# Potential and importance of metalloproteinases and interleukins in inflammation and metastasization in non-small cell lung cancer

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**Abstract:** It is now known that, in lung cancer, while there is a loss of the standard biomarkers, such as the EGFR or ALK rearrangements, there is also a loss of standard immune related biomarkers, such as the PD-1 and PD-L1. These findings resulted in new immunotherapies, already approved and which offer new options in the treatment of this cancer. Although the current overview is far better than decades ago, lung cancer continues to be the deadliest cancer of all. Thus, there is still a lack of biomarkers that can be used in order to revert that situation, whether through the finding of new therapeutic options or through the finding of new biomarkers which may help guide the treatment of the patient. Markers of inflammation and metastasization, such as metalloproteinases (MMPs) and its inhibitors or interleukins (ILs), may play that role as they are very important in carcinogenesis. In the cancer environment, MMPs and its inhibitors are often related to the inflammation, metastasis, angiogenesis and tumour growth. Their influence has a wide range and they can both promote or combat the development of cancer, although usually MMPs are related to bad prognosis and cancer development whereas its inhibitors are related to the opposite. Many of these have already been studied in the immune and tumour microenvironment, as is the case of MMP-2, MMP-7 or MMP-9, and the possibility for them to have a more critical role in this cancer is still being studied. Although the targeting of these biomolecules as a treatment option may not have the desired results, there is still room to study their importance in the prognosis and treatment response. As for ILs, these crucial components of the immune system response also have dual roles in lung cancer, since it is believed that not only they can enhance tumour cell recognition by effector cells, but also they can be used by the tumour in its microenvironment to edit, modulate and evade the immune system response. As it happens with the MMPs and its inhibitors, targeting these molecules or use them as therapy may not be the best option, but their potential role as prognostic biomarkers still stands.

**Keywords:** Lung cancer; non-small cell lung cancer (NSCLC); immune system; personalised medicine; metalloproteinases (MMPs); tissue inhibitors of metalloproteinases (TIMPs); interleukins (ILs)

## Introduction

The relation between cancer and the immune system has been studied over the years. In the past, it was observed that some tumours were infiltrated by many cells of the immune system and that those infiltrates were supposedly related to the attempt, by the host immune system, to eradicate the tumour (1). Nowadays, it is clear that the tumour associated inflammation has a paradoxical effect of enhancing carcinogenesis, and its progression is considered as an enabling characteristic of cancer (1). However, immunosurveillance is also present and the immune system acts against transformed cells, but eventually that combat interaction fades and components of the immune system are also capable of hosting an immune microenvironment that anchors the tumour and gives room for it to grow and to develop (1-5).

Lung cancer continues to be the deadliest cancer of all and non-small cell lung cancer (NSCLC) represents the majority of cases (6). There are many genetic alterations associated and many risk factors, such as the tobacco smoke (6-16). Nowadays, precise and personalised medicine has become top priority. The discovery of the loss of standard biomarkers allows us, today, to have more targeted therapies and immunotherapies that enhance our options fighting this cancer (17-19). It becomes crucial to approach the right patient, at the right time, with the right drug, dose and schedule (18). However, drug resistance still remains an obstacle to surpass that limits the effectiveness of targeted therapies. Therefore, the finding of new biomarkers not only can be used as part of new therapies, but also allow us to understand and evaluate which patients can benefit from which therapy (20,21).

In this review, the role of some biomolecules will be explored. Special attention will be paid to the metalloproteinases (MMPs), the tissue inhibitors of metalloproteinases (TIMPs) and the interleukins (ILs). These markers of inflammation and metastasization play important roles in carcinogenesis, given that the MMPs and the TIMPs have already been associated with metastasization, angiogenesis or tumour growth; while ILs are important intermediates of our immune system and have already been associated with inflammation and carcinogenesis (22,23). Due to their relationship with carcinogenesis and metastasization as well as with inflammation, their potential to be used in clinical practice as prognosis or treatment response markers remains possible. Hence, this review will also consider not only

what these biomolecules are, but also their importance and function in the NSCLC.

## Immune system, tumour microenvironment and NSCLC

The tumour development is usually associated with inflammation which can become a chronic condition later on (2). The immune system escape is a fundamental hallmark of cancer and the interaction between the tumour and the immune system suggests that there is an elimination of the nascent tumour cells by the immune system, followed by an equilibrium stage where the immune system reaction can control the tumour progression, and finally by the escape stage when tumour cells are able to resist the immune system effect by immunoediting and evading immune system response (2-4). During lung cancer progression, the tumour cells interact with the host and create the microenvironment where local cancer-antigen-specific immune system response is shaped (5). Some elements of the immune system acquire changes that enable the tumour to avoid destruction and those elements may play a role in future targeted therapies or immunotherapies (5).

In NSCLC, besides an infiltration of different types of immune system cells, there is also an expression of inflammatory molecules that are normally related to tumour progression, evasion of the immune system response, invasion and metastasis (2), as well as an expression of immune inhibitory molecules in the tumour microenvironment (17). A feature of almost all cancers and their microenvironments is the leukocyte infiltration, which includes mast cells, Natural Killer (NK) cells, T and B lymphocytes and, in particular, the tumour-associated macrophages (TAMs) (2). These infiltrates are associated with angiogenesis and other processes that can be prejudicial to the host and that are essential for the tumour microenvironment to endure and for the tumour to progress (2).

Macrophages are one of the most important immune system cells in the immune and tumour microenvironment. There are two pathways for the macrophages to differentiate, the M1 or the M2 activation (24). When macrophages go into M1 differentiation they become effector cells, whereas M2 macrophages are capable of suppressing the immune system response, promote tissue remodelling and angiogenesis (24). These M2 macrophages are usually present in the tumour microenvironment, indicating an immune suppressing environment (24). In

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the acute response, macrophages, dendritic cells and mast cells are effectors capable of mediating this response by releasing cytokines, reactive oxygen species and other mediators (2,25). Direct T-cell recognition of tumour cells requires the presence of antigenic peptides, usually presented by major histocompatibility complex (MHC) molecules (17). In a significant number of lung cancer cases, there is a deregulation of components, which are part of this machinery, via genetic and epigenetic alterations, leading cancer cells to avoid death by the T cell activity (17). Suppressed presenting antigen molecules are one of the mechanisms that lead to the immune response escape and, in some cases, it can be reversed by interferon-γ (IFN-γ) (26). This is important in the immunotherapy context, as there is a possibility that NK cells and T cells can be activated within the tumour microenvironment and as they are a source of IFN-γ (17). According to some studies, cancer cells can also evade the immune system response by immunoediting and inducting antigen-specific tolerance (27,28). The immunosurveillance in lung cancer is effective in early stages of carcinogenesis, but eventually gets inhibited and the tumour progresses (29). The low antigenicity of lung cancer cells leads to a passive escape from the immune response (29). The cytotoxic lymphocytes are suppressed in the tumour microenvironment and follow regulatory mechanisms which inhibit the recognition of tumour antigens by the antigen presenting cells (29). The population of regulatory T cells (Tregs) and the expression of Foxp3 in tumour cells and tumour infiltrating lymphocytes are increased and associated with a suppressed immune system (29).

## Immunotherapy and NSCLC

In NSCLC, there have been recent developments on immunotherapies. An example of this are the anti-cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) inhibitors, like ipilimumab. This consists in a monoclonal antibody (mAb) that blocks CTLA-4 which, in turn, is responsible for balancing the costimulatory signals delivered by CD28 during T-cell activation (17). The CTLA-4 is expressed exclusively on T cells, where it regulates the amplitude of the early stages of the T cells activation. It counteracts the costimulatory receptor CD28, as both have identical ligands: CD80 and CD86 (30). This inhibitor, however, is only approved for the treatment of advanced melanoma and its combination with nivolumab, an anti-programmed cell death-1 (PD-1) inhibitor, is being investigated in NSCLC (31).

The anti-PD-1 inhibitors are another option in the treatment of NSCLC. In this case, the mAb blockades the PD-1 checkpoint that targets PD-1, blocking the ligation with its major ligand programmed cell death-ligand 1 (PD-L1) whose overexpression in the tumour is related to better clinical activity of these immunotherapies (17,32,33). As for the PD-1 expression in NSCLC patients, PD-1 negative expression patients, who undergo this treatment, have a similar overall survival when compared with patients treated with chemotherapy (33). In addition, patients with high expression of PD-1 achieve better results with regard to their survival (33). While nivolumab was the first anti-PD-1 checkpoint inhibitor to be approved for metastatic NSCLC patients, pembrolizumab has also been approved for metastatic NSCLC patients whose tumours express PD-L1 (31). The PD-1 is an immune checkpoint normally expressed by the activated T cells, activated Tregs, B cells and even NK cells, but which can also be found on myeloid cells of the stroma surrounding the tumour (17,30,34). It is clear that in comparison with the CTLA-4 this molecule modulates later stages of the immune system response (30). The PD-1 binds to its ligands PD-L1 or programmed cell death-ligand 2 (PD-L2) which are expressed on antigen presenting cells, but can also be found in tumour cells, being an important escape mechanism for them (30). One aspect that must be taken into consideration is the safety use of these therapies, as they can represent general immune system changes in the patients. According to data from many clinical trials, it seems that the CTLA-4 inhibitors are generally safe to use, with manageable side effects related to their mechanism of stimulating the immune system response (35). The therapies involving anti-PD-1 agents seem to be generally similar between each other and the side effects are, in general, manageable (35).

Recently, the immune checkpoint molecule lymphocyte-activation gene 3 (LAG-3) (CD223) and receptor killer-cell immunoglobulin-like receptor (KIR) were reported as possible targets (36). Early data suggest that LAG-3 inhibits T-cell activity, but it modulates T cell activation as well (36). Preclinical data suggests that the anti-tumour activity of PD-1 may work in synergy with LAG-3 (36,37). Lirilumab, a mAb that blocks the interaction between KIR on NK cells and their ligands, can eventually be an option to be used in lung cancer (36). Another option in the treatment of lung cancer is the use of therapeutic vaccines, in which the host's immune system is forced to recognise tumour antigens, to increase anti-tumour activity by T cells, and to inhibit pathways connected with proliferation, growth, angiogenesis

and many other important processes in carcinogenesis (19,38-40). There are two kinds of vaccines: the tumour cell vaccines which are made from autologous or allogeneic tumour cells, and the antigen-based vaccines which are developed to facilitate the exposure of the host's immune system to tumour-specific antigens (38). Theoretically, the use of vaccines is very effective in minimal residual disease, but there is also a possibility to use them as an adjuvant treatment after resection in an early stage NSCLC, or even as a second line treatment following the chemotherapy and radiation for the advanced stage disease (38).

The incorporation of immunotherapeutic agents, either together or combined with chemotherapy, targeted therapy and radiotherapy, is at the same time a possibility and a challenge (30,41). In an early stage disease, immunotherapy may be combined with a first-line treatments or used as an adjuvant treatment after surgery (42). The combination strategies using chemotherapy, radiation therapy, targeted agents and immunotherapies may become an option against NSCLC, especially when metastasized, but all these possibilities are currently being studied (30). The study of epidermal growth factor receptor (EGFR) or anaplastic lymphoma kinase (ALK) rearrangements may give the patient more options for his treatment (43). Another important aspect is the treatment selection for NSCLC patients, since not all of them will benefit from all therapies (44). Therefore, the finding and study of biomarkers are essential to identify patients who will benefit from these therapies which, in turn, may lead to considerable improvements towards a more personalised and precise medicine (17).

## Markers of inflammation and metastasization

### MMPs and TIMPs

MMPs are classified as a calcium dependent, $Zn^{2+}$ containing endopeptidases and can be found in soluble or membrane-bound forms (45). There are 23 human MMPs divided into different groups: collagenases, gelatinases, stromelysins, matrylisins, elastases, membrane-type MMPs and others (45-48). They participate in extracellular matrix (ECM) degradation, but they are also important in tissue homeostasis, host defence, tissue remodelling and repairing, inflammation and many other processes (46,49-51). The proteolytic activity of these molecules is usually very low in healthy tissues, their transcription levels can be upregulated by inflammatory cytokines and growth factors, and their expression can also be regulated by hormones, tumour promoters, cell-to-cell and cell-to-ECM interactions (46). The regulation of their activity is crucial and there are three levels of regulation already reported and studied, namely gene transcription, proenzyme activation and inhibition of enzyme activity (22). The activation requires proteases to remove the propeptide domain, since MMPs are normally secreted as inactive zymogens termed pro-MMPs (22,49,52). Finally, their inhibition can be direct and specific with the TIMPs or non-specific by α1 proteinase inhibitor and α2 macroglobulin (22). Their natural inhibitors, the TIMPs, are specific inhibitors that regulate and control their activities in tissues, and can bind to the MMPs in order to regulate their activity (49,53). There are four TIMPs (TIMP-1-4) identified in vertebrates and their expression is usually upregulated during tissue remodelling (49,53). TIMPs can form complexes with pro-MMPs, so to regulate their action. For example, TIMP-2, TIMP-3 or TIMP-4 can bind with pro-MMP-2 and TIMP-1 or TIMP-3 bind with pro-MMP-9 (53). It is also important to note that the interaction of TIMP-2 with pro-MMP-2 is part of an activation mechanism of pro-MMP-2 into functional MMP-2 and is actually mediated by another MMP, the MMP-14 (53).

In the immune system landscape, MMPs and TIMPs can modify chemotactic agents which are essential for the migration of immune system cells, such as neutrophils (51). For example, MMP-9 processes IL-8 to increase its potency, MMP-2 can inactivate monocyte chemotactic protein-3 (MCP3) which is a cell-attracting chemokine, and MMP-7 mediated cleavage releases C-X-C motif chemokine ligand 3 (CXCL3) which attracts neutrophils to the site of infection (54-56). In neutrophils, MMP-9 is usually stored and ready to release after stimulation with IL-8 or tumour necrosis factor (TNF) (51,57). In mice, TIMP-1$^{-/-}$ neutrophils migrated more rapidly to the site of infection and the immune system response was stronger and amplified; in other study, the neutrophils were suppressed by TIMP-3 (58,59). Another immune system response is given by the macrophages. The M1 or M2 differentiation, described above, is crucial for the type of response given (51). The mRNA levels of MMP-1, MMP-2, MMP-3, MMP-7, MMP-10, MMP-14 and MMP-25 were found to be upregulated and increased in *in-vitro* M1 macrophages by comparison with the M2 macrophages, suggesting that these MMPs may be important in the M1 differentiation (60).

Elevated expression and activity of MMPs are commonly observed in acute or chronic inflammation, as they can

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regulate inflammation (61). MMPs can exhibit either pro-inflammatory or anti-inflammatory activities, depending on the characteristics and context. For example, MMP-9 is capable of potentiate the activation of pro-inflammatory cytokines and chemokines, while MMP-2 can process monocyte chemoattractant proteins and, by doing so, it generates chemokine receptor antagonists with anti-inflammatory properties (55,61). MMP-14 was identified as an important MMP for the regulation of pro-inflammatory cytokines, such as the IL-12 and IL-6 as well as the anti-inflammatory IL-10; it can also cleave and inactivate chemokines which are involved in inflammation (51). In the inflammation of the airways, the ECM degradation generates peptides, which exhibit chemotactic activity through the activation of CXC chemokine receptor on neutrophilic granulocytes, and the MMP-12 cleavage of neutrophil elastase can generate neutrophil attracting peptides (46,62). MMPs can also compromise the T cell activity, since several MMPs can cleave transforming growth factor β (TGF-β) to its active form which inhibits T cell response (64). MMP-9 produced by tumour cells can cleave IL-2Rα on the surface of activated T cells and suppress their activity and their proliferative capacity (63).

In the cancer landscape, MMPs are believed to facilitate metastasis by breaking down physical barriers provided by ECM or by the basement membrane, but their action in carcinogenesis is important in many other processes, such as tumour growth, apoptosis and angiogenesis (22,64). MMPs are able to regulate tumour growth by many mechanisms: they can release cell membrane bound precursors of growth factors, they are able to modulate the bioavailability of growth factors sequestered by ECM proteins, and they can indirectly modulate proliferative signals (22). Many MMPs are capable of activating cell surface bound TNF-α which promotes cell survival via NF-κB pathway (46). MMP-8, MMP-9 or MMP-12 can modulate the activity of CXCL11, which is a Th1 attracting chemokine, whose receptor (CXCR7) is expressed in many types of tumour cells and activates signalling pathways that promote cell survival and growth (65). However, MMPs are also able to inhibit growth by activating TGF-β, generating proapoptotic molecules, such as the Fas ligand (22,66). They may, critically, disrupt the balance between growth and antigrowth signals in tumour microenvironment, due to their potential to influence bioavailability and functionality of multiple important factors regulating growth (52). In the apoptotic process, MMPs also have dual roles. Antiapoptotic actions are mediated by the cleaving of the Fas ligand, proteolytic shedding of tumour associated MHC complex class I related protein and activation of serine, threonine kinases or protein kinase B (22). The MMPs' apoptotic actions involve the changing of ECM composition by their ability to cleave adhesion molecules and, eventually, lead the cell to apoptosis (22,67). MMPs interfere with the induction of apoptosis in malignant cells and can even lower the impact of chemotherapy on the tumour (52). MMPs also play a dual role in Angiogenesis, as they can either promote or inhibit it. They are able to promote endothelial cell migration and trigger the angiogenic switch (61). Pro-angiogenic MMPs are normally produced by stromal cells or inflammatory leukocytes (64). They can promote angiogenesis by degradation of basement membrane and other ECM components, by releasing ECM bound pro-angiogenic factors [such as the vascular endothelial growth factor (VEGF)], and by triggering integrin intracellular signalling (22,68). They are capable of inhibiting angiogenesis by cleaving plasminogen, which releases angiostatin; by cleaving collagen, which produces endostatin; and by shedding cell surface bound plasminogen activator receptors, which are required for endothelial cell invasion (22,52,67-70).

The proteolytic activity of MMPs is essential to allow the malignant cell to break physical barriers during expansion and intravasation, extravasation and invasion at distant sites (67). Hence, MMPs are strongly associated with metastasization (67). During invasion, MMPs can be found in structures called invadopodia, which represent the site where ECM degradation will take place (22,67). MMP-14 and MMP-1, -2 or -9 are examples of MMPs that degrade a variety of ECM molecules and facilitate invasion (22,67). Many proteolytic enzymes are fundamental for tumour cells to access blood vessels and lymphatic system; MMP-2 and MMP-9 can both degrade components of basement membrane, enhancing the invasion process (22). It is also described that MMPs are important factors for circulatory tumour cells to extravasate and establish colonies (22). The cell movement is also associated with MMPs, as they can regulate cell-cell and cell-ECM interactions during migration (67). The production of peptides, such as collagen and laminin, can also promote cell migration, due to the degradation of ECM components or integrins that act as substrates for MMPs (67,71-73). MMP-2 and MMP-14 degrade laminin 5 and trigger motility of the cell (22). Epithelial-mesenchymal transition (EMT) has also been associated with high expression levels of MMPs, such as MMP-2,-7,-9 among others (66,67). Their activity

is important in this crucial process for the metastasization to occur (22). For example, MMP-7 is capable of cleaving E-cadherin disrupting cell adhesion and leading to the EMT and migration (22,67,74). It is also described that the EMT can be induced by TGF-β and that MMP-28 can cause its proteolytic activation and lead to EMT (22,76). E-cadherin can be cleaved by MMP-3 and MMP-7 and it triggers EMT (22). In general, studies show that overexpression of some MMPs conferred tumour cells with more tumorigenicity and invasiveness (64).

Usually, high MMPs activity is correlated with tumour progression, but the balance between them and their inhibitors is crucial. Deregulation of TIMPs may result in an increase of MMPs activities and, thereby, in a more invasive potential for tumour cells. Upregulation of TIMPs can actually inhibit tumour invasion and metastasis (61). However, their action is not only associated with tumour suppressing activities, although it is reported that TIMPs commonly act as tumour suppressive agents and are normally dysregulated in cancer landscape, which can justify the conflicting data reports (61).

In NSCLC, many studies have been developed with promising results, demonstrating that these biomolecules can be useful and potentially be considered biomarkers, as they can eventually predict clinical outcomes, such as prognosis or drug response, which demonstrates their importance in both carcinogenesis, as described above, and in the future of personalised medicine. High expression in tumour and peritumoural cells of MMP-2 was associated with increased risk of tumour recurrence and with poor prognosis (76). Its overexpression in patients at an early stage was also observed and was related to poor prognosis, as well as to invasion and metastasis (77,78). Its serum levels are usually higher in patients with metastases than in patients with localised disease being, thereby, associated with poor prognosis, and can eventually be used to monitor the progression of the disease (79). High MMP-7 expression in NSCLC is common and was already associated with poor response to platinum-derived chemotherapy, showing potential to be used in these patients, as a predictive drug response marker for the resistance to chemotherapy (52,80,81). The expression and activity of MMP-9 is usually upregulated and it seems to be related to the clinical stage of the patient, tumour growth, disease progression and aggressive tumour behaviour, leaving open the possibility for it to be used as a prognosis marker and a therapeutic target (81-85). TIMPs are also important due to their relationship with MMPs, and their importance in NSCLC has also been

studied over the years. TIMP-1 mRNA expression levels in tumour tissue are usually higher by comparison with normal lung tissue (86). Its expression has also been associated with decreased survival, low recurrence-free survival and poor prognosis, which can possibly happen due to the context and other TIMP-1 activities besides MMP inhibition (87-90). The role of this TIMP in NSCLC is still to be fully discovered and additional studies will provide a better insight into its functions in cancer development (87-90). As TIMP-2 expression indicates a favourable prognosis in patients with NSCLC, it may be a protective factor that could help predict the outcome of chemotherapy or targeted inhibitory antibody therapies (91).

MMPs were already looked upon as potential therapeutic targets, but their dual role is one of the possible reasons why the outcome of some clinical trials with MMP inhibitors was not desirable (46). In NSCLC, a phase III trial with marimastat, a synthetic MMP inhibitor based on hydroxamate which acts as a zinc-binding group, observed no significant difference, in progression-free survival or overall survival, between the group with the inhibitor and the group with the placebo (61). However, prinomastat, an alternative zinc-binding non-peptide MMP inhibitor, demonstrated antitumor activities in various preclinical studies, including lung cancer models (61). In particular, in NSCLC the anti-tumour efficacy in chemoresistant human NSCLC was observed with prinomastat as a single agent or when combined with chemotherapy (61). On the contrary, a phase III trial with a combination of taxol and carboplatin for advanced NSCLC patients was not effective (61). Nevertheless, the possibility of being a good option in early stages remains unknown (61). A different therapeutic approach is the use of nanoparticles micelles containing paclitaxel conjugated to a hydrophilic moiety via an MMP-2 cleavable peptide (92). These nanoparticles were effective in NSCLC xenografts by comparison with paclitaxel or micelles alone (92). This efficacy may be justified due to the accumulation of micelles at the tumour site, by virtue of the enhanced permeability and retention (EPR) effect and of the peptide cleavage by MMP-2 leading to active cell penetrating paclitaxel (92). In lung cancer carcinoma cells, resistance to different platinum compound or cisplatin after exposure to MMP-7 was related to increased levels of anti-apoptotic protein BCL2, which means that this MMP inhibition may be used as adjunct to chemotherapy for those who show some resistance (92).

In summary, there are many processes involved in carcinogenesis, where MMPs play important roles and their

**Table 1** Summary of the processes of carcinogenesis and MMPs associated with them (62,68,94)

| Process | MMPs |
|---|---|
| Cancer cell invasion | MMP-1; MMP-2; MMP-9; MMP-28 |
| Cancer cell proliferation | MMP-1; MMP-2; MMP-3; MMP-7; MMP-9; MMP-11; MMP-14; MMP-19 |
| Tumour angiogenesis | MMP-1; MMP-2; MMP-3; MMP-8; MMP-9; MMP-10; MMP-11; MMP-13 |
| Cell adhesion, migration and EMT | MMP-1; MMP-2; MMP-3; MMP-7; MMP-9; MMP-13; MMP-14; MMP-28 |
| Immune surveillance | MMP-1; MMP-2; MMP-3; MMP-7; MMP-8; MMP-9; MMP-11; MMP-14 |

EMT, epithelial-mesenchymal transition; MMPs, metalloproteinases.

activity can influence them, as shown in *Table 1* (61,67,93).

## ILs

It is known that the mediators of inflammation and immune system response are, in general, very important in the tumour microenvironment. The inflammation registered has many tumour promoting effects, as it promotes angiogenesis, tumour growth. progression and metastasis, thereby altering the immune response (25,94). One of those mediators are cytokines, which have dual roles: they have influence in changing the balance from an active and tumour eliminating immunosurveillance status to a tumour promoting microenvironment, and they can also have tumour suppressing effects (95). In this context, attention will be paid to one major class of cytokines, the ILs, crucial components of both the adaptive and innate immune system response (96,97). They are considered to be cellular messenger molecules, because they allow cells of the immune system to communicate between each other and to create coordinated, specific responses to an antigen (23,98). They act in a paracrine or autocrine way and the response of a single cell to them depends on the ligands involved, the receptors expressed on the cell surface and the many cascades that can be involved (99). Almost all ILs are also capable of modulating growth differentiation and activation during immune system response and they can have both pro-inflammatory or anti-inflammatory functions (23,99-101).

During tumorigenesis, ILs can stimulate immune system effector cells and stromal cells at the tumour site, in order to enhance the tumour cell recognition by the cytotoxic effector cells (23). They are also involved, directly or indirectly, in many mechanisms of the tumour development, such as immunoediting, immunosurveillance, inflammation and EMT mechanisms (23). The role of ILs in immunosurveillance and immunoediting is crucial in cancer landscape and may lead to new opportunities

and possibilities of novel therapeutic approaches in the future, but due to their activity and function in the immune system and many molecular pathways, their use for cancer treatment may also have some limitations, such as severe immunological adverse conditions (23,102).

In the NSCLC landscape, the roles of many ILs and their importance in this cancer have been studied over the years, as shown by the examples listed in *Table 2* (103-118).

Anti-inflammatory ILs can also inhibit angiogenesis, growth and invasion, as is the case of the IL-4, usually produced by T cells, mast cells, basophils and is capable of inducing antibody production by B cells, (104). High levels in NSCLC support the hypothesis that Th2 cytokines associated with the inhibition of lymphocyte activation are usually higher in these patients and Th17 related to cytokines are also increased by comparison with controls (103,119). However, this interleukin and its characteristics were also associated with potential inhibitory features that may act against the tumour (104,105). In fact, some ILs may exhibit anti-tumour roles. IL-24 can disrupt stromal cell-derived factor 1 (SDF-1)/CXCR4 signalling pathway and, by doing so, it can inhibit lung tumour cell migration and invasion; and when combined with CXCR4 inhibitors, it exhibits anti-metastatic activities (115). IL-27 has also been associated with anti-tumour effects, such as anti-proliferation and anti-angiogenesis, as well as with immune system stimulation (116). The study of IL-33, associated with the induction of Th2 cytokines, can inhibit infection and inflammation, this supports the hypothesis of a tumour suppressor microenvironment present in cancer patients, at least at early stages of the development, with plasma levels of this interleukin being inversely associated with lung cancer progression (117). It is also known that Treg cells Foxp3+ are capable of producing IL-35 and that this interleukin is required for T-reg mediated immunosuppression, as well as it is important in T cell proliferation suppression and it can induce naïve T cells to convert into Treg cells (118). In

**Table 2** Importance and action of ILs in lung cancer with focus on NSCLC

| ILs | Activity | References |
|---|---|---|
| IL-4 | The circulating levels were found to be higher in NSCLC patients prior to surgery treatment; the study of polymorphisms has also shown that high levels of this IL might me related to the presence of a polymorphism and that IL-4 can act as an anti-angiogenic regulator and inhibit expression of MMP-9 (associated with cell invasion); furthermore, IL-4 can inhibit the production of Prostaglandin $E_2$ ($PGE_2$), which is associated with angiogenesis and invasion in NSCLC cells | (104-106) |
| IL-5 | IL-5 deficiency in mice offered protection to the lung from metastasis | (107) |
| IL-6 | High levels of this IL have been associated with tumour progression, poor response to chemotherapy and poor survival outcome in NSCLC patients treated with chemotherapy; polymorphisms have also been studied and possibly associated with the patients' prognosis; although in very early stages of tumour development it may have a tumour suppressing activity | (108-111) |
| IL-10 | High levels of this IL were observed in NSCLC patients prior to surgery treatment | (104) |
| IL-12 | The expression of this IL by adipose-derived mesenchymal stem cells can inhibit adenocarcinoma cells migration and invasion, thus inhibiting tumour growth, invasion and migration | (112) |
| IL-17 | This IL can induce EMT and promote lung cancer cell migration and invasion via NF-κB pathway; in lung cancer, the levels of this IL were found to be increased and correlated with advanced stage disease, increased number of metastatic sites and poor survival; it has also been associated with angiogenesis, as the levels of IL-17 were found to be associated with the levels of VEGF | (113-115) |
| IL-24 | This IL was associated with the inhibition of lung tumour cell migration and invasion | (116) |
| IL-27 | It can promote the anti-tumour action of intratumoral myeloid cells and it deregulates stemness genes suppressing the self-renewal potential in squamous cell carcinoma cells | (117) |
| IL-33 | High levels of IL-33 were found in the initial stages of lung cancer; the plasma levels of this IL were reported to be inversely associated with lung cancer progression | (118) |
| IL-35 | Circulating levels of this IL were found to be higher in NSCLC patients and are positively related with the tumour's TNM stage and lymph node metastasis and negatively related with their survival | (119) |

NSCLC, non-small cell lung cancer; VEGF, vascular endothelial growth factor; ILs, interleukins.

NSCLC, circulating levels of this interleukin are increased and associated with poor overall survival (118).

On the other hand, pro-inflammatory ILs, such as the IL-6 which can be produced by many immune system cells in acute or chronic inflammation, can act as a growth and an antiapoptotic factor (109,120). High levels of this interleukin have been associated with a poor prognosis, therapeutic resistance and metastatic disease (120-122). However, it may also have an anti-tumour activity, at least at very early stages of tumour development induced by K-Ras (110). Another example is given by IL-17, a vital interleukin in chronic inflammation, but also very important in the pathophysiology of cancer itself (112,114). It is capable of inducing EMT and of promoting lung cancer cell migration and invasion via NF-κB pathway (112). It has been also associated with angiogenesis, with a more advanced stage of the disease and with worse overall survival (113,114).

ILs associated with allergic and infectious diseases, such as the IL-5, have also been studied in mice lung metastasis models (106). It was suggested that IL-5 may facilitate metastasis and colonisation through the recruitment of eosinophils, which produce C-C motif chemokine 22 (CCL22), leading to the recruitment of Treg cells to its location and, therefore, to a regulation of the immune system in the tumour microenvironment (106).

Although the relationship between ILs and MMPs has also been studied, more insight is needed so to clarify and support these and other relations, specifically in the NSCLC landscape, as most of the studies rely on several types of *in vitro* and *in vivo* studies. Clinical studies focusing on the levels of ILs and MMPs and their relationship will give more insight in this matter and on the clinical importance of these findings. IL-1 is believed to play an important role in tumour promotion and metastasis through the regulation and promotion of expression of MMPs (23,102). For example, Il-1β may upregulate the expression of MMP-13 (23).

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In bladder cancer cell lines IL-5 was associated with invasion and MMP-9 expression (102). The stimulation of IL-6 can also induce an elevated expression of MMP-2 and MMP-9 in nasopharyngeal carcinoma cell lines (123). In lung cancer cell lines, IL-6 was reported to regulate both the expression of mRNA and the protein levels of MMP-10 (124). To be noted that high levels of IL-6 were also related to high expression of MMP-3, MMP-13, thereby enhancing the migration abilities of these cells (125). IL-8 and IL-17 enhance the activity of MMP-2 and MMP-9 which, in turn, increases the metastatic activity and as for IL-17, this IL was already associated with the expression and activity of MMPs; it induces the expression of MMP-9 *in vivo* and *in vitro* and its inhibition in tumour sites may suppress MMP-9 (23,114,126,127) and IL-17A, in a gastric cancer cell line, promoted an elevated expression of MMP-2 and MMP-9 as well as a diminishing expression of TIMP-1 and TIMP-2 levels, therefore enhancing invasion (128). High levels of IL-23 have also been associated with an upregulation of MMP-9, related to an increase of angiogenesis, which appears to be important in tumour-promoting pro-inflammatory processes (98). High expression of IL-32 is also associated with cell migration and invasion *in vitro*, as it can upregulate the expression of MMP-2 and MMP-9 (129). It is also interesting to note that MMPs can also potentiate the activation of cytokines, due to their activity. For example, MMP-9 can potentiate the activation of pro-inflammatory cytokines, such as TNF, IL-1β, IL-6, IL-8, and chemokines (62).

As aforementioned, ILs have important roles in carcinogenesis. Their influence in processes, such as inflammation, angiogenesis or metastasis, is crucial and very important in order to regulate the tumour microenvironment and the immune system response. They can exhibit either pro or anti-tumour effects, and some of them may have potential to be considered as a prognosis marker or even a therapeutic agent with therapeutic potential. Their immunomodulatory functions are essential in immunosurveillance and their roles can difficult their targeting, as it may change the overall function and homeostasis of the patient's immune system.

## Conclusions

The relationship between the immune system and NSCLC has been studied over the years. The tumour development seems to be associated with a local inflammation which can later on become chronic. Although at the beginning was believed that the immune system acted against the tumour, from a certain moment onwards that reaction was altered, modulated, and eventually it was concluded that the immune microenvironment helps the tumour to expand and develop. The current knowledge on the immune checkpoints have already enabled us to have immunotherapies showing promising results in this cancer. The finding of the loss of standard biomarkers allowed the scientific community not only to develop new targeted therapies, but also to have a better insight on the NSCLC patients and their prognosis or treatment response. However, this cancer continues to be the deadliest. As such, the finding of new biomarkers, which can help understand which patients have the better treatment response or their prognosis, is particularly important. Here, the markers of inflammation and metastasization are potential candidates to be looked upon as important biomarkers in this cancer. Metalloproteinases and their inhibitors as well as many ILs have already been studied and many of them were associated with poor prognosis or differences in the treatment response. Despite the potential, there are still many obstacles and limitations in incorporating these biomarkers into clinical practice since, as seen above, MMPs, TIMPs and ILs play dual roles in carcinogenesis, having pro or anti-tumoural effects. The future perspective in this area certainly involves a better knowledge on the MMPs, TIMPs and ILs mechanisms and moments of action. Therefore, it is important to understand what triggers the tumour cells to evade the immune system response and how are they able to use its intermediates and their surrounding environment to actually develop and expand. The complex networks in which these markers are involved are also a problem, in order to fully understand their action and what triggers their reaction. Nonetheless, what truly stands for the potential of these markers is the possibility to use them as markers of prognosis or of treatment response. Eventually, the development of new studies, which gather a considerable number of NSCLC patients, focused on understanding the importance of MMPS, TIMPs and ILs in the prognosis and treatment response of NSCLC patients, will improve our knowledge on the subject and will clearly show if they can be used in clinical practice. Although the potential of some MMPs, TIMPs and ILs has already been exploited and studied, with promising results seeming to offer a possibility of their use in the future, new studies are needed to clarify and actually verify the results that have been reported.

## Footnote

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