# Research progress on tumour-associated macrophages in gastric cancer (Review)

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**Abstract.** Tumour-associated macrophages (TAMs) are immune cells that are present in large numbers in the tumour immune microenvironment. TAMs are important for the occurrence, development, invasion, metastasis and immune escape of tumours. TAMs have become a novel therapeutic target and prognostic indicator in the individualised treatment of patients. Studies have reported that the number of TAMs can predict the size, stage and metastasis of gastric cancer. Therefore, in-depth examination of TAMs may be important for high-risk screening, early diagnosis and prognostic judgment of patients with gastric cancer. The present review examined the research progress of TAMs in gastric cancer on the basis of previous literature studies. Moreover, this review systematically evaluated the three major aspects of the differentiation of macrophages, the tumour-promoting mechanism of TAMs in gastric cancer and the relationship between TAMs and treatment of gastric cancer. Finally, this review aimed to provide a reference for investigating the prognostic indicators and treatment targets of patients with gastric cancer.

**Contents**



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## 1. Introduction

Gastric cancer is the third leading cause of cancer-related mortality worldwide (1). The incidence of gastric cancer is lowest in Northern Europe and Northern America, and remains highest in Eastern and Central Asia and Latin America (1-3). In all confirmed cases of gastric cancer, >1/3 of cases occur in China. Moreover, the incidence and mortality rates of gastric cancer in China rank second amongst all cancer types and are second only to lung cancer (4). The age-standardised 5-year survival rate of gastric cancer in the Chinese population was only 35.9% in 2010-2014 (5). Although immune checkpoint blocking therapy can improve the survival rate of some patients with gastric cancer (6), not all patients can benefit from this immunotherapy. Numerous patients with gastric cancer have problems that should be addressed, such as hyperprogressive diseases (7-12), low efficiency of a single drug (13-15) and treatment-related adverse events (TRAEs) (16-26).

For example, immune checkpoint inhibitors cause imbalances in immunological tolerance, resulting in inflammatory side effects which are called immune-related adverse events (irAEs). Masuda *et al* reported that the development of irAEs was closely associated with clinical responses of patients with advanced gastric cancer in nivolumab monotherapy (27). Park *et al* revealed that irAEs may predict overall survival (OS) as well as progression-free survival (PFS) and represent meaningful biomarkers across different types of cancer including gastric cancer (28).

All of these issues aforementioned may be associated with the complex regulation of the tumour immune microenvironment, as the immune contexture can convey important information associated with prognosis and therapeutic

responsiveness (29-35). The tumour immune microenvironment is composed of innate immune cells, adaptive immune cells and cytokines (CKs), amongst others. These immune components form a complex regulatory network. Neutrophils secrete tumour-promoting factors (36), while T cells and NK cells secrete antitumour factors (37,38). Moreover, regulatory T cells (Tregs), regulatory B cells and myeloid-derived suppressor cells (MDSCs) secrete immunosuppressive cytokines (CKs) (37). It has been revealed that macrophages secrete antitumour factors and tumour-promoting factors, depending on their state of differentiation (39,40).

A previous study (41) has reported that the prognosis of colorectal cancer is positively correlated with high-density macrophages. It has also been revealed that the prognosis of most tumours, such as liver cancer and breast cancer, is inversely associated with high-density macrophages (41). However, the correlation between macrophages and the prognosis of bone tumours, prostate cancer, lung cancer and gastric cancer remains controversial; therefore, these cancer types have a strong research value. For example, Zhang *et al* observed that the higher the number of tumour-associated macrophages (TAMs), the worse the prognosis of patients with gastric cancer (42). Another meta-analysis also supported this conclusion (43). However, Wang *et al* reported that the more TAMs, the more favourable the prognosis of patients with gastric cancer, and that patients with diffuse-type gastric cancer had a higher macrophage infiltration density compared with patients with intestinal-type gastric cancer (44). These research results indicated that TAMs are a research hotspot in the field of gastric cancer. The present review systematically examined the research progress of TAMs in gastric cancer in recent years, based on the three major aspects of the differentiation of macrophages, the tumour-promoting mechanism of TAMs in gastric cancer and the relationship between TAMs and treatment of gastric cancer.

## 2. Differentiation of macrophages

Macrophages originate from monocytes in the blood circulation (45), and are important participants in the innate immune response. Given their high plasticity, macrophages primarily exist in two different states of differentiation (46) (Fig. 1).

M1-type macrophages are activated by interferon-γ (IFN-γ), lipopolysaccharide (LPS) and Toll-like receptor (TLR) ligands. These macrophages can secrete CKs, such as IL-6, IL-12, IL-23 and TNF-α, and these CKs exert pro-inflammatory, cytotoxic and antitumour effects (46,47). On the other hand, M2-type macrophages are activated by IL-4 and IL-13. These macrophages secrete CKs, such as IL-10 and transforming growth factor-β (TGF-β), which possess anti-inflammatory and tumour-promoting effects (47,48). With regard to phenotype, M1-type macrophages highly express CD64, CD68, CD86 and major histocompatibility complex (MHC) 2 (46,49), while M2-type macrophages lowly express MHC2 and feature the expression of CD163, CD200 receptor (CD200R) and CD206 (46,49).

$CD204^+$ macrophages in the stroma are receptors for M2-type macrophages. It has been reported that an increase in the number of these macrophages may be associated with the occurrence of gastric cancer (50). However, the correlation between M2-type macrophages and the prognosis of gastric cancer is currently controversial. For example, Kim *et al* revealed that high-density M2-type macrophage infiltration was associated with favourable disease-free survival (DFS) in patients with gastric cancer (51). However, Park *et al* revealed that high-density M2-type macrophage infiltration was associated with poor DFS in patients with gastric cancer (52). Based on a previous study, high-density M2-type macrophages are also associated with poor OS in patients with gastric cancer (53).

Under the action of IL-4, IL-10 and IL-13, macrophages can be recruited around tumour cells and eventually differentiated into TAMs (Fig. 1). Macrophages co-cultured with gastric cancer cells likely differentiate into M2-type TAMs (54). M2-type TAMs have evident immunosuppressive effects on diffuse-type and genomically stable-type gastric cancer (55). Furthermore, M2-type TAMs can promote peritoneal metastasis of gastric cancer via the epidermal growth factor receptor signalling pathway in the abdominal cavity of gastric cancer patients with peritoneal metastasis (56).

A major feature of the tumour microenvironment of gastric cancer is the chronic inflammation caused by *Helicobacter pylori* (Hp) infection. This feature is the classic determinant of gastric cancer (57). It has been revealed that Hp can damage the immune response of M1-type macrophages and lead to the differentiation of M2-like macrophages, thereby promoting reactive oxygen species-induced macrophage apoptosis (58). Another major feature of the tumour microenvironment of gastric cancer is hypoxia. For one thing, macrophages and hypoxia serve an important role in regulating the invasive ability of gastric cancer cells *in vitro* (59). For another thing, hypoxia decreases the percentage of M1-type macrophages by targeting microRNA (miR)-30c and mTOR in human gastric cancer (60). Furthermore, the upregulation of endothelin-2 and vascular endothelial growth factor (VEGF) can mediate the accumulation of TAMs in gastric cancer in hypoxic areas, ultimately promoting the differentiation of M1-type macrophages into M2-type macrophages (50).

## 3. Tumour-promoting mechanism of TAMs in gastric cancer

*TAMs promote angiogenesis in gastric cancer.* When gastric cancer cells are stimulated by a hypoxic environment, macrophages can be recruited in the tumour microenvironment of gastric cancer and are differentiated into TAMs (61). On the one hand, TAMs can promote the activation of the hypoxia-related signalling pathways and increase the activity of matrix metalloproteinases (50,61). Moreover, TAMs facilitate the formation of microvessels in gastric cancer (50,61). On the other hand, the expression of vasohibin-1 tissue is significantly and positively correlated with the expression of VEGF-A in gastric cancer. TAMs can upregulate vasohibin-1 to promote angiogenesis in gastric cancer (62). In addition, thymidine phosphorylase expressed by TAMs can promote angiogenesis in gastric cancer (63).

M2-type TAMs serve an important role in the angiogenesis of gastric cancer. M2-type macrophage culture medium treated with high-mobility group protein B1 (HMGB1) can promote the angiogenesis of human gastric cancer MKN-45

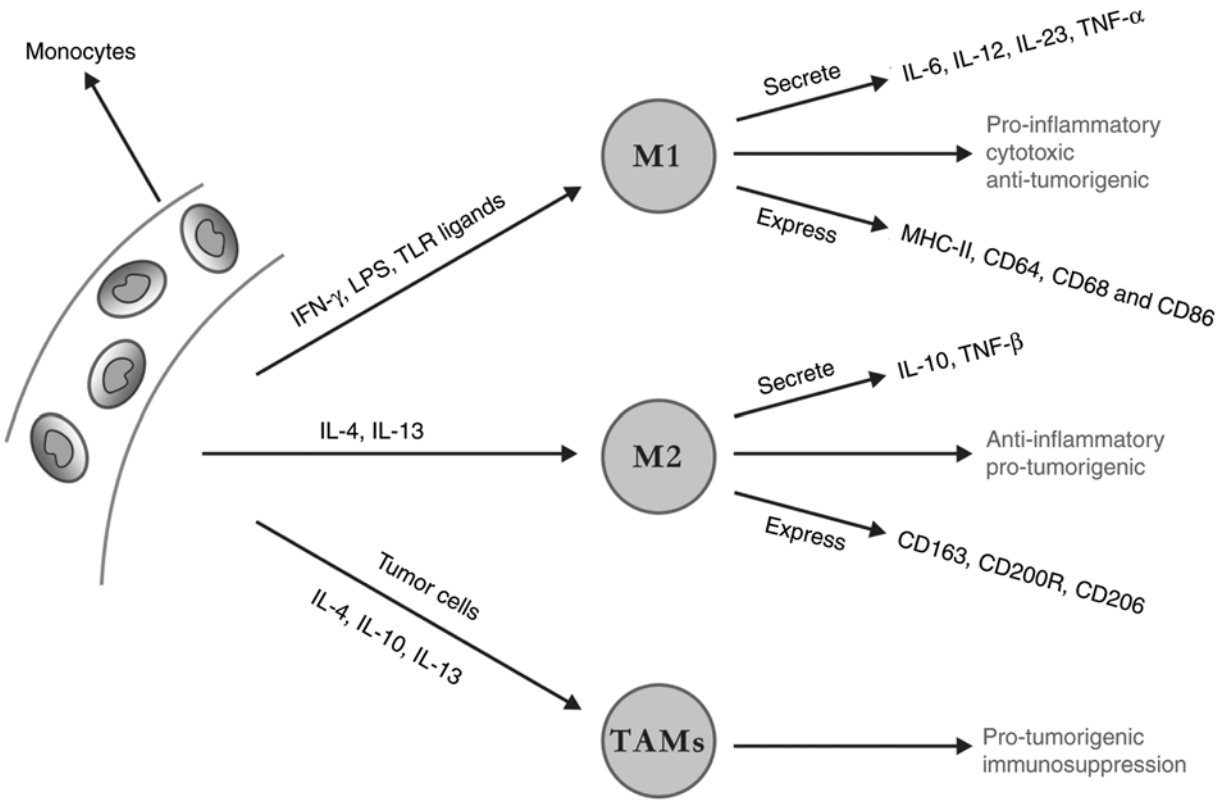


Figure 1. Macrophage differentiation and their roles. M1-type macrophages are activated by IFN-γ, LPS or TLR ligands. M1-type macrophages can secrete pro-inflammatory cytokines and serve anti-tumorigenic roles. M2-type macrophages are activated by IL-4 and IL-13, and can secrete anti-inflammatory cytokines and serve pro-tumorigenic roles. TAMs exert pro-tumorigenic roles. IFN, interferon; LPS, lipopolysaccharide; TRL, Toll-like receptors; TAMs, tumour-associated macrophages.

cell line *in vitro*. It has also been revealed that $CD163^+$ TAMs in gastric cancer are associated with the increased density of microvessels in cancer nests, tumour stroma and tumour invasive margins, indicating that M2-type TAMs can promote angiogenesis in gastric cancer (52).

*TAMs promote the invasion and metastasis of gastric cancer.* Invasion and metastasis are important causes of poor prognosis of patients with gastric cancer (64-73). These processes represent a multistep biological cascade that leads to widespread dissemination of gastric cancer cells in various tissues (74). TAMs can induce the expression of transcription factor forkhead box Q1 (FOXQ1) (75) and TGF-β1 (76) to promote the epithelial-mesenchymal transition (EMT), invasion and metastasis of gastric cancer cells. The coexistence of TAMs and TGF-β is associated with tumour aggressiveness, which can be an independent prognostic factor for gastric cancer (50). Moreover, the cytoskeleton rearrangement during EMT is an important mechanism of tumour invasion and metastasis. TAM-derived exosomes can activate the PI3K/AKT signalling pathway, thereby mediating the transfer of apolipoprotein E from TAMs to gastric cancer cells, and ultimately induce the cytoskeletal rearrangement and metastasis of gastric cancer (77,78) (Fig. 2). In addition, the expression of chemokine CXCL12 is closely associated with the recruitment of M2-type TAMs in tumour invasive margins. It has been suggested that CXCL12 may be involved in the invasion of gastric cancer (52). C-X-C Motif Chemokine Ligand 8 (CXCL8), ADAM metallopeptidase domain (ADAM) 8, ADAM9, C-C motif chemokine ligand (CCL) 5, secreted phosphoprotein 1, semaphorin 4D, TIMP metallopeptidase inhibitor 3, T-cell immunoglobulin mucin family member 3 (Tim-3) and Vinculin-2 are also indicated to be involved in the invasion and metastasis of gastric cancer cells caused by TAMs (79-82).

TAMs can spread through the lymphatic vessels of patients with gastric cancer, thus promoting the invasion and metastasis of gastric cancer cells (83). The interaction between lymph node-derived lymphatic endothelial cells and TAMs in gastric cancer may be an important initial step in the progression of lymphangiogenesis to lymph node metastasis (54). HMGB1 is also associated with the lymph node metastasis of gastric cancer. HMGB1 can activate the receptor for advanced glycosylation end products to increase the tumour-promoting activity of M2-type macrophages and enhance the invasive ability of the co-cultured human gastric cancer MKN-45 cells (84).

*TAMs in gastric cancer promote chemotherapy resistance.* Cisplatin is a commonly used drug for the treatment of advanced gastric cancer. However, long-term medication can result in resistance. In addition to increased drug efflux and enhanced anti-apoptotic effects caused by genetic changes in tumour cells, the protection of the tumour microenvironment on tumour cells can lead to drug resistance. Considering that the overexpression of miR-21 has no effect on the ATP-binding cassette transporter gene of gastric cancer cells in the tumour

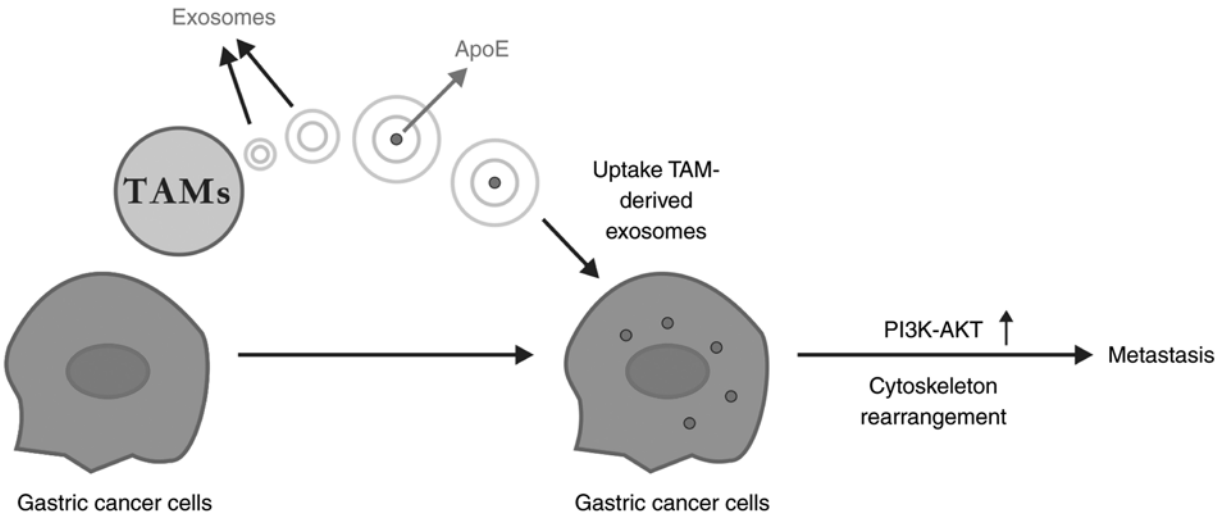


Figure 2. PI3K/AKT signalling pathway mediates TAM-derived exosomal ApoE-induced metastasis. Exosomes secreted by TAMs transfer ApoE into gastric cancer cells, leading to PI3K/AKT signalling pathway activation, cytoskeletal rearrangement and metastasis of gastric cancer cells. TAMs, tumour-associated macrophages; ApoE, apolipoprotein E.

microenvironment, TAM-derived exosomes can transport miR-21 from M2-type TAMs to gastric cancer cells. This extracellular transport can downregulate PTEN and enhance the activity of AKT, thereby increasing the survival rate of gastric cancer cells (85). Thus, targeted therapy of miR-21 extracellular transport caused by TAM-derived exosomes may improve the resistance of patients with gastric cancer to cisplatin.

## 4. TAMs in gastric cancer and immune checkpoint

Programmed death protein 1 (PD-1) and its two ligands programmed death-ligand 1 (PD-L1) as well as programmed death-ligand 2 (PD-L2) serve as an immune checkpoint axis which can suppress T-cell proliferation in carcinoma (86,87). While the prognosis of gastric cancer remains poor, PD-1 and PD-L1/PD-L2 are promising prognostic biomarkers (88).

*TAMs in gastric cancer and PD-1/PD-L1.* PD-1/PD-L1 signalling pathway has become the hot spot of current immunotherapies for gastric cancer. D'Ignazio *et al* observed a higher number of $CD68^+$ macrophages with a lower number of $CD163^+$ macrophages and the inhibition of the PD-1/PD-L1 in gastric and colorectal patients treated with enteral immunonutrition (89). Consequently, there is an intricate relationship between macrophages and the PD-1/PD-L1 signalling pathway during the progression and treatment of gastric cancer.

PD-1 is one of the best-studied and most clinically successful immune checkpoint drug targets. Kono *et al* performed double immunohistochemical staining of PD-1 and CD68 in gastric cancer tissue and found numerous $PD\text{-}1^+CD68^-$ tumour infiltrating cells (90). They also determined the frequency of $PD\text{-}1^+$ macrophages in gastric cancer tissue by flow cytometry. Flow cytometric analysis revealed that $PD\text{-}1^+$ macrophages in gastric cancer express more CD206, indicating that these $PD\text{-}1^+$ macrophages exhibited an M2-type profile. Similarly, Wang *et al* revealed that $PD\text{-}1^+$ TAMs express an M2-type surface molecule, such as a significant increase in the expression of CD206, and a clear decrease in the expression of an M1-type surface molecule including CD64 (91).

PD-L1 is a key protein upregulated by tumour cells to suppress the immune response. $PD\text{-}L1^+$ TAMs were revealed to account for approximately 50% of all $PD\text{-}L1^+$ cells in gastric cancer (92). Harada *et al* performed immunohistochemical staining of PD-L1, CD68 and CD163 in 217 gastric adenocarcinoma tissue specimens from the tissue microarrays (93). These authors observed that M2-type TAMs could promote the expression of PD-L1 in gastric cancer cells. Moreover, the expression of PD-L1 in gastric adenocarcinoma cells was examined, and a high density of $CD68^+$ cells and $CD163^+$ cells was identified (CD68, $P=0.0002$; CD163, $P<0.0001$; the P-value indicated that the correlation between the expression of PD-L1 and CD163 was closer). In addition, Huang *et al* also identified $CD206^+$ macrophages to be most relevant to high PD-L1 expression (92).

To summarize, both PD-1 and PD-L1 are markedly more closely associated with M2-type TAMs in gastric cancer. Targeting M2-type TAMs may represent an effective approach to modulate the activity of anti-PD-1/PD-L1 agents and combined M2-type TAM-centered strategies should be developed to maximize the efficacy of anti-PD-1/PD-L1 agents in gastric cancer.

*TAMs in gastric cancer and PD-L2.* PD-L2 is a less-studied ligand of PD-1 in gastric cancer. Nakayama *et al* revealed that IFN-γ (which can activate M1-type macrophages), and also to a lesser extent, IL-4 (which can activate M2-type macrophages and TAMs) could upregulate PD-L2 expression in gastric cancer cells (94). Thus, correlation analysis was conducted between PD-L2 proteins and CD proteins from M1-type TAMs as well as M2-type TAMs in gastric cancer, by our research group. Public genomic data sets from The Cancer Genome Atlas (TCGA; https://portal.gdc.cancer.gov) (95) were analysed and TCGA RNA-Seq data of gastric adenocarcinoma were first assessed. As indicated in Figs. 3 and 4, the correlation between the expression of PD-L2 and CD163 was

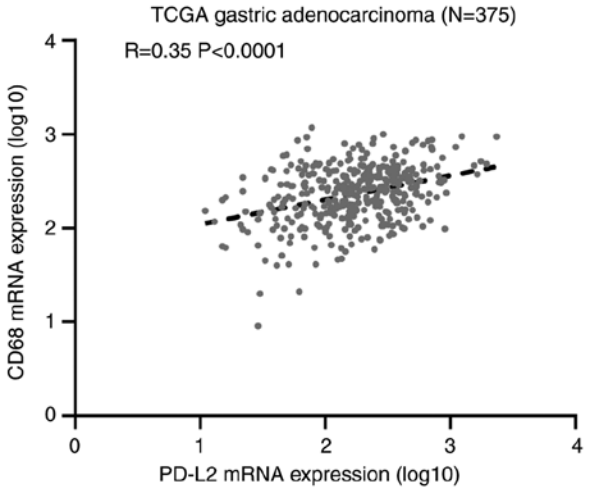


Figure 3. Correlation assay between PD-L2 and CD68 expression in gastric adenocarcinoma from TCGA datasets. PD-L2, programmed death-ligand 2; TCGA, The Cancer genome Atlas.

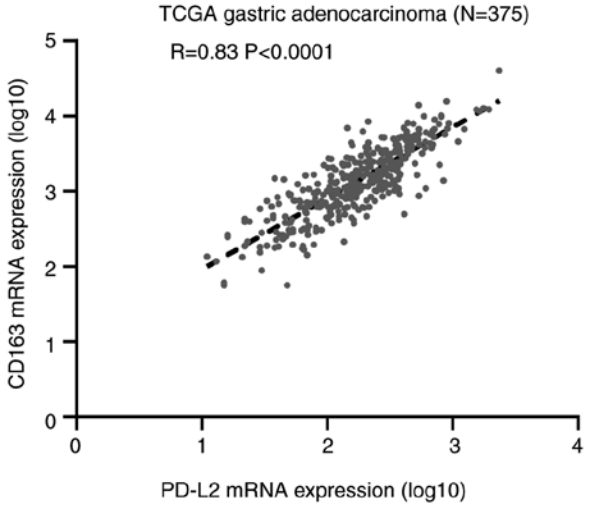


Figure 4. Correlation assay between PD-L2 and CD163 expression in gastric adenocarcinoma from TCGA datasets. PD-L2, programmed death-ligand 2; TCGA, The Cancer genome Atlas.

closer. Hence, PD-L2 was revealed to be significantly more closely associated with M2-type TAMs in gastric cancer and its expression should be considered when determining the optimal immunotherapy for gastric cancer.

## 5. TAMs affect the immune response of patients with gastric cancer

*PD-1$^+$ TAMs in gastric cancer impair CD8$^+$ T cells via IL-10.* TAMs express PD-1 at a significantly higher level compared with that in the surrounding healthy tissues. Wang *et al* provided a new insight into possible manipulation of PD-1$^+$ TAM-mediated immunosuppression in gastric cancer (91). These authors reported that TAMs from patients with gastric cancer shared markedly increased PD-1 levels, which promoted tumour progression by impairing the antitumour functions of CD8$^+$ T cells. Moreover, PD-1$^+$ TAMs possessed stronger immunosuppressive activity of CD8$^+$ T-cell function compared with PD-1$^-$ TAMs. When PD-1$^+$ TAMs interacted with PD-L1$^+$ cells, IL-10 was produced in large quantities to induce the dysfunction of CD8$^+$ T cells and impaired the anti-tumour immune response. These results indicated that PD-1 signal immunotherapies may function through a direct effect on PD-1$^+$ TAMs.

*Lipid-accumulated TAMs in gastric cancer reduce phagocytic potency and upregulate PD-L1.* Previous studies have addressed the important role of lipids in immune cells, including myeloid-derived suppressor cells and dendritic cells (96-98). Luo *et al* provided evidence that lipid accumulation also presents in TAMs (99). They demonstrated that the effect of lipid accumulation conferred the M2-type polarization of TAMs in gastric cancer. On the one hand, lipid-accumulated TAMs in gastric cancer reduced phagocytic potency against tumour cells. On the other hand, lipid-accumulated TAM upregulated PD-L1 expression, which blocks antitumour T-cell responses to support their immunosuppressive functions. There is an abundance of lipids in the tumour microenvironment of gastric cancer that can be acquired by TAMs. Increased serum lipid levels are present in patients with gastric cancer and favour tumour progression. Thus, exploring the mechanisms of lipid-laid TAMs holds potential for the development of therapeutic interventions in gastric cancer. Moreover, these authors also revealed that the PI3-kinase-γ (PI3K-γ) signalling pathway may contribute to the intrinsic lipid generation in TAMs in the murine gastric cancer cell line MFC, and the reduced lipid accumulation in TAMs may be due to the dominant M1-type TAMs after PI3K-γ inhibitor treatment. To sum up, targeting of PI3K-γ signalling pathways in TAMs may provide a novel potential approach to improve the long-term survival of patients with gastric cancer.

*Dendritic cell-specific intercellular adhesion 3-grabbing non-integrin (DC-SIGN)$^+$ TAMs in gastric cancer promote an immunoevasive tumour microenvironment.* DC-SIGN is one of the most widely researched C-type lectin receptors, and these are mainly expressed on certain macrophages and dendritic cells. Liu *et al* identified that DC-SIGN$^+$ TAMs were highly infiltrated in patients with gastric cancer and this high infiltration of DC-SIGN$^+$ TAMs was closely associated with a higher ratio of Foxp3$^+$ Tregs/CD8$^+$ T cells (100). These CD8$^+$ T cells in the high DC-SIGN$^+$ TAMs subgroup failed to exert antitumour immunity. There were decreased expression levels of IFN-γ, granzyme B and perforin, as well as increased expression levels of PD-1 and CTLA-4 in the tumour microenvironment of gastric cancer, suggesting that DC-SIGN$^+$ TAMs in gastric cancer could promote an immunoevasive tumour microenvironment. Conclusively, DC-SIGN$^+$ TAMs may be independent prognosticators for gastric cancer and could improve the therapeutic strategy of fluorouracil-based adjuvant chemotherapy and immune checkpoint inhibitors.

*TAMs in gastric cancer impair NK cells via TGFβ1.* The percentage of NK cells in tumour tissue is significantly decreased in advanced gastric cancer, and this low percentage of NK cells positively correlates with poor OS of patients with gastric cancer. Peng *et al* investigated the relationship between macrophages and NK cells in tumour tissue from patients with

gastric cancer, and their results demonstrated a role for TAMs in NK-cell functional impairment (101). On the one hand, TAMs in gastric cancer suppressed the expression of Ki-67, IFN-γ and TNF-α in NK cells. On the other hand, TAMs in gastric cancer isolated from tumour tissue produced higher TGFβ1 (a known inhibitor of NK cell function) compared with those from non-tumour tissues, and flow cytometric analysis revealed that TGFβ1 was absent on the surface of TAMs in gastric cancer, suggesting that TAMs in gastric cancer may secrete TGFβ1 to mediate NK-cell functional impairment. To further confirm this hypothesis, an antibody against TGFβ1 was added to the coculture system of TAMs in gastric cancer and NK cells. Eventually, these authors demonstrated that TGFβ1 blockade subsequently attenuated TAM-mediated suppression of Ki-67, IFN-γ and TNF-α expression in NK cells. In conclusion, blockade of TGFβ1 could restore the function of NK cells and could be a useful therapeutic strategy for patients with gastric cancer.

## 6. TAMs and treatment strategies of gastric cancer

The treatment of gastric cancer involves surgical resection, chemotherapy, radiation therapy and immunotherapy (102). The current overall treatment strategy for gastric cancer is a comprehensive treatment based on surgery. Furthermore, radical gastrectomy is the only radical treatment for gastric cancer. Although various therapies have developed in recent years, the mortality rate of gastric cancer remains high as the early stage of this cancer type is asymptomatic (103). Thus, traditional treatments must be improved, and novel treatment regimens should be developed.

*Methionine enkephalin (MENK).* MENK is an endogenous opioid penta-peptide (104). MENK at a suitable range of concentrations not only possesses immunotherapeutic activity (105-107), but also promotes the polarization of TAMs from M2-type to M1-type.

Wang *et al* identified that human gastric cancer cell lines HGC27 and SGC7901 expressed opioid receptor (OGFr) (49). These authors revealed that MENK upregulated the expression of OGFr, while it inhibited proliferation and induced HGC27 as well as SGC7901 cell line apoptosis by blocking the PI3K/AKT/mTOR signalling pathway. They also demonstrated that MENK increased the expression levels of CD64 and TNF-α, but decreased the expression levels of CD206 and IL-10, suggesting that MENK could exert its antitumour function by inducing TAM polarization from the M2-type to M1-type in gastric cancer. These findings may provide evidence to improve the clinical treatment of gastric cancer. However, Wang *et al* did not indicate specific opioid receptor subtypes. Which subtype opioid receptor (Mu, Delta Kappa) involved here should be further investigated.

*Sophoridine.* Sophoridine is an alkaloid extracted from seeds of *Sophora alopecuroides L.*, which has anti-arrhythmia function (108) and antitumour activities (109).

Zhuang *et al* demonstrated that sophoridine upregulated IL-12α and TNF-α, while it downregulated IL-10 and CD206 via the TLR4/IRF3 signalling pathway in the tumour microenvironment of gastric cancer, suggesting that sophoridine promoted TAMs in gastric cancer to polarize towards the M1-type, as well as suppressed M2-type polarization (109). As is well known, $CD8^+$ T cells are a major antitumour factor (110). Sophoridine-treated TAMs could increase the cytotoxic function of $CD8^+$ T cells and the percentage of gastric cancer cell lysis by upregulating granzyme B and perforin, and downregulating PD-1 and Tim-3. Furthermore, the C-C motif chemokine receptor 2 (CCR2)/CCL2 signalling pathway is considered to be associated with macrophage infiltration into the tumour microenvironment (111,112). Sophoridine could also inhibit macrophage infiltration into the tumour microenvironment of gastric cancer by downregulating the expression of CCR2 (109). Therefore, Chinese medicine may have important implications in gastric cancer treatment, and sophoridine may be a potential therapeutic candidate.

*Emactuzumab in combination with selicrelumab.* The most significant signalling pathway associated with TAM recruitment and proliferation is CSF-1/CSF-1 receptor (CSF-1R), vital to the transition from M1-type TAM into M2-type TAM (50). The anti-CSF-1/CSF-1R signalling pathway can reduce the infiltration of M2-type macrophages into tumour tissues (50). Emactuzumab is a monoclonal antibody directed against CSF-1R expressed by macrophages (113). Selicrelumab is a selective agonistic cluster of differentiation 40 (CD40) monoclonal antibody (114), which has been tested clinically along with tremelimumab (115).

Machiels *et al* evaluated the phase Ib study of selicrelumab in combination with emactuzumab in 37 advanced solid tumour patients including 3 patients with gastric carcinoma (116). They revealed that the best objective clinical response was stable disease in 40.5% of patients. The most frequently TRAEs were infusion-related reactions (75.7%), fatigue (54.1%), facial edema (37.8%), increase in aspartate aminotransferase (35.1%) and creatinine phosphokinase (35.1%). Selicrelumab in combination with emactuzumab demonstrated a manageable safety profile and triggered $CD8^+$ T-cell increase and a decrease of TAMs in the solid tumour. However, this combination therapy did not translate into objective clinical responses.

*Nanoparticle albumin-bound (nab)-paclitaxel in combination with ramucirumab.* Paclitaxel is one of the most effective antineoplastic agents for the treatment of numerous forms of cancer (117). Nab-paclitaxel was developed to improve paclitaxel solubility and does not need premedication to avoid infusion-related reactions associated with solvent-based paclitaxel (118). Ramucirumab is the first targeted drug approved by the U.S. Food and Drug Administration for the treatment of advanced gastric cancer, after failure of previous chemotherapy (119). The inhibition of ramucirumab on the VEGF receptor 2 can reduce the immune infiltration of TAMs and the release of CKs and chemokines, as well as inhibit the proliferation and reproduction of gastric cancer cells and improve the clinical prognosis of patients with gastric cancer (50).

Bando *et al* conducted a single-arm phase II study to investigate the efficacy and safety of nab-paclitaxel plus ramucirumab combination therapy in patients with advanced gastric cancer in refractory to first-line chemotherapy (120). It was demonstrated that the overall response rate of this combination therapy for pre-treated patients with advanced

gastric cancer was 54.8%. The median PFS was 7.6 months, and the toxicities were manageable. Moreover, the main grade 3/4 TRAEs included decreased neutrophil count (76.7%), decreased white blood cell count (27.9%), anaemia (11.6%), decreased appetite (7.0%), hypertension (4.7%), proteinuria (4.7%) and febrile neutropenia (4.7%). No treatment-related mortalities occurred. It was determined that dose modification of nab-paclitaxel due to febrile neutropenia may decrease the cumulative dose of nab-paclitaxel. Correspondingly, treatment continuation may be longer. However, in general, nab-paclitaxel in combination with ramucirumab demonstrated favourable activity and a manageable safety profile. Therefore, this combination therapy may be a promising treatment option for previously treated patients with advanced gastric cancer.

*Lenvatinib in combination with pembrolizumab*. It has been reported that the response rates with pembrolizumab (a PD-1 inhibitor) treatment were limited to ~15% in patients with advanced gastric cancer who had a PD-L1 combined positive score of ≥1 (14). The development of novel combination therapies is required to improve the treatment response rates. Lenvatinib, a multi-kinase inhibitor, increased the infiltration of $CD8^+$ T cells and decreased TAMs levels, as well as enhanced the activation of the IFN signalling pathway and the antitumour function of PD-1 inhibitors (121).

Kawazoe *et al* conducted a single-arm phase II study to investigate the efficacy and safety of lenvatinib plus pembrolizumab combination therapy in patients with gastric cancer in the first-line or second-line settings (122). These authors identified that the objective response rate of this combination therapy was 69% and the median PFS was 7.1 months. The main grade 3 TRAEs included hypertension (38%), proteinuria (17%) and decreased platelet count (7%). No grade 4 TRAEs and treatment-related mortalities occurred. Although all patients required at least one dose reduction of lenvatinib owing to proteinuria and serious adverse effects of anti-angiogenic therapies, such as gastric haemorrhage and gastric perforation, lenvatinib in combination with pembrolizumab demonstrated promising antitumour function and manageable toxicities.

## 7. Future challenges

One reason for the controversy between TAMs and gastric cancer prognosis is the absence of histological sites. Park *et al* reported that $CD163^+$ TAMs in the tumour stroma and tumour invasive margins were associated with not only size, depth of invasion, TNM staging, lymph node metastasis and lymphatic invasion of gastric cancer, but also with poor OS and DFS of patients with gastric cancer (52). Moreover, TAMs in cancer nests are associated with histological types and poor DFS, but not with OS. M2-type TAMs in the tumour stroma and tumour invasive margins have a stronger influence on the progression and poor prognosis of gastric cancer compared with the M2-type TAMs in the cancer nest. In another study, Wang *et al* revealed that, while macrophages in healthy tissues and adjacent tissues had no effect on the prognosis of patients with gastric cancer, the greater the number of the combination of macrophages and Tregs in the tumour tissue, the higher the survival rate of patients with gastric cancer. Therefore, TAMs at different histological sites may have different effects on the progression and prognosis of patients with gastric cancer. Thus, future in-depth investigations of TAMs in gastric cancer must consider the differences caused by various histological sites (53).

The prognostic effects of different histological types of TAMs on gastric cancer are significant. For example, Kawahara *et al* observed that high-density TAMs were significantly associated with the poor prognosis of patients with intestinal-type gastric cancer but not with the survival of patients with diffuse-type gastric cancer (63). In another study, Liu *et al* conducted a multivariate survival analysis of 598 patients with gastric cancer (123). These authors reported that $CD163^+$ M2-type TAMs were independent prognostic factors. Moreover, it was revealed that expression levels of $CD163^+$ M2-type TAMs was low in signet-ring cell carcinoma and mucinous adenocarcinoma, and was high in poorly differentiated adenocarcinoma. However, the high-density M2-type TAM infiltration in signet-ring cell carcinoma and mucinous adenocarcinoma indicated a favourable prognosis. Therefore, the prognostic significance of M2-type TAMs in gastric cancer in different histological types should be further clarified.

## 8. Conclusions

TAMs serve a significant role in the development of gastric cancer. The tumour-promoting mechanism of TAMs in gastric cancer involves angiogenesis, invasion, metastasis, chemotherapy resistance and immune tolerance. TAMs also demonstrated a favourable application potential in the prognostic evaluation and treatment of patients with gastric cancer. With the continuous optimisation of technology and progression of research, the findings of TAMs will gradually enter the clinical field and provide references for the individualised treatment of patients with gastric cancer.

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## Availability of data and materials

Not applicable.

## Authors' contributions

ZZ searched the literature and drafted the manuscript. ZY and HZ assisted with the critical revision of the manuscript. QW, XJ and JW were involved in the conception of the study. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

Not applicable.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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