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REVIEW ARTICLE

The tumour immune microenvironment in oesophageal cancer

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Oesophageal cancer (OC) is an inflammation-associated malignancy linked to gastro-oesophageal reflux disease, obesity and tobacco use. Knowledge of the microenvironment of oesophageal tumours is relevant to our understanding of the development of OC and its biology, and has major implications for understanding the response to standard therapies and immunotherapies, as well as for uncovering novel targets. In this context, we discuss what is known about the TME in OC from tumour initiation to development and progression, and how this is relevant to therapy sensitivity and resistance in the two major types of OC. We provide an immunological characterisation of the OC TME and discuss its prognostic implications with specific comparison with the Immunoscore and immune-hot, -cold, altered-immunosuppressed and -altered-excluded models. Targeted therapeutics for the TME under pre-clinical and clinical investigation in OCs are also summarised. A deeper understanding of the TME will enable the development of combination approaches to concurrently target the tumour cells and TME delivering precision medicine to OC patients.

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BACKGROUND

Oesophageal cancer (OC) is the seventh most common cancer and the sixth leading cause of cancer-related mortality worldwide.[1] Although outcomes have improved, the prognosis remains poor compared with other solid tumour types: the 5-year overall survival (OS) rate for OC is approximately 45–50% for OC patients who can be treated with curative intent, and 15–25% for all other patients.[2] OC is often viewed as a single entity, but two main pathological subtypes—oesophageal squamous cell carcinoma (OSCC) and oesophageal adenocarcinoma (OAC)—which show distinct aetiologies and characteristics exist.[3] OSCC previously accounted for over 90% of cases of OC in the USA, but a decrease in smoking is thought to be responsible for the declining incidence of this subtype in Western countries over past decades;[4] other risks include alcohol consumption, human papilloma virus infection, nutritional deficiencies, oesophageal achalasia and the rare inherited disorder tylosis (hyperkeratosis).[2] In marked contrast, the incidence of OAC as well as adenocarcinoma of the oesophagogastric junction (GOJ), has markedly increased in the West, in parallel with an increased prevalence of gastro-oesophageal reflux disease (GORD), obesity, and *Helicobacter pylori* (*H. pylori*) infection.[4] Barrett's oesophagus (BO), characterised pathologically by specialised intestinal metaplasia (SIM) and developing as a result of long-term reflux of acid and bile, is a pathologically defined precursor of OAC, with a malignant progression potential of approximately 0.12% per annum.[5] It can progress through identifiable sequences from SIM through low-grade dysplasia and high-grade dysplasia to invasive OAC.[5]

An evolving theme in the understanding of carcinogenesis and established tumour biology is the key role of a dynamic tumour microenvironment (TME).[6] The TME comprises a complex collection of components, including stromal cells with immunosuppressive features, such as cancer-associated fibroblasts (CAFs), immune cells, such as tumour-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs) and regulatory T (T_{reg}) cells, in addition to extracellular matrix (ECM), blood vessels and soluble mediators;[7] other non-immune factors within the TME, such as cancer stem cells (reviewed elsewhere), also play pivotal roles in tumour initiation, progression and therapy resistance.[8,9] Importantly, the TME also contains several anti-tumour components, including immune cell subsets such as cytotoxic T lymphocytes, T helper type 1 (T_H1) cells, natural killer (NK) cells, and M1-type macrophages and their associated cytokines (Table 1).[10] A fine balance exists between the pro-tumour and anti-tumour factors within the TME, the outcome of which profoundly influences whether an efficient anti-tumour immune response will be mounted to eradicate the growing tumour or whether the tumour will progress, evading anti-tumour immunity.[11] Furthermore, certain cells can display both pro- and anti-tumour effects, depending on evolving factors such as tumour stage or interaction with other TME components.[12]

Inflammation, altered metabolism, angiogenesis and hypoxia all influence the evolving and dynamic tumour–TME interaction as well as its outcome, impacting the key hallmarks of cancer in addition to the response, and resistance, to standard therapies.[12] The wide spectrum of responses across solid tumours to therapies that target elements of the TME, in particular the immune cell phenotype, highlights how complex this interface is between the tumour cell and the TME.[11] Tumours have been described as ecological systems, with constant dynamic crosstalk with the TME influencing their development and growth.[13] In this context, the role of the TME with respect to OC is poorly understood. Although studies have contributed substantially to our existing knowledge of the TME in OC[14–18] a lot of unanswered questions remain,

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Table 1. Role of immunosuppressive and anti-tumour immune cell subsets within the TME in OCs.

Immune cell type	Function in the TME
CTL	Destroys antigen-specific tumour cells.[78–81]
NK cell	Destroys MHC-I devoid cells.[160]
T_H1	Contributes to anti-tumour immunity enhancing CTL function.[78–81]
CAF	Promotes lung, bone marrow and liver metastasis via secretion of CXCL12 that binds CXCR4 and CXCR7 on OAC and OSCC cells enhancing tumour cell growth, angiogenesis, invasion and migration.[45] Remodels the ECM via secretion of MMP and LOX enzymes promoting tumour progression, invasion and migration.[56]
MDSC	Secretes immunosuppressive mediators (iNOS, TGF-β and arginase,[49]) activates fibroblasts, induces angiogenesis, polarises $CD4^+$ T cells toward a T_{reg} phenotype and inhibits NK cell cytotoxicity.[51]
Neutrophil	Induces direct DNA damage in oesophageal epithelium via secretion of ROS which activates pro-survival PI3K/Akt, NF-κB and ERK1/2 signalling pathways in oesophageal epithelium leading to inhibition of apoptosis, promotion of proliferation, invasion, metastasis, angiogenesis and immune evasion.[27]
Obese adipocyte	Recruits and polarises macrophages in the omentum to an M2-like phenotype which promotes systemic low-grade inflammation promoting tumour initiation and progression via secretion of pro-tumour IL-6 and TNF-α.[33] Secretes leptin that increases proliferation and inhibits apoptosis in oesophageal epithelium via Akt, MAPK and STAT pathways.[35]
TAM	Induces direct DNA damage in oesophageal epithelium via secretion of ROS which activates pro-survival PI3K/Akt, NF-κB and ERK1/2 signalling pathways in oesophageal epithelium leading to inhibition of apoptosis, promotion of proliferation, invasion, metastasis, angiogenesis (secretion of thymidine phosphorylase[47]) and immune evasion.[27] Secretes IL-6 and TNF-α that promote anti-apoptotic pathways and TNF-α activates oncogenes.[33] Secretes COX-2 that promotes cancer stem cell-like activity, proliferation, apoptotic resistance, angiogenesis, metastasis and immunosuppression.[52]
T_H2 cell	Secretes IL-10, IL-4, IL-5 and IL-13 cytokines dampening anti-tumour immunity promoting progression from BO to OAC.[161]

BO Barrett's oesophagus, *CAFs* cancer-associated fibroblasts, *COX-2* cyclo-oxygenase-2, *CXCL* chemokine (C-X-C motif) ligand, *CXCR* CXC chemokine receptor, *CTL* cytotoxic T lymphocyte, *ECM* extracellular matrix, *ERK* extracellular signal-regulated kinase, *iNOS* inducible nitric oxide synthase, *IL* interleukin, *LOX* lysyl oxidases, *MAPK* mitogen-activated protein kinase, *MDSC* myeloid-derived suppressor cell, *MMP* matrix metalloproteinase, *NF-κB* nuclear factor κB, *NK cell* natural killer cell, *OAC* oesophageal adenocarcinoma, *OSCC* oesophageal squamous cell carcinoma, *PI3K* phosphatidylinositol 3-kinase, *ROS* reactive oxygen species, *STAT* signal transducer and activator of transcription, *T_H1 cell* T helper type 1 cell, *T_H2 cell* T helper type 2 cell, *T_{reg}* T regulatory cell, *TGF-β* tumour growth factor-β, *TNF-α* tumour necrosis factor-α.

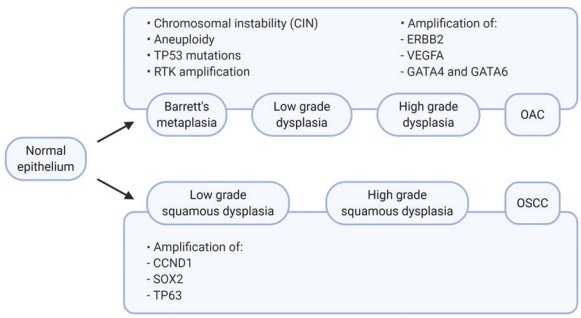
hindering the development of tailored therapeutic approaches for treating patients with OC. In this article, we outline current knowledge with respect to the TME in OC carcinogenesis, disease prognosis and response to standard therapies, as well as in the context of novel approaches to drug targeting of this disease.

GENOMIC CHARACTERISTICS OF OC

The Cancer Genome Atlas (TCGA) network studies on oesophageal and gastric cancer (GC) have made several key findings and advanced our genetic and molecular understanding of the two subtypes of OC.[19] First, OSCC and OAC show distinct patterns of somatic copy number mutations and the associated genes amplified (Box 1).[19] For instance, OSCC frequently displays genomic amplifications of *CCND1*, *SOX2* and/or *TP63* genes, whereas in OAC, *ERBB2*, *VEGFA*, *GATA4* and *GATA6* are more commonly amplified.[19] Second, OAC more closely resembles GOJ adenocarcinoma, as well as gastric adenocarcinoma, with the dominant intestinal pathology type being predominantly chromosomally instable (CIN), featuring aneuploidy, mutation of *TP53*, and amplification of several genes encoding receptor tyrosine kinases; by contrast, OSCC tumours appear more closely related to squamous carcinomas from other organs than to OAC.[19] TCGA analysis suggests that, in a modern paradigm of adenocarcinoma of the oesophagus, gastroesophageal junction and stomach, a unifying hypothesis might exist[19] such that, although different factors such as GORD and *H. pylori* can fuel inflammation, metaplasia and carcinogenesis, the genomic, mutational, molecular, and immune features are largely similar between intestinal OAC, GOJ and gastric adenocarcinoma.[19] The Asian Cancer Research Group reported the most common phenotype as microsatellite stable and *TP53* inactive, similar to the CIN variant of gastric adenocarcinoma.[19] There is no clear inherent genetic predisposition to OAC, with only 7% of BO and OAC cases were determined to be familial in a European cohort.[20]

Box 1 The genetic steps of origin and progression of OSCC and OAC

TCGA studies revealed that OSCC is enriched for CCND1 and SOX2 and/or TP53 gene amplifications. Whereas, OAC is enriched for ERBB2, VEGFA, GATA4 and GATA6 genomic amplifications. OAC typically has a CIN phenotype with aneuploidy, TP53 mutations and RTK amplifications.



INFLAMMATION AND THE TME IN OESOPHAGEAL CARCINOGENESIS

Several factors have been observed to play a role in the development of OC, with GORD and obesity being central to OAC carcinogenesis,[21] while smoking and alcohol consumption underlie much of the aetiology for OSCC. In this context, GORD and obesity-driven inflammation generate a pro-tumorigenic microenvironment consisting of pro-inflammatory M2-type macrophages, neutrophils, MDSCs and T_H2 cells, as well as pro-inflammatory mediators that include interleukin (IL)-1β, IL-8, IL-6, reactive oxygen species (ROS) and tumour-promoting T_H2 cytokines (Fig. 1).

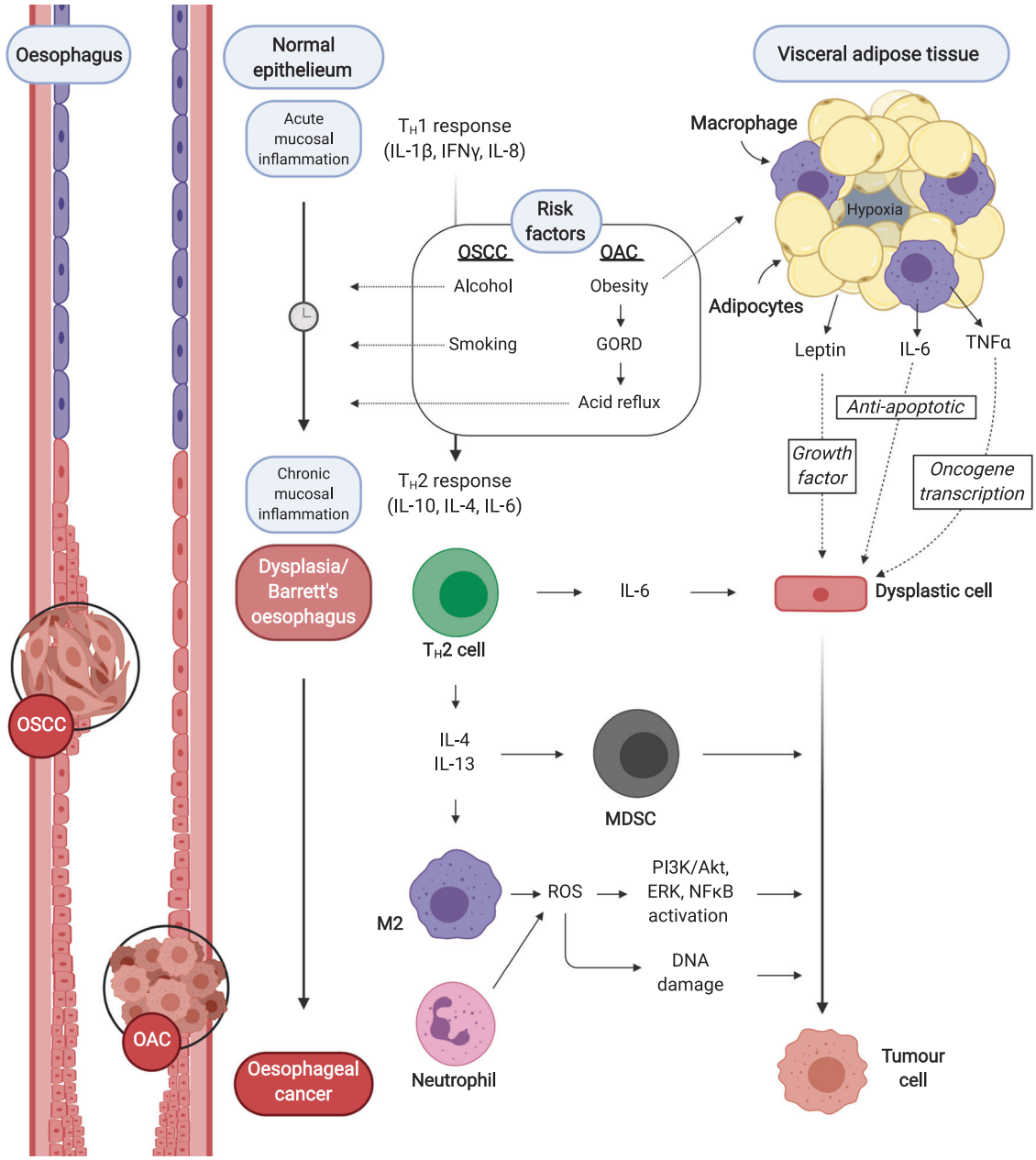


Fig. 1 Inflammation and the TME in oesophageal carcinogenesis. Exposure to risk factors causes acute mucosal inflammation primarily mediated by T_H1 cells. With chronic exposure, inflammation shifts from a T_H1 cell response to a T_H2 cell response, which drives local inflammatory changes in the oesophageal epithelium establishing precursor lesions known as dysplasia. Transformation of dysplastic lesions to invasive carcinoma is driven by local and systemic factors. Locally, elaboration of T_H2 cell-mediated inflammatory pathways culminates in recruitment of myeloid-derived suppressor cells (MDSCs), M2 macrophages and neutrophils leading to pro-survival pathway activation and mutation-inducing DNA damage. Obesity is considered a systemic carcinogenic factor which promotes pro-tumorigenic inflammation via release of growth factors such as leptin and cytokines including IL-6 and TNF-α from adipose tissue and macrophages, respectively.

GORD

In response to acid and bile reflux in GORD, inflammation progresses from being acute, with a distinct inflammatory, immune and molecular phenotype, to a chronic inflammatory or metaplastic phenotype, such as SIM, which has characteristics including mutational changes consistent with neoplasia.[22] In murine models of GORD, T cells infiltrate the submucosal layers of the oesophagus prior to other immune cells and before inflammation manifests.[23] In humans, increased levels of IL-1β, interferon (IFN)-γ and IL-8 mRNA are found in oesophagitis compared with normal oesophageal squamous epithelium.[23] In BO tissue, an increase in the levels of T_H2 cells, which secrete IL-10, IL-4 and IL-6, is seen.[23,24] Collectively, these results highlight the plasticity of the inflammatory profile throughout OAC progression, as well as the key role of T cells.[23,24] In a rat model of GORD and severe oesophagitis, M1-type macrophages were recruited to epithelial and stromal cells, where they activated STAT3 signalling prior to infiltration of M2-type macrophages and progression to OSCC and OAC.[25] The reciprocal interaction of T cells, macrophages and MDSCs is also evident in human studies of OAC and OSCC, which have revealed elevated levels in OAC and OSCC tumours of the T_H2 cytokines IL-4 and IL-13, which mediate the recruitment of M2 macrophages and MDSCs.[26]

ROS, mainly derived from macrophages and neutrophils in response to anti-inflammatory T_H2 responses induced by GORD cause direct DNA damage in the normal oesophageal epithelium which, if chronically sustained, can induce neoplasia.[27] In addition, ROS activates pro-survival pathways including phosphatidylinositol 3-kinase (PI3K)/Akt, nuclear factor (NF)-κB and extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK)1/2 in epithelial cells, promoting carcinogenesis and tumour development by inhibiting apoptosis, evading the immune system and promoting proliferation, invasion, metastasis and angiogenesis—all of which are hallmarks of cancer.[27] NF-κB, a 'master' transcription factor in epithelial cells, is central to inflammation in cancer[27] and high levels of NF-κB-induced IL-8 and IL-1β were found in BO epithelium and were further increased in OAC.[28] The expression of IL-8 positively correlated with progression from BO to OAC.[28] IL-8 recruits neutrophils, induces immune cell infiltration and promotes angiogenesis, tumour cell stemness, cell survival, migration and metastasis.[27] The dynamic relationship between NF-κB and IL-8 is a prime example of the reciprocal crosstalk observed between factors of the TME working in tandem to promote inflammation, angiogenesis, metastasis and other tumorigenic processes.[27,28]

Obesity

Obesity is strongly associated with the development of OAC, by the promotion of GORD, chronic systemic and tissue-specific low-grade inflammation as well as altered metabolism.[29] GORD induces localised and chronic inflammation in the lower-third of the oesophagus, whereas obesity is responsible for generating systemic low-grade inflammation that abrogates cancer immune surveillance systemically via inhibition of NK cell-mediated destruction of tumour cells.[30] Additionally, the increased abundance of visceral adipose tissue in obese individuals results in an increase in intra-abdominal pressure on the stomach forcing the gastric contents into the lower part of the oesophagus and promoting the development of GORD.[31] Obesity might also promote the progression of Barrett's-associated SIM to dysplasia and OAC.[29] Adipose tissue, particularly visceral or central fat, can contribute to pro-tumorigenic inflammation, in particular when associated with the metabolic syndrome phenotype, which includes type 2 diabetes mellitus, hypertension, hyperlipidaemia and non-alcoholic fatty liver disease.[32] Excessive adiposity can drive hypoxia and has immunoregulatory effects, being associated with macrophage recruitment, M2 polarisation, and recruitment of immune cells with the subsequent production of pro-inflammatory pro-tumour cytokines, including IL-6 and TNF-α.[21] IL-6 promotes anti-apoptotic pathways and TNF-α activates oncogenes, amongst other effects.[33] Increased levels of growth factors—in particular, vascular endothelial growth factor (VEGF) and the satiety hormone leptin—might also influence carcinogenesis.[34,35] For instance, leptin in BO patients is associated with an increased risk of OAC.[34] The leptin receptor (ObR) is upregulated in patients with OAC and correlates with an advanced tumour stage and involvement of the lymph nodes.[35] Leptin promotes tumour development and progression by increasing proliferation and inhibiting apoptotic pathways mediated through Akt, MAPK and STAT.[35] Other factors that link the adipose microenvironment and the TME include CAFs, MDSCs, angiogenesis, and altered metabolism, which have been shown in other cancer-types (reviewed in ref. [21]).

THE TME, IMMUNE SUPPRESSION AND TUMOUR PROGRESSION

Once a tumour is established, a complex interplay of cellular and non-cellular factors within the TME favours a predominantly immunosuppressive, tumour-promoting local environment,[36] (Fig. 2). This is not to say that anti-tumour factors do not exist in this system but, rather, that they are being outcompeted by pro-tumour signals and might even be physically excluded from the tumour bed by stiffening of the ECM and high tumour interstitial fluid pressure causing anomalous hydrodynamic blood flow.[37,38] ECM components can also sequester important T cell-recruiting chemokines, preventing the formation of a functional chemokine gradient necessary to guide T cells to the TME.[39] Stiffening of the ECM also impedes immune cell infiltration and activation and favours metastatic dissemination by driving epithelial to mesenchymal transition in tumour cells mediated through integrin-receptor binding and activation of pro-survival and proliferation signalling pathways in tumour cells.[36,38]

Key immunosuppressive cells that have a role in tumour progression include CAFs, TAMs and MDSCs, whereas soluble mediators, such as the cyclo-oxygenase (COX)-2–prostaglandin E (PGE)-2 axis and membrane-tethered enzymes that remodel the ECM are also important.[36] An important mechanism of immunosuppression within the TME is the upregulation of inhibitory immune checkpoint ligands and receptors on the surface of tumour cells and immune cells respectively, which through binding to their cognate receptors or ligands on anti-tumour T cells, NK cells and antigen presenting cells dampen anti-tumour function of these cell types and can polarise their phenotypes toward an immunosuppressive and regulatory phenotype promoting immunosuppression as well as tumour progression.[40,41]

Immunosuppressive cells: CAFs, TAMs and MDSCs

CAFs build and remodel the ECM, secreting an array of factors that recruit immunosuppressive immune cells including TAMs and neutrophils to the TME, that create an immunosuppressive milieu that repels effector anti-tumour T cells.[42] CAFs promote the release of multiple growth factors, inflammatory cytokines and pro-metastatic mediators including transforming growth factor-β (TGF-β), hepatocyte growth factor, fibroblast growth factor, VEGF, IL-6, CXC-chemokine ligand (CXCL12), Wnt2, periostin and podoplanin, hence promoting angiogenesis, tumour cell proliferation, invasion, metastasis, immunosuppression and immune evasion.[43,44] The secretion by CAFs of CXCL12, which binds to CXCR4 and CXCR7 on tumour cells,[45] is responsible for promoting metastasis to the lungs, bone marrow and liver in OAC and OSCC by enhancing tumour cell growth, angiogenesis, invasion and migration.[45] In addition, in a cohort of OAC patients ($n = 183$), 93% of cases contained CAFs with a myofibroblastic (α-SMA-positive) phenotype, which correlated significantly with poor survival.[46] Primary CAFs isolated from OAC tumours promoted the invasion of OAC cells as demonstrated in vitro using Transwell migration assays and in vivo using murine xenograft models.[46] This effect was mediated by the secretion, by CAFs, of the matricellular protein periostin, which binds to αvβ3 and αvβ5 integrins on the surface of OAC cells, activating PI3K–Akt signalling to mediate OAC cell invasion.[46]

TAMs secrete proteases and growth factors that are capable of stimulating invasion, angiogenesis, inflammation, metastasis and immunosuppression, thereby promoting tumour progression.[47] TAMs secrete IL-10, TGF-β and arginase 1 which have well-characterised immunosuppressive functions and include inhibition of T cell activation, differentiation, proliferation, and effector functions, as well as inhibition of T cell and NK cell cytotoxicity and promotion of Treg cell expansion.[48] In addition, TGF-β inhibits the expression of co-stimulatory molecules and IL-12 by dendritic cells (DCs) and subsequent DC maturation and migration.[48] OSCC cells secrete macrophage chemoattractant protein-1 (MCP-1) to recruit TAMs, which, in turn, promote angiogenesis via the secretion of proangiogenic factors such as thymidine phosphorylase.[47]

The pro-inflammatory cytokines IL-6, PGE-2, IL-1β and VEGF secreted by tumour cells or immunosuppressive immune cell types including TAMs, Tregs and CAFs activate and expand MDSC populations in tumours.[15,49] MDSCs potently suppress anti-tumour

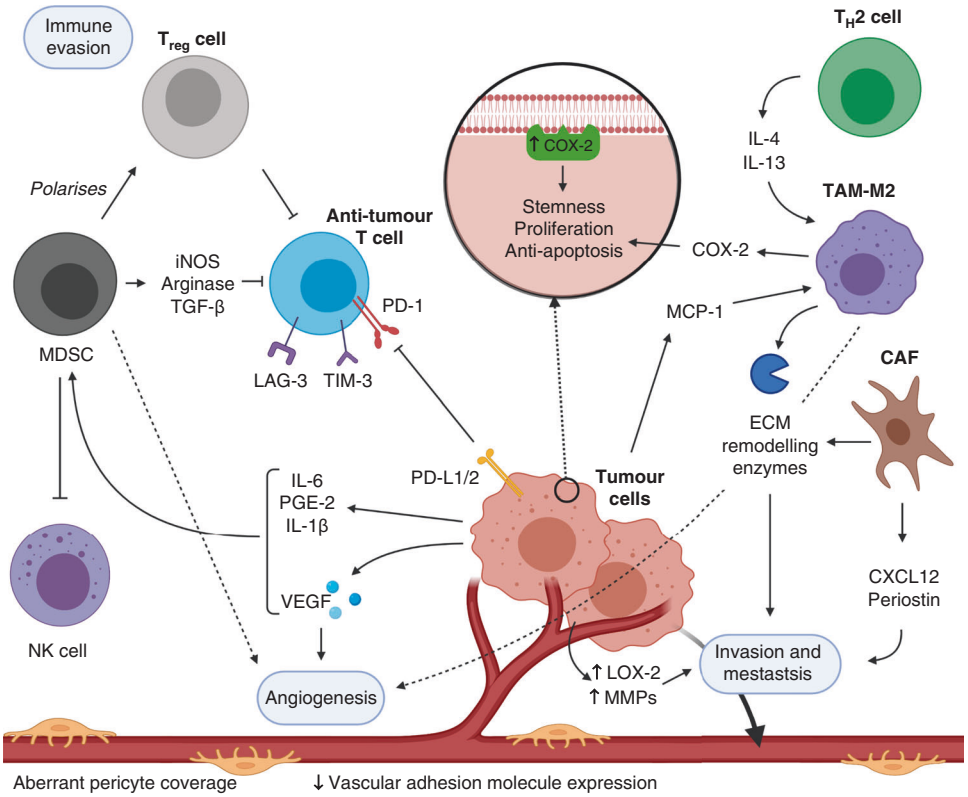


Fig. 2 The TME, immune suppression and tumour progression. Many cellular and non-cellular components of the TME contribute to tumour progression. Interactions between such constituents promote key hallmarks of cancer including cancer cell proliferation, immune evasion, angiogenesis, invasion and metastasis. CAFs cancer-associated fibroblasts, COX-2 cyclooxygenase-2, CXCL chemokine (C-X-C motif) ligand, ECM extracellular matrix, IL interleukin, iNOS inducible nitric oxide synthase, LAG-3 Lymphocyte-activation gene 3, LOX-2 lysyl oxidase-2, MCP-1 macrophage chemoattractant protein-1, MDSC myeloid-derived suppressor cells, MMPs matrix metalloproteinases, NK cell natural killer cell, PD-1 Programmed cell death protein 1, PD-L1/2 Programmed death-ligand 1/2, PGE-2 Prostaglandin E_2, ROS reactive oxygen species, TAM tumour-associated macrophage, TGF-β tumour growth factor-β, T_H2 cell T helper type 2 cell, TIM-3 T cell immunoglobulin and mucin domain-containing protein 3, T_{reg} T regulatory cell, VEGF vascular endothelial growth factor.

immunity through the secretion of inducible nitric oxide synthase (iNOS), which inhibits MHCII expression on T cells and induces T cell apoptosis, and arginase, which limits the availability of arginine, thereby suppressing T-cell activation.[49] The production of TGF-β by MDSCs can inhibit NK cell cytotoxicity, NKG2D expression, IFN-γ production and induce NK cell anergy mediated by membrane-bound TFG-β on the surface of MDSCs.[50] As well as their role in immunosuppression, MDSCs can promote tumorigenesis[51] through the activation of fibroblasts and the induction of angiogenesis.[51] Indeed, MDSCs were greatly expanded in the p120-catenin deficient mouse model for oral SCC, in which they activated fibroblasts to induce desmoplasia, suggesting an important role for MDSCs in OSCC tumorigenesis.[49]

COX-2, MMPs and LOX

Soluble factors within the TME are of significant therapeutic relevance, with most studies to date focusing on the COX-2–PGE-2 pathway.[52] COX-2 promotes cancer-stem-cell-like activity, proliferation, apoptotic resistance, angiogenesis, metastasis and immunosuppression.[52] Sharma et al., demonstrated that COX-2 suppresses host anti-tumour immunity via inhibition of DC function which was restored with the use of COX-2 inhibitors.[53] DCs cultured in tumour conditioned media and pulsed with tumour-specific peptides demonstrated a reduction in antigen processing and presentation machinery, decreased IL-12 secretion and expression of CD11c, DEC-205, MHC class I antigen, MHC class II antigen, CD80, and CD86 maturation markers.[53] However, when DCs were treated with COX-2 inhibited-tumour conditioned media there was no abrogation of DC function, highlighting an important role for COX-2 in preventing activation of innate and subsequent activation of adaptive anti-tumour immunity.[53] COX-2 protein expression is elevated in epithelial cells in BO and OAC, as well as being induced by bile acid exposure as a consequence of severe reflux, particularly in patients with BO.[54] By inducing the production of PGE-2, COX-2 promotes inflammation in OAC and BO,[27] and the level of COX-2 expression in OSCC tissues was reported to positively correlate with dysplasia.[55]

Tissue remodelling within the ECM mediated through enzymes including matrix metalloproteinases (MMPs) and lysyl oxidases (LOX) expressed and released by stromal cells—in particular, immunosuppressive CAFs—has a key role in tumour progression, invasion and migration.[56] LOX-2 expression was significantly increased in 92% of OSCC tumours,[57] and MMP-7, MMP-9 and MMP-13 are overexpressed in OSCC and positively associated with tumour staging.[58]

Fast growing tumour cells rapidly deplete the available oxygen and essential nutrients (glucose, glutamine, arginine, tryptophan) within the TME, depriving anti-tumour immune cells from nutrients and oxygen leading to tumoural hypoxia.[59] The hypoxic and hostile TME compensates through the activation of pro-angiogenic pathways generating new yet defective tumour vasculature in an attempt to replenish oxygen levels and nutrients to the TME.[60] This tumour vasculature, is characterised as a chaotic network of tortuous and leaky blood vessels comprising dysfunctional endothelial cells with a decreased expression of adhesion molecules that are required for immune cell extravasation to the tumour, and aberrant pericyte coverage, which prevents the trafficking of anti-tumour immune cells into the TME contributing

to immunosuppression within the TME.[61] The disrupted tumour vascular has poor oxygen perfusion due to its tortuous and leaky structure which ultimately enhances hypoxia within the TME.[60] Hypoxia can inhibit tumour cell differentiation and promote maintenance of an aggressive cancer-stem-like phenotype.[62] Additionally, hypoxia leads to a switch toward a glycolytic phenotype in tumour cells increasing glucose uptake and starving anti-tumour immune cells of glucose which is essential for anti-tumour T cell effector functions.[63] Increased glycolysis also results in acidification of the TME due to a build-up of lactate, a key by-product of glycolysis, within the microenvironment which has been shown to promote expansion and survival of several immunosuppressive cell types such as Treg cells, MDSCs and TAMs and inhibit NK cell function.[63,64] Tumour hypoxia profoundly impacts the TME, in part via upregulation of hypoxia-inducible factor 1-α (HIF1-α).[65] Knockdown of HIF1-α in OSCC cells decreased their proliferation in vitro and delayed the formation of murine OSCC xenografts compared with wild-type controls (wild-type: 8.4 ± 2.1 days versus HIF1-α knockdown: 6 ± 1.2 days) as well as inhibiting vasculogenic mimicry and tumorigenicity in vivo.[66]

Inhibitory immune checkpoints

Inhibitory immune checkpoints play a key role in immunosuppression, but might also be important in tumour progression.[40] The expression of the immune checkpoint receptor lymphocyte-associated gene 3 (LAG-3) was identified in 10.5% of OAC tumours and found to correlate positively with the percentage of $CD4^+$ and $CD8^+$ tumour-infiltrating T cells.[67] In a study of 165 patients with OAC, patients with TILs expressing LAG-3 had a longer median OS of 70.2 months compared with 26.9 months in non-expressors.[67] LAG-3 is often expressed on exhausted anti-tumour T cells[68] therefore, tumours with LAG-3 expressing TILs may identify patients who had immunologically visible tumours whereas, patients that lacked LAG-3 expressing TILs may reflect an immunologically ignorant or 'silent' tumour that failed to generate an anti-tumour immune response.[67] In a study of 183 patients with OSCC, the expression of another immune checkpoint receptor, T-cell immunoglobulin and mucin domain-3 (TIM-3), was associated with a high density of $CD8^+$ TILs and the expression of programmed cell death protein-1 (PD-1), another immune checkpoint receptor.[69] As T-cell expression of LAG-3 and TIM-3 often denotes T-cell exhaustion, the presence of LAG-3- and TIM-3-expressing T cells might reflect an immunologically visible tumour with ongoing attempted anti-tumour immune responses,[40] indicating a potential role for the expression of novel immune checkpoints in tumours for stratifying patients with immunologically visible tumours that may perhaps harbour a pre-existing anti-tumour immune response and may benefit from immune checkpoint blockade.

Increased levels of the immune checkpoint ligands PD-L1 and PD-L2 have been detected in both OAC and OSCC tumours.[70,71] Increased PD-L1 expression correlated positively with an increased depth of tumour invasion whereas increased PD-L2 expression correlated negatively with $CD8^+$ T cell infiltration in OSCC.[70] Tumour-expressed PD-L1 and PD-L2 was detected in 2% and 51.7% of OAC tumours, respectively.[72] However, immune cell-expression of PD-L1 was observed in 18% of OAC cases.[72] This study demonstrated that PD-L1 was preferentially expressed on immune cells, whereas, PD-L2 was preferentially expressed on tumour cells in OAC, suggesting that the ligands for PD-1 receptor may have cell-type specific roles in mediating immune evasion and suppression withing the OAC TME. PD-L2 was also detected on BO epithelium but not in non-BO oesophagitis, and the T_H2 cytokines IL-4 and IL-13, which play a key pathogenic role in the progression of BO to OAC, upregulated PD-L2 on OAC cells in vitro.[72] Other studies suggest that PD-L1 and PD-L2 have distinct functions in regulating type-1 and type-2 immune responses, and are upregulated on TAMs by T_H1 and T_H2 cytokines, respectively.[73] These results suggest that the T_H2-like inflammatory environment in BO and OAC might contribute to the tumour expression of PD-L2 and implicate a potential therapeutic role in OAC for immune checkpoint blockers that target the PD-1/PD-L1/PD-L2 axis in OAC.

THE IMMUNE INFILTRATE, THE TME AND PROGNOSTIC IMPACT

Because an active anti-tumour immune response, characterised by immune cell infiltration into tumours, is linked with improved survival outcome in solid tumours,[74] it follows, therefore, that tumours that cannot completely evade the immune system have less lethal phenotypes than those that can. Further knowledge of what mediates this incomplete evasion could provide valuable information on factors affecting patient survival.[74]

The Immunoscore in patients with colorectal cancer

This paradigm is well developed for colorectal cancer (CRC), but not for OC.[75,76] The concept of the prognostic benefit of lymphocytic invasion of tumours has evolved from early observations to a current model that describes the 'immune contexture' of CRC based on the density, location, organisation and functional orientation of TILs, called the Immunoscore.[75,76] To obtain the Immunoscore, cells in the tumour core and the invasive margin that express CD3, CD8 and CD45RO (a marker of memory T cells) are enumerated and a score from 0 to 4 (I0–I4) is assigned, whereby 0 refers to a low density of positive cells in both regions, and 4 refers to a high level of positive cells in both regions.[75,76] In large volume series, less than 5% of patients with CRC with a high (I4) score relapsed, compared with over 70% of patients with a low (I0/I1) score.[75,76] The Immunoscore has been validated in a larger international study of 2681 tumours[77] and a meta-analysis of 8 studies including 4689 CRC patients.[75–77] Moreover, for stage II CRC, the Immunoscore might possess a prognostic ability that exceeds that of the TNM Classification of Malignant Tumours AJCC/UICC staging classification.[75–77]

The prognostic potential of the measurement of immune cells in OC

The studies in CRC therefore provide an intriguing insight into how the lymphocytic compartment of the TME might influence clinical outcomes.[75–77] Such a concept could, theoretically, apply to OC, but this has yet to be studied sufficiently in large cohorts and therefore this hypothesis is speculative and unproven. There are, however, data suggesting that such a parallel with CRC might exist,[78–81] and the measurement of TILs has revealed prognostic significance in both OAC[78–81] and OSCC[82,83] studies. CD8, CD3, CD4 and FoxP3 are the most common markers used to assess lymphocytic infiltration.[78–83] A meta-analysis revealed a significant prognostic potential for CD3 ($n = 248$ from two studies, HR = 0.51, 95% CI = 0.32–0.7) and CD8 ($n = 203$ HR = 0.55, 95% CI = 0.3–10.8).[80] The prognostic impact of FoxP3 expression in tumours is less clear, with a meta-analysis counterintuitively showing an association of this T_{reg} cell marker with improved OS for OAC (two studies, $n = 252$, OR = 0.51, 95% CI = 0.33–0.79, $P = 0.002$), CRC and head and neck cancers.[84] A 2020 study also demonstrated a prognostic value for CD45RO, PD-1 and the inducible costimulatory molecule ICOS in several tumour types, including OAC.[81] Furthermore, the presence of $TIM\text{-}3^+$ TILs was demonstrated to be an independent risk factor for recurrence-free survival (RFS) and OS in OSCC,[69] and subgroup analysis revealed that the $TIM\text{-}3^+$ $PD\text{-}1^+$ CD8 low group had a worse RFS and OS compared with $TIM\text{-}3^-$ $PD\text{-}1^-$ CD8 high group.[69]

The MHC class II antigen presentation molecules—for example, HLA-DR—are also of prognostic interest, as patients with high expression in the epithelium of OACs were shown to survive over twice as long as those with lower than median expression,[85] an

effect also seen in other gastrointestinal cancers.[86,87] This result suggests that immune markers upstream of adaptive immunity, particularly those involved in adaptive immune priming, might also have an important role to play in prognosis.[88]

Although T cells have been the principal focus of studies on immune cells with prognostic potential in the TME, the abundance of myeloid cells in OC tumours also appears to have a prognostic potential (albeit a negative one), with a high density of macrophages expressing CD163 or CD204 associated with a worse OS in OSCC and OAC patients compared with patients with low levels of these cells.[89] Additional studies reported that TAMs expressing high levels of CD204 positively correlated with an increased depth of tumour invasion, lymph and blood vessel invasion, advanced clinical stage and lymph node metastasis.[90]

Other TME factors with prognostic potential

Other components of the TME in addition to immune cell populations have also demonstrated prognostic potential in OC.[91,92] The IL-6/STAT3 pathway is central to inflammation within the TME, and high levels of both tumoural and serum IL-6 were positively associated with a shorter OS in OSCC.[91] IL-6 tumoural expression was also positively associated with metastasis in OSCC,[91] and levels of HIF1-α and vascular mimicry negatively correlated with OS, lymph node metastasis, serosa infiltration and TNM staging.[92] The 5-year-survival rate was 7.14% (5/70) in cohorts with high HIF-lα expression and 57.78% (52/90) in cohorts with low HIF-lα expression, respectively.[92]

With respect to prognosis, a systematic review and meta-analysis of immunohistochemical biomarkers in resected OAC tissue assessed the cumulative prognostic capacity of COX-2, epidermal growth factor receptor (EGFR), human epidermal growth factor receptor (HER)−2, Ki67, leucine-rich repeat-containing G-protein coupled receptor 5 (LgR5), p53 and VEGF.[80] This meta-analysis showed significant prognostic potential for COX-2 ($n = 382$ from 3 studies, HR = 2.47, 95% CI = 1.15–3.79) and EGFR ($n = 642$ from two studies, HR = 1.65, 95% CI = 1.14–2.16).[80] The receptor tyrosine kinase EGFR is one of the main upstream modulators of COX-2 in cancer cells, thereby highlighting the importance of this pathway in mediating pro-tumour inflammation.[52] Members of the MAPK family and NF-κB are also key upstream modulators of COX-2 in cancer cells.[93] This highlights the key role of COX-2 in tumour progression as multiple tumour-promoting pathways converge to induce COX-2 expression in OAC cells. A subsequent meta-analysis of 25 studies on 2,465 patients with OAC or OSCC also showed that overexpression of COX-2 was associated with poor survival (HR = 1.60, 95% CI = 1.32–1.94, $P < 0.001$) as well as depth of invasion, lymph node metastasis, distant metastasis and TNM stage.[93] The co-expression of MMP-7, MMP-9 and MMP-13 in early-stage OSCC tumours correlated with tumour cell differentiation, vessel permeation and lymph node metastasis, and identified a poor prognostic cohort compared with those negative for co-expression (13 months versus 58 months).[58]

In summary, although research is still ongoing and, relative to CRC, in its infancy, a similar scoring model based on the presence of TILs, alongside the broader use of methods to characterise the TME, might uncover useful characteristics that could be an adjunct to standard TNM classification and create considerations for management pathways or novel therapies in OC. In the CRC setting, differentiation of 'hot' (highly infiltrated by immune cells), 'altered' and 'cold' (not infiltrated) tumours corresponded to a 2-year risk of relapse of 10%, 50% and 80%, respectively.[94] The 'altered' state was further subdivided into 'altered-immunosuppressed' or 'altered-excluded', to differentiate between tumours where some degree of immune cell infiltration is apparent but not optimal for prognostic benefit, and tumours where lymphocytes are only seen at the edge of the tumour but have failed to physically access the tumour core, respectively.[94] As the majority of OSCC and OAC tumours typically show some evidence of lymphocytic infiltration,[79,81,95] these tumours would broadly be classified as altered-immunosuppressed-type tumours. This tumour type features lymphocytic infiltration but also contains high levels of soluble immunosuppressive factors, such as TGF-β, IL-10 and VEGF.[96] In this context, a meta-analysis of 30 studies with over 2,000 participants showed that elevated VEGF expression was associated with poor survival in patients with OSCC (HR = 1.81, 95% CI = 1.57–2.10) but not OAC.[96] The reasons for this are unclear, VEGF plays a key role in driving angiogenesis, however, there are several other pro-angiogenic signalling pathways that promote angiogenesis, which may play a more predominant role in OAC compared with OSCC and include several signalling axes such as TGF-β-TGF-βRII, angiopoietin-1/2-Tie-2, PDGF–PDGFR, FGF–FGFR, notch and neuropillin signalling pathways which all converge to promote pro-survival and pro-angiogenic processes in tumour cells (reviewed in ref. [97]). Alternatively, VEGF-mediated sprouting angiogenesis is not the key mechanism for generating tumour vasculature, other mechanisms include vessel co-option and vascular mimicry (reviewed in ref. [98]) which may be more important for generating tumour vasculature in OAC. Additionally, several studies have highlighted that VEGF is an important player in promoting immunosuppression via inhibition of DC function, inhibition of T cell recruitment to the TME, promotion of T cell exhaustion and proliferation of MDSCs.[99,100] Therefore, it has been postulated that the therapeutic benefits of blocking VEGF are as a result of the inhibition of immunosuppression mediated by VEGF as opposed to the role of VEGF in promoting angiogenesis.[99,100] There are several cell types and soluble mediators that we discuss in this review which promote immunosuppression via similar mechanisms to VEGF and therefore, the role of VEGF in OAC may be redundant, but more important in OSCC.

THE TME AND THERAPEUTIC RESPONSE

Standard therapies for OCs include surgery, chemotherapy and radiation.[101,102] For patients who present with locally advanced OAC, either chemotherapy alone or combined with radiation therapy prior to radical surgery is given.[101] For locally advanced OSCC, either combination chemotherapy and radiation prior to surgery, or high-dose radiation with chemotherapy represent equivalent therapeutic options.[101] A deeper understanding of the TME in OCs, however, would facilitate the development of predictive biomarkers of the response to chemotherapy or radiation therapy, which would represent a significant therapeutic benefit, distinguishing patients who are likely to benefit from these approaches from those patients who might be harmed either by the side effects of treatment or a delay to surgery.

Response to chemotherapy

The infiltration of TAMs into OSCC tumours, recruited by tumour-derived MCP-1, is associated with a poor response to chemotherapy.[47] Additionally, increased levels of MDSCs in OSCC[49] and OAC[103] patients correlated positively with therapeutic resistance and advanced disease stage.[49] In terms of stromal factors, COX-2 expression in OSCC tissues was reported to predict resistance to chemotherapy,[104] and high levels of both tumoural and serum IL-6 were positively associated with a poor response to neoadjuvant chemoradiotherapy in OSCC patients.[91] In a study of 196 patients with OSCC, the density of $FOXP3^+$ cells in the residual tumour (or its scar) correlated with a poor pathological response to chemoradiation, as determined by tumour regression grade, and with predicted poor cancer-specific survival.[105]

Response to radiotherapy

Radiotherapeutic resistance in OC is polymodal and can be mediated by cancer-cell-intrinsic mechanisms that include alterations in DNA repair, cellular energetics and growth signalling, as

well as alterations in the TME. The majority of radiation-induced DNA damage that results in cell death during radiotherapy is thought to be mediated by oxygen free radicals, which are generated by radiation and the presence of oxygen within the TME.[106] Consequently, hypoxia is a key mediator of radiation resistance in several cancer types, including OCs.[107] Murine OAC subcutaneous xenograft studies also demonstrated the predictive role of hypoxia within the TME in radiotherapy response.[108] Mice received 5 Gy irradiation for 5 consecutive days and tumoural hypoxia was measured by a ^{18}F-FAZA PET/CT scan.[108] Radiation was less effective in hypoxic tumours compared with normoxic tumours, determined by the extent of tumour regression.[108] A systematic review also determined that intra-tumoural HIF-1α overexpression was an independent prognostic factor for treatment response.[109]

Novel approaches

Although chemotherapy is the first-line treatment for patients presenting with metastatic or recurrent disease, novel approaches have been developed and approved based on tumour cell or TME characteristics, such as tumoural expression of HER2[110] and the expression of PD-L1 on OAC cells and immune cells.[111] In addition, pembrolizumab was approved in 2020 for the treatment of patients with unresectable or metastatic tumour mutational burden (TMB)-high solid tumours.[112] Novel agents include Herceptin (trastuzumab;[101,113] anti-HER2 mAb[110]) and Cyramza (ramucirumab; anti-VEGFR2 mAb; blocks the binding of VEGF-A, -C and -D).[114] Herceptin in combination with cisplatin and capecitabine/5-FU is used for the first-line treatment of metastatic HER2$^+$ OGJ and GC.[110] The Phase 3 trial ToGA ($n = 594$), which led to the FDA approval of this regimen, demonstrated that the median OS was 13.8 months in the chemotherapy-trastuzumab arm versus 11.1 months in the chemotherapy-only arm, respectively.[113] Data from this trial showed HER2 positivity in 32.2% (65/202) of OGJ cases versus 21.4% (451/2,112) of GC cases, highlighting only a small proportion of OGJ and GC patients express the target for trastuzumab and perhaps there may be other therapeutics more suitable for patients whose tumours were negative for HER2 expression.[115] The Phase 3 RAINBOW trial demonstrated that combining ramucirumab with paclitaxel significantly prolonged survival compared with paclitaxel alone in advanced OGJ and GC tumours (9.63 versus 7.26 months, respectively).[116]

THE TME AND IMMUNOTHERAPY

The TME in OC, as discussed above, is rich in immune cells and is therefore considered an attractive target for immunotherapy. The anti-PD-1 monoclonal antibody (mAb) pembrolizumab is FDA approved for the treatment of advanced or recurrent OGJ[111] in patients who fail to respond to first- and second-line chemotherapy and have a PD-L1$^+$ combined positive score (CPS; the total number of PD-L1 staining cells (tumour cells, lymphocytes, macrophages) divided by the total number of viable tumour cells, multiplied by 100) of ≥1.[111] The Phase 2 study KEYNOTE-059 demonstrated that pembrolizumab induced an objective response rate (ORR) of 11.6% (30/259 patients) and conferred a greater median duration of response (16.3 versus 6.9 months) in patients with a PD-L1$^+$ CPS ≥ 1 (57.1% or 148/259 had PD-L1$^+$ tumours).[111] Pembrolizumab has also been approved by the FDA as second-line therapy for patients with locally advanced or metastatic OSCC with a CPS of ≥10[117] after the KEYNOTE-181 Phase 3 trial showed an improvement in OS in these patients.[117] The Phase 3 ATTRACTION-3 study demonstrated the OS benefit (10.9 versus 8.4 months, $P = 0.019$) of nivolumab, another mAb that targets PD-1, regardless of PD-L1 status, in OSCC, leading to the FDA approval (in June 2020) of this agent for the second-line treatment of advanced, metastatic or recurrent OSCC.[118]

Does the TME explain the limited benefit of Immunotherapy?

It seems somewhat paradoxical that an inflammation-associated cancer such as OAC, which also has a high TMB relative to other malignancies[119] (similar to that of non-small lung cancer (NSCLC) and malignant melanoma), has so far demonstrated largely disappointing response rates to immune-targeted approaches in comparison with these aforementioned malignancies.[120] A key issue is whether the TME of OCs negatively impacts on responses to PD-1/PD-L1 blockade. Zeng et al., demonstrated that the TME in GC, could stratify responders and non-responders to PD-1 blockade.[121] Given the underlying genetic and molecular similarities of gastric adenocarcinomas and adenocarcinomas of the oesophagus,[19] this may be a promising approach to identify OC patients likely to benefit from immune checkpoint blockade. Using the TMB and an immune scoring system to predict the therapeutic outcome is an attractive approach, as strongly evident from the results of studies of NSCLC, in which the pre-treatment TMB positively correlated with the pathological response.[122]

A genomic analysis of the TME of 1,524 patients with GC identified three distinct phenotypes associated with the response to immune checkpoint blockade.[121] A so-called high TME score subtype was characterised by immune activation and enriched for genes involved in the response to T-cell activation and to viruses and IFN-γ, and predicted a positive response to PD-1 blockade.[121] There was also a significant positive correlation between the high TME score and the TMB.[121] By contrast, a low TME score subtype was characterised as immunosuppressive and was enriched for genes involved in ECM remodelling, epithelial–mesenchymal transition, cell adhesion and angiogenesis.[121] Given the genetic and molecular similarities between OAC, OGJ and gastric adenocarcinomas, this study lends important insight into the composition of the TME and its correlation with response to ICB. Similar studies in OAC and OSCC are certainly warranted.

In a study of genomic, transcriptomic and immune data from 323 patients with CIN subtypes of OC and GC, CD8$^+$ T cells predominated at the invasive margin while TAMs showed tumour-infiltrating capacity.[95] The presence of CD8$^+$ T cells in the invasive margin may suggest the ability of the host to mount an anti-tumour immune response, however, the exclusion of CD8$^+$ T cells from the tumour core indicates an immune-excluded tumour in OC patients with CIN subtypes. A clear role for TGF-β in mediating T exclusion from tumours has been established in CRC and urothelial cancer where, blockade of TGF-β induced T cell infiltration in immune-excluded tumour types and synergised with PD-L1 blockade in murine studies.[123,124] However, it remains to be determined whether TFG-β plays a role in generating the T cell-excluded tumours observed in CIN OCs and certainly warrants further studies. Given the pleiotropic roles of TAMs in mediating immunosuppression and the high level of tumour-infiltrating TAMs observed in 'cold' CIN immune-excluded OCs, therapeutic targeting of TAMs may represent a novel precision medicine approach for this particular subtype in OCs. In GC, Epstein-Barr virus positive (EBV$^+$) tumours and tumours showing microsatellite instability (MSI) have intense T-cell infiltration and high PD-L1 expression[125] and respond well to immune checkpoint inhibitors.[126] However, CIN and the genome stable subtype (largely GCs with diffuse histology) showed fewer T cells[125,127] and poor responses to immune checkpoint inhibitors.[126] Further studies are warranted to determine if EBV$^+$/MSI and CIN/genome-stable TME subtypes correlate with the response to immune checkpoint inhibitors in OC. Notably, marked heterogeneity existed in T-cell infiltration in GC, with samples from Western patients often being more 'hot' (that is, showing a considerable extent of T-cell infiltration) compared with those from Asian patients.[95] Immunologically 'cold' CIN tumours were enriched for MYC activity and harboured amplified *CCNE1*.[95] Another multi-omic OAC dataset ($n = 511$) found a three-way association between hypermutation, Wnt pathway activation (associated with T-cell exclusion) and loss

of genes involved in immune signalling, such as β2-microglobulin, a component of MHC-I.[128] Accordingly, these studies suggest an acquired mechanism through which OAC prevents the immune surveillance in the TME that is induced by a high TMB, potentially offering one means by which the response to checkpoint blockade can be subverted. These studies highlight the close interlinked relationship between intrinsic genetic and genomic tumour features and their potential role in influencing the composition of the TME. For example, the above studies showed that CIN OC tumours corresponded with 'cold' and 'immune-excluded' tumour types with tumour-infiltrating TAMs. Whereas 'hot' tumours consisting of TILs were most common in OAC patients from Western regions, where obesity is a major cause for the development of OAC and arises as a consequence of chronic insult to the oesophagus by GORD which likely contributes to a high TMB often accompanied with a 'hot' tumour type.

FUTURE PROSPECTS FOR THE TME AS A THERAPEUTIC TARGET

When optimising treatment strategies, the evolution and ecology of tumours should ideally be considered, moving away from the idea that tumours stem from one aberrant cell and instead considering a model of cancer as a dynamic multi-cellular network, influenced by competing factors, such as pro- and anti-tumour cells, growth factors and their inhibitors, and other resources that are essential for growth.[129] The TME, or at least its immune component, presents one such complex prognostic and therapeutic target, given the heterogeneity of tumour infiltration by immune cells and immune cell plasticity, as evidenced by the modest outcomes to date with immunotherapy in the OC setting.[40] Figure 3 highlights the therapeutics targeting TME components in OCs that are currently undergoing preclinical and clinical development, and the current clinical trials targeting elements of the TME in OCs are summarised in Table 2.

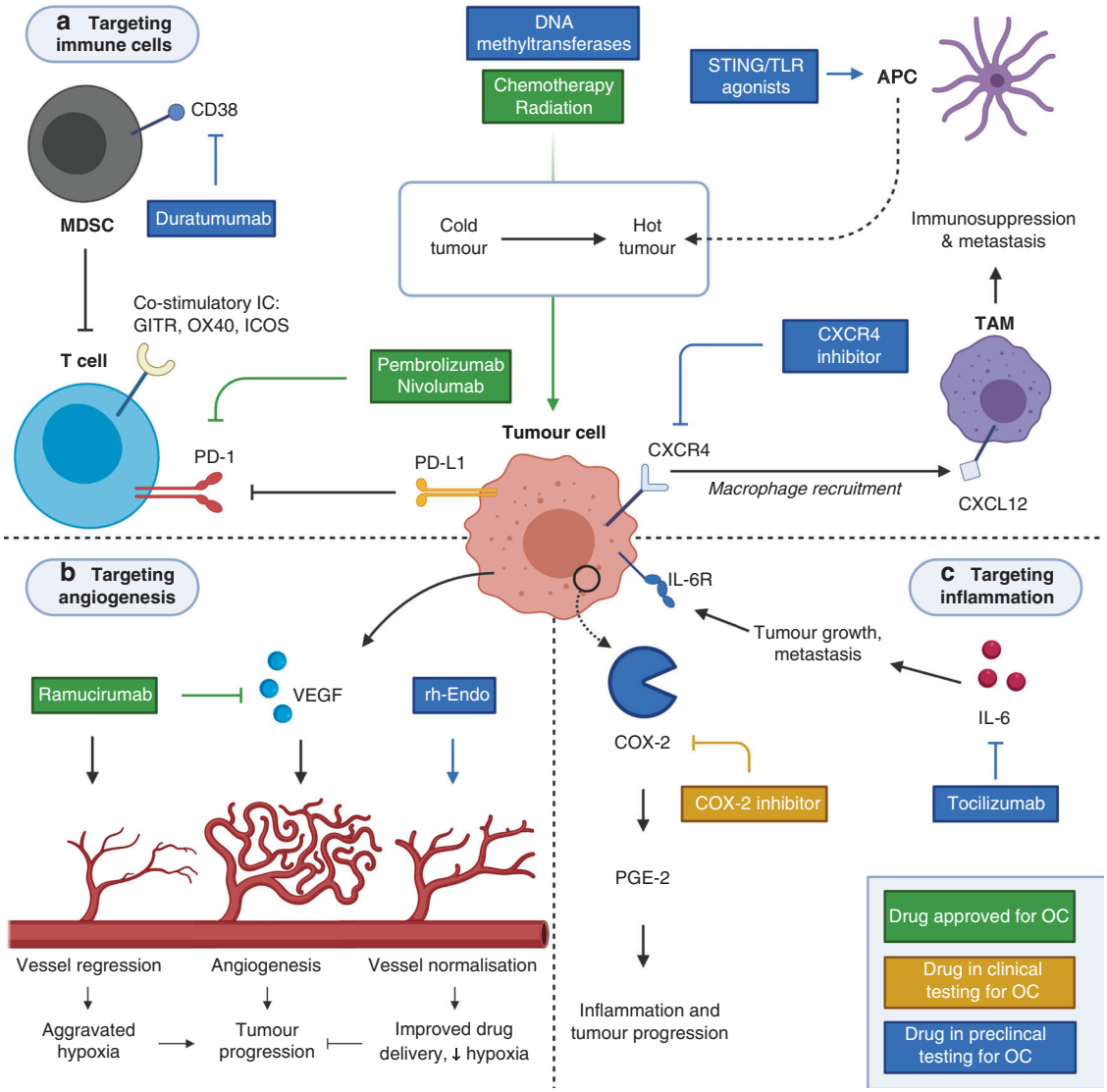


Fig. 3 Drug targets in the TME. a Targeting suppressive immune cells and inhibitory immune checkpoints can re-invigorate exhausted anti-tumour immunity. Immunologically 'cold' tumours can be converted to 'hot' tumours using conventional therapies (chemo/radiotherapy) as well as novel therapies including epigenetic modulators (DNA methyltransferases) and STING/TLR agonists. **b** Angiogenesis is driven by hypoxia-induced VEGF release by tumour cells and can be targeted using ramucirumab and recombinant human endostatin (rh-Endo) with varying degrees of success. **c** Targeting inflammatory mediators can impact on cancer progression. APC antigen-presenting cell, COX-2 cyclo-oxygenase-2, CXCL chemokine (C-X-C motif) ligand, CXCR chemokine (C-X-C motif) receptor, IL interleukin, MDSC myeloid-derived suppressor cell, OC oesophageal cancer, PD-1 Programmed cell death protein 1, PD-L1 Programmed death-ligand 1, PGE-2 Prostaglandin E_2, rh-Endo recombinant human endostatin, STING stimulator of interferon genes, TAM tumour-associated macrophage, TLR Toll-like receptor, VEGF vascular endothelial growth factor.

Table 2. Summary of clinical trials testing agents targeting the TME in OCs.

TME target	Trial	Cancer-type	Treatment regimen	Clinical outcome
Angiogenesis	RAINBOW Phase 3 *(led to FDA approved)*	Advanced OGJ and GC	Paclitaxel versus Cyramza (ramucirumab, VEGFR2 mAb) + paclitaxel.	OS: 7.26 months (paclitaxel) versus 9.63 months (paclitaxel + ramucirumab).[116]
	NCT01368419 Phase 2	OSCC and OAC	Endostatin (interferes with bFGF and FGF-2 pro-angiogenic functions) + IR (2 Gy/30 fractions).	Recruiting, data pending.
	NCT01747551 Phase 2	OAC, OGJ and GC	FOLFOX (5-FU + oxaliplatin + leucovorin) + bevaxizumab (anti-VEGF-A).	Data pending.
	Phase 2	OSCC	Endostatin + CRT.	PFS: 9.5 months (CRT, $n = 31$) versus 16.5 months (CRT + endostatin, $n = 32$). 1-year and 2-year OS rates: 67.7% (CRT) versus 78.1% (CRT + endostatin) and 45.1% (CRT) versus 56.2% (CRT + endostatin), respectively.[146]
Immune checkpoints	NCT02625610 (JAVELIN Gastric 100) Phase 3	Advanced OGJC, GC	Avelumab versus oxaliplatin and capecitabine/5-FU.	mOS: 10.4 months (avelumab) versus 10.9 months (oxaliplatin and capecitabine/5-FU) (HR: 0.91 [0.74–1.11]; $P = 0.18$), ORR: 13.3% versus 14.4%.
	NCT02625623 (JAVELIN Gastric 300) Phase 3	Advanced OGJC, GC	Avelumab versus irinotecan + paclitaxel.	mOS: 4.6 months (avelumab) versus 5 months (irinotecan + paclitaxel) (HR: 1.1 [0.9–1.4]; $P = 0.81$), ORR: 2.2% versus 4.3%.
	NCT02267343 (ATTRACTION-2) Phase 3	Advanced OGJC, GC	Nivolumab versus placebo.	mOS: 5.3 months (nivolumab) versus 4.14 months (placebo) (HR = 0.63 [0.51–0.78], $P < 0.0001$), 12-month OS: 26.2% vs 10.9%.
	NCT02569242 (ATTRACTION-3) Phase 3	Advanced OSCC	Nivolumab versus paclitaxel/docetaxel.	mOS: 10.9 months (nivolumab) versus 8.4 months. (paclitaxel/docetaxel) (HR: 0.77 [0.62–0.96], $P = 0.019$).
	NCT02370498 (KEYNOTE-061) Phase 3	Advanced OGJC, GC	Pembrolizumab versus paclitaxel.	mOS: 9.1 months (pembrolizumab) versus 8.3 months (paclitaxel) (HR: 0.82 [0.66–1.03], $P = 0{\cdot}0421$).
	NCT02564263 (KEYNOTE-181) Phase 3	Advanced OAC, OSCC	Pembrolizumab versus paclitaxel/docetaxel.	No difference in OS in ITT group (HR 0.89; [0.75–1.05], $P = 0.056$) In CPS >10 cohort mOS: 9.3 months (pembrolizumab) versus 6.7 months (paclitaxel/docetaxel) (HR: 0.69 [0.52–0.93], $P = 0.0074$).
	NCT02494583 (KEYNOTE-062) Phase 3	Advanced OGJC, GC	Pembrolizumab versus pembrolizumab, cisplatin and 5-FU/capecitabine or cisplatin and 5-FU/capecitabine.	Pembrolizumab noninferior to chemotherapy (mOS: 10.6 (pembrolizumab) versus 11.1 months (cisplatin and 5-FU/capecitabine), HR: 0.91 [0.69–1.18]). Pembrolizumab + chemotherapy not superior to chemotherapy (mOS: 12.5 months (pembrolizumab + cisplatin and 5-FU/capecitabine) versus 11.1 months (cisplatin and 5-FU/capecitabine); HR: 0.85 [0.70–1.03], $P = 0.05$).
Immune components (other)	NCT01929486 Phase 1a/1b	Solid tumours including OCs	Mogamulizumab (anti-CCR4 mab, depletes Tregs).	Data pending.
	ORIENT-15 Phase 3	OSCC	Siltuximab (anti-IL-6 mab) or placebo in combination with cisplatin, 5-FU and paclitaxel.	Recruiting, data pending.

5-FU 5-fluorouracil, *bFGF* basic fibroblast growth factor, *CCR4* C-C chemokine receptor 4, *CPS* combined positive PD-L1 score, *CRT* chemoradiotherapy, *FGF2* fibroblast growth factor-2, *GC* gastric cancer, HR, hazard ratio, *ITT* intention to treat, *IL-6* interleukin-6, *IR* irradiation, *mOS* median overall survival, *mab* monoclonal antibody, *ORR* objective response rate, *OAC* oesophageal adenocarcinoma, *OC* oesophageal cancer, *OSCC* oesophageal squamous cell carcinoma, *OGJ* oesophagogastric junctional adenocarcinoma, *OS* overall survival, *PFS* progression free survival, T_{reg} T regulatory cell, *VEGF-A* vascular endothelial growth factor-A.

Targeting immune cells to boost anti-tumour responses

As immune checkpoint inhibitors appear to only benefit patients with 'hot' tumours,[130] one approach to extend the range of patients who might benefit from these agents involves attempting to convert immunologically 'cold' tumours into 'hot' tumours by strategic combination therapy. This might involve, for instance, using priming doses of radiation or chemotherapy to enhance responses to immunotherapy.[40,131] Radiation and chemotherapy can induce DNA damage in cancer cells, consequently increasing their immunogenicity through the generation of neoantigen-yielding nonsynonymous mutations.[132] Therefore, immunostimulatory radiation and chemotherapy regimens are a promising approach for converting 'cold' CIN OAC tumours to 'hot' tumours, thus increasing the likelihood of success of concomitant immune checkpoint blockade.[133]

The modulation of oncogenic signalling and genetic and epigenetic pathways regulating the expression of T cell-recruiting chemokines can give rise to T-cell-excluded tumours,[134,135] in which T cells are seen to collect around the periphery of the tumour but not within it. However, this observation does suggest that the tumour is immunogenic and that the host is able to mount a T-cell-mediated immune response.[134,135] Therefore, strategies to induce T cell infiltration into T cell-excluded tumours to generate a 'hot' TME—through epigenetic modulation of T_H1-derived chemokines or blockade of Wnt–β-catenin signalling—are being investigated.[133] Epigenetic silencing is often responsible for the loss of expression of β2-microglobulin and T-cell-recruiting chemokines[136] and, accordingly, epigenetic modifiers are promising multipurpose agents that can convert both 'cold' and 'T cell-excluded' tumours to 'hot' tumours. Among epigenetic modifiers are DNA methyltransferases, which can induce the re-expression of β2-microglobulin, T-cell-recruiting chemokines and tumour-associated antigens to facilitate the activation of tumour-specific T cells, T-cell trafficking to the tumour and increased tumour immunogenicity.[136]

In the case of altered-immunosuppressed tumours, which appear to be the predominant OC phenotype, immunosuppressive TME factors, including macrophages, MDSCs and Treg cells, could be pharmacologically targeted.[11] For example, the CXCL12/CXCR4 axis facilitates macrophage recruitment to the TME,[137] and CXCR4 expression has been detected in OAC and OSCC tumour tissues as well as on the surface of OSCC cells in vitro.[137] Notably, the expression of CXCR4 was significantly higher in corresponding metastatic tumour tissue in lymph nodes compared with the primary tumour and CXCR4-expressing OSCC cells had a stronger migration capacity than CXCR4-negative OSCC cells in vitro.[138] The CXCL12/CXCR4 axis might therefore represent a key pathway in OSCC that promotes lymph node metastasis and recruits immunosuppressive macrophages into the TME.[138] Although therapeutic blockade of CXCR4 in murine models of breast[139] and prostate[140] cancer significantly reduced tumour burden and metastasis, blockade of this axis has yet to be tested in preclinical and clinical studies of OC.

$CD38^+$ MDSCs—the expression of CD38 being induced by a number of tumour-derived factors—showed an immunosuppressive and tumour-promoting phenotype in the p120-catenin deficient mouse model of OSCC, probably due to the increased expression of iNOS, which inhibits MHC-II expression on T cells and induces T cell apoptosis.[141] Expanded $CD38^+$ MDSCs were present in the peripheral circulation of OSCC patients with advanced stage disease.[141] Therapeutic targeting of CD38 in vivo using a crosslinking antibody, duratumumab (Darzalex)—an approach that is FDA approved for the treatment of multiple myeloma[142]—reduced OSCC tumour growth in murine studies.[141]

A further approach is to indirectly boost anti-tumour responses—for example, by activation of stimulator of interferon genes (STING) pathway, Toll-like receptors or antigen-presenting cells.[136] Co-stimulatory immune checkpoints (GITR, OX40, and ICOS) and co-inhibitory immune checkpoints (TIGIT, LAG3 and TIM3) also present plausible targets for cancer immunotherapy, by stimulating and reinvigorating anti-tumour immunity, respectively.[143]

Targeting angiogenesis to normalise the tumour vasculature

An anti-angiogenic approach to normalise the tumour vasculature, aiming to increase pericyte coverage, enhance tumour vessel perfusion, decrease vascular permeability and reduce hypoxia,[144] also has therapeutic potential, and could enhance the efficacy of other treatment modalities, including combinations of radiation therapy with chemotherapy, by facilitating efficient drug delivery, infiltration of anti-tumour immune cells and delivery of oxygen.[144] Indeed, an anti-angiogenic compound, recombinant human endostatin (rh-Endo), normalised the vasculature of OSCC tumours and decreased hypoxia, as demonstrated by a decrease in VEGF and HIF1-α expression,[145] and pre-treatment with rh-Endo prior to irradiation significantly reduced tumour growth in an OSCC xenograft compared with rh-Endo administered on its own.[144] Furthermore, a Phase 2 trial demonstrated that rh-Endo in combination with chemoradiotherapy significantly increased OS and PFS (median PFS: 16.5 months versus 9.3 months, respectively) in OSCC compared with chemoradiotherapy alone.[146]

The lack of clinical benefit to date of anti-angiogenic drugs in OCs could potentially be explained by excessive pruning of the tumour vasculature by anti-angiogenic agents in a dose- and time-dependent manner, resulting in increased hypoxia, tumour cell migration, inflammation, enhancement of a stem-like phenotype and immunosuppression in the TME.[65] An alternative approach to enhance treatment efficacy involves scheduling the optimal targeting of anti-angiogenic agents alongside conventional regimens using knowledge of the therapeutic window during which the vessels initially become normalised, although an insight into how long they remain in that state is required.[65] Murine models growing a human brain tumour that were treated with a VEGFR2 tyrosine kinase inhibitor demonstrated that the 'normalisation window' was short-lived (approximately 6 days) and was defined by an increase in tumour oxygenation, which increased the tumoural perfusion of oxygen to enhance the efficacy of radiation therapy.[65]

Targeting soluble pro-inflammatory molecules

The inhibition of STAT3 in OSCC and OAC cells induces apoptosis and decreases survival, migration and proliferation in vitro.[147] The inhibition of IL-6 in OSCC tumour-bearing mice also decreased tumour growth, angiogenesis, epithelial–mesenchymal transition, enhanced radiosensitivity, DNA damage and attenuated STAT3 activation.[91] Currently, a Phase 2 trial in patients with advanced or metastatic pancreatic cancer to assess whether or not combining an IL-6 inhibitor (tocilizumab) with chemotherapy enhances the efficacy of chemotherapy (NCT02767557) is ongoing. The results from this trial will help inform trial design investigating the efficacy of tocilizumab in combination with chemotherapy and/or chemoradiotherapy in OCs.

A meta-analysis of 9 studies with over 5,000 participants shows that COX inhibition leads to a reduced risk of OAC development in BO patients[148] and it has been suggested that administration of COX-2 inhibitors in a preoperative setting could reduce the risk of metastasis in cancer patients via inhibition of the multimodal pro-tumorigenic effects of COX-2, which include promotion of tumour cell proliferation, apoptotic resistance, cancer-stem-cell-like activity, angiogenesis and immunosuppression.[52] Overexpression of COX-2 in urinary cancer cells in vitro resulted in an increase in invasiveness and angiogenic ability by activation of VEGF, urokinase-type plasminogen activator, and MMP-2, whereby, treatment with a selective COX-2 inhibitor reversed this pro-metastatic phenotype identifying a specific role for COX-2 in promoting metastasis.[149] Further supporting a therapeutic role for COX-2 inhibitors in OCs, blockade of COX-2 dampened

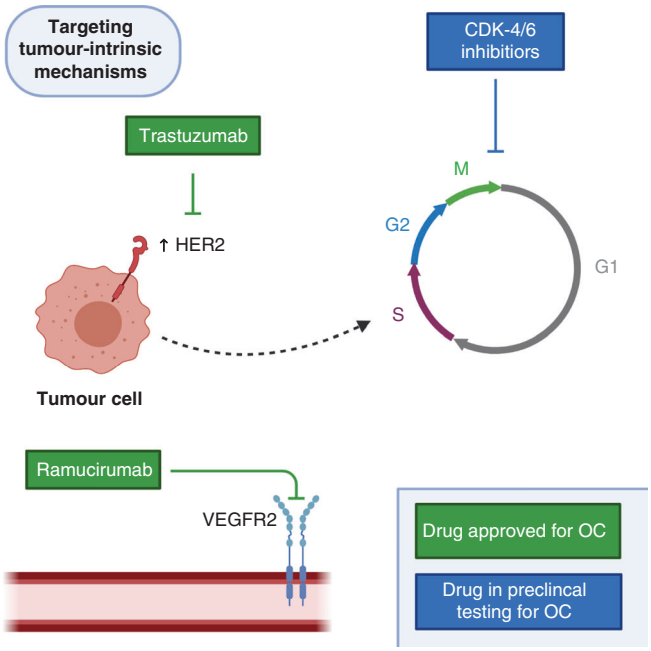


Fig. 4 Targeting tumour-intrinsic mechanisms. Trastuzumab and ramucirumab target HER2 and VEGFR2, respectively. Cyclin-dependent kinase (CDK)-4/6 inhibitors are a novel class of targeted therapy which block cell-cycle progression in the middle of the G1 phase and prevent cancer cell progression.

inflammation and induced apoptosis of BO and OAC cells.[150] Additional studies using in vitro and in vivo models of OSCC demonstrated that COX-2 inhibition decreased the production of PGE-2 and inhibited cell growth and tumour progression.[55]

Targeting tumour-intrinsic mechanisms

Mounting evidence suggests that tumour-intrinsic features, including the molecular and genetic composition of a tumour itself, substantially influence whether a tumour will be immune-hot, immune-cold, altered-immunosuppressed or altered-excluded.[133] Figure 4 highlights the therapeutics targeting tumour-intrinsic features in OCs that are currently undergoing preclinical and clinical development. In other cancer types, activating mutations in oncogenic pathways including WNT–β-catenin, MAPK, JAK-STAT3 and NF-κB are responsible for generating a 'cold' or 'altered-excluded' or 'altered-immunosuppressed' TME via the release of mediators that block T cell recruitment or via repression of T cell-recruiting chemokines.[133] Both OSCC and OAC tumours show overactivation of STAT3 and NF-κB signalling, perhaps suggesting a key role for these pathways in the generation of immune-altered (immunosuppressed or excluded) TME.[25,26,28,35] Additionally, MSI tumours, which are typically accompanied by a 'hot' TME, are common in OAC patients of Western origin, which is probably attributed to the accumulation of mutations resulting from obesity-associated inflammation.[95,151]

Ultimately, therapeutic approaches designed on the basis of both the genetic composition of the tumour and the composition of the TME, integrating both TCGA and Oesophageal Cancer Clinical and Molecular Stratification (OCCAMS) studies, offer the potential to deliver tailored precision medicine to OSCC and OAC patients.[19,95,128]

OSCC tumours are enriched in cyclins D and E, HER2 and VEGFA amplifications.[19] Cyclin-dependent kinase (CDK)-4/6 inhibitors, which block the activity of cyclin D1, are in clinical trials for the treatment of other tumours such as breast cancer and non-breast malignancies, but have yet to be tested in OCs.[152] However, the CDK inhibitor PD-0332991 reduced OSCC cell growth, invasion and migration, and enhanced cisplatin and 5-FU toxicity in vitro.[153] In addition, PD-0332991 potently inhibited OSCC cell growth and lung metastasis in murine studies.[153] Future trials in OC patients will be important to determine the clinical efficacy of CDK inhibitors.

Therapeutics targeting HER2 and VEGFA have been FDA approved for the treatment, in combination with chemotherapy, of OGJ tumours.[110,116] However, the efficacy of these targeted therapies is limited to an increase in OS of merely several months,[110,116] which implies that the dynamic TME has adapted, and more tailored therapeutic approaches will be required to boost the efficacy of these therapies.

CONCLUSION

Although OCs are not as well characterised as many other tumour types, and considerably less is known about the activity and impact of immune cells within the TME on OCs compared with CRC,[154] evidence continues to emerge that the TME is responsible for shaping anti-tumour immunity[11] and is relevant to the therapeutic response in this tumour type.[15,155] A reasonable conclusion from the data to date is that improved combination strategies are needed to target the TME as well as the tumour cells directly.[11] The addition to conventional regimens of therapeutics that target features of the TME has yielded some promising results to date, although the increases in median survival are marginal and often short-lived, which might highlight the dynamic and adaptive properties of the TME.[110,116] It appears, however, that the TME requires a window in which to evolve and develop resistance through other mechanisms, and this window might be therapeutically exploitable. Hence, the sequential use of several TME-targeted therapeutics in a rational and timely manner in which the TME never receives the window of opportunity to evolve is an attractive approach.

Although a multitude of studies have demonstrated that a wide variety of immunosuppressive cells and physical TME features are responsible for mediating treatment resistance, the contribution of each is unclear.[95,125–127] The most likely scenario is the existence of several redundant pathways that mediate therapeutic resistance. Consequently, more holistic studies providing a comprehensive characterisation of integrated key cellular components and soluble mediators in the TME in a cohort of OC patients at different stages of the disease are required. Multi-omic and systems biology approaches will be useful in this regard, as well as ecological models of cancer.[129] Considering the tumour as an evolving ecosystem subject to complex selective pressures has allowed application of ecological models to multi-omic data to predict tumour behaviour. A recent consensus conference has proposed a framework of tumour classification based on evolutionary and ecological indices.[156] Other ecological models such as species distribution modelling,[157] evolutionary game theory and refinement of cancer growth models to consider all the effects rather than simple logistic growth, as reviewed by Korolev, Xavier and Gore,[158,159] have been proposed to quantitatively describe the complex relationship between tumour cells and their microenvironment. Such models aim to classify a wealth of dynamic TME information into actionable indicators to facilitate tailored cancer treatment. These strategies will be key in identifying rational therapeutic targets to combine with current standards of care to overcome resistance and prolong survival in OC patients at specific stages of the disease.

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AUTHOR CONTRIBUTIONS

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