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Gastric cancer immunosuppressive microenvironment heterogeneity: implications for therapy development

Tadahito Yasuda[1,*], **Y. Alan Wang**[1,*]

[1]Brown Center for Immunotherapy, Department of Medicine, Melvin and Bren Simon Comprehensive Cancer Center, Indiana University School of Medicine, Indianapolis, IN 46202, USA

Abstract

Although immunotherapy has revolutionized solid tumor treatment, durable responses in gastric cancer (GC) remain limited. The heterogeneous tumor microenvironment (TME) facilitates immune evasion, contributing to resistance to conventional and immune therapies. Recent studies have highlighted how specific TME components in GC acquire immune escape capabilities through cancer-specific factors. Understanding the underlying molecular mechanisms and targeting the immunosuppressive TME will enhance immunotherapy efficacy and patient outcomes. This review summarizes recent advances in GC TME research and explores the role of the immune-suppressive system as a context-specific determinant. We also provide insights into potential treatments beyond checkpoint inhibition.

GC is highly lethal and is ranked as the fifth most frequently diagnosed cancer globally and the fourth leading cause of cancer death. The prognosis for patients with GC is poor and the 5 year survival rate is <10% [1,2]. Surgery followed by adjuvant chemotherapy or chemoradio-therapy is the standard-of-care treatment for half of GC patients who are eligible for surgery [3,4]. However, even after resection, ~60% of patients experience a relapse locally or with distant metastases [5]. Moreover, many patients who are diagnosed with disseminated tumors are unable to receive surgery. Peritoneal metastasis is common for GC, and occurs in nearly one third of patients at the time of diagnosis [6,7].

The development of GC is a complex process influenced by a range of internal and external factors including genetics, infection, dietary habits, and environmental pollution. In particular, there are disease-specific factors that contribute to GC progression, such as driver genes in the malignant type of GC, chronic inflammation caused by *Helicobacter pylori* infection, and the influence of malignant ascites – the accumulation of fluid within the abdominal cavity typically caused by metastatic tumor cells [8,9]. Immunosuppression is a common hallmark of cancer. Although immune checkpoint blockade (ICB) immunotherapy has revolutionized cancer, its response rate in advanced GC remains limited [10,11].

*Correspondence: tayasuda@iu.edu (T. Yasuda) and yaw@iu.edu (Y.A. Wang).

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This lack of response is likely due to our limited knowledge about the tumor immune microenvironment (TIME) that is influenced by GC-specific factors.

TIME comprises various components, such as immune cells and stromal cells, which secrete cytokines and other factors through interaction with each other that impact on cancer progression and metastasis. Complex interactions between the immune and stromal components influence the TIME, leading to the development of GC [12,13]. Recent advances in single-cell RNA sequencing (scRNA-seq) technology are advancing our understanding of tumor heterogeneity and the TIME in GC. Moreover, emerging single-cell techniques are providing opportunities to study the spatial organization of the TIME, which may help to identify novel therapeutic approaches and improve patient outcomes [14–16].

Genomic drivers of the TME related to immunosuppression

GC is a heterogeneous disease with marked phenotypic diversity, including a variety of molecular subtypes. Traditionally, GC classification has been based on histological features, dividing the histological phenotype into gland-forming adenocarcinoma (intestinal-type) and highly infiltrating cells (diffuse-type) according to Lauren's classification. Intestinal-type GC tends to occur in older patients and in males, whereas diffuse-type GC occurs more frequently in younger patients and in females [17]. The diffuse type is known for its aggressive behavior and resistance to conventional therapy, and hence poor prognosis [18]. However, these classification systems have little clinical utility in guiding patient therapy. The importance of classifying GC based on mutations began to be recognized with the introduction of The Cancer Genome Atlas (TCGA) classification which categorized GC into four subtypes: Epstein–Barr virus-positive (EBV), microsatellite instability (MSI), genomically stable (GS), and chromosomal instability (CIN) [19,20]. Among these subtypes, MSI-high GC and EBV-related GC often have an increased level of immune infiltration and are considered to be more amenable to immune modulation [19,21]. However, these two subtypes are rarely represented in patients with advanced GC. For advanced GC it is necessary to develop a deeper understanding of the molecular basis and contextual factors underlying this disease (Figure 1).

Whole-genome and whole-exome sequencing (WES) studies have revealed subtype-specific unique mutational signatures (*RHOA*, *MUC6*, *CTNNA2*, *GLI3*, *RNF43*, and others) in addition to previously known mutations (*TP53*, *ARID1A*, and *CDH1*) [22]. *RHOA* mutations occur in 15–25% of diffuse-type tumors but not in intestinal-type tumors [19,23,24]. *RHOA* Y42C, the most common *RHOA* mutation in diffuse-type GC, represents a gain-of-function oncogenic event. *RHOA* Y42C expression with loss of the canonical tumor-suppressor *CDH1* induces tumor proliferation and more aggressive metastatic potential in a genetically engineered mouse model [25,26]. The *RHOA* Y42C mutation mediates PI3K–AKT–mTOR pathway activation and is associated with abundant regulatory T cells (Tregs) and low $CD8^+$ T cells [27]. Recently, large-scale genomic analysis of 1335 GC cases identified subtype-specific drivers, including *PIGR* and *SOX9*, which were significantly enriched in the diffuse subtype of the disease [28] and in tumor cells derived from malignant ascites [29]. PIGR plays a crucial role in the transcytosis of soluble immunoglobulin A within epithelial cells and is closely linked to the maintenance

of polar integrity and mucosal immunity [30]. Although PIGR has been reported to be associated with tumor metastasis through epithelial–mesenchymal transition (EMT) [31], its clinical significance and oncogenic role in GC remain unclear. *SOX9* is one of the most highly upregulated *SOX* genes in GC, and elevated expression is observed in both primary and metastatic GC tissue. SOX9 is crucial for the initiation of tumorigenesis from stem or progenitor cells. In a prospective 10 year study involving 1152 patient samples with intestinal metaplasia, a well-recognized premalignant condition often associated with GC development, *SOX9* was identified as one of the 26 key driver genes linked to intestinal metaplasia. *SOX9* mutation promotes the expansion of stem cells [32], and SOX9-positive gastric stem cells modulate biased symmetric cell division, leading to malignant transformation of gastric stem cells. Moreover, elevated expression of SOX9 is associated with GC recurrence and poor prognosis [33]. Previous studies have also implicated SOX9 in WNT and KRAS signaling which is associated with immune silencing in other tumor types [34–36]. Furthermore, downregulation of SOX9 in tumor cells enhanced $CD8^+$ T cell responses while decreasing the levels of CCL2 and IL10 produced by M2 macrophages in animal models [37].

Taken together, these findings emphasize the complex interplay between genomic drivers and the TIME in GC, A deeper understanding of these molecular alterations and their impact on immune regulation holds promise for the development of novel therapeutic strategies to overcome immunosuppression and improve outcomes for patients with advanced GC.

Biological heterogeneity of GC TME landscape

The TME is a complex system that can both suppress immune responses and enhance tumor progression, and is largely influenced by the stromal components within it. Tumor cells can mobilize these stromal components to inhibit multiple steps of T cell activation, resulting in immunosuppression. This process is often referred to as ‘stromal components of immunosuppression’ and is a major obstacle to effective antitumor immune responses (Figure 2). scRNA-seq technologies and spatial transcriptomics (ST) have provided unprecedented insights into the TME of GC, revealing specific cellular and transcriptional features that distinguish the GC TME from normal gastric tissue in both human and mouse models [38–45] (Box 1).

T cells

The immunosuppressive microenvironment of GC is characterized by T cell exhaustion [46,47]. Exhausted $CD8^+$ tumor-infiltrating lymphocytes (TILs) cells are increased and IRF8, a transcription factor that regulates CD8 T cell differentiation, is downregulated in primary surgical tissue samples from patients with advanced GC [48]. Analysis of scRNA-seq data of primary and metastatic tumors revealed specific transcriptional signatures in exhausted $CD8^+$ T cells that were tumor- and metastatic site-specific [49]. The score of the 20 enriched genes signature from lymph node-derived exhausted $CD8^+$ T cells was significantly higher in primary GC with lymph node metastasis compared to non-lymph node metastasis. As such, this score could serve as a predictor of lymph node metastasis, as validated in a GC cohort [50].

$CD8^+$ T cell activation can also impact on patient responses to neoadjuvant chemotherapy such as 5-fluorouracil plus oxaliplatin-based neoadjuvant chemotherapy. Responses can be heterogeneous among patients, with some showing little or no response [51]. The TME of non-responders comprises of increased LAG3-expressing exhausted T cells, decreased levels of tumor-associated macrophages (TAMs), reduced M1 macrophage repolarization, and increased B cell infiltration [52]. Moreover, some non-responders with MSI-high (MSI-H) GC had a heterogeneous TME that included abundant exhausted T cells [53]. Trials such as ATTRACTION2 [10] and CheckMate032 [54] that compared first-line chemotherapy with second-line immunotherapy in patients with unresectable advanced or recurrent GC have demonstrated the effectiveness of immunotherapy following neoadjuvant chemotherapy.

Integrated analysis through WES and whole-transcriptome sequencing (RNA-seq) of peritoneal carcinomatosis specimens in patients with GC revealed the existence of two major molecular subtypes, namely ‘mesenchymal-like’ and ‘epithelial-like’, according to differences in responses to chemotherapy. Patients with the less responsive ‘mesenchymal-like’ subtype exhibited higher expression levels of several immune checkpoint proteins, including T cell immunoglobulin and mucin domain-containing protein 3 (TIM-3), its ligand galectin-9, V-domain Ig suppressor of T cell activation (VISTA), and transforming growth factor-β (TGF-β) [55]. Malignant ascites often contain a variety of growth factors, cytokines, chemokines, and other soluble factors which can modulate the interaction between the tumor cells and the peritoneal microenvironment. Understanding the complex interplay between malignant ascites and peritoneal TME is a crucial area of investigation in GC biology [8,56]. Additional scRNA-seq studies on malignant cells from ascites identified two subtypes, gastric-dominant (mainly gastric cell lineages) and GI-mixed (with mixed gastric and colorectal-like cells). Moreover, immune deconvolution analysis combined with other public databases suggests that the better clinical outcomes found with GI-mixed tumors could be partially associated with an effective immune response against the tumor, including higher levels of B cells and M1 macrophage polarization, lower levels of fibroblasts and M2-like macrophages, and elevated cytolytic activity [57].

Myeloid lineage cell types

TAMs originate from either infiltrating monocytes or tissue-resident macrophages. TAMs play an important role in tumorigenesis through the enhancement of angiogenesis, migration, and remodeling [58–60]. TAMs are one of the most abundant immune components in GC, but their heterogeneity makes them difficult to characterize. Recent studies have used CD163 and CD206 marker profiles on TAMs through multiplex immunohistochemistry of 56 human GC cases to reveal seven predominant populations (two M1-like and five M2-like TAM populations). An increased density of $CD163^+$ ($CD206^-$) TAMs is associated with upregulated immune signaling and improved patient survival. $CD206^+$ M2-like TAMs have been identified as a population of TAMs that express high levels of PD-L1 [61]. Other studies have suggested that IL-10-producing M2 TAMs facilitate immune evasion and are related to poor prognosis [62,63]. In addition, TAM-derived CXCL8 can inhibit $CD8^+$ T cell function by inducing the expression of PD-L1 on macrophages in GC [64]. Moreover, PD-L1 expression on TAMs and tumor cells appears to

better stratify GC patients with worse prognosis than PD-L1 expression in GC tumor cells alone [65,66].

Chronic infection with *H. pylori* is the main cause of distal GC and intestinal-type GC, and almost 90% of distal GC cases are caused by *H. pylori* infection [9,67]. In chronic inflammatory conditions, bacterial infection in cooperation with PGE2 signaling through the PGE2 receptor subtype 4 (EP4) promotes the upregulation of CCL2, which recruits macrophage to gastric mucosa [68]. Macrophage-derived tumor necrosis factor-α (TNF-α) reciprocally promotes WNT signaling in tumor cells, which contributes to gastric tumorigenesis [69]. Moreover, in human gastric tissues, elevated levels of phosphorylated EGFR were observed throughout the histologic cascade from gastritis to carcinoma [70]. EGFR signaling is also found in macrophages in response to *H. pylori*-activated NF-κB and MAPK1/3 pathways to induce cytokine production, leading to persistent inflammation and GC [71].

TAMs in the peritoneal cavity are also important for peritoneally disseminated GC. Intraperitoneal TAMs in patients with GC with peritoneal dissemination were polarized to the M2 phenotype and contributed to tumor proliferation and progression [72,73]. Cavity-resident macrophages within the peritoneum have high levels of TIM4, which can mediate sequestration of CD8 T cells away from tumor targets and limit antitumor activity during peritoneal metastasis [74]. Overall, these findings highlight the importance of characterizing TAMs in GC and suggest that targeting TAMs and TIM4 could be a promising therapeutic strategy for the disease.

Myeloid-derived suppressor cells (MDSCs) are the most commonly described cells arising during chronic inflammation and cancers, and they suppress the antitumor functions of T cells and natural killer (NK) cells [75]. Gastric-specific overexpression of human interleukin-1β (IL-1β) in transgenic mice mimics *H. pylori*-induced chronic inflammation and recruits MDSCs to initiate stepwise preneoplastic transformation toward GC [76]. The S100A8/A9 heterodimer, a hallmark of MDSCs, upregulates CXCL1 expression in GC cells through the TLR4/p38-MAPK/NF-κB pathways, and tumor-derived CXCL1 reciprocally induces polymorphonuclear (PMN)-MDSC accumulation. In addition, PMN-MDSCs exert immunosuppression through S100A8/A9, which leads to $CD8^+$ T cell exhaustion that is dependent on the intrinsic TLR4/AKT/mTOR pathway in GC cells [77]. A study investigating the role of immunosuppressive MDSCs observed that PD-L1-expressing GC organoids exhibit resistance to nivolumab when cocultured with PMN-MDSCs *in vitro*. Spatial profiling on primary tissue samples obtained from patients with GC confirmed the presence of infiltrating MDSCs within cancerous tissues. These cells expressed markers associated with PMN-MDSCs, such as *ARG1*, *CD66B*, *VISTA*, and *IDO1* [78]. Understanding the interplay of GC cells and its associated TME, as well as the role of MDSCs and their mechanisms of action, may provide important insights into the development of new therapeutic strategies for GC.

Neutrophils are abundant in GC. They associate with disease progression and negatively correlate with patient survival [79,80]. Indeed, the neutrophil-to-lymphocyte ratio (NLR) in TME, which can be predicted using computerized tomography (CT)-based radiomics,

can be used to evaluate response to immunotherapy [81] because a high NLR value is associated with non-responders. Moreover, neutrophil extracellular traps (NETs) are net-like structures composed of DNA–histone complexes and proteins released by activated neutrophils in response to infection [82]. NETs are thought to prevent bacterial and fungal dissemination. In advanced cancer, tumor-derived inflammatory factors stimulate neutrophils in the omentum to release NETs, and these can contribute to tumor recurrence or metastasis [83]. Abdominal infectious complication after gastrectomy stimulates neutrophils to release NETs both in peripheral blood and the abdominal cavity. Those NETs could facilitate GC metastasis, which is dependent on TGF-β signaling [84]. Furthermore, NETs in the omentum can generate extracellular traps that function as an immunosuppressive premetastatic niche [85].

Tumor-associated neutrophils (TANs) are generally involved in tumor-promoting inflammation by driving angiogenesis, metastasis, and immunosuppression [86,87]. Tumor-derived granulocyte-macrophage colony-stimulating factor (GM-CSF) activates TANs and induces PD-L1 expression on TANs via Janus kinase–signal transducer and activator of transcription 3 (JAK–STAT3) signaling. TANs suppress the function of T cells in a PD-L1-dependent manner [80].

GC cells also develop mechanisms to overcome immunotherapy through TME remodeling. For example, CXCL5 is upregulated in patient-derived GC cells in response to anti-PD1 therapy and recruits TANs to exert immunosuppression in a humanized animal model. Blocking the CXCL5/CXCR2 axis involved in TAN recruitment enhances the efficacy of anti-PD-1 immunotherapy *in vivo* [88]. From these perspectives, the intricate interplay between neutrophils and cancer cells underscores their crucial roles in GC pathogenesis, immunotherapy response, and metastatic processes, indicating their potential for therapeutic intervention.

Cancer-associated fibroblasts (CAFs)

CAFs are the most abundant stromal cell type in the TME. CAFs facilitate tumor invasion or drug resistance in GC [89,90], as well as immunosuppression, by inducing a chronic inflammatory state and secreting immunomodulatory cytokines. The tumor-promoting and immunosuppressive functions of CAFs make them ideal therapeutic targets. Several distinct CAF clusters have been found in GC, including immunomodulatory CAFs (iCAFs). iCAFS secrete CXCL1, CXCL3, CXCL5, and CXCL8 that recruit PMN-MDSCs to create an inflammatory state. Moreover, treatment of mice with the small-molecule multi-kinase inhibitor, regorafenib, significantly reduced the iCAF-like subtype. It is not clear whether and how other CAFs may also support GC progression [91]. ST could be a useful approach to study CAFs, especially for fibrotic tumor types such as pancreatic ductal adenocarcinoma (PDAC) and diffuse-type GC [91–95]. Recently, ST was used to assess the effect of regorafenib on CAF heterogeneity in a GC mouse model, which demonstrated that regorafenib treatment reverses the immunosuppressive microenvironment caused by the fibrotic stroma [91].

Systemic inflammation is a common finding in advanced cancer patients, particularly in GC patients with peritoneal metastasis. A recent study using single-cell proteomics

found that malignant ascites contain p16-positive CAFs that express senescence-associated secretory phenotype (SASP) factors which promote the proliferation and invasion of neighboring cancer cells [96]. CAF-derived SASP factors also stimulate angiogenesis by polarizing infiltrating macrophages to a proangiogenic M2 phenotype and contribute to an immunosuppressive microenvironment [97–99]. Collectively, these findings suggest that the CAFs and their intricate interactions with myeloid cells should be explored further for therapeutic interventions.

Nervous system

The gastrointestinal (GI) tract is equipped with a distinctive intrinsic nervous system known as the enteric nervous system (ENS), often referred to as the ‘second brain’. This intricate network of neurons acts independently or in conjunction with the central nervous system (CNS) to modulate the diverse functions of the gut in health and disease [100,101]. The extensive crosstalk between nerves and GI cancer cells results in innervation during tumor progression [102].

Serotonin, chemically known as 5-hydroxytryptamine (5-HT), is a monoamine neurotransmitter synthesized by enterochromaffin (EC) cells in the gut mucosa that is distributed to GI neurons. It plays a role in immune regulation in GC. In GI cancer, including GC, peripheral serotonin derived from platelets can modulate the TIME [103]. Serotonin enhances the expression of PD-L1 on mouse and human cancer cells *in vitro*, whereas the expression of other immune-relevant genes such as MHC-I remains unchanged, a process known as serotonylation. Serotonin concentrations in metastases of patients with abdominal tumors negatively correlate with the number of tumor-infiltrating T cells [104].

Metabolic heterogeneities of the TME

Tumor cells are able to obtain necessary nutrients from their nutrient-poor environment and utilize them to thrive and proliferate [105]. This metabolic interaction affects not only the progression of GC but also contributes to the formation of the TIME [106,107]. The competition of nutrients leads to metabolic reprogramming, which in turn leads to immune tolerance by depriving CD8 T cells of glucose and inhibiting their metabolism through the CD155/TIGIT signaling pathway [108]. Bioinformatic analyses have revealed that higher levels of antitumor-associated immune infiltration are typically accompanied by increased tumor glucose metabolism. Patients with such immune profiles tend to be responsive to immunotherapy, and metabolic status can serve as a potential predictive marker for the treatment response of GC [109]. Indeed, the GC-specific genomic driver, the *RHOA* Y42C mutation, can lead to increased production of free fatty acids under low-glucose conditions through activation of the PI3K–AKT–mTOR signaling pathway. These fatty acids are more effectively consumed by Treg cells, impairing the immune response against GC, and suggesting that metabolic differences associated with molecular features could provide a valuable tool to aid in selecting specific treatment approaches for patients with GC [27].

Furthermore, the integrated analysis of mass spectrometry imaging-based spatial metabolomics and lipidomics with ST revealed a significant decrease in glutamine levels in peritumoral lymphoid tissue (PLT) compared to distant lymphoid tissue (DLT), as well

as higher expression of the *GLS* gene and the glutamine transporter gene *SLC1A5* in PLT, suggesting excessive utilization of glutamine. Glutamine plays a pivotal role in modulating immune cells, including regulating macrophage polarization and the activation of T cells for antitumor activity within the TIME. In addition, the study identified low histamine expression and a lack of infiltrating immune cells. Histamine can enhance T helper type 1 responses and promote myeloid cell differentiation to suppress cancer formation. These findings offer valuable insights into the precise spatial mapping of metabolite, lipid, and gene expression signatures within TIME [110].

Effective immune therapy to heterogeneous GC TME

Chemotherapy remains the most fundamental and accessible component in the preoperative treatment of GC. Advances in immune checkpoint inhibitors are showing promise in treating GC. Combinations of PD-1 inhibitors, including nivolumab (CheckMate 649) [111,112], sintilimab (ORIENT-16) [113,114], and pembrolizumab (KEYNOTE-859) [115], with standard chemotherapy have demonstrated a significant improvement in survival outcomes compared to chemotherapy alone for patients with advanced HER2-negative GC in several Phase 3 trials. Ipilimumab, a cytotoxic T lymphocyte antigen 4 (CTLA-4) inhibitor, and nivolumab employ distinct but complementary mechanisms to restore antitumor T cell function and induce *de novo* antitumor T cell responses [116]. However, their combination in CheckMate 649 did not enhance overall survival compared to chemotherapy alone [117]. Nonetheless, the response rates observed in the ATTRACTION2 or KEYNOTE062 studies appeared to be higher than those for nivolumab or pembrolizumab monotherapy, even in the PD-L1-low population, suggesting a potential role for CTLA-4 inhibition [10,118]. Recent exploratory biomarker analysis from CheckMate 649 suggests potential benefits of nivolumab plus ipilimumab in Treg-enriched patients [119]. However, only a minority of patients with GC demonstrate durable responses to PD-1/PD-L1 or CTLA-4 inhibitor treatment. This limited response can be attributed to GC genomic status related to MSI-H and high tumor mutation burden or to the heterogeneity of the TME.

CAFs with high fibroblast activation protein (FAP) expression have been identified as predictors of poor clinical prognosis in GC [93]. Therefore, direct approaches to targeting activated CAF markers, such as FAP, have been explored in several malignant cancers such as PDAC and colon cancer. However, a Phase 2, single-arm clinical trial combining gemcitabine with the FAP inhibitor talabostat showed no significant benefits compared to historical gemcitabine monotherapy in patients with metastatic PDAC [120,121]. Similarly, a Phase 2 trial of talabostat in metastatic colon cancer also reported no objective responses [122]. Considering that CAFs play a role in shaping complex immune microenvironment [123], future strategies should focus on immune modulation within the TIME by blocking CAF-mediated immunosuppression rather than on targeting specific CAF clusters.

Regorafenib is an oral multi-kinase inhibitor designed to target angiogenic (VEGF, TIE-2), stromal (PDGF-β), and oncogenic (RAF, RET, and KIT) receptor tyrosine kinases (Table 1). A recent Phase 3 clinical trial (INTEGRATE IIa; NCT02773524) revealed that regorafenib as a monotherapy improved overall survival compared to placebo [124]. An ongoing Phase 3 clinical trial (INTEGRATE IIb; NCT04879368) is investigating the potential of regorafenib

in GC treatment to assess the clinical benefits of regorafenib in combination with nivolumab as compared to traditional chemo-therapy alone [125].

Other multi-kinase inhibitors such as cabozantinib or lenvatinib are also being used in clinical trials in combination with ICB. Each inhibitor reported a different impact on the TIME. Cabozantinib selectively depletes MDSCs and reduces their immunosuppressive role in GC [126]. Clinical trials combining cabozantinib with ICB agents such as durvalumab (CTLA-4), tremelimumab (PD-L1), and pembrolizumab (PD-1) have shown promise in trials (NCT03539822 [127] and NCT04164979). In addition, the multi-kinase inhibitor lenvatinib can modify the TIME by reducing TAMs. When combined with PD-1 blockade, it showed enhanced antitumor activity via stimulating IFN signaling [128]. A Phase 2 trial (NCT03609359) showed that the combination of lenvatinib plus pembrolizumab has promising antitumor activity with an acceptable safety profile in patients with advanced GC [129]. Currently, Phase 3 trials to assess the efficacy and safety of lenvatinib plus pembrolizumab plus chemotherapy compared to chemotherapy alone are ongoing (NCT04662710).

There are also ongoing Phase 1/2 clinical trial focusing on myeloid cells in GC treatment. One such trial is exploring the potential of BI-1607, a CD32b inhibitor that targets inhibitory Fcγ receptor (FcγR) proteins expressed on immune cells, including macrophages. This inhibitor is being tested in combination with trastuzumab in HER2-positive GC patients (NCT05555251). In addition, myeloid checkpoint inhibitors, such as the CD47 inhibitor evorpacept, have shown promise in Phase 2/3 clinical trials. This inhibitor, when combined with standard chemotherapy, also offers a potential treatment strategy for patients with advanced HER2-positive GC (NCT05002127). In cases of peritoneal metastasis in GC, recent studies support the significance of inhibiting CD47. Coexpression of galectin-3 with CD47 in peritoneal metastatic cells is associated with diffuse type GC and tumor recurrence. Targeting both galectin-3 and CD47 significantly enhances reprogramming of TAMs and phagocytosis leading to an increased T cell response and suppressed tumor growth in a mouse peritoneal metastasis model [130].

A recent study involving exploratory analysis from the KEYNOTE-061 trial [131] utilizing RNA sequencing data revealed that the monocytic (M)-MDSC signature exhibited adverse relationships with overall survival for pembrolizumab (PD-1), whereas such associations were absent in the case of chemotherapy [132]. It is noteworthy that therapeutic strategies targeting MDSCs have also been investigated in PDAC and other cancers. CXCR2, a receptor on MDSCs, facilitates their recruitment to the tumor site expressing its ligands. Inhibition of CXCR2, either alone or in combination with PD-1-based ICB, has demonstrated promising outcomes in terms of extending overall survival in animal models of CRC [133] and PDAC [134]. Furthermore, clinical trials evaluating CXCR1/2 inhibitors have been conducted in patients with metastatic PDAC, melanoma, prostate, and colon cancer, providing additional support for the potential of targeting MDSCs in GC.

Chimeric antigen receptor (CAR)-T therapy has also generated great interest in the field of GC. This innovative approach involves the activation of an individual’s own T cells to effectively target and eliminate tumors. CARs are synthetic receptors that

enable T cells to recognize tumor-associated antigens (TAAs). In contrast to conventional treatment methods, CAR-T therapy can overcome the intertumoral heterogeneity observed among patients. Although CAR-T therapy has demonstrated remarkable clinical efficacy in various hematologic malignancies, its effectiveness in treating solid tumors remains limited primarily owing to the increased heterogeneity of antigens in solid tumors and the presence of a more potent TIME [135–138].

Clinical trials of CAR-T therapy in GC are currently underway. CLDN18.2 is the gastric-specific isoform of the tight junction protein CLDN18. It is highly expressed in multiple cancers, especially in cancers of the digestive system, making it a potential target for antitumor therapy. Previous preclinical data indicated that CT401 – genetically engineered autologous T cells expressing the CLDN18.2-targeted CAR – had antigen-specific antitumor effects on GC without any harmful effect in animal models [139]. Based on these results, a clinical trial using CT401 was well tolerated and had encouraging efficacy in previously treated patients with CLDN18.2-positive advanced GC in a Phase 1 study (NCT03874897) [140]. This treatment is currently being tested in a Phase 2 clinical trial (NCT04581473). Another effective target for CAR-T is CDH17 (also known as LI-cadherin), a cell adhesion protein. CDH17 is mainly expressed in GI tract and is also a marker of GI system adenocarcinomas. CDH17CAR-T eradicated CDH17-expressing GC in tumor xenograft or autochthonous mouse models without any harm to the normal intestinal epithelial cells [141].

Taken together, it is imperative to explore future strategies targeting the components responsible for immunosuppression in the TME. In addition, combination therapy with conventional ICB or chemotherapy is will also be essential to effectively address the heterogeneous GC TME. When considering therapeutic approaches for peritoneal metastasis in GC, the direct delivery of drugs through ascites, such as catheter-based intraperitoneal chemotherapy, has shown effectiveness in reaching free cancer cells or peritoneal tumors [8]. Consequently, CAR-T therapy holds significant promise in addressing GC-related ascites, with the potential for high efficacy.

Concluding remarks and future perspectives

The complex interactions between immune cells, stromal cells, and tumor cells within the TIME create an environment that promotes tumor growth, metastasis, and immune evasion. Moreover, understanding the specific contributions of myeloid lineage cell types, as well as of CAFs, will be crucial for developing targeted therapeutic strategies. Although current treatment options for GC, such as ICB therapy and chemotherapy, have shown some effectiveness, the heterogeneity of the TIME presents a significant challenge. By targeting these immunosuppressive components, it may be possible to enhance the efficacy of immunotherapy and improve patient outcomes. Future research should particularly focus on unraveling the heterogeneity of myeloid lineage cell types in GC, which are not well understood compared to CAFs, and on identifying novel therapeutic targets within these cell populations.

In addition, advances in single-cell resolution and ST techniques offer exciting opportunities to gain deeper insights into the TIME. Integrating ST with other genomic and proteomic techniques can provide a better understanding of tumor cell heterogeneity, interactions with neighboring cells, and the functional consequences of gene expression profiles. Although ST currently serves as a valuable technology, most studies primarily complement existing knowledge and provide descriptive insights at this stage. However, the integration of ST with other approaches is expected to significantly improve our ability to explore novel targets for GC treatment in the future.

Moreover, CAR-T therapy emerges as a promising approach to overcome the heterogeneity observed among patients. CAR-T therapy has demonstrated remarkable clinical efficacy in hematologic malignancies. However, its effectiveness in treating solid tumors has been limited owing to increased antigen heterogeneity and the presence of a more potent TIME. Although CAR-T for CLDN18.2 showed a high response rate, the durability was short and approximately half of patients required readministration of CAR-T. By gaining a better understanding of immune system interactions within the TME, personalized treatment approaches can be developed, ultimately leading to improved prognosis and outcomes for patients with GC. Continued research using sophisticated animal models in this field holds great promise for advancing the field of immunotherapy and benefiting patients afflicted by this devastating disease (see Outstanding questions).

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Box 1. The single-cell approach for deciphering the TME

Several studies utilizing scRNA-seq analysis suggest that the immunosuppressive immune cell subtypes are completely different depending on the depth of analysis, even in the same tumor tissue [142,143]. Spatial transcriptomics (ST), a novel approach that incorporates spatial information into transcriptome analysis, holds great potential for unraveling the complex and heterogeneous microenvironment. ST can enhance our understanding of the interactions between tumor cells and immune contexts by providing spatial information [38,41,45].

One limitation of the ST technique is the lack of single-cell resolution, requiring the need for deconvolution. As a result, integrating ST with other conventional technologies becomes valuable in cancer research. By complementing conventional scRNA-seq, ST offers new insights into cellular interactions and signaling pathways within the TME [93,144]. Faced with these challenges, some ST platforms operating at the single-cell level have gradually gained traction. For instance, technologies such as NanoString CosMx [15,145], Vizgen MERSCOPE [146], and 10× Genomics Xenium [16] have emerged as single-cell spatial solutions designed to capture targeted transcripts. Moreover, to capture a wider area, Stereo-seq has been developed to study the biological process in a mouse model [14]. These cutting-edge innovations hold immense potential to reveal the complexity of the spatial and functional tissue architecture, thereby enhancing our understanding of intercellular interactions at an unprecedented level of resolution (Figure I). In more recent developments, the integration of mass spectrometry imaging-based spatial metabolomics and lipidomics with ST has allowed a hierarchical visualization of intratumor metabolic heterogeneity and cell metabolic interactions [110].

By combining ST with other genomic and proteomic technologies, we can better understand the heterogeneity of tumor cells, their interactions with neighboring cells, and the implications of their gene expression profiles. Although ST currently serves as an informative technique, ST studies remain complementary to existing knowledge and provide descriptive insights for novel hypothesis generation and testing. We anticipate that integration of ST with experimental validation will have a significant impact on novel treatment approaches and biomarkers for the early detection of GC metastasis.

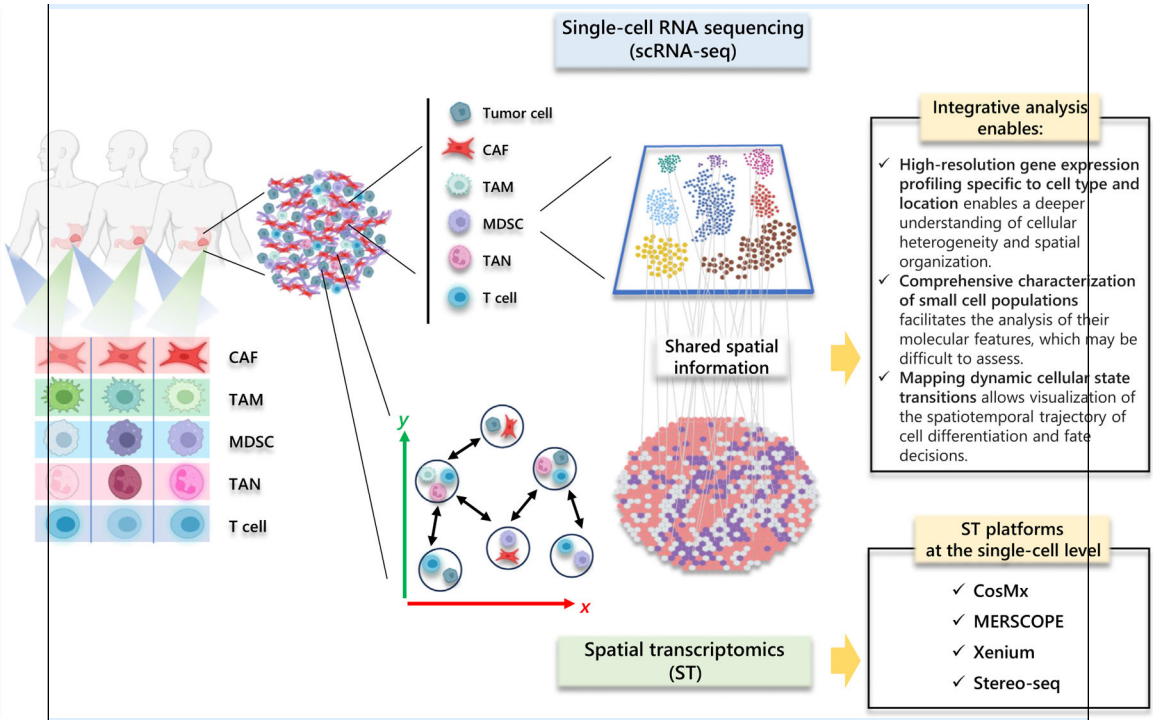

Figure I. Single-cell spatial technology and GC heterogeneity.

Integrative analysis combining scRNA-seq and ST allows high-resolution gene expression profiling specific to cell type and location, thereby facilitating comprehensive characterization of small cell populations and mapping of dynamic cellular state transitions. Moreover, some ST platforms operating at the single-cell level have gained prominence.

Highlights

Gastric cancer (GC) is a heterogeneous intractable disease with marked phenotypic diversity, including a variety of molecular subtypes. This substantial phenotypic diversity of GC facilitates immune evasion and modulation, contributing to its resistance to conventional and immune therapies.

Recent technological advances of single-cell analysis and spatial transcriptomics have allowed a deeper understanding of tumor microenvironment (TME) heterogeneity that marks a potential turning point, leading to the exploration of novel strategies beyond checkpoint inhibition, including cellular immunotherapy.

Clinical trials in advanced GC patients are targeting TME components associated with resistance to standard chemotherapy and/or checkpoint inhibitor treatment with the aim of overcoming therapy resistance.

Outstanding questions

How do driver genes specific to the diffuse-type GC impact on the progression of GC and its immune micro-environment? Can we target these driver genes?

Can cutting-edge innovations such as ST platforms operating at the single-cell level provide insights that go beyond current understanding of heterogeneity of the TME?

Can a deeper understanding of the heterogeneity among myeloid lineage cell types, for example, as well as their behavior within the TME, lead to new therapeutic strategies?

Is cellular immunotherapy such as CAR-T therapy a potentially effective option for addressing the distinctive peritoneal metastasis observed in GC?

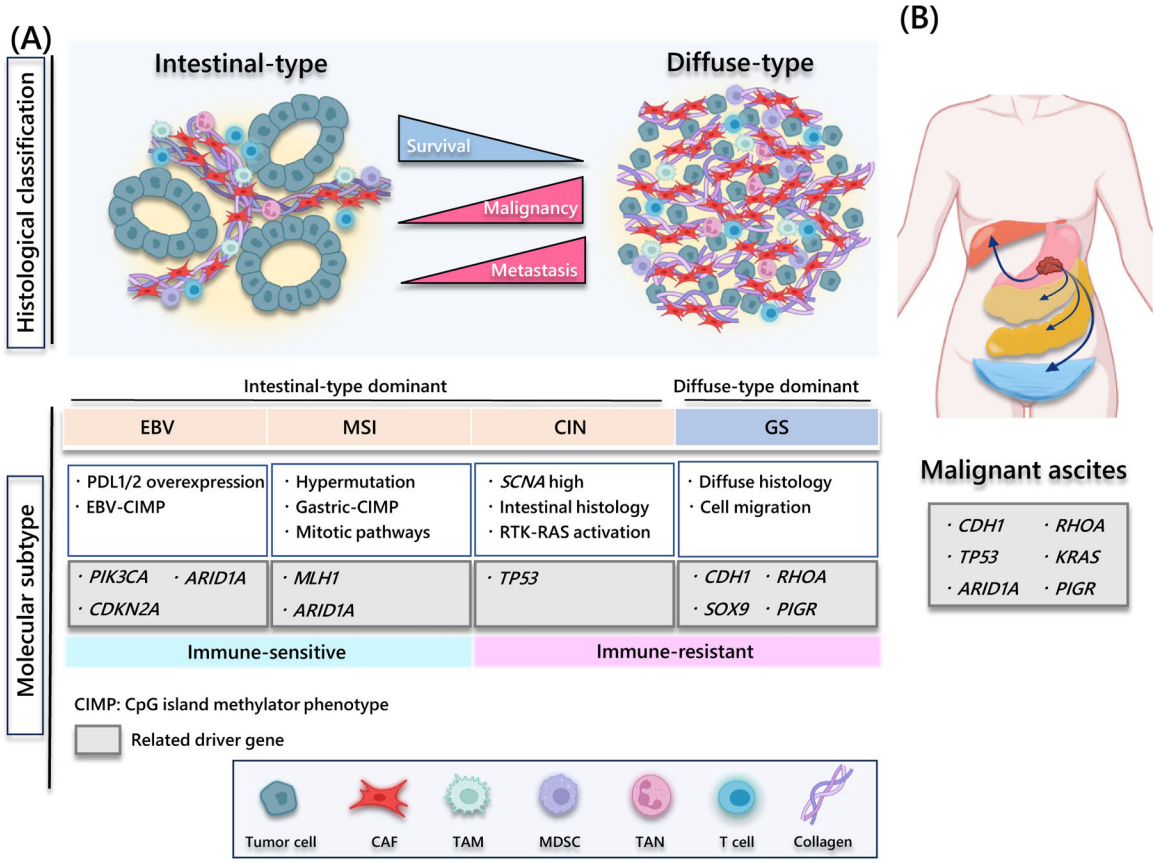

Figure 1. Summary of histological classification and molecular subtypes of gastric cancer (GC).

(A) Histological classification includes two types: intestinal-type and diffuse-type. Molecular subtyping has identified four subtypes: EBV, MSI, CIN, and GS. The majority of patients in the EBV, MSI, and CIN subtypes exhibit an intestinal-type dominance, whereas patients in the GS subtype show a diffuse-type dominance. Each subtype is associated with specific driver genes. (B) Malignant ascites from metastatic cancer cells display distinct driver genes. Abbreviations: CAF, cancer-associated fibroblast; CIN, chromosomal instability; EBV, Epstein–Barr virus-positive; GS, genomically stable; MDSC, myeloid-derived suppressor cell; MSI, microsatellite instability; TAM, tumor-associated macrophage; TAN, tumor-associated neutrophil.

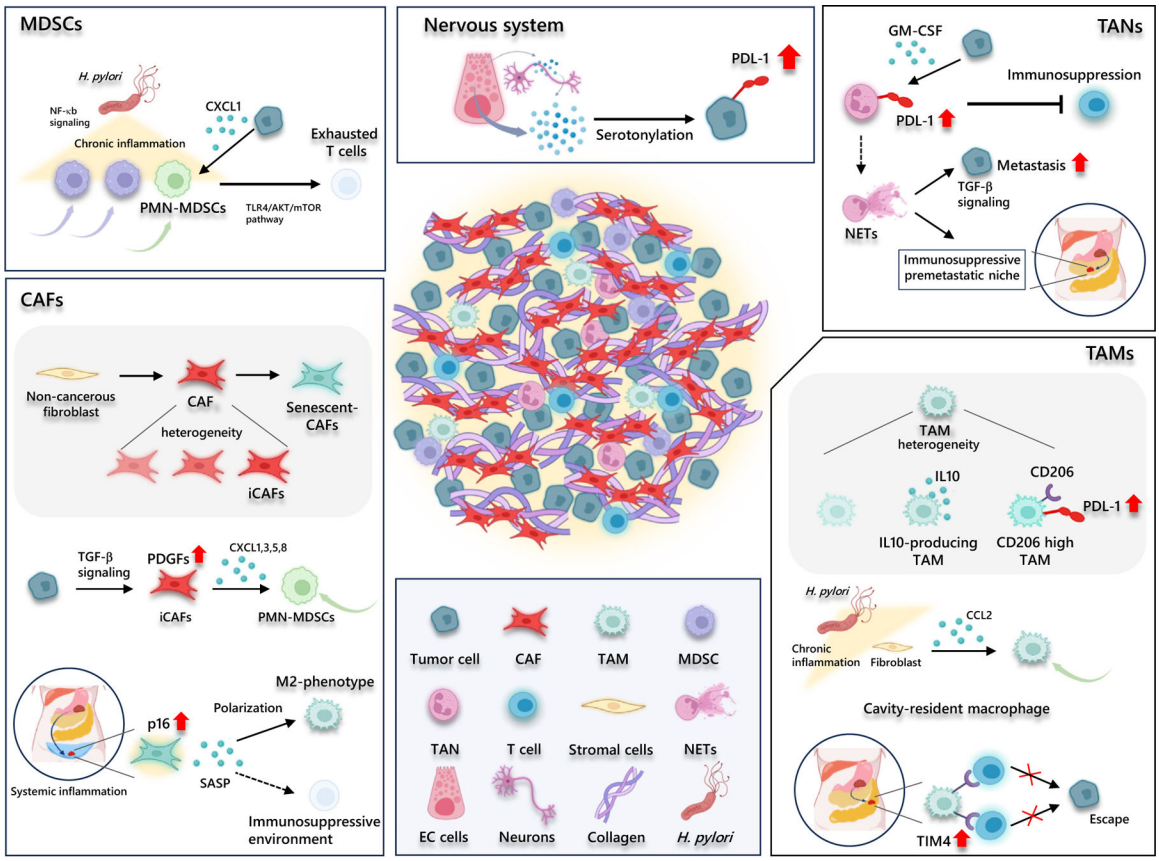

Figure 2. The tumor microenvironment (TME) is a complex system influenced by stromal components.

The TME consists of diverse components, including TAMs, MDSCs, TANs, CAFs, and nerves. TAMs exhibit a heterogeneous population, and CD206 highly expressing macrophages enhance the expression of PD-L1. TAMs can be recruited by CAFs secreting CCL2 during chronic inflammation caused by *Helicobacter pylori* infection. Furthermore, cavity-resident macrophages in patients with metastasis express TIM4, which sequesters $CD8^+$ T cells away from tumor targets. MDSCs are recruited during chronic inflammation, and PMN-MDSCs are induced by tumor-derived CXCL1. PMN-MDSCs lead to exhaustion of $CD8^+$ T cells through the TLR4/AKT/mTOR pathway. TANs enhance the expression of PD-L1 through tumor-derived GM-CSF. In addition, NETs generated in the omentum can serve as an immunosuppressive premetastatic niche. CAFs also exhibit a heterogeneous population, and iCAFs recruit MDSCs by secreting inflammatory cytokines. Senescent CAFs that are observed in malignant ascites of patients with systemic inflammation exhibit a SASP. Senescent CAFs induce the polarization of infiltrating macrophages toward an M2 phenotype, creating an immunosuppressive environment. The nerve system assists in enhancing the expression of PD-L1 through serotonylation. Abbreviations: CAF, cancer-associated fibroblast; iCAF, immunomodulatory CAF; EC cell, enterochromaffin cell; MDSC, myeloid-derived suppressor cell; PMN-MDSC, polymorphonuclear MDSC; NET, neutrophil extracellular trap; SASP, senescence-associated secretory phenotype; TAM, tumor-associated macrophage; TAN, tumor-associated neutrophil.

Table 1.

Selected clinical trials investigating the immune TME in gastric cancer

Drug combination	Mechanism of additional agents	Chemotherapy	ICB	Phase	*N*	Population	Clinical trial
GEN-001 Avelumab	GEN-001: targeting the microbiome	No	PD-L1	2	50	PDL1-positive GC	NCT05419362
Evorpacept (ALX148) Trastuzumab Ramucirumab Paclitaxel	Evorpacept (ALX148): CD47 inhibitor	Yes	No	2/3	450	HER2-positive metastatic GC	NCT05002127
BI-1607 Trastuzumab	BI-1607: CD32b inhibitor	No	No	1/2	116	HER2-positive solid tumors including advanced GC	NCT05555251
CYNK-101 Pembrolizumab (PD-1) Trastuzumab Recombinant human IL-2 Cyclophosphamide Fludarabine Mesna	CYNK-101: drives NK cells	Yes	PD-1	1/2	52	HER2-positive solid tumors including advanced or metastatic GC	NCT05207722
Cyclophosphamide Neoantigen peptide vaccine Pembrolizumab Sargramostim	Sargramostim: recombinant GM-CSF	Yes	PD-1	1	36	Malignant solid tumors including advanced GC	NCT05269381
PF-07062119 Anti-PD1 Anti-VEGF	VEGF inhibitor	No	PD-1	1	130	Malignant solid tumors including metastatic GC	NCT04171141
Q702 Pembrolizumab	Q702: selective TK inhibitor	No	PD-1	1/2	120	Malignant solid tumors including advanced GC	NCT05438420
Regorafenib Avelumab	Regorafenib: multi-kinase inhibitor	Yes	No	1/2	747	Malignant solid tumors including advanced GC	NCT03475953
Regorafenib	Regorafenib: multi-kinase inhibitor	No	No	3	250	Malignant solid tumors including advanced GC	NCT02773524
Regorafenib	Regorafenib: multi-kinase inhibitor	No	PD-1	3	450	Malignant solid tumors including advanced GC	NCT04879368
Cabozantinib Durvalumab Tremelimumab	Cabozantinib: multi-kinase inhibitor	No	PD-L1 CTLA4	1/2	117	Malignant solid tumors, including advanced GC	NCT03539822
Cabozantinib Pembrolizumab	Cabozantinib: multi-kinase inhibitor	No	PD-1	2	20	Malignant solid tumors including metastatic GC	NCT04164979
Lenvatinib Pembrolizumab	Lenvatinib: multi-kinase inhibitor	No	PD-1	2	29	Malignant solid tumors including advanced GC	NCT03609359
Lenvatinib Pembrolizumab Oxaliplatin Leucovorin (or levoleucovorin) 5-Fluorouracil (5-FU) Capecitabine	Lenvatinib: multi-kinase inhibitor	Yes	PD-1	3	890	Malignant solid tumors including metastatic GC	NCT04662710
Claudin 18.2 CAR-T PD-1 monoclonal antibody Chemotherapy	CAR-T targeting claudin 18.2	Yes	PD-1	1	123	Malignant solid tumors including GC	NCT03874897
CT041 autologous CAR-T Physician's choice (paclitaxel or irinotecan	CT041 autologous CAR-T targeting claudin 18.2	Yes	PD-1	1/2	192	Malignant solid tumors including advanced GC	NCT04581473

Drug combination	Mechanism of additional agents	Chemotherapy	ICB	Phase	*N*	Population	Clinical trial
or apatinib or anti-PD-1 antibody)							
Claudin 18.2 CAR-T	CAR-T targeting claudin 18.2	No	No	1	30	Malignant solid tumors including advanced GC	NCT05472857
IM92 CAR-T	CAR-T targeting IM92	No	No	1	6	Malignant solid tumors including GC	NCT05275062