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Chemokine-targeted therapies: An opportunity to remodel immune profiles in gastro-oesophageal tumours

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ABSTRACT

Immunotherapies are transforming outcomes for many cancer patients and are quickly becoming the fourth pillar of cancer therapy. However, their efficacy of only ~25% in gastro-oesophageal cancer has been disappointing. This is attributed to factors such as insufficient patient stratification and the pro-tumourigenic immune landscape of gastro-oesophageal tumours. The chemokine profiles of solid tumours and the availability of effector immune cells greatly influence the immune infiltrate, producing 'cold' or 'immune-excluded' tumours in which immunotherapies are unable to reinvigorate the immune response. Other biological functions for chemokines have emerged, such as promoting cell survival, polarising T cell responses, and supporting several hallmarks of cancer. Therefore, chemokine networks may be exploited with therapeutic intent to mobilise and polarise anti-tumour immune cells, with further utility as combination treatments to augment the efficacy of current cancer immunotherapies. Few studies have demonstrated the clinical benefit of chemokine-targeted therapies as monotherapies, and this review proposes their consideration as combination treatments. Herein, we explore the anti-tumour and pro-tumour implications of chemokine signalling in gastro-oesophageal cancer and discuss their value as prognostic and predictive biomarkers in response to treatment.

1. Current therapeutic landscape in gastro-oesophageal cancer

Gastro-oesophageal cancers (GOC) have a global incidence of 17.4 cases per 100,000, with a mortality rate of 13.3 cases per 100,000 [1]. An estimated 640,100 cases of oesophageal cancer and over 1 million cases of gastric cancer were recorded in 2020 [1]. Five-year overall survival (OS) rates for oesophageal and gastric cancers remain as low as 5%, depending on disease stage at clinical presentation [2,3]. Current standard of care practices for treating oesophageal cancer include neo-adjuvant chemoradiation therapy prior to surgical resection of tumour (oesophagectomy) [4]. A typical chemoradiation regimen includes carboplatin and paclitaxel with radiation therapy and such a regimen is also suitable for carcinomas of the gastro-oesophageal junction [5]. Response rates to standard of care chemoradiation therapy in oesophageal cancer have been reported in the range of 43.8%–47.1% [6]. In gastric cancers, the current standard of care measures includes surgical resection (gastrectomy) followed by adjuvant chemotherapy or chemoradiation therapy [7]. A typical adjuvant chemotherapy regimen for gastric cancer includes 5-fluorouracil and cisplatin [8]. Response rates to standard of care adjuvant chemotherapy in gastric cancers have been reported as approximately 20% [8].

Oesophageal squamous cell carcinoma (OSCC) is the main histological subtype of oesophageal cancer, representing approximately 90% of all oesophageal cancer diagnoses worldwide [9]. Oesophageal adenocarcinoma (OAC) is the dominant subtype in western regions including North America, Europe and Oceania, which account for approximately 46% of global OAC diagnoses [9]. Gastric adenocarcinoma represents 90–95% of gastric cancer diagnoses worldwide, with the remainder including gastrointestinal stromal tumours, neuroendocrine tumours, and primary lymphomas [10].

Known risk factors contributing to the development of GOC include excessive alcohol consumption, tobacco smoke and obesity [11]. The chronic inflammatory condition gastro-oesophageal reflux disease is a major risk factor for developing OAC [12]. Chronic acid reflux causes the metaplastic conversion of squamous epithelial cells to a columnar conformation, a pre-malignant condition known as Barrett's Oesophagus (BO), with an annual conversion rate to OAC of approximately 0.2–0.3% [12,13]. Following this period of metaplasia, the progression to carcinoma can occur and follows a stepwise sequence of low-grade dysplasia to high grade dysplasia and finally progressing to OAC [12].

Approximately 50% of oesophageal cancer patients present with

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List of abbreviations

Bcl-2	B cell lymphoma 2
BO	Barrett's Oesophagus
CCL	Chemokine (C-C motif) ligand
CCR	Chemokine (C-C motif) receptor
CAF	Cancer-associated fibroblast
CAR	Chimeric antigen receptor
CTL	Cytotoxic T lymphocyte
CXCL	Chemokine (C-X-C motif) ligand
CXCR	Chemokine (C-X-C motif) receptor
CX_3CL	Chemokine (C-X_3-C motif) ligand
CX_3CR	Chemokine (C-X_3-C motif) receptor
DC	Dendritic cell
EMT	Epithelial mesenchymal transition
FoxP3	Forkhead box protein 3
GOC	Gastro-oesophageal cancer
ICI	Immune checkpoint inhibitor
IFN	Interferon
IL	Interleukin
MAPK	Mitogen-activated protein kinase
MDSC	Myeloid-derived suppressor cell
MHC	Major histocompatibility complex
MMP	Matrix metalloproteinase
NK	Natural killer
OAC	Oesophageal adenocarcinoma
OS	Overall survival
OSCC	Oesophageal squamous cell carcinoma
PD-1	Programmed death 1
PD-L1	Programmed death ligand 1
PI3K	Phosphoinositol 3 kinase
RFS	Recurrence-free survival
TAM	Tumour-associated macrophage
TGF	Transforming growth factor
TME	Tumour microenvironment
TNF	Tumour necrosis factor
VEGF	Vascular endothelial growth factor

advanced metastatic disease [14]. Furthermore, the overall 5-year survival rate for metastatic patients remains at 5%, compared to 20% in non-metastatic patients [2]. Further complicating matters, only 30–40% of these patients demonstrate an overall response to chemotherapy [15], thus warranting novel clinical strategies, such as immunotherapy to improve survivorship. The most prevalent metastatic patterns of oesophageal cancers include liver, distant lymph node, lung, bone and brain, accounting for 37%, 29%, 16%, 15% and 3% of cases, respectively [14]. The median OS rates associated with such metastases lie between 4 and 10 months [14]. In gastric cancer; liver, bone, lung, and brain metastases account for 71%, 15%, 12% and 2% of cases, respectively [15]. Median OS rates with these metastases are less than 5 months [14].

Immune checkpoint inhibitors (ICIs) present a promising strategy for improving chemotherapy response rates, with the anti-programmed death-1 receptor (PD-1) monoclonal antibody pembrolizumab increasing the 12-month OS by approximately 20% when used with platinum-based chemotherapy, compared to platinum-based chemotherapy alone in non-small cell lung cancer [16]. However, the efficacy of ICIs is heavily limited by the immune composition within the tumour microenvironment (TME) [17]. 'Hot' tumours exhibit high levels of T cell infiltration and molecular markers indicative of immune activation, such as pro-inflammatory cytokines, allowing the host to elicit an effective anti-tumour immune response and demonstrate higher response rates to ICIs [17]. While oesophageal and gastric cancers develop from a background of inflammation, the immune landscape of these cancers has been described as immunosuppressive and highly fertile for malignant growth [18], therefore treatment with ICIs fails to re-establish an active immune response within the bulk tumour [19].

Chemokines are a family of low molecular weight proteins that govern leukocyte migration by binding to cognate G protein-coupled chemokine receptors on their surface [20]. The entire chemokine system consists of approximately 50 chemokine ligands and 20 chemokine receptors, which are categorized into four subfamilies; -XC-, -CC-, -CXC- and -CX_3C-, based on the orientation and number of two N-terminal cysteine residues [20]. Chemokines and their receptors are widely expressed across tumour cells, immune cells and stromal cells within the TME [20]. Table 1 below outlines the currently known chemokine ligand and receptor pairs in humans.

Chemokines hold strong governance over the immune composition of the TME by driving chemotaxis of leukocytes expressing their respective receptors [21]. As such, targeting these chemokine networks may be employed to selectively recruit anti-tumour immune cells and convert immunologically inert contextures towards a 'hot' profile, ultimately increasing response rates to ICIs and promoting tumour eradication in GOC patients [21].

Table 1
The chemokine superfamily in humans. Four subfamilies of classical chemokine receptors and their respective ligands. Adapted from Ref. [20].

-XC- chemokine receptors	-XC- chemokine ligands
XCR1	XCL1, XCL2
-CC- chemokine receptors	**-CC- chemokine ligands**
CCR1	CCL3, CCL5, CCL7, CCL8, CCL14, CCL15, CCL16, CCL23, CCL26
CCR2	CCL2, CCL7, CCL8, CCL11, CCL13, CCL16, CCL26
CCR3	CCL3, CCL5, CCL7, CCL11, CCL13, CCL14, CCL15, CCL18
CCR4	CCL17, CCL22, CCL24, CCL26, CCL28
CCR5	CCL3, CCL4, CCL5, CCL7, CCL8, CCL14, CCL16
CCR6	CCL20
CCR7	CCL19, CCL21
CCR8	CCL1, CCL16, CCL17
CCR9	CCL25
CCR10	CCL27, CCL28
-CXC- chemokine receptors	**-CXC- chemokine ligands**
CXCR1	CXCL6, CXCL7, CXCL8
CXCR2	CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, CXCL8
CXCR3	CXCL4, CXCL9, CXCL10, CXCL11, CXCL13
CXCR4	CXCL12
CXCR5	CXCL13
CXCR6	CXCL16
CXCR7	CXCL11, CXCL12
-CX_3C- chemokine receptors	**-CX_3C- chemokine ligands**
CX_3CR1	CX_3CL1, CCL26

In this review, the far-reaching and multifaceted biological effects of chemokines on immunity and malignancy in GOC is discussed. The development and challenges of chemokine-targeted therapeutic strategies to fine tune the immunological parameters of the TME towards a favourable, anti-tumour environment with clinical benefit to patients is also outlined.

2. Role of chemokines in anti-tumour immunity

The anti-tumour immune response is heavily reliant on the infiltration of effector natural killer (NK) cells and T cells, and their cytokine

production and cytotoxic functions [21]. The TME of OAC exhibits an anti-tumour chemokine profile but this is paralleled by compromised migratory capacity of circulating T cells [22]. Such impaired migratory capacity of T cells may diminish the anti-tumour immune response, facilitating immune escape and subsequent tumour progression, and suggests that therapeutically recruiting such cells to the TME may present challenges. In contrast, we have reported that the migratory capacity of NK cells in OAC patients is not impaired and that chemokine-targeted therapies may have utility in boosting their movement to the tumour [23].

2.1. Innate immunity

Natural killer (NK) cells are potent killers of tumour cells and form a crucial component of the anti-tumour response [24]. Lower intratumoural frequencies of NK cells are associated with poorer prognoses in GOC patients and therefore, therapies to boost their movement to these tumours is a promising concept [25,26]. Furthermore, in the setting of obesity associated OAC, we have reported the erroneous migration of NK cells to the extratumoural tissues of the omentum and liver, and their significantly altered viability and effector function within these tissues [23]. Therefore, novel chemokine-targeted approaches are needed to limit misdirected NK cell chemotaxis and promote their survival and infiltration of GOC tumours. CX_3CL1-CX_3CR1 signalling has been linked with NK cell recruitment to gastric adenocarcinoma tumours, supported by a positive correlation between intratumoural CX_3CL1 expression and NK cell infiltration [27]. However, the significant abundance of this chemokine in the omentum of GOC patients is likely to complicate and possibly overpower its role in recruiting NK cells to tumours in obese cancer patients [28]. In fact, CX_3CL1 is a key driver of CTL chemotaxis to OAC omentum and subsequent phenotypic alteration, suggesting that this adipochemokine is detrimental to anti-tumour immune cell trafficking to the tumour [28].

$CD1a^+$ DC trafficking to OSCC tumours is mediated by CCL2 and CCL20 [29], whilst in gastric cancer tissues, expression of the CXCR3 chemokine receptor was correlated with enhanced intratumoural DC infiltration [30]. Conventional dendritic cells (DCs) are important mediators of both innate and adaptive immune responses by directly phagocytosing tumour cells and subsequent antigen presentation and stimulation of effector T cells [31]. DCs are also crucial in priming cytotoxic responses in NK cells, $CD8^+$ T cells and T_H1 cells by secreting high levels of IL-12 to induce pro-inflammatory cytokine production [31]. DCs also exist as an immunosuppressive 'immature' phenotype, characterised by reduced capacity for phagocytosis and antigen presentation, coupled with reduced costimulatory molecule expression [31]. Nonetheless, the chemokine signalling pathways involved in positioning immature DCs in the GOC microenvironment remain unknown and further research into this area may uncover a potential drug target to enhance the anti-tumour profile of infiltrating leukocytes in these tumours.

2.2. Adaptive immunity

Cytotoxic T lymphocytes (CTLs) are potent tumour killers and a crucial component of the anti-tumour immune response [32]. The chemokines CCL4, CCL5, and CXCL10 have been identified as key drivers of CTL recruitment to the tumour in OSCC [33, 34, Fig. 1A], while high levels of CXCR4 expression on the surface of gastric cancer cells is associated with higher frequencies of tumour-infiltrating CTLs [35]. Although studies have shown that GOC tumours are enriched with CTLs [36], these populations are often exhausted by an immunosuppressive milieu within the TME, brought forward by a vast array of pro-tumour immune cells [37]. By changing the migratory patterns of these cells, chemokine-targeted therapies may hold therapeutic value in alleviating the inhibition imposed on CTLs and should be explored further in GOC.

Type 1 $CD4^+$ helper T (T_H1) cells are a second pivotal component of

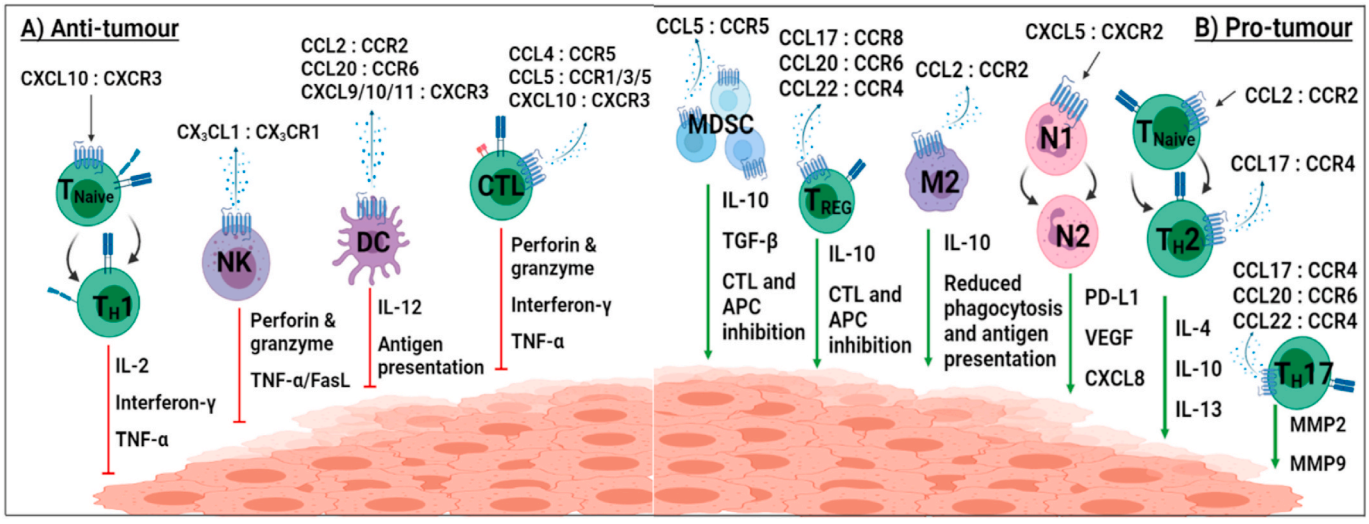

Fig. 1. Immune subpopulations influenced by chemokines in GOC. *A.* CX_3CL1 recruits NK cells which induce apoptosis in tumour cells via granzyme expulsion and death ligand engagement [27]. CXCL10 recruits $CXCR3^+$ T_H1 cells to the TME, which secrete interferon-γ to upregulate antigen presentation and drive inflammation [22,38]. CXCL10 also promotes differentiation of naïve T cells toward an anti-tumour T_H1 phenotype in a CXCR3-dependent manner [39]. CCL4, CCL5 and CXCL10 recruit CTLs to the TME, which induce apoptosis in tumour cells and promote inflammation by secreting interferon-γ [33,34]. CCL2, CCL20 and CXCR3 ligands regulate DC trafficking, which generate antigen-specific cytotoxic T cells and secrete IL-12 to enhance their anti-tumour capacity [29,30]. *B.* CCL17, CCL20 and CCL22 promote T_{REG} migration to the TME, which suppress effector T cells and antigen-presenting cells through immune checkpoint signalling and IL-10 secretion [55,58]. CCL17 recruits T_H2 cells via CXCR4 signalling, exerting immunosuppressive effects on anti-tumour T cells via IL-10 secretion [22]. CXCL5 promotes N2 neutrophil polarisation, thus promoting tumour growth by stimulating the expression of VEGF and PD-L1 to drive angiogenesis and T cell anergy, respectively [51, 52]. CCL5 recruits MDSCs to the TME, which secrete IL-10 to suppress CTLs and antigen-presentation [61]. MDSCs also secrete IL-4 and TGF-β to maintain pools of immunosuppressive T_H2 and T_{REG} cells, respectively [60]. CCL17, CCL20 and CCL22 recruit T_H17 cells to the TME, which upregulate MMPs to confer a pro-metastatic phenotype in surrounding cancer cells [63,64]. Black arrows indicate the polarising role of chemokines on immune cell phenotypes. Blue arrows indicate the chemotactic effects exerted by chemokines on each immune cell type. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the anti-tumour immune response and it is established that their recruitment to OAC tumours is mediated by the CXCL10-CXCR3 chemokine network [22,38]. Chemokines have previously been implicated in the polarisation of $CD4^+$ T cells [39]. Groom et al. outlined a role of the CXCL10-CXCR3 axis in promoting T_H1 polarisation, whereby CXCL10-knockout murine dendritic cells displayed an 80% decrease in the frequency of IFN-γ^+ TNF-α^+ T_H1 cells relative to wild-type and CXCL9 knockout cells [39]. To date, the chemokine-mediated polarisation of $CD4^+$ T cells in GOC has not been fully investigated, thus warranting additional studies to uncover the polarising role of CXCL10 and other chemokines in these malignancies.

3. Role of chemokines in pro-tumour immunity

Unlike anti-tumour immunity, the degree of immunosuppression exerted within the TME greatly favours the adaptation of malignant cells, providing a means to evade immune destruction and sustain tumour cell proliferation [40]. Chemokines can influence the escape of tumour cells from the anti-tumour immune response by recruiting immunosuppressive immune cell populations into the TME [[41], Fig. 1B].

3.1. Innate immunity

M2 macrophages, also known as tumour-associated macrophages (TAMs), are characterised by their anti-inflammatory and pro-tumour properties [42]. M2 macrophages are distinguished from their M1 counterparts by reduced capacity for phagocytosis and antigen presentation, repression of pro-inflammatory cytokines (IL-1, IL-6 and TNF-α) and increased secretion of anti-inflammatory cytokines (IL-4, IL-10, IL-13 and TGF-β) [42].

Trafficking of macrophages to the TME is linked with the CCL2-CCR2 axis in both oesophageal and gastric cancer [43,44]. OSCC tumours with high expression of CCL2 positively correlated with increased macrophage counts, approximately 2.5-fold higher than $CCL2^{Low}$ expressing tumours [43]. A study by Ohta et al. in gastric cancer reported that $CCL2^{High}$ tumours displayed macrophage counts approximately 1.6-fold higher than $CCL2^{Low}$ tumours [44]. In a CCL2-knockout murine model of OSCC, inhibition of the CCL2-CCR2 axis demonstrated a 10% reduction in tumour infiltrating TAMs, further supported by a 60% reduction in tumour incidence across the sample population [45]. Yamamoto et al. concluded that OAC patients with high infiltrate of M2 macrophages had 5-year OS rates of 57.2% relative to 71% in the low infiltrate cohort [46].

While CCL2 is a key driver of TAM accumulation in GOC tumours and an attractive therapeutic target, the drivers of M2 macrophage polarisation in GOC have not been fully elucidated [43–46]. CXCL12-CXCR4 signalling has demonstrated interesting phenomena in influencing the behaviour of macrophages in the TME of multiple myeloma [47], breast [48], and oral squamous cell carcinomas [49] by shifting the secreted cytokine profile towards this M2 subtype, including IL-10, IL-4 and IL-13. These pathways should be explored in the context of GOC to identify a potential therapeutic opportunity for these patient cohorts.

Like macrophages, neutrophils exhibit polarised phenotypes associated with anti-tumour (N1) and pro-tumour immunity (N2) [50]. A study by Mao et al. found that CXCL5 could polarise neutrophils towards a pro-tumour profile in gastric cancer by activating ERK and p38 mitogen-activated protein kinase (MAPK) pathways [51]. This subset of gastric cancer-infiltrating neutrophils displayed higher expression patterns of PD-L1, vascular endothelial growth factors (VEGFs), IL-23, IL-8 and IL-6, which collectively promote tumour cell growth, angiogenesis and metastasis [51]. Neutrophils are recruited by the CXCL5-CXCR2 and CXCL8-CXCR2 axes in gastro-oesophageal cancers and future studies are needed to elucidate the utility of CXCR2 antagonism in this setting [51,52].

3.2. Adaptive immunity

Type 2 $CD4^+$ helper T lymphocytes (T_H2) exert opposing effects on the immune landscape of the TME relative to their T_H1 counterparts by upregulating T_H2 cytokines such as IL-10, IL-4 and IL-13 [53], and it is established that they are recruited to OAC tumours via the CCL17-CCR4 signalling axis [22]. Interestingly, a role for CCL2 and CXCL11 in polarising T cells toward a T_H2 phenotype has previously been elucidated, where T cells isolated from draining lymph nodes of CCL2 knockout mice displayed a significant reduction in IL-4, IL-5 and IL-10 secretion [54].

Regulatory T cells (T_{REGs}) are recruited by the chemokines CCL17, CCL20 and CCL22 in oesophageal cancer [55]. These immunosuppressive T cells elicit their effects via PD-1 and cytotoxic T lymphocyte antigen-4 (CTLA-4) immune checkpoint signalling, IL-10 and transforming growth factor β (TGF-β) secretion, and IL-2 sequestration [56]. As such, T_{REGs} have been regarded as prominent pro-tumour cells in the TME [56].

Yue et al. identified the overexpression of IL-33 in tumour tissue in 74% of OSCC patients and established a positive correlation between IL-33 and CCL2 levels in OSCC cells [57]. Knockdown of IL-33 expression significantly reduced the expression of CCL2 in OSCC cells *in vitro* [57]. A corollary to this study indicated a strong positive correlation between IL-33 mRNA expression and Forkhead box P3 (FoxP3) mRNA, the master transcription factor dictating T_{REG} differentiation [57]. Overall, this study suggests that CCL2 may recruit T_{REGs} to the TME in OSCC, whose expression is induced by IL-33 in OSCC cells.

CCL2 and CCL17 have been implicated in positioning T_{REGs} within the gastric cancer TME and *anti*-CCL2 and *anti*-CCL17 neutralising antibodies significantly reduced T_{REG} migration *in vitro* [58]. Furthermore, CCL2 and CCL17 levels within the gastric tumours were also found to be 20% higher than the adjacent normal gastric mucosa, suggesting that blockade of these chemotactic signals may have therapeutic potential to alleviate immune suppression in gastric cancer patients [58]. Within OAC tumours, we have reported both the infiltration of IL-10-producing T cells and an abundance of the T_{REG}-recruiting chemokine CCL20, suggesting that CCL20 receptor antagonists might have utility to limit tumour infiltration by T_{REG} cells and enhance anti-tumour responses in these patients [22,59].

Myeloid-derived suppressor cells (MDSCs) are a notorious pro-tumour subtype of leukocytes descending from immature myeloid progenitors [60]. MDSCs produce tumour-promoting cytokines including IL-10, TGF-β and IL-4, whilst also expressing PD-L1 which can inhibit anti-tumour NK cell and T cell functions [60]. MDSCs are also implicated in mediating immunotherapy resistance and metastatic priming of distal sites [60].

The CCL5-CCR5 axis recruits MDSCs to the TME in gastric cancer, elucidated in a study by Yang et al. [61]. Although little is known about the role of chemokine networks in recruiting MDSCs in oesophageal cancers, downregulation of CXCL1 expression with metformin reduced MDSC populations of the TME in murine xenograft models of OSCC and specific targeting of this chemokine pathway should be explored further [62].

The CCL17-CCR4, CCL22-CCR4 and CCL20-CCR6 chemokine signalling axes recruit T_H17 cells to the TME in OSCC [63]. Studies have implicated T_H17 cells in conferring a pro-metastatic phenotype in OSCC cells by upregulating the expression of MMP-2 and MMP-9 via IL-17A/NF-κB-dependent signalling mechanisms [64]. To date, the chemokine networks which govern T_H17 recruitment to gastric tumours are yet to be uncovered.

4. Chemokines as promoters of the hallmarks of cancer

The hallmarks of cancer are ten characteristics exhibited by malignant cells which ultimately describe their propensity to accumulate in large densities and invade surrounding tissues, irrespective of intrinsic

regulatory mechanisms and a functioning immune response [65]. Chemokines display both indirect and direct effects in driving these hallmarks of cancer, either through recruitment of deleterious immune cells or inducing adverse biological responses downstream of their receptors' signalling axes [66] as outlined in Fig. 2. To date, chemokines have been implicated in supporting nine of these hallmarks, as outlined below.

4.1. Sustaining proliferative signalling and evasion of cell death

A study by Wang et al. demonstrated that CXCL1 and CXCL2 activate MAPK signalling pathways via CXCR2 in OSCC biopsy-derived cell lines [67]. Activation of these pathways promotes growth, proliferation, and survival of cells, which may advance tumour progression if homeostatic levels are exceeded [68]. The authors suggest that these signalling axes persist in a continuous autocrine manner, providing malignant cells with an inexhaustive supply of mitogenic signalling to drive tumorigenesis [67]. CXCR7 overexpression was previously linked to the initiation and progression of gastric cancer, whereby the CXCL12-CXCR7 signalling axis in gastric cancer cells promoted the phosphorylation of p38, ERK1/2 and JNK MAPKs and subsequent tumour cell proliferation [69].

4.2. Promoting angiogenesis

Activation of the CCR7 signalling axis in gastric epithelial cells upregulates VEGF-C expression in gastric cancer [70]. VEGF-C binds to VEGFR2 and VEGFR3, thus stimulating the proliferation and migration of endothelial cells from proximal blood and lymphatic vessels towards the bulk tumour mass [71]. *Helicobacter pylori*-positive gastric cancers exhibit elevated expression levels of CXCL8 within gastric epithelial tissues, a known neutrophil chemoattractant [72]. Furthermore, the increased density of neutrophils correlated with the upregulated expression of pro-angiogenic VEGFs and MMPs, implicating CXCL8 as a druggable immunotherapeutic target [72].

4.3. Activating invasion and metastasis

Chemokines contribute to the 'molecular barcode' theory in the context of tumour metastasis, orchestrating the organ-specific dissemination of malignant cells to distant secondary locations based on compatibility between chemokine and chemokine receptor expression profiles [73].

Small interfering RNA knockdown of CXCR7 in OSCC cells has previously been shown to decrease the expression of pro-epithelial mesenchymal transition (EMT) and pro-metastatic genes including MMPs, zinc finger E-box binding homeobox 2, zinc finger protein SNAI2 (Slug) and c-Myc [74]. The upregulation of Slug and downregulation of zinc finger E-box binding homeobox 2, permits the loosening of cellular junctions via loss of e-cadherin, thus forming a mesenchymal-like conformation with increased capacity for invasion and dissemination [74].

In gastric cancer cells, the CXCL12-CXCR4 axis has been implicated in driving locoregional lymph node metastasis [78]. The study found that CXCL12 and CXCR4 expression was significantly higher in metastatic lymph node tissues in comparison to the primary tumour tissues [75]. Additionally, gastric epithelial CXCR4 expression was shown to increase proportionally alongside TNM stages in gastric cancer [75]. Furthermore, gastric cancer patients with high levels of intratumoural CXCR4 expression had 30% lower 5-year OS compared to those with low intratumoural CXCR4 expression [75]. CXCR4 has also been implicated in promoting liver metastasis in gastric cancers through the engagement of CXCL12 with CXCR4 expressed on gastric cancer cells [76]. Collectively, these studies suggest that CXCR4 may be an attractive therapeutic

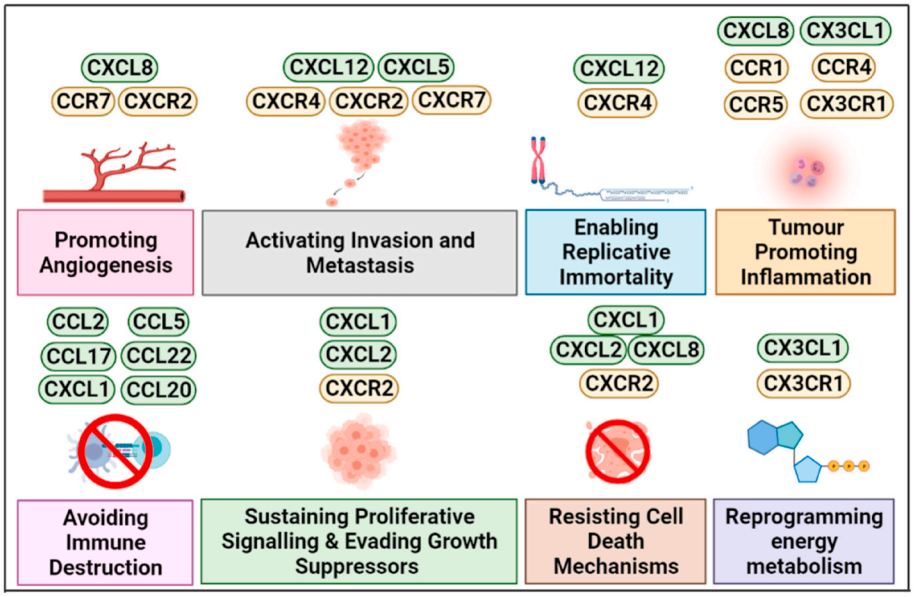

Fig. 2. Role of chemokines in promoting the hallmarks of cancer. *Promoting angiogenesis*: CXCL8 promotes angiogenesis by recruiting $CXCR2^+$ neutrophils, which provide the growing neoplasm with pro-angiogenic mediators [72]. Activation of CCR7 signalling also upregulates VEGFs to promote angiogenesis and lymphangiogenesis [70]. *Avoiding immune destruction*: CCL17, CCL20 and CCL22 recruit T_{REGs} to suppress CTLs and antigen presentation via immune checkpoint signalling and IL-10 secretion, facilitating immune escape and tumour progression [55]. CCL2 recruits TAMs expressing PD-L1, TGF-β and IL-10 to suppress and exclude effector T cells from the TME [78]. CXCL1 and CCL5 may recruit MDSCs to further promote immune evasion via IL-10 and TGF-β secretion [61,62]. *Activating invasion and metastasis*: CXCL12 orchestrates organ-specific metastasis by recruiting $CXCR4^+$ cancer cells from the primary tumour [76]. CXCL5 upregulates Snail and Slug in cancer cells to initiate EMT and also polarises $CXCR2^+$ neutrophils towards a N2 phenotype capable of inducing pro-metastatic properties in cancer cells [51]. CXCR7 signalling upregulates MMPs, Snail and Slug in cancer cells to induce EMT [74]. CAF-derived CXCL12 enhances the invasion capacity of gastric cancer cells [94]. *Sustaining proliferative signalling*: CXCL1 and CXCL2 activate MAPK pathways vis CXCR2 to upregulate cell cycle progression and subsequent tumorigenesis [67]. *Resisting cell death mechanisms*: CXCL1 and CXCL2 activate pro-survival PI3K-Akt signal transduction networks by binding CXCR2, allowing cancer cells to evade cell death mechanisms by counteracting intrinsic pro-apoptotic signals [69]. CAF-derived CXCL8 activates pro-survival PI3K-Akt pathways in cancer cells to overcome the cytotoxic effects of chemotherapy [93]. *Tumour-promoting inflammation:* CX_3CL1 draws CX_3CR1^+ $CD8^+$ memory T cells away from the bulk tumour mass to the omentum, thus creating a bias towards immunosuppressive immune cells in the TME to facilitate tumour growth [28]. An abundance of $CCR4^+$ T_H2 cells in BO creates a pro-tumour phenotype in the oesophageal TME, potentially contributing to the development of OAC [22]. CXCL8 promotes tumorigenic inflammation by recruiting neutrophils with pro-angiogenic and pro-metastatic roles in gastric cancer [72,84,85]. *Enabling replicative immortality:* CXCL12-CXCR4 signalling may induce the expression of telomerase reverse transcriptase to elongate telomere sequences for the delaying of cellular senescence [90]. *Reprogramming energy metabolism:* CX_3CL1-CX_3CR1 signalling upregulates hypoxia-inducible factor 1α to shift energy metabolism towards glycolysis [91].

target for preventing lymph node metastasis, due to its multi-faceted role in driving several organ-specific metastases.

CXCL5 binds with CXCR2 on gastric cancer cells, thus inducing the expression of zinc finger protein SNAI1 (Snail) and Slug transcription factors to initiate the EMT [51]. Engagement of CXCL5 with CXCR2 expressed on neutrophils promotes their migration to the TME and upregulates the secretion of IL-6 and IL-23, key signatures of the N2 profile [51]. Gastric cancer cells co-cultured with these CXCL5-activated neutrophils displayed elevated expression of pro-EMT vimentin, Slug, Snail and v-cadherin, which reversed when treated with IL-6 and IL-23 neutralising antibodies [51]. Furthermore, this study highlights the secondary indirect roles of chemokines in driving gastric cancer cell metastasis via recruitment of immunosuppressive N2 neutrophils to the TME. The authors reported significantly higher expression levels of CXCL5 in gastric cancer tissue compared with adjacent normal tissues [51], thus highlighting CXCL5 as an attractive therapeutic target in preventing gastric cancer metastasis.

Collectively, these studies show a tightly regulated mechanism of metastasis whereby chemokine ligands promote plasticity and invasiveness in cancer cells expressing their respective receptors. This is followed by migration of such cells along a concentration gradient towards a hotspot of specific chemokine ligands where they eventually take up residence to form secondary colonies. Such chemokine-governed metastasis indicates why GOC tumour cells have a higher propensity to metastasize explicitly within lung, hepatic, brain, bone, and lymph node organs.

4.4. Avoiding immune destruction

T_{REG} cells are recruited to the TME by CCL20, CCL17 and CCL22 [55]. T_{REGs} promote immune evasion primarily through three mechanisms [77]. T_{REGs} express the IL-2Rα receptor which binds and depletes the T cell mitogen IL-2 with significantly higher affinity than the standard IL-2R, thus diminishing the clonal expansion of effector $CD8^+$ and $CD4^+$ lymphocytes [77]. T_{REGs} also secrete IL-10, IL-35 and TGF-β which repress antigen presentation, T_H1 associated pro-inflammatory cytokine secretion and induce T_{REG} proliferation, respectively [77]. Contact-dependent mechanisms of T_{REG} inhibition are mediated through lymphocyte activation gene 3, CTLA-4 and PD-L1, which associate with MHC II, CD28 and PD-1, respectively [77]. Ligation with these receptors activates the Src homology region 2 domain-containing phosphatases to reverse costimulatory signalling and retain tolerogenic subpopulations of effector immune cells to suppress the immune response, creating a favourable environment for tumour proliferation [77]. Therefore, antagonism of CCL20, CCL17 and/or CCL22 receptors may attenuate immunosuppression within the GOC TME and facilitate a more potent anti-tumour immune response.

The potential of CCL2 as a druggable target has already been presented here. In oesophageal cancer, CCL2-CCR2 axis has previously been shown to drive tumour immune evasion by recruiting $PD\text{-}L1^+$ TAMs [78]. Activation of signal transducer and activation of transcription 3/c-Myc pathways via CXCL8 signalling has been shown to upregulate PD-L1 expression in gastric cancer cells also [78]. TAMs secrete TGF-β which stimulate T_{REG} differentiation, providing a source of immunosuppressive IL-10 within the TME [42]. Collectively, these mechanisms repress the cytotoxicity of anti-tumour T cells and promote their exclusion from the TME, thus providing further justification to assess targeting CCL2-mediated accumulation of TAMs in GOC to augment anti-tumour immunity.

4.5. Evading growth suppressors

In tumour cells, chemokine signalling can activate oncogenic signalling pathways such as the pro-survival phosphoinositol-3-kinase/protein kinase B (PI3K/Akt) network [79]. Alongside driving tumorigenesis, activation of these anti-apoptotic factors can confer resistance to chemotherapy and radiotherapy by inhibiting the DNA damage checkpoint circuitry [80,81].

Activation of the PI3K/Akt pathway counteracts pro-apoptotic signals through the phosphorylation and stabilisation of B cell lymphoma-2 (Bcl-2), thus permitting Bcl-2 homologous antagonist/killer and Bcl-2 associated X protein sequestration to prevent the mitochondrial-dependent pathway of apoptosis [81]. This phenomenon can advance tumour progression if pro-survival effects over-encumber the intrinsic pro-apoptotic signals. Pan-CXCL antagonism demonstrated a 50% reduction of Bcl-2 mRNA in human OSCC cells *in vitro*, implying a prominent pro-survival role driven by the CXC family [79].

4.6. Tumour-promoting inflammation

The potential for tumours to exploit the inflammatory response is largely accredited to the chemokine network, allowing for the construction of a microenvironment which is highly fertile for neoplastic growth through the selective recruitment of leukocytes displaying pro-tumour phenotypes, or exclusion of anti-tumour populations [21]. In obesity-associated OAC, our group have reported that the omentum and liver are enriched in inflammatory chemokines and cytokines that facilitate erroneous recruitment of significant numbers of anti-tumour immune cells to these extratumoural tissues [82,83]. Furthermore, we have demonstrated that OAC-patient derived T cells preferentially migrate to the liver and omentum via the CCR1 pathway and that CCR1 antagonism can facilitate a significant reduction in such migration, suggesting a potential therapeutic strategy to redirect effector T cell populations to the oesophageal TME and promote tumour eradication [82]. We have also reported that the $CX_3CL1\text{-}CX_3CR1$ pathway governs the recruitment and phenotypic switching of $CD8^+$ memory T cells to the omental tissue of OAC patients, effectively depriving the oesophageal TME of a sufficient anti-tumour immune response [28]. Therefore, chemokine receptor antagonists present several opportunities to prevent the accumulation of inflammatory and anti-tumour T cells in extratumoural compartments and increase their availability to respond to the chemotactic cues of the TME.

Recent evidence from our group has suggested a role for chemokines in the conversion from BO to OAC, whereby reduced frequencies of circulating anti-tumour $CCR5^+$ T_H1 cells are met with increased pro-tumour $CCR4^+$ T_H2 counterparts in BO and OAC patients [22]. Interestingly, we also reported that CCR4 antagonism significantly reduced the migration of pro-tumour T_H2 cells in BO, suggesting that targeting CCR4 in the clinical setting may prove a viable strategy for re-instating anti-tumour immunity and potentially impeding the conversion from BO to adenocarcinoma [22].

CXCL8 is a pro-inflammatory chemokine strongly associated with neutrophilic migration in *H. pylori*-related gastric cancers [72]. Furthermore, a strong correlation between neutrophil infiltration and gastric cancer cell proliferation was previously elucidated, suggesting that neutrophils can stimulate pro-survival pathways in gastric epithelial cells to support tumour growth, although precise mechanisms remain unclear [84]. High levels of intratumoural CXCL8 have also been shown to upregulate the expression of PD-L1 on TAMs in gastric cancer, corresponding with impaired infiltration and functionality of anti-tumour $CD8^+$ T cells [85]. Moreover, CXCL8 contributes to tumorigenic inflammation in gastric cancer by promoting the influx of pro-tumour neutrophils and abrogating $CD8^+$ T cell responses [84,85], and antagonism of its receptor CXCR2 remains an attractive therapeutic target to improve anti-tumour immunity.

Alongside chemokine involvement, studies have shown that gastrointestinal pathogens can also induce tumourigenic inflammation. Epstein-Barr virus infections promote a T_H1 cytokine profile by suppressing the activity of SH2-domain containing protein 1a, a negative regulator of the signalling lymphocytic activation molecule-ERK-interferon-γ pathway [86]. On the other hand, the latent membrane protein-1 has also been shown to upregulate CCL17 and CCL22 production by B

cells, a known chemoattractant for the T_H2 subpopulation [87], thus warranting further investigation to confirm its utility as a prognostic marker in cancers. *Helicobacter pylori* is a gram-negative bacteria found in the gastric cavity of approximately 50% of the world's population, which causes chronic inflammation and is the strongest known risk factor for gastric cancer [88]. The neutrophil-activating protein is one of *H. pylori*'s many virulence factors and has been implicated in upregulating IFN-γ production and repressing IL-4 production in $CD4^+$ T cells by activating Toll-like receptor 2-dependent signalling pathways, thus skewing their profile towards a pro-inflammatory T_H1 phenotype [89].

4.7. Enabling replicative immortality

The CXCL12-CXCR4 signalling axis has been shown to delay the replicative senescence of endothelial progenitor cells by inducing the expression of human telomerase reverse transcriptase through the PI3K-Akt signalling network [90], however this phenomenon requires further validation in cancers to deduce the potential of re-instating cellular senescence by targeting chemokine pathways.

4.8. Reprogramming energy metabolism

CX_3CL1 has previously been shown to upregulate the expression of hypoxia inducible factor 1-α in human pancreatic ductal adenocarcinoma cells *in vitro* by activating PI3K/Akt and MAPK pathways downstream of CX_3CR1 [91]. The same study found that treating human pancreatic ductal adenocarcinoma cell lines with recombinant CX_3CL1 increased both glucose uptake and lactate production in a dose-dependent manner [91]. Ultimately, this study provides evidence to suggest that the chemokine system is indeed capable of reprogramming cellular energy metabolism by activating hypoxia inducible factor 1-α to drive this glycolytic switch, however this phenomenon requires further clarification in GOC.

4.9. Cancer-associated fibroblasts

While little is known of the chemokine networks which govern the recruitment of cancer-associated fibroblasts (CAFs) to GOC tumours, CAFs are known to secrete an array of chemokines involved in promoting several immune-independent hallmarks of cancer [92]. Zhai et al. found that CXCL8 derived from CAFs could induce a chemo-resistant phenotype in gastric cancer cells *in vitro* by activating pro-survival PI3K-Akt and NF-κB related signalling networks to withstand the cytotoxic effects exerted by cisplatin therapy [93]. Moreover, these findings were paralleled by a dramatic increase in the IC_{50} of cisplatin [93], suggesting that targeting CAF-derived CXCL8 may be an attractive therapeutic target to re-instate chemosensitivity in gastric cancer patients.

Sugihara et al. demonstrated that $CXCL12^{High}$ CAFs isolated from primary gastro-oesophageal junctional adenocarcinoma tissues could invoke a pro-metastatic phenotype in patient-derived gastro-oesophageal junctional adenocarcinoma cells when co-cultured together [94]. The team subsequently subjected these cells to a matrigel invasion assay and reported a six-fold increase in invasive cells relative to those cultured in the absence of CAFs [94], thus highlighting CAF-derived CXCL12 as a potential druggable target to impede tumour progression in GOC.

5. Therapeutically targeting chemokines in GOC

Currently, most of the evidence portraying chemokine antagonists as viable anti-cancer therapeutics in upper gastrointestinal malignancies remains in the pre-clinical setting. As of 2021, only two chemokine receptor antagonists have been studied in phase I clinical trials; LY2510924 [NCT02737072] and BKT140 [NCT01010880], both targeting the CXCR4 receptor in solid tumours and multiple myeloma, respectively. Fig. 3 depicts the biological implications achieved by chemokine ligand and receptor antagonists in pre-clinical studies of GOC. Table 2 summarizes current pre-clinical evidence of chemokine receptor antagonists in GOC.

5.1. Pan-CXCL antagonism

The efficacy of the pan-CXCL antagonist UNBS5162 to reduce the growth of human OSCC cells has been demonstrated *in vitro* [79]. Treatment of OSCC cells with UNBS5162 yielded a 35% decrease in cell viability and a 48% reduction in colony count [79]. The authors hypothesised that the anti-tumour effects exerted by CXCL antagonism are primarily derived through inhibition of the pro-survival PI3K-Akt transduction network, conveyed by an approximate 50% reduction in Bcl-2 expression relative to untreated cells [79]. Ultimately, this suggests that CXC chemokine networks activate anti-apoptotic signalling circuitry such as the PI3K-Akt pathway to drive tumorigenesis in GOC and further exemplifies the anti-neoplastic potential of chemokine-targeted therapies [79].

5.2. CXCR2 knockdown and antagonism

Despite the pro-tumourigenic role of CXCR2, pre-clinical evaluation of the CXCR2 antagonist SB332235 failed to demonstrate a significant reduction in OAC cell proliferation; however, the invasive potential of OAC cells was significantly reduced in a dose-dependent manner [95].

Another CXCR2 antagonist, SB225002, has previously been shown to reduce the phosphorylation of ERK1 and ERK2 MAPK signal transducers in OSCC and significantly reduced the proliferation of OSCC cells by 50% [67]. In addition, a study by Wu et al. demonstrated a decrease in phospho-ERK1 and phospho-ERK2 expression using siRNA knockdown of CXCR2 in OSCC cells and resulted in a significant reduction in tumour volume in mice [96]. Another notable finding of Wu et al.'s investigation included a dramatic decrease in invasiveness within CXCR2 knockdown cells [96]. Collectively, these studies portray CXCR2 as an attractive therapeutic target for perturbing tumour cell growth and invasiveness in oesophageal cancers.

5.3. CXCR4 inhibition and antagonism

The evidence supporting the role of CXCR4 in tumour metastasis and its potential TAM-promoting effects place it as an attractive therapeutic target in GOC. Moreover, CXCR4 expression has been detected in 84.6% of 214 OSCC samples and overexpression of this chemokine receptor has also been identified in advanced gastric cancers [97,98]. CTCE-9908 is a low molecular weight peptide capable of eliciting competitive inhibition of CXCR4 by binding with higher affinity than CXCL12 [99]. In a murine xenograft model of gastroesophageal junctional adenocarcinoma, CTCE-9908 inhibition of CXCR4 demonstrated a mean tumour weight reduction of 57% relative to untreated mice [99]. Underlying this phenomenon, the authors suggest the existence of transactivation networks between CXCR4 and human epidermal growth factor receptor 2 (HER2) which drive cell proliferation, based on positive correlations between their expression patterns in OC xenograft models [99]. However, further investigation is warranted to confirm the true interplay between the CXCR4 axis and pro-survival transduction networks in GOC.

AMD3100 targets the CXCL12-CXCR4 axis via CXCR4 antagonism [100]. When treated with AMD3100 *in vitro*, the OSCC cell line exhibited a 25% reduction in proliferation index [96]. Translated within an *in vivo* murine xenograft model of OSCC, AMD3100 reduced the tumour volume by approximately 50% relative to untreated control mice [100].

The efficacy of the AMD3100 CXCR4 antagonist and anti-HER2 trastuzumab were examined in murine xenograft models of $HER2^+$ metastatic OSCC, demonstrating approximate mean tumour weights of 0.1 g (AMD3100/trastuzumab), 0.3 g (trastuzumab alone), 0.5 g (AMD3100 alone) and 0.8 g (control) [101]. AMD3100 monotherapy

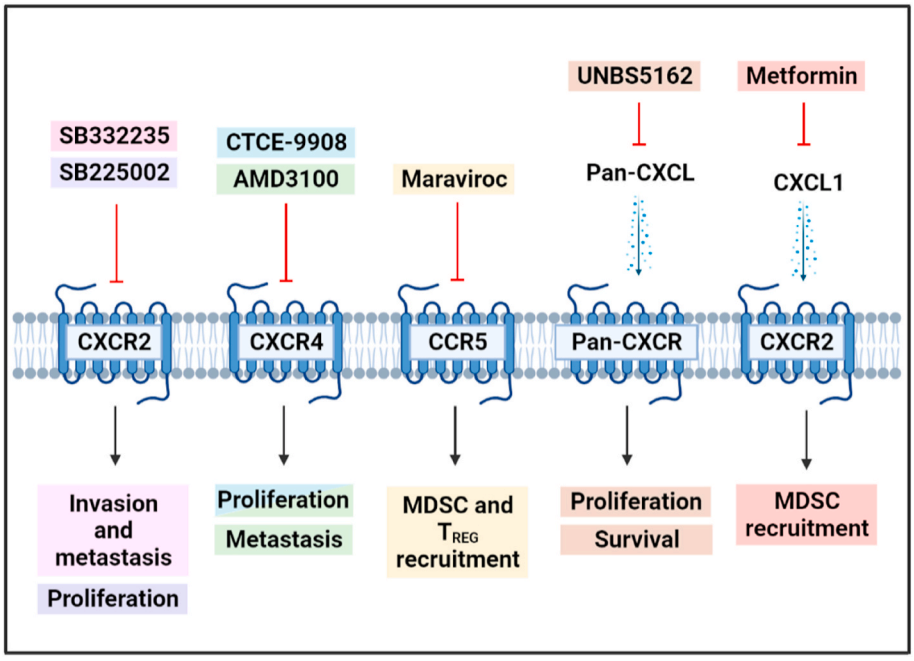

Fig. 3. Therapeutic targeting of chemokine signalling axes in GOC. SB332235: Antagonism of CXCR2 with SB332235 reduces the invasiveness and metastatic potential of OAC cells [95]. SB225002: Antagonism of CXCR2 with SB225002 exerts anti-proliferative effects in OSCC cells [67]. *AMD3100*: Antagonism of CXCR4 with AMD3100 reduces proliferation and metastasis in OSCC cells [98]. *CTCE-9908*: Antagonism of CXCR4 with CTCE-9908 reduces tumour cell proliferation in OSCC xenograft models [99]. *Maraviroc*: Antagonism of CCR5 with Maraviroc reduces tumour cell proliferation, MDSC recruitment and T_{REG} recruitment in gastric cancer xenograft models [61]. *UNBS5162*: Targeting CXCLs with UNBS5162 decreases proliferation and survival of OSCC cells [79]. *Metformin*: Targeting CXCL1 with Metformin reduces the migration of pro-tumour MDSCs in OSCC [65].

Table 2
Chemokine ligand and receptor antagonists. Preclinical evidence of chemokine antagonists as monotherapies or combination therapies in GOC, all numerical values are approximated.

Target	Drug	Cancer Type	Treatment Efficacy
Pan-CXCL	UNBS5162	OSCC	35% decrease in cell viability [79]
CXCR2	SB332235	OAC	90% reduction in invasiveness [95]
CXCR2	SB225002	OSCC	50% reduction in cell viability [67]
CXCR4	CTCE-9908	OSCC xenograft	57% reduction in tumour weight [97]
CXCR4	AMD3100	OSCC	50% reduction in tumour volume [98]
HER2/ CXCR4	Trastuzumab + AMD3100	OSCC xenograft	80% reduction in liver metastasis [99] 87.5% reduction in tumour weight [100]
CCR5/PD-1	Maraviroc + pembrolizumab	GC xenograft	53% reduction in tumour weight [61] 300% increase in CD4/CD8 cell ratio [61]
CXCL1	Metformin	OSCC	150% decrease in MDSC recruitment [62]

failed to reduce liver micrometastases in contrast to trastuzumab, which inhibited both liver and lung metastasis significantly, represented by a 75–100% decrease in human glyceraldehyde-3-phosphate dehydrogenase mRNA when assessed by real-time polymerase chain reaction [101]. Trastuzumab achieved superior inhibition of both liver and lung metastasis in contrast to AMD3100 monotherapy, but its clinical use may be hindered by the low incidence rate of HER2 overexpression in oesophageal cancers relative to CXCR4 [101].

5.4. *CCR5 antagonism*

Yang et al. examined the combinational approach of the CCR5 antagonist, Maraviroc, with anti PD-1 immune checkpoint inhibition in a murine model of gastric cancer [61]. In this study, CCR5 inhibition alone reduced the accumulation of MDSCs and T_{REGs} by approximately 25% relative to control mice [61]. Maraviroc and *anti*-PD-1 alone yielded tumour weight reductions of approximately 16% and 26%, respectively [61]. Used in combination, a synergistic reduction of 53% is observed [61]. This increase in therapeutic efficacy is largely accredited to the reinvigoration of $CD8^+$ and $CD4^+$ and MDSC repression, further supported by a three-fold increase in tumour infiltrating $CD8^+$ and $CD4^+$ T cells relative to control mice [61]. Indeed, this study highlights the synergistic therapeutic potential between immune checkpoint inhibitors and chemokine receptor antagonists in promoting anti-tumour immunity, demonstrating potential for translation into clinical studies.

5.5. *CXCL1 inhibition*

In a murine xenograft model of OSCC, treating OSCC cells with 10 mM of metformin showed a three-fold reduction in CXCL1 mRNA expression relative to untreated cells, in addition to a 1.5-fold reduction in pro-tumour MDSC populations after 24 h [62]. Metformin promotes the phosphorylation of adenosine monophosphate-activated protein kinase, resulting in the activation of dachshund homologue 1 [25]. Dachshund homologue 1 is an intrinsic inhibitor of nuclear factor kappa B, thus reducing the transcription of CXCL1 [62]. However, more specific targeted approaches such as the antagonism of CXCL1 might prove useful in the reduction of MDSC-mediated immune suppression within the GOC TME.

5.6. *CX_3CR1 antagonism*

As an exclusive receptor for CX_3CL1 and CCL26, CX_3CR1 is an appealing therapeutic target as there is less redundancy in this pathway compared to other chemokine axes; a feature of the chemokine system which has presented a major challenge for their therapeutic utility [102, 103].

CX_3CL1 mediates the homing of CX_3CR1^+ $CD8^+$ memory T cells to the omentum in OAC patients, effectively driving inflammation of the adipose tissue and depriving the oesophageal TME of a crucial anti-tumour immune population [24]. CX3CL1 also modulates the phenotype of these $CD8^+$ memory T cells and alters their migratory and adhesion molecule expression [28]. Therefore, antagonism of CX_3CR1 may therefore serve as a promising strategy to improve anti-tumour immunity in OAC by re-directing the omental migration of these immune cells and localizing their accumulation in the tumour to elicit their

cytotoxic effects. Although current evidence is limited in the GOC space, CX_3CR1 antagonism has demonstrated encouraging results in breast [104] and pancreatic cancers [105] for impeding the invasion and migration of tumour cells, and its biological effects must be further characterised in GOC to support its therapeutic utility in these cancers.

5.7. Off-target effects of chemokine receptor antagonists

While targeting the chemokine system to immobilise pro-tumour immune cells may seem a plausible strategy to enhance the anti-tumour immune profile of the TME, these populations of cells are crucial for maintaining homeostatic levels of inflammation [106]. Therefore, systemically impeding their migration may impair the crucial regulation and resolution of immune responses and ultimately contribute to pathological inflammation [106]. For example, the CCL20-CCR6 chemokine pathway is an important mediator of immune tolerance by trafficking T_{REG} cells to sites of inflammation to promote resolution [107]. Additionally, systemically administered drugs that modulate crucial chemotactic networks may also impair the migratory capacity of leukocytes and increase patient susceptibility to pathogenic infections. Such pathways include CX_3CL1-CX_3CR1 and CCL2-CCR2, which recruit NK cells and DCs, respectively, to mediate innate immunity [27,29]. While such off-target effects of systemically administered chemokine receptor antagonists may pose a significant developmental hurdle, their consideration in pre-clinical and clinical studies will help to mitigate and manage them.

5.8. Chemokine-targeted therapies in combination with immune checkpoint inhibitors

While Yang et al.'s study outlined above in Section 5.6 remains the first of its kind to evaluate the therapeutic utility of chemokine receptor antagonism in combination with ICIs to our knowledge [61], numerous pre-clinical studies outside of the GOC space have achieved optimistic results with major translational significance from co-targeting CXCR4 and PD-1 as outlined below.

Antagonism of CXCR4 with AMD3100 in a murine xenograft model of human triple-negative breast cancer resulted in an approximate 50% reduction in intratumoural IL-10 and TGF-β mRNA levels when quantified through quantitative polymerase chain reaction [108]. Subsequent treatment of these mice with an *anti*-PD-1 ICI yielded an 80% reduction in tumour volume compared to just 40% in mice treated with the *anti*-PD-1 ICI alone [108]. A latter study of AMD3100 in a murine xenograft model of human ovarian cancer reported that mice treated with a combination of AMD3100 and *anti*-PD-1 displayed a median overall survival of approximately 75 days compared to 62 days in mice treated with *anti*-PD-1 alone, underpinned by an 80% increase in the frequency of intratumoural IFN-γ^+ $CD4^+$ and $CD8^+$ T cells relative to those treated with *anti*-PD-1 alone [109]. Treatment with the AMD3100 CXCR4 antagonist with *anti*-PD-1 was also proven superior to *anti*-PD-1 alone in a murine xenograft model of human glioblastoma, whereby 60% of mice receiving combination therapy survived 50 days following implantation, versus 30% in those receiving *anti*-PD-1 alone [110].

Collectively, these studies show that chemokine receptor antagonists are capable of synergizing with ICIs by alleviating the immunosuppressive milieu within the TME and increasing the availability of cytotoxic T cells to these drugs to re-invigorate their effector functions. While clinical trials are yet to evaluate the utility of chemokine-targeted therapies in combination with ICIs, numerous studies in the pre-clinical setting have formed firm rationale to advance future immunotherapy-based treatment strategies in GOC. With novel ICIs such as anti-LAG-3 Relatlimab [NCT03044613], bi-specific anti-TIM-3/*anti*-PD-1 RO7121661 [NCT04785820] and anti-TIM-3 INCAGN02390 [NCT03652077] currently under investigation in clinical trials for the treatment of upper gastrointestinal cancers, an opportunity has surfaced for chemokine-targeted therapies to potentially transform outcomes for GOC patients harbouring tumours with an immune-cold phenotype.

6. Prognostic and predictive roles of chemokines in the therapeutic response

As the recruitment and polarisation of anti-tumour and pro-tumour immune cells is shaped by different cytokine and chemokine signatures, profiling the GOC TME may provide more insights into its immunological composition. There is ample evidence to suggest that chemokines and their receptors may serve as predictive and prognostic biomarkers in both the pre-operative and adjuvant settings and might be used as a tool to plan treatment pathways and predict adverse clinical outcomes (Table 3).

6.1. CCL4

CCL4 recruits $CCR5^+$ CTLs in OSCC [33]. Furthermore, intratumoural expression of CCL4 in OSCC patients demonstrated 5-year OS of 50% and 25% in $CCL4^{High}$ and $CCL4^{Low}$ cohorts, respectively [34]. Noble et al. established a link between CTL infiltration and overall response to neoadjuvant chemoradiation therapy in OAC, with $CD8^{High}$ and $CD8^{Low}$ patient cohorts displaying 5-year OS of approximately 65% and 17%, respectively [111]. These studies collectively suggest that the CCL4 may serve as a prognostic factor for both OS and therapeutic response in OAC and OSCC, acting as a surrogate marker for CTL infiltration.

6.2. CCL5

CCL5 recruits anti-tumour CTLs through the CCR5 axis in OSCC [33]. Liu et al. concluded that higher intratumoural expression of CCL5 correlated with a 10% increase in 5-year OS in OSCC patients (60% in $CCL5^{High}$ versus 50% in $CCL5^{Low}$) [33]. Patients with elevated CCL5 also displayed increased CD8 and granzyme B expression, typical indicators of the CTL subset [33]. In contrast, serum levels of this chemokine were deemed detrimental to the survival of gastric cancer patients, signified by 5-year OS of approximately 40% and <5% in $CCL5^{High}$ and $CCL5^{Low}$ cohorts, respectively [112]. Importantly, these contrasting studies indicate the differential chemokine profiles of the blood and tumour, and between different cancer subtypes in the GOC space.

Table 3
Prognostic and predictive value of chemokine ligands and receptors in GOC. Clinical outcomes relating to high and low expression profiles of various chemokine ligands implicated in GOC.

Chemokine Ligand	Location	Cancer Type	Outcome ($Expression^{High}$)	Outcome ($Expression^{Low}$)
CCL22	Serum	Gastric carcinoma	50% 5-year OS [115]	20% 5-year OS [115]
CXCL8	Serum	Gastric carcinoma	55% 5-year recurrence free survival [52]	70% 5-year recurrence free survival [52]
CCL4	Intratumoral	OSCC	50% 5-year OS [34]	25% 5-year OS [34]
	Intratumoral	OAC	65% 5-year OS [111]	17% 5-year OS [111]
CCL5	Intratumoral	OSCC	60% 5-year OS [33]	50% 5-year OS [33]
	Intratumoral	Gastric carcinoma	<5% 5-year OS [112]	40% 5-year OS [112]
CCL20	Intratumoral	OSCC	55% 5-year OS [110]	50% 5-year OS [110]
CXCL10	Intratumoral	OSCC	55% 5-year OS [112]	50% 5-year OS [112]
	Intratumoural	Gastric carcinoma	45% 5-year OS [114]	30% 5-year OS [114]
CXCR3	Intratumoral	Gastric carcinoma	55% 5-year OS [117]	25% 5-year OS [117]

6.3. CXCL10

CXCL10 recruits $CXCR3^+$ CTLs and T_H1 cells in GOC [33,39]. Furthermore, OSCC patients with intratumoural $CXCL10^{High}$ expression displayed 5-year OS of 60% compared with a 50% 5-year OS in the $CXCL10^{Low}$ cohort [113]. In gastric cancer, intratumoural $CXCL10^{High}$ expression conveyed 5-year OS of approximately 45% in contrast to 30% in $CXCL10^{Low}$ cells [114]. These studies support the use of CXCL10 as a promising biomarker for survival in GOC patients based on CTL infiltration.

6.4. CCL20

CCL20 is a known chemoattractant for pro-tumour T_{REG} cells in OSCC [55]. When studying the effects of CCL20-CCR6 signalling in OSCC, Liu et al. failed to indicate substantial differences in prolonged OS between $CCL20^{High}$ and $CCL20^{Low}$ cohorts, with 5-year OS rates of 55% and 50%, respectively [34]. Interestingly, patients with high levels of both CCL4 and CCL20 expression conveyed 5-year OS of approximately 40% relative to the 60% recorded in the $CCL4^{High}$ $CCL20^{Low}$ cohort, indicative of a pro-tumourigenic role of CCL20 in OSCC [34]. Overall, CCL20 alone may not be a suitable marker for predicting survival in OSCC patients and might be best paired with a strong anti-tumour chemokine to form a predictive chemokine signature.

6.5. CCL22

CCL22 recruits pro-tumour T_{REG} cells in oesophageal and gastric cancers [55]. In gastric cancer, the intratumoural expression of CCL22 has demonstrated 5-year OS rates of 40% and 60% in $CCL22^+$ and $CCL22^-$ patients, respectively [115]. Such patients had received adjuvant 5-fluorouracil-based chemotherapy following gastrectomy [115]. This study also outlined the role of CCL22 in predicting chemotherapy response, with $CCL22^-$ patients experiencing 5-year OS of approximately 50% relative to 20% in the non-chemotherapy $CCL22^+$ cohort [115]. CCL22 expression in gastric cancer tissues was also found to be two-fold higher in patients with recurrent tumours versus those who did not relapse [115]. Furthermore, these data suggest that CCL22 may pose as a pre-operative diagnostic marker for peritoneal metastases and a predictive marker for the subsequent recurrence of curable disease in patients.

6.6. CXCL8

Neutrophils are recruited by CXCL8 and CXCL5 in gastro-oesophageal cancers [51,52]. A study by Ogura et al. identified serum CXCL8-CXCR2 expression in 33% of OSCC patients, half of which received neoadjuvant chemotherapy or chemoradiation therapy [52]. $CXCL8^+$ $CXCR2^+$ patients exhibited post-operative 5-year recurrence free survival (RFS) rates of approximately 15%, in contrast to 60% in the combined non-double positive cohorts [52]. Interestingly, the $CXCL8^+/CXCR2^-$ and $CXCL8^-/CXCR2^+$ cohorts revealed a 10% decrease in 5-year recurrence free survival relative to the $CXCL8^-/CXCR2^-$ patients [52]. CXCL8 is also capable of binding CXCR1, thereby potentially recruiting pro-tumour $CXCR1^+$ neutrophils to compensate for reduced CXCR2-dependent signalling, ultimately highlighting the need to consider the redundancy amongst chemokine receptors in such studies. The 5-year disease specific survival rate of $CXCL8^+/CXCR2^+$ patients was found to be approximately 30% lower than the other cohorts [52]. Conversely, CXCL8 expression has been shown to impede RFS in gastric cancer patients, indicated by 5-year RFS of 55% and 70% in $CXCL8^{High}$ and $CXCL8^{Low}$ patients, respectively [116]. Collectively, these studies portray CXCL8 as a potential marker for predicting the likelihood of recurrence in GOC patients.

6.7. CXCR3

High expression levels of CXCR3 in gastric cancer tissues has been associated with lower levels of CD146 immunostaining, implicating this chemokine receptor in the impedance of M2 macrophage accumulation in the TME [117]. 5-year OS rates were identified as approximately 55% and 25% in $CXCR3^{High}$ and $CXCR3^{Low}$ treatment naïve patients, respectively [117]. Therefore, CXCR3 may be an attractive biomarker for predicting survival in gastric cancer patients, based on its inverse association with immunosuppressive M2 macrophage infiltration.

6.8. Prognostic value of the microbiota in GOC

Immunohistochemical analyses of patient-derived oesophageal cancer tissues has revealed higher expression levels of CCL20 in patients harbouring *Fusobacterium nucleatum* DNA than those without [118]. Moreover, patients with *F. nucleatum* DNA had 5-year OS rates of 50%, in comparison to 70% in those without [118]. CCL20 is a known chemoattractant for immunosuppressive T_{REG} cells and tumour-fuelling T_H17 cells, which may contribute to poorer outcomes [55,63]. In gastric cancer, increased expression of CXCL1, CXCL2, CXCL6 and CXCL8 chemokine ligands were reported in patient-derived gastric cancer cells when co-cultured with *F. nucleatum* [119]. In line with the outcomes observed in oesophageal cancer, gastric cancer patients with confirmed *F. nucleatum* infection displayed 5-year OS rates of approximately 55% in contrast to 80% in those without *F. nucleatum* infection [119].

Overall, these studies suggest that the local microbiota holds strong governance over the chemokine signature of proximal tissues and thus is highly influential on the immune infiltrate of tumours and subsequent response to treatment. Furthermore, this phenomenon may raise questions surrounding the feasibility of dietary interventions as prophylactic chemopreventative measures, due to the firm link between the human microbiome and dietary patterns [120].

7. Future perspectives of chemokine-targeted therapies in GOC

The capacity for chemokine-targeted therapies to remodel the immune signature of the TME holds significant value in potentiating multiple branches of immunotherapy alongside ICIs. For example, one major limitation to the success of chimeric antigen receptor T (CAR T) cell therapies in combatting solid malignancies is the presence of an immunosuppressive milieu within the TME which can exclude CAR T cells from the tumour parenchyma and inhibit their cytotoxic potential [121]. Moreover, the capability of chemokine-targeted therapies to inhibit the infiltration of immunosuppressive populations such as MDSCs and T_{REG} cells [61] residing in the microenvironment therefore serves as a potential strategy to promote CAR-T cell accumulation within the TME to elicit their cytotoxic responses. Likewise, combining chemokine-targeted therapies with other forms of immunotherapy such as ICIs and DC vaccines may uncover a synergistic role in promoting effector T cell infiltration of tumours and ultimately overcome the recalcitrant nature of 'immune cold' tumours to further fortify immunotherapy as the fourth pillar of cancer treatment.

8. Conclusion

The pivotal role of chemokines in positioning and polarising immune cells in cancer is paralleled by their role in tumour initiation and progression. From shaping the anti-tumour and pro-tumour immune response to influencing individual hallmarks of cancer, a vast array of potential drug targets lies within the chemokine system to convert the TME to a favourable, immune-active contexture. Although chemokine antagonists have shown promising results across numerous pre-clinical studies, these drugs are yet to be evaluated in clinical investigations for GOC. Future studies should focus towards using humanised models rather than *in vitro* cell line assays to recapitulate the complexity of the

human TME and extratumoural compartments when assessing the efficacy and pharmacokinetics of chemokine receptor antagonists before costly clinical trials are conducted. This is particularly true in obesity-associated cancer such as OAC, in which immune responses and migratory pathways are severely dysregulated. By promoting tumour infiltration of anti-tumour immune cells, chemokine-targeted therapies hold huge potential to improve the response rates to conventional chemotherapy, radiotherapy, and immunotherapy. While the redundancy of the chemokine system presents challenges, these can be overcome by dual antagonism, effective receptor coverage, better preclinical data, and combination therapies with other targeted immunotherapies such as ICIs. This review uncovers the breadth of opportunities within the chemokine network to improve treatment efficacy and transform outcomes for the growing number of GOC patients globally.

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Author contributions

CO'D wrote the manuscript and performed the literature search and data analysis. MJC developed the concept for the review, proofread and provided intellectual insights, and edited all drafts of the manuscript from initiation until completion. MD, NED, and JL co-developed the concept for the review and proofread and edited the manuscript.

Declaration of competing interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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