type mice, but were abolished by blockade of eosinophil accumulation in the tumours[126]. Similarly, induction of IL-4 expression in rat C6 gliomas resulted in eosinophil infiltration, decreased vascularization and tumour necrosis that was independent of adaptive immunity[127]. Eosinophil-mediated tumour rejection has also been demonstrated towards IL-4-secreting D5.40 mouse mammary adenocarcinoma cells[128]. Under these experimental conditions, eosinophil infiltration and antitumour immunity were dependent on $CD8^+$ T cells but independent of IL-5.

Interleukin-33. The epithelial cell-derived cytokine IL-33 plays a fundamental role in promoting type 2 immunity in allergic responses and parasitic infections[49], and experimental data suggest that IL-33 may also be important in tumour immunity. Transgenic expression or exogenous delivery of IL-33 in metastatic models of B16-F10 melanoma and LLC, as well as in other established tumour models, resulted in increased antitumour immunity characterized by increased nuclear factor-κB (NF-κB) signalling, as well as proliferation, activation and infiltration of $CD8^+$ T cells, NK cells and eosinophils[71,129]. In the B16-F10 model, intranasal delivery of IL-33 increased CCL11 expression and ST2-dependent eosinophil recruitment into the lung, preventing pulmonary metastasis[71]. IL-33 can also delay metastatic progression of ovarian cancer to the peritoneal cavity in a mouse model. This effect was mediated, at least in part, by eosinophils[130].

Therapeutic inhibition of dipeptidyl peptidase 4 (DPP4) using sitagliptin resulted in induction of IL-33, elevation of CCL11-mediated eosinophil infiltration, degranulation and tumour cell cytotoxicity leading to reduced tumour growth in mice[131] (FIG. 4). Collectively, it appears that IL-33 can promote direct and indirect antitumorigenic activities of eosinophils. Indirectly, IL-33 can induce eosinophils to stimulate recruitment of $CD8^+$ T cells via secretion of CXCL9 and CXCL10. Directly, IL-33 can induce eosinophil degranulation and granule-dependent tumour cell cytotoxicity[131]. The precise settings in which therapeutic use of IL-33 could be used to amplify antitumorigenic activities of eosinophils requires further investigation, especially as IL-33 has been shown to have dual functions and can promote tumour growth[132].

Myeloid colony-stimulating factors. The haematopoietic cytokine granulocyte–macrophage colony-stimulating factor (GM-CSF) has profound effects on multiple immune cells and provides a link between innate and adaptive immune responses by regulating DC development and T cell activation[133]. GM-CSF has been incorporated into cancer treatment regimens, and multiple studies have evaluated the utility of GM-CSF as monotherapy and as an adjuvant in association with cancer vaccines or in combination with chemotherapy[134]. In a mouse study of implanted irradiated tumour cells engineered to secrete GM-CSF, cytokine secretion had a potent and long-lasting antitumour effect that was dependent on $CD4^+$ and $CD8^+$ T cells and involved the accumulation of eosinophils and macrophages[135]. Eosinophilia has also been correlated with GM-CSF activation of DCs in humans. Retrospective analysis of data from three phase III trials using sipuleucel-T (DCs loaded with a fusion protein containing a prostate cancer tumour antigen and GM-CSF) revealed improved prostate cancer-specific survival and antigen-specific immune responses. These immune responses included a transient increase in peripheral blood eosinophilia, which correlated with activated T cells secreting IFNγ, IL-5 and CCL17, a chemokine that is associated with T_H2 cell responses[136]. Eosinophils have also been associated with positive antitumour immune response following combination therapy using gemcitabine and docetaxel chemotherapy with or without GM-CSF and low-dose IL-2 (REF.[137]). Although eosinophils may serve as biomarkers of IL-2 response, they may also act as antitumour effector cells or as immunomodulatory cells that enhance DC recruitment and activation through secretion of granule proteins, such as EDN, which may act as an alarmin[138].

Targeting eosinophils for therapy

Over the past 20 years, numerous eosinophil-targeting therapies, including monoclonal antibodies to IL-5, IL-5Rα, eotaxins and Siglec-8, have been developed for the treatment of asthma, atopic dermatitis and other eosinophil-associated disorders (reviewed elsewhere[139]). Currently, there is no evidence to support an increased prevalence of neoplasia in recipients of eosinophil-depleting therapies[140,141]. Nonetheless, vigilance is required as many of the disorders being treated with these agents are chronic and will require lifelong therapy. The long-term effects of eosinophil depletion on the prevalence of cancer will likely be determined in the next few decades as more patients are treated with these new drug regimens.

Conclusions

In this Review we hope to raise the awareness of basic cancer research scientists and oncologists to a relatively unexplored yet important cell in the TME. Eosinophils have potent capabilities to impact local immunity and tissue remodelling during homeostasis and disease. In the TME, there is currently more evidence to suggest that eosinophils display either direct or indirect antitumorigenic activities. However, there is also evidence of tumour-promoting effects, and the precise signals and mechanisms that direct the tumour-promoting or tumour-suppressive phenotypes of eosinophils require further investigation. Assessing the roles of eosinophils in experimental models that mimic human disease, where opposing prognostic value has been attributed for eosinophils, may provide important clues to understanding their diverse nature. In addition, careful monitoring for the development of tumours in patients with chronic eosinophil-associated disorders who are receiving targeted therapies that deplete eosinophils or impair their function and/or migration is essential. Finally, bulk and single-cell RNA sequencing and proteomic methods should be exploited to help dissect the diverse phenotypic landscape of tumour-infiltrating eosinophils under physiological conditions and following therapy.

In summary, there is ample evidence suggesting that eosinophils play a role in the TME and can affect the prognosis and response to therapy in cancer. In this context, a better understanding of the mechanisms involved could provide new ways to enhance the efficacy of existing ICB and/or possibly facilitate the generation of new treatment modalities.

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