Eosinophils: The unsung heroes in cancer?

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Prolonged low-grade inflammation or smoldering inflammation is a hallmark of a cancer. Eosinophils are components of the immune microenvironment that modulates tumor initiation and progression. Although canonically associated with a detrimental role in allergic disorders, these cells can induce a protective immune response against helminthes, viral and bacterial pathogens. Eosinophils are a source of anti-tumorigenic (e.g., TNF-α, granzyme, cationic proteins, and IL-18) and protumorigenic molecules (e.g., pro-angiogenic factors) depending on the milieu. In several neoplasias (e.g., melanoma, gastric, colorectal, oral and prostate cancer) eosinophils play an anti-tumorigenic role, in others (e.g., Hodgkin’s lymphoma, cervical carcinoma) have been linked to poor prognosis, whereas in yet others they are apparently innocent bystanders. These seemingly conflicting results suggest that the role of eosinophils and their mediators could be cancer-dependent. The microlocalization (e.g., peritumoral vs intratumoral) of eosinophils could be another important aspect in the initiation/progression of solid and hematological tumors. Increasing evidence in experimental models indicates that activation/recruitment of eosinophils could represent a new therapeutic strategy for certain tumors (e.g., melanoma). Many unanswered questions should be addressed before we understand whether eosinophils are an ally, adversary or neutral bystanders in different types of human cancers.

Introduction

Eosinophils, first identified in peripheral blood and named by Paul Ehrlich in the 1870 s, are present in all classes of vertebrates and it has been estimated that they have emerged, long before the development of adaptive immunity. Human eosinophils derive from CD34+CD117+ pluripotent hematopoietic stem cells in the bone marrow, where they complete their maturation and subsequently enter the circulation where they represent ≥ 1% of leukocytes. Upon activation, these cells synthesize and release a plethora of biologically active mediators that individually have potential positive or negative effects on various target cells. Eosinophils act as sentinels of the surrounding environment, with the capacity to rapidly perceive tissue insults and initiate biochemical programs of inflammation or repair.

Eosinophils and their mediators have been canonically associated with a detrimental role in allergic diseases, but these cells can induce a protective immune response of the host against helminthes, viral and microbial pathogens. Interestingly, epidemiological and experimental studies indicate an inverse association between IgE-mediated allergies and cancer, suggesting a tumor-protective effect of IgE.

The initiation and progression of cancer are the result of multi-step processes characterized by the accumulation of driver gene mutations and epigenetic alterations. The immune system recognizes and eliminates mutant cells constantly generated. However, immune-resistant cancer cells can slip through this system and proceed to develop tumors. Normal microenvironment (innate and adaptive immune cells, fibroblasts, blood and lymphatic vessels, and extracellular matrix) maintains tissue homeostasis and is a barrier to cancer development. Incorrect signals (chemokines, cytokines, reactive oxygen species, lipid mediators, etc.) from an aberrant microenvironment alter tissue homeostasis and initiate/promote tumor growth. Smoldering inflammation is a hallmark of cancer. Several innate and adaptive immune cells, such as macrophages, mast cells, lymphocytes, neutrophils, natural killer (NK) T cells and eosinophils, are stromal components of the inflammatory microenvironment that influence the development of experimental and human tumors.

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Basic biology of eosinophils

Eosinophils represent a minority of peripheral blood leukocytes and have been erroneously neglected for decades. Immunologists and oncologists are now appreciating that these cells produce a plethora of mediators such as cationic proteins [major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil peroxide (EPX), and eosinophil-derived neurotoxin (EDN)], cytokines, chemokines, angiogenic, and lipid mediators. In addition, eosinophils can adhere to activated endothelial cells, leave the bloodstream and concentrate at inflammatory sites. Resident eosinophils in the intestine, uterus, lung, and adipose tissue are increasingly seen as homeostatic cells with critical roles in normal development and/or morphogenesis. This function is thought to occur through secretion of cytokines and growth factors such as TGF-β, involved in tissue remodelling and immune homeostasis. Moreover, human eosinophils play a major role in the modulation of a wide spectrum of innate and adaptive immune cells, including several subsets of lymphocytes, macrophages, mast cells, basophils, neutrophils, dendritic and plasma cells, epithelial and fat cells.

Interleukin-5 (IL-5) is the most important growth, differentiation, and activating factor for human eosinophils. This cytokine acts on target cells by binding to the specific IL-5 receptor (IL-5R), which consists of an IL-5 Receptor α (IL-5Rα) subunit and common receptor β subunit (βc). The βc subunit is a signal-transducing molecule shared with the receptors for IL-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF). IL-5 is mainly produced by type-2 innate lymphoid cells (ILC2), Th2 cells, mast cells, invariant NKT cells, and eosinophils themselves.

IL-5, together with IL-3, and GM-CSF, is crucial for supporting the maturation of human eosinophils in the bone marrow and GATA-1 is a transcription factor critical for eosinophils development. IL-5, along with IL-3 and GM-CSF, mediates eosinophil survival by NF-kB-induced Bcl-xL, which inhibits apoptosis. Increasing evidence indicates that IL-33 is required for basal eosinophil homeostasis. This cytokine regulates eosinophils at multiple levels: during their maturation in the bone marrow, activation of mature cells, and probably development/activation of eosinophil progenitors within tissue. In addition, IL-33 can directly activate eosinophils inducing up-regulation of the adhesion molecule CD11b and the activation marker CD69, expression of pro-inflammatory cytokines and chemokines, superoxide anion production and degranulation.

Eosinophils have the propensity to leave the bloodstream and migrate into inflamed tissues including tumor microenvironment (TME). Migration of eosinophils is mediated by the adhesion of several integrins expressed by activated eosinophils to endothelial cells. The attraction of eosinophils into inflamed tissues is mediated by eotaxins (eotaxin-1/CCL11, eotaxin-2/CCL24, eotaxin-3/CCL26), and RANTES/CCL5 that activate the CCR3 receptor highly expressed on human eosinophils. However, tissue accumulation of eosinophils not only relies on recruitment of from blood stream, but is also the result of in situ eosinophilopoiesis and self turnover in response to survival/differentiation factors produced by neighboring cells, including stem cells. Thus, local production of IL-5, IL-33 and presumably other cytokines, can increase eosinophils survival and sustain regional eosinophilopoiesis.

Eosinophil recruitment at tumor sites

Eosinophils are present into the TME of several human solid and hematological tumors and in experimental tumor models. The precise mechanisms underlying eosinophils infiltration of tumors remain largely undefined; indeed, it is a complex process that depends on a combination of cytokines, adhesion molecules and chemokines. Eotaxin-1/CCL11, eotaxin-2/CCL24, and RANTES/CCL5, produced by human solid and hematological tumors, can activate CCR3 on eosinophils. Alarmins or damage-associated molecular patterns (DAMPs) potentially triggering eosinophils recruitment include the high-mobility group box 1 protein (HMGB1), IL-1β, and IL-33. HMGB1 is a highly conserved and ubiquitously expressed protein that has both nuclear and extracellular functions in cancer. HMGB1 can be released either passively by necrotic and damaged cells or by active mechanism triggered upon immune cell activation. Once released in the extracellular space, HMGB1 can mediate inflammation, cell migration, proliferation and differentiation. Extracellular HMGB1 acts as a chemoattractant for eosinophils through the activation of toll-like receptor (TLR)-2 and TLR-4, or the receptor for advanced glycation end products (RAGE). IL-33, a member of IL-1 family, is mainly expressed by epithelial and endothelial cells and it is associated with allergic disorders, inflammation and infection. In the precursor form IL-33 is a transcriptional regulator factor but in the active form is released by stressed, damaged and necrotic cells in the extracellular space where it acts as an alarmin. The minimal IL-33 receptor (IL-33R) complex consists of IL-1R4, also known as ST2, and IL-1R3, also known as IL-1RAcP. The IL-33R complex is more sophisticated in mast cells. IL-33 is expressed in several human cancers and can attract eosinophils directly via the ST2 receptor or indirectly through the activation of ILC2 and mast cells that in turn produce IL-5. Lastly, extracellular adenosine triphosphate (ATP) is also recognized as a DAMP that is implicated in adaptive immune responses following immunogenic chemotherapy. ATP can act as a potent chemoattractant for eosinophils in vitro and in vivo activating eosinophils effector responses through binding to P2Y purinergic receptors.

Lung cancer cells can release IL-5, explaining eosinophilia occasionally associated with this tumor. Eosinophils can be recruited by vascular endothelial growth factors (VEGFs) and angiopoietin 1 (Ang1) produced by tumor and immune cells through the engagement of VEGF receptors (VEGFR-1/VEGFR-2) and Tie2, respectively expressed by human eosinophils.

Eosinophil infiltration of cancers can also be mediated by the production of chemotactic factors by tumor-infiltrating immune cells. Macrophages and mast cells can contribute to eosinophil recruitment through the production of VEGF receptors and Adenosine, produced by tumor cells, potentiates the production of angiogenic factors from human macrophages and mast cells. Moreover, histamine and PGD2, released by
controls eosinophil development in the bone marrow. Recent evidence indicates that IL-33 precedes IL-5 in regulating eosinophilopoiesis. This process of proliferation and differentiation is driven by transcription factors (e.g. GATA-1). IL-5, together with IL-3 and GM-CSF, controls eosinophil development in the bone marrow. Recent evidence indicates that IL-33 precedes IL-5 in regulating eosinophilopoiesis via the activation of the IL-33R receptor, ST2. Circulating human eosinophils selectively express IL-5Rα, CCR3, EMR1, CRTH2, and Siglec-8. Eosinophil can leave the bloodstream and target the bone marrow, liver, and spleen for elimination. In some tissues, eosinophils can be phagocytosed by macrophages. Eosinophils express a wide spectrum of integrins (α and β) and can roll and adhere to VCAM-1 and ICAM-1 on activated endothelial cells. This interaction favors the chemotactic activity of several chemokines (eotaxin-1/CCL11, eotaxin-2/CCL24, eotaxin-3/CCL26, and RANTES) that activate the CCR3 receptor highly expressed on eosinophils. This explains the propensity of eosinophils to leave the bloodstream and migrate into inflamed tissues and certain tumors. Several cells (fibroblasts, epithelial and endothelial cells, smooth muscle cells, T cells, macrophages, and eosinophils itself) are a major source of these chemokines. IL-5, IL-33 and presumably other cytokines locally produced by both immune cells (eosinophils, ILC2, T cells, and mast cells) in tumor microenvironment and by tumor cells can prolong the life span of eosinophils at site of tumor growth.

activated mast cells27 can mediate eosinophil migration through the activation of CRTH229 and H4 receptor,34 respectively. IL-4 secreted by Th-2 cells may also promote indirectly, through induction of local production of CCL11, eosinophils recruitment to the tumor site.95,96 In summary, a plethora of factors produced by cancer and immune cells can attract and/or activate eosinophils in TME.

Roles of eosinophils in cancer

The heterogeneity of different subsets of immune cells (e.g. monocytes, macrophages, T helper cells, mast cells, neutrophils, NK, NKT cells),97–99 their plasticity26,99–101 and their reciprocal interactions28 have complicated the comprehension of the role of the TME in tumor initiation and development.26 Despite eosinophils are readily identified by their specific morphology and staining characteristics, their phenotype, function and morphology can change upon induction of inflammation and aberrant microenvironment,35,102,103 including the TME.104 Several studies have attempted to identify the contributory functions of eosinophils in tumor growth control. In the majority of human and murine experimental studies, eosinophils appear to play an anti-tumorigenic role (Table 1). In clinical cancers, the presence of eosinophils at either tumor site or in peripheral blood is a favourable prognostic factor for most cancers, although evidence for a pro-tumorigenic role for eosinophils is reported (Table 2). Only few studies reported a non-contributing role of eosinophils in experimental105 and human tumors.106,107 We performed a retrospective analysis exploiting public repository databases of microarray data from cancer patients biopsies (GEO, https://www.ncbi.nlm.nih.gov/geo/) in order to correlate the expression levels of Siglec-8, EPX and Charcot-Leyden crystal galectin (CLC/Galectin-10), three eosinophil-specific markers108–110 in function of the survival rate in different types of cancer. To this aim, we used SurvExpress (http://bioinformatica.mty.itesm.mx/SurvExpress), an online biomarker validation tool to correlate multiple gene expression data to cancer patients survival rate.111 These findings evidence an anti-tumoral role of eosinophils in most cancer types analyzed (skin melanoma, colorectal, breast and gastric), a pro-tumoral action in other tumors (blood, lung and ovarian) and no effect in brain and bladder cancer (Fig. 2, Supplementary Table 1).

In several human tumors such as gastric,112,113 colorectal,114–116 nasopharyngeal,117 oral,118,119 laryngeal120 and breast cancer,121 eosinophils appear to be anti-tumorigenic. By contrast, in Hodgkin’s lymphoma122,123 and cervical cancer,124 infiltration of eosinophils is associated with poor prognosis. In addition, blood eosinophilia has been associated with positive response to immunotherapy and is generally correlated with longer survival in advanced melanoma patients, whereas it is an unfavorable prognostic factor in T-cell leukemia/lymphoma.128 These apparently conflicting results are intriguing and suggest that the role of eosinophils and their mediators in human tumors could be cancer-specific. Several initial mouse studies have implied eosinophils in anti-cancer responses, although a clear role for these cells was not directly demonstrated.95,105,129 In experimental studies a protective role of eosinophils was found in several tumors such
as colon carcinoma,\textsuperscript{130,131} melanoma,\textsuperscript{53,95,132,133} Hodgkin’s lymphoma,\textsuperscript{134} hepatocellular carcinoma,\textsuperscript{135} prostate cancer\textsuperscript{136} and fibrosarcoma.\textsuperscript{137} Thus, studies addressing potential functions of eosinophils in experimental and human tumors have provided conflicting results.\textsuperscript{138–140}

Two recent studies have examined the role of eosinophils and their mediators in tumor initiation and rejection. Carretero and collaborators have elegantly demonstrated that eosinophils produce several chemokines (CCL5, CCL9, CXCL10) that are essential for the attraction of CD8$^+$ T cells in TME in a model of melanoma.\textsuperscript{132} Moreover, eosinophils favor macrophage M1 skewing through the production of IFN-$\gamma$ and TNF-$\alpha$. M1 macrophages amplify Th1 responses, providing a positive loop in the anti-tumor response.\textsuperscript{141} In addition, M1 macrophages produce nitric oxide (NO), reactive oxygen species (ROS), IL-1$\alpha$, and TNF-$\alpha$, which are important components of the anti-tumor arsenal. Carretero et al. also demonstrated that eosinophils contribute to normalization of tumor vasculature, thus promoting tumor rejection. Lucarini and collaborators have investigated the role of IL-33/ST2 axis in a model of melanoma.\textsuperscript{53} This effect was associated with intratumoral accumulation of eosinophils and CD8$^+$ T cells and with local and systemic activation of NK and CD8$^+$ T cells. Moreover, IL-33 caused ST2-dependent eosinophil recruitment in the lung that prevented pulmonary metastasis after intravenous injection of melanoma cells. In addition, depletion of eosinophils by treatment with an anti-Siglec-F antibody abolished the protective effects of IL-33 against both tumor growth and metastasis formation. Functional studies revealed that IL-33-activated eosinophils exert both an accessory role, promoting the recruitment of tumor-reactive CD8$^+$ T cells at the tumor site and a direct cytotoxic effect against target melanoma cells, suggesting a dual mechanism for eosinophil-mediated antitumoral activity. Collectively, these two studies illuminate some of the immunologic mechanisms through which eosinophils play an anticancer role and open the way to the reconsideration of eosinophils in the development of new cancer immunotherapies. The roles of eosinophils in human and experimental tumors are summarized in Fig. 3.

### Does the role of eosinophils in tumors vary according to their microlocalization?

In addition to quantitative and qualitative cell composition, the spatial distribution of immune cells at the tumor site may affect tumor outcome.\textsuperscript{142} Moreover, different stages of tumors can be associated with qualitative and quantitative changes in different types of immune cells in the periphery and center of tumors.\textsuperscript{134,144} For example, mast cells in perilesional stroma of melanoma play a protective role.\textsuperscript{145} In NSCLC, mast cell infiltration of tumor islets confers a survival advantage independently of tumor stage.\textsuperscript{146,147} By contrast, peritumoral mast cells were associated with a better prognosis only in stage I NSCLC.\textsuperscript{148} In pancreatic ductal adenocarcinoma (PDAC) mast cell density in the intratumoral border zone, but not the peritumoral or the intratumoral center zone, was associated with a worse prognosis.\textsuperscript{149} In patients with cutaneous lymphoma only the density of peripheral mast cells correlated with disease progression.\textsuperscript{150} Collectively, these findings suggest that the microlocalization of immune cells is an important aspect in the initiation and progression of several tumors.

Tumor-associated eosinophils within the necrotic or perivascular areas of tumors have been reported.\textsuperscript{64,95} The number of peritumoral eosinophils has a significant favourable impact on prognosis of colorectal cancer patients.\textsuperscript{50} However, these studies did not examine differences between the periphery and the center of tumors. A novel technique allows a simultaneous single-cell analysis of the immune landscape of tumor microenvironment.\textsuperscript{151} It has been found that lung adenocarcinoma and non-involved lung tissue were equally enriched in eosinophils and neutrophils whereas other immune cells (e.g. CD8$^+$ T cells and macrophages) showed marked differences. Thus, this

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#### Table 1. Anti-tumorogenic activity of eosinophils in experimental tumors.

| Type of Cancer | Model | Eosinophil detection | Observed effects |
|---------------|-------|----------------------|------------------|
| Colon         | Human Colo-205 cell line | May-Grünewald-Giemsa Anti-EPX, Anti-MBP | Eosinophils induce direct tumor cell killing through adherence and release of cytotoxic granules in vitro (Gatault, Delbeke et al. 2015; Legrand, Driss et al. 2010) |
| Fibrosarcoma  | Mice implanted with MCA-induced fibrosarcoma cells | Carboil’s chromotrope-hematotoxyl Anti-EPX | Eosinophils protect from tumor growth in vivo and induce direct tumor cell killing in vitro (Simson, Ellyard et al. 2007) |
| Hepatocellular | Mice implanted with MH134 hepatocellular carcinoma cells | Anti-EPX | Eosinophils inhibit tumor growth in vivo and exert tumor citotoxicity in vitro (Kataoka, Konishi et al. 2004) |
| Hodgkin’s lymphoma | Human T-cell and B-cell lymphoma cell lines | N.I. | ECP is cytotoxic for Hodgkin’s lymphoma tumor cells in vitro (Gilmelius, Rubin et al. 2011) |
| Melanoma      | Mice implanted with B16.F10 melanoma cells | Anti-Siglec-F | Eosinophils inhibit tumor growth and lung metastasis in vivo and induce tumor cell killing in vitro (Carretero, Sectigli et al. 2015) |
|               | Mouse model of experimental lung melanoma B16.F10 metastasis | Anti-EPX | Eosinophil infiltration into the lung prevents tumor metastasis in vivo (Ikutani, Yanagibashi et al. 2012) |
|               | Mouse model of experimental lung melanoma B16.F10 metastasis | Carboil’s chromotrope-hematotoxyl; Anti-MBP | Influx of degranulating eosinophils into the lung prevents tumor metastasis in vivo and eosinophil lysates are cytotoxic for tumor cells in vitro (Mattes, Hulett et al. 2003) |
| Prostate      | Human DU 145 and PC-3 cell lines | N.I. | Activated eosinophils from allergic and asthmatic individuals inhibit tumor cell growth in vitro (Furbert-Harris, Parish-Gause et al. 2003) |

EPX: eosinophil peroxide; MPB: major basic protein; ECP: eosinophil cationic protein; N.I.: not indicated.
Eosinophils and tumor angiogenesis

Angiogenesis, the formation of new blood vessels, is an essential process for supplying essential nutrients and oxygen to growing malignant tissues. Immune cells are an essential process for supplying essential nutrients and oxygen to growing malignant tissues. Immune cells are an important source of pro-angiogenic factors. Human eosinophils produce several pro-angiogenic factors such as VEGF-A, fibroblast growth factor (FGF-2), CXCL8/IL-8 and osteopontin. Several studies have highlighted the association in human tumors between increased eosinophil density and angiogenesis by evaluating the expression of VEGF-A.

The VEGF-A gene can be alternatively spliced to form the pro-angiogenic VEGF-A165 and the anti-angiogenic VEGF-A165b. In certain tumors, the anti-angiogenic VEGF-A165b isoform is dominant. In addition, human neutrophils can produce both pro- and anti-angiogenic isoforms of VEGF-A. The results on VEGF-A in cancer should be confirmed differentiating between these two isoforms. The different pro- and anti-angiogenic isoforms of VEGFs produced by eosinophils in cancer have not been investigated.

Lymphangiogenesis, the formation of new lymphatic vessels, is important for the development of metastasis. Activated eosinophils play a relevant role in metastasis insurgence. Eosinophils have been detected in metastatic lymph nodes of cancer patients and the production of lymphangiogenic factors (e.g. VEGF-C and VEGF-D) by eosinophils should be further addressed. Human eosinophils produce matrix metalloproteinases (e.g. MMP-9) which regulate the digestion of extracellular matrix (ECM) favoring the invasive and metastatic behavior of cancer cells in TME.

Eosinophil extracellular traps and cancer

Several immune cells including human neutrophils, mast cells, and eosinophils can release granular and nuclear contents in the extracellular space in response to various stimuli (Extracellular Trap cell death, ETosis). Increasing evidence indicates that neutrophil ETosis (NET) plays a role in cancer. NETs can accelerate the metastatic process by entrapping tumor cells that can travel through vessels and lead to seeding in other organs. Degranulation and release of extracellular eosinophil secondary granule proteins in situ have been reported in both murine and human cancers. Functional in vitro assays have confirmed that eosinophils can mediate direct tumor cell killing via release of cytotoxic granules, including Granzymes. It is reported that eosinophils exploit ETosis for their main activities during airway inflammation. The role of eosinophil ETosis in cancer deserves further investigation.
Eosinophils as accessory cells in cancer

Besides exerting direct cytotoxic effects against cancer cells, eosinophils may participate to the anti-tumor response as accessory/immunomodulatory cells. In experimental melanoma models, tumor-associated eosinophils were shown to express chemokines (CCL5, CXCL9, CXCL10) promoting the recruitment of tumor-reactive CD8 T cells that mediate tumor rejection. In addition, eosinophils may cooperate with other immune cells such as macrophages, mast cells, NK cells and dendritic cells to induce anti-tumor responses. Eosinophils may also affect local T cell responses by modulating the balance of Th1 and Th2-related cytokines. Finally, eosinophils may serve as non-professional antigen presenting cells (APC). Resting eosinophils do not constitutively express MHC class-II or co-stimulatory markers. Upon activation by certain cytokines or other inflammatory stimuli, eosinophils can up-regulate these molecules and stimulate primed CD4 T cell responses in vitro and in vivo. Whether eosinophils function as APC at the tumor site remains to be determined.

IgE, atopy and cancer

Eosinophils and their mediators play a pivotal role in several allergic disorders. Epidemiological studies have suggested an inverse association between allergic diseases or IgE and certain tumors. It has been suggested that one of the protective role of IgE/atopy in cancer could be mediated in part by hypereosinophilia associated with several allergic disorders.

Role of eosinophils in cancer immunotherapy

Initial attempts to stimulate the immune system in cancer patients with IL-2 showed blood eosinophilia caused by increased plasma concentration of IL-5. It has been suggested that tumor-associated eosinophils in IL-2-treated patients exert cytotoxic effects on cancer cells. More recently, cancer
immunotherapy with monoclonal antibodies targeting immune checkpoints (CTLA-4, PD-1 and PD-L1) has yielded clinical benefits, including durable responses, to a percentage of patients with different malignancies.\textsuperscript{188–190} Interestingly, daily practice with ipilimumab (anti-CTLA-4) in patients with melanoma has shown that early increase in peripheral blood eosinophils is associated with improved survival.\textsuperscript{191,192} Importantly, baseline peripheral blood eosinophilia is associated with a better clinical outcome in melanoma patients treated with ipilimumab\textsuperscript{126,193} and pembrolizumab.\textsuperscript{127}

**Outstanding questions and conclusions**

Studies on eosinophils biology are routinely conducted under physiological pH and normoxia. By contrast, the metabolic phenotype of tumors is characterized by low pH and areas of either hypoxia or normoxia.\textsuperscript{90} Tumor-associated macrophages (TAMs) in normoxic tumor tissues express M1 markers whereas those in hypoxic tissues express M2 markers.\textsuperscript{194} These aspects caution against the over interpretation of results from \textit{in vitro} studies of eosinophils at physiological conditions. It will be important to investigate how hypoxic conditions influence the proteomic and lipidomic phenotypes of eosinophils. Eosinophils can migrate to draining lymph nodes where they can act as APC.\textsuperscript{195,196} An analysis of eosinophils in tumor-draining lymph nodes (TDLNs) and in tertiary lymphoid structures of tumors is missing. It has been reported basophils in TDLNs of patients with pancreatic ductal adenocarcinoma correlates with reduced survival.\textsuperscript{197} The role of eosinophils in TDLNs, in tertiary lymphoid tissues and at metastatic sites of different tumors remains to be explored.

The anti- or pro-tumorigenic role of eosinophils in different human tumors appears to be cancer-specific. As shown for tumor-associated macrophages (M1 and M2)\textsuperscript{99} and tumor-associated neutrophils (N1 and N2),\textsuperscript{100,101} subpopulations of eosinophils are recently begun to emerge\textsuperscript{198} and could play different, even opposite effects in various types of tumors. In this respect, it has been proposed that eosinophils may be distinguished into E1 or E2 based on the balance of Th1/Th2 cytokine expression patterns.\textsuperscript{53,109,132} Alternatively, although eosinophils are relatively short lived, different TMEs could alter their phenotype. Indeed, eosinophils, like other immune cells, are endowed with phenotypic and functional plasticity depending on environmental factors which may vary in composition in the different cancer microenvironments.\textsuperscript{104} Gene expression profiling has demonstrated that several individual human cancers (e.g. melanoma, gastric, lung and breast cancers) are heterogeneous with a spectrum of molecular changes.\textsuperscript{199–203} The complex heterogeneity (spatial, temporal, intratumoral, intertumoral) of the TME adds an additional layer of complexity.\textsuperscript{151,204,205} Simultaneous single-cell analysis of the immune landscape of TME of different subtypes of human cancers defined by genetic markers can greatly expand our knowledge of the role of eosinophils in tumor initiation and progression.

Tumor cells evade host immune attack by expressing several checkpoints such as programmed cell death-1 protein (PD-1) and its ligands (PD-L1 and PD-L2) which inhibit PD-1\textsuperscript{+} lymphocytes in TME.\textsuperscript{206} Monoclonal antibodies targeting the PD-1/PD-L1 pathway unleash anti-tumor immunity and have revolutionized the management of a wide spectrum of malignancies. Certain cancer cells (e.g. melanoma) express also PD-1, in addition to PD-L1, providing an additional tumor intrinsic mechanism enhancing the protumorigenic effect of PD-1/PD-L1 axis.\textsuperscript{207} Human eosinophils express PD-L1 and, to a lesser extent, PD-L2.\textsuperscript{208} An important task will be to investigate the role of PD-1/PD-L1 axis of eosinophils in tumor microenvironment.

All the above implies that elucidation of the roles of eosinophils in different human tumors will demand studies of increasing complexity beyond those assessing merely eosinophil density and microlocalization. Fig. 4 schematically illustrates some of the possible mechanisms by which eosinophils and their mediators may promote tumor regression or, vice versa, tumor initiation.

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**Figure 4.** Possible mechanisms by which eosinophils and their mediators play a protumorigenic or an antitumorigenic role. Eosinophils in TME can promote tumor initiation and progression through the release of ROS, angiogenic factors, metalloproteinase-9, and the induction of epithelial-to-mesenchymal transition. Eosinophils can exhibit antitumor activity through direct tumor cell cytotoxicity mediated by ROS, granzyme, TNF-\(\alpha\), eosinophil cationic proteins (ECP, EPX, MBP), and IL-18. IFN-\(\gamma\) produced by eosinophils favors M1 polarization of TAMs. Eosinophils can also exert antitumor activity indirectly through the attraction of CD8\(^+\) T cells via the production of CCL5, CXCL9, and CXCL10.
and progression in experimental and clinical tumors. These apparently controversial effects might reflect differences in stage, grade, and subtypes of tumors, different methods employed to identify eosinophils (e.g. Giemsa, cationic proteins), different microanatomical compartments analyzed (i.e. peritumoral vs intratumoral) in the various studies or perhaps subtypes of tumor-associated eosinophils. Therefore, many fundamental questions need to be addressed before we understand whether eosinophils are an ally, adversary or innocent bystander in human cancers.

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Author contributions
GV, MRG, and SL conceived and designed the review. All the authors contributed intellectually and to the writing of the submitted version of the manuscript.

Conflict of interest statement
The authors declare that the research was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

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