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REVIEW ARTICLE

Diffuse Gastric Cancer: A Comprehensive Review of Molecular Features and Emerging Therapeutics

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Abstract

Diffuse-type gastric cancer (DGC) accounts for approximately one-third of gastric cancer diagnoses but is a more clinically aggressive disease with peritoneal metastases and inferior survival compared with intestinal-type gastric cancer (IGC). The understanding of the pathogenesis of DGC has been relatively limited until recently. Multiomic studies, particularly by The Cancer Genome Atlas, have better characterized gastric adenocarcinoma into molecular subtypes. DGC has unique molecular features, including alterations in *CDH1*, *RHOA*, and *CLDN18-ARHGAP26* fusions. Preclinical models of DGC characterized by these molecular alterations have generated insight into mechanisms of pathogenesis and signaling pathway abnormalities. The currently approved therapies for treatment of gastric cancer generally provide less clinical benefit in patients with DGC. Based on recent phase II/III clinical trials, there is excitement surrounding Claudin 18.2-based and FGFR2b-directed therapies, which capitalize on unique biomarkers that are enriched in the DGC populations. There are numerous therapies targeting Claudin 18.2 and FGFR2b in various stages of preclinical and clinical development. Additionally, there have been preclinical advancements in exploiting unique therapeutic vulnerabilities in several models of DGC through targeting of the focal adhesion kinase (FAK) and Hippo pathways. These preclinical and clinical advancements represent a promising future for the treatment of DGC.

Key Points

Diffuse-type gastric cancer (DGC) is a clinically aggressive subtype of gastric adenocarcinoma with poor clinical prognosis and generally inferior response to currently approved therapies.

Claudin 18.2-based and FGFR2b-directed therapies have shown significant promise in phase II and III clinical trials, and capitalize on unique biomarkers enriched in DGC.

Preclinical models of DGC have been developed based on known pathogenic alterations in *CDH1*, *RHOA*, and *CLDN18-ARHGAP26* fusions, and identify the focal adhesion kinase and Hippo pathways as unique therapeutic vulnerabilities.

1 Introduction

Gastric cancer is the fifth most common cancer and fourth leading cause of cancer mortality worldwide [1]. Traditionally, gastric adenocarcinoma has been classified according to the Laurén classification, with three major histologic subtypes: diffuse-type, intestinal-type, and mixed, which account for approximately 30%, 50%, and 20% of cases, respectively [2, 3]. There are numerous contrasting clinicopathologic features between diffuse-type gastric cancer (DGC) and intestinal-type gastric cancer (IGC) (Table 1). DGC is histologically characterized by poorly differentiated, poorly cohesive cells, and frequent presence of signet ring cells (Fig. 1a, b) [4, 5]. In contrast, IGC is histologically characterized by tubular or glandular structures that appear similar to adenocarcinomas arising from the intestines (Fig. 1c). Clinically, DGC is associated

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with a worse prognosis and increased risk for peritoneal spread compared with IGC [6–9], and DGC is more prevalent in young patients and women (Table 1) [3, 10, 11]. While rates of IGC have decreased over recent decades, the incidence of DGC has been increasing [12, 13].

Traditional risk factors for gastric cancer include *Helicobacter pylori* infection, smoking, obesity, and alcohol; however, there are noteworthy differences between the relative risk of these factors for DGC versus IGC (Table 1) [14]. *Helicobacter pylori* infection is associated with a far greater risk of developing IGC than DGC [15], while smoking and obesity are associated with an increased risk of developing IGC but not for DGC [16, 17]. Heavy alcohol use (> 4 drinks per day) is associated with modestly increased risk of both IGC and DGC, with a larger effect size in the IGC subtype [18]. Notably, there are important racial disparities pertaining to the incidence of DGC, which includes higher proportion of DGC in Asian, Hispanic White, and Native American populations compared with the non-Hispanic White population [19–21].

DGC is a relatively rare but more clinically aggressive subtype of gastric cancer and there are significant unmet needs in this patient population. The objectives of this review are to highlight major molecular and genetic alterations of DGC, discuss currently approved therapies for gastric cancer that may be insufficient for the DGC population, and highlight several promising therapeutics and targetable molecular pathways that may yield substantial clinical benefit for patients with DGC.

2 Genetic and Molecular Landscape of Diffuse-Type Gastric Cancer (DGC)

2.1 Molecular Classification

The first major next-generation sequencing (NGS) study for gastric cancer involved comprehensive analysis in The Cancer Genome Atlas (TCGA), which analyzed 295 patients with early-stage gastric cancer [22]. This study established four major subtypes of gastric cancer, consisting of Epstein–Barr virus positive (EBV), microsatellite instability (MSI), chromosomal instability (CIN), and genomic stability (GS). There was significant enrichment of DGC in the GS cohort, with 73% of cases classified as DGC. Within the GS cohort, there was enrichment of *CDH1* mutations (37%), *RHOA* mutations (15%), and *CLDN18-ARHGAP26* fusions (15%). Clinically, the patients with GS gastric cancer had the least benefit from adjuvant chemotherapy [23].

The Asian Cancer Research Group (ACRG) performed a similar NGS study for gastric cancer for 300 patients after total or subtotal gastrectomy [24]. This study established four subtypes: MSI, microsatellite stable/TP53 active (MSS/TP53$^+$), microsatellite stable/TP53$^-$ (MSS/TP53$^-$), and microsatellite stable/epithelial–mesenchymal transition (MSS/EMT). The MSS/EMT cohort had the highest proportion of DGC, with over 80%, and worst overall survival, which was replicated when applied to other large datasets, including TCGA. The MSS/EMT cohort also had increased rates of recurrence and peritoneal seeding. Mutationally, the MSS/EMT cohort was characterized by somatic mutations in *TP53* (33%) and *ARID1A* (13.9%), and amplifications in *CCNE1* (12.2%). There were notable differences between the MSS/EMT group and GS, including far lower rates of *CDH1* mutations in the MSS/EMT group of only 2.8%. In the ACRG cohort, DGC was seen in all subtypes besides

Table 1 Key differences in clinicopathologic characteristics of diffuse-type and intestinal-type gastric cancer

Features	Diffuse-type gastric cancer	Intestinal-type gastric cancer	References
Prevalence	~ 30%	~ 50%	Chen et al. [3]
Age	Younger	Older	Chen et al. [3], Lee et al. [11], Díaz Del Arco et al. [199]
Sex	Female incidence = male incidence	Male predominant	Chen et al. [3], Lee et al. [11], Díaz Del Arco et al. [199]
Genetic risk factors	Strong	Weak	Blair et al. [55], Thrift et al. [14]
Environmental risk factors (*Helicobacter pylori* infection, smoking, alcohol, obesity)	Modest	Strong	Thrift et al. [14], Parsonnet et al. [15], Sasazuki et al. [16], Jang et al. [17], Rota et al. [18]
Pathology characteristics	Poorly cohesive, signet ring cells	Tubular, papillary	WHO Classification [5]
Type of metastasis	Peritoneal	Liver	Koemans et al. [9]
Prognosis	Worse	Better	Chen et al. [3], Petrelli et al. [6]

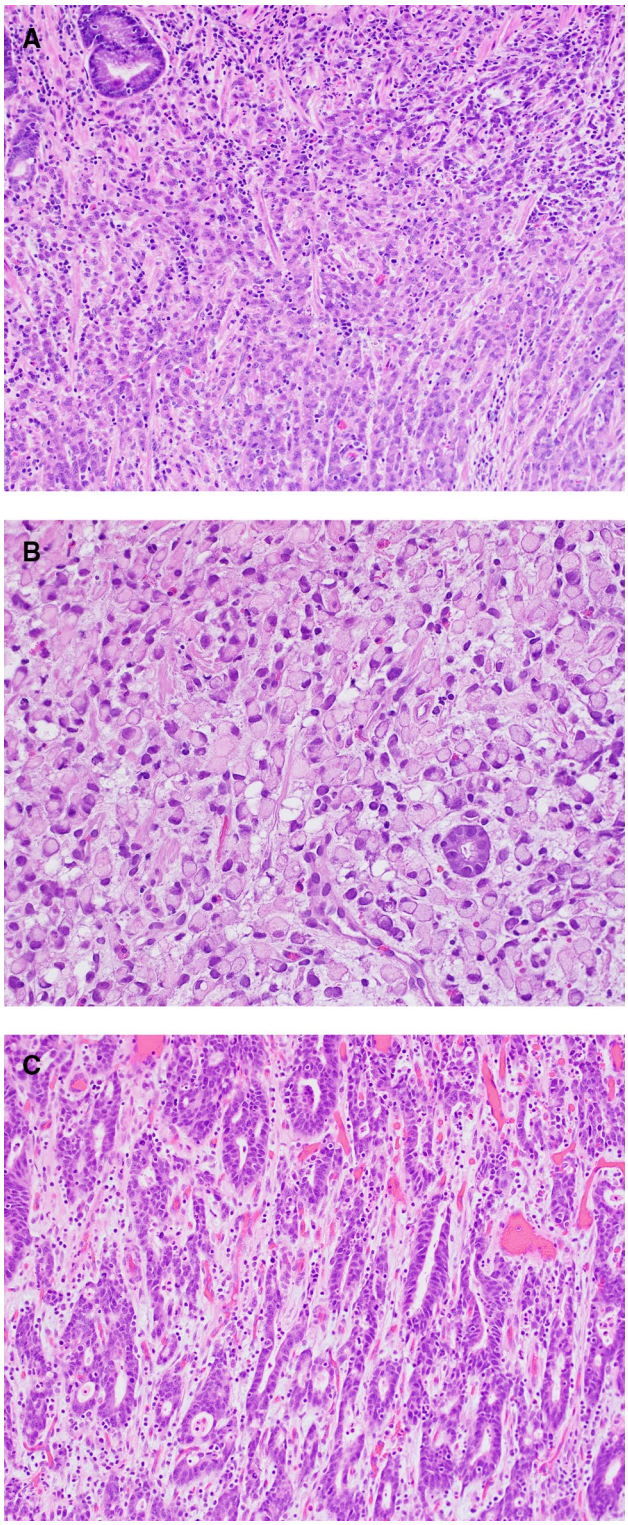

Fig. 1 Representative images of diffuse-type and intestinal-type gastric cancer. **a** Diffuse-type gastric adenocarcinoma at × 200 magnification. Tumor is composed of small nests of poorly cohesive tumor cells. Hematoxylin and eosin-stained sections. **b** Diffuse-type gastric adenocarcinoma with signet ring cells at × 400 magnification. Tumor composed of single, poorly cohesive neoplastic cells with intracytoplasmic mucin and eccentrically located nuclei (signet ring cells). Hematoxylin and eosin-stained sections. **c** Intestinal-type gastric adenocarcinoma at × 200 magnification. Tumor is composed of irregularly shaped glands with areas of cribriform architecture. Hematoxylin and eosin-stained sections

MSS/EMT, which differed from preferential clustering of DGC in the GS subtype in TCGA.

Oh et al. performed unsupervised hierarchical clustering analysis using gene expression data from 93 patients who underwent gastrectomy that identified two phenotypes—mesenchymal and epithelial [25]. The mesenchymal phenotype, enriched for DGC histology, was characterized by high genome integrity, increased EMT-promoting pathways, and proteins such as upregulation of the transforming growth factor (TGF)-β and hedgehog pathways, decreased E-cadherin and α-cadherin, chemotherapy resistance, and worse clinical outcomes. The survival analysis was validated with multiple large gastric cancer cohorts.

Another study by Lei et al. performed unsupervised hierarchical clustering analysis using gene expression data from 248 gastric adenocarcinoma patients [26]. Three major subtypes were identified, i.e. mesenchymal, proliferative, and metabolic. The mesenchymal subtype was characterized by high levels of EMT and cancer stem cells pathways, low E-cadherin, low copy number alterations, and sensitivity to phosphatidylinositol 3-kinase (PI3K) inhibitors. Furthermore, diffuse-type histology was highly enriched in the mesenchymal subtype compared with the proliferative and metabolic subtypes.

Ho et al. created an integrative classification of mesenchymal subtype gastric cancer (Mes-GC) to merge the histopathologic features of DGC, the transcriptomic features of the mesenchymal subtype, and the genomic features of GS gastric cancer [27]. The TCGA, ACRG, Oh et al., and Lei et al. datasets underwent nearest template prediction and differential gene expression analysis to develop a 993-gene consensus gene signature. The Mes-GC tumors were enriched for diffuse-type histology, few genomic alterations, and poor response to chemotherapy and immunotherapy. For Mes-GC tumors, transcriptional enhancer activator domain 1 (TEAD1) was identified as a master regulator of oncogenic transcription programs.

Additionally, there are several proposed molecular subtypes within DGC itself. Ge et al. performed detailed proteomic analysis on paired tumor and nearby normal tissue of 84 DGC patients who underwent total or subtotal gastrectomy [28]. The investigators established three clusters of proteins, referred to as PX1, PX2, and PX3. PX1 was enriched in cell cycle-related processes, PX2 was enriched in cell cycle-related processes and EMT, and PX3 was enriched with immune response pathways. The PX1 and PX2 cohorts benefited from adjuvant chemotherapy, while

PX3 did not. Further analysis revealed this clinical benefit was irrespective of genetic alterations. Kim et al. utilized RNA sequencing to identify a molecular signature in 150 gastric tissue samples, including 107 DGCs [29]. The distinct subtypes of DGC were intestinal-like (INT) and core diffuse-type (COD). The INT subtype had molecular characteristics overlapping with the TCGA MSI and EBV subtypes and improved response to immune checkpoint inhibition. The COD subtype had worse prognosis, improved response to adjuvant chemotherapy, molecular characteristic overlap with the TCGA GS subtype, and was characterized by increased expression of insulin-like growth factor 1 (*IGF1*) and neurexophilin and PC-esterase domain family member 2 (*NXPE1*).

These molecular classifications highlight the heterogeneity within both gastric cancer and DGC itself. There are ongoing investigations to target the most enriched pathways in DGC.

2.2 Signaling Pathway Abnormalities

The most common mutations unique to DGC are mutations in *CDH1*, *RHOA*, and their regulators. There are several in vivo models of DGC that have been generated with alterations in these pathways (Fig. 2).

CDH1 is a tumor suppressor gene that encodes for epithelial cadherin, a key transmembrane glycoprotein involved in cell–cell adhesion, polarity maintenance, and cellular signaling [30, 31]. The loss of function of *CDH1* in gastric cancer results in loss of cell adhesion, decreased contact inhibition, and altered cell migration leading to tumor invasiveness and metastasis [32–34]. There is significant crosstalk signaling with the Hippo, Wnt, and Rho GTPase pathways, as highlighted in Fig. 2 [35]. The most common genomic alteration in sporadic DGC is through mutations or methylation that inactivates *CDH1* [36–38]. In hereditary diffuse gastric cancer (HDGC), the most common alteration is germline *CDH1* mutations [39].

RHOA is a gene that encodes for Ras homolog family member A, a small GTPase in the Rho family of GTPases, and is involved in actin organization, cell migration, and cell cycle signaling [40–42]. Aberrant signaling in the RhoA pathway can further result in resistance to anoikis, tumor migration and invasion, and resistance to chemotherapy [43, 44]. There remains controversy about gain-of-function versus loss-of-function mutations in *RHOA*, but this may depend on the mutation [43, 45]. The most common *RHOA* mutation is *RHOA* Y42C, which has strong evidence of gain-of-function status [46]. A key downstream target of RHOA includes Rho kinase (ROCK), a serine/threonine kinase, which leads to further signaling that affects actin cytoskeleton reorganization, resulting in subsequent cell migration, invasion, and metastases (Fig. 2) [47, 48]. A preclinical model of DGC with *CDH1* loss and *RHOA* Y42C mutation induced DGC in vitro and in vivo [46].

Mutations in key regulators of RhoA signaling may also lead to DGC. The most common genetic alteration is fusion between tight junction protein Claudin 18 (CLDN18) and Rho GTPase-activating proteins (ARHGAP), particularly ARHGAP26. *CLDN18-ARHGAP* fusions occur independently of mutations in *CDH1* and *RHOA* and have a poorly cohesive pattern characteristic of DGC [22, 49]. This exclusivity suggests that *CLDN18-ARHGAP* fusions lead to dysregulation in cell adhesion, migration, and signaling in a manner similar to that in pathogenic *CDH1* and *RHOA* mutations (Fig. 2). *CLDN18-ARHGAP* fusions are unique to gastric cancer, enriched in young-onset gastric cancer, and associated with a higher proportion of signet rings, more advanced disease, increased metastases, and poor response to chemotherapy [49–52]. Functionally, the *CLDN18-ARHGAP26* fusions are implicated in loss of epithelial integrity and cellular invasion due to disruption of wild-type CLDN18, which is a key tight junction protein [53]. A preclinical model of DGC with *CLDN18-ARHGAP26* fusion with *TP53* loss induced DGC in vitro and in vivo [54].

These molecular alterations lead to several signaling pathway activations and dependencies, which are unique opportunities for potential targeted therapy and will be further discussed in the 'Emerging Treatments for Diffuse-Type Gastric Cancer' section.

3 Hereditary Diffuse Gastric Cancer (HDGC)

3.1 Introduction and Genetic Mutations Associated with HDGC

HDGC is an autosomal dominant cancer syndrome characterized by an increased risk of DGC and lobular breast cancer due to a germline mutation in the E-cadherin gene *CDH1* [55]. Other germline mutations in *CTNNA1*, *BRCA2*, *ATM*, *PALB2*, *SDHB*, *STK11*, and *MSR1* may also lead to HDGC [39]. HDGC has previously been estimated to confer a lifetime risk of gastric cancer of 70% for men and 56% for women, although this was likely overestimated due to ascertainment bias [39]. More recent studies estimate a lifetime gastric cancer risk of approximately 40% for men and 30% for women [56, 57]. Overall, HDGC is estimated to account for 1–3% of all gastric cancer cases [58].

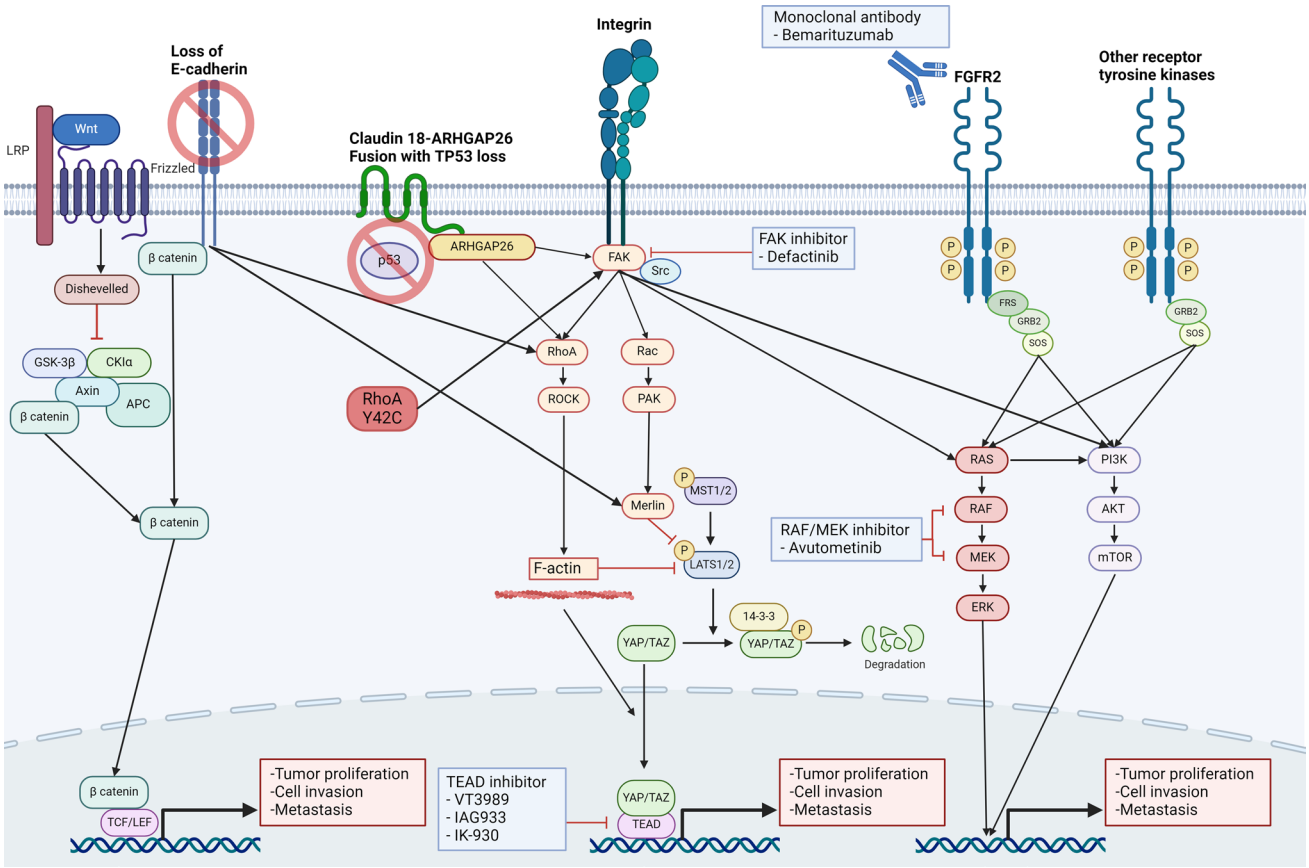

Fig. 2 Key signaling pathways in diffuse-type gastric cancer. Key signaling pathways in diffuse gastric cancer include the focal adhesion kinase, E-cadherin, Wnt, Hippo, and FGFR2/other receptor tyrosine kinase pathways. There is signaling crosstalk between several of these pathways. The downstream effects of aberrant signaling of these pathways lead to tumor proliferation, cell invasion, and metastasis. Two preclinical models of DGC include gain-of-function *RHOA* Y42C with *CDH1* loss and *Claudin 18(CLDN18)-ARHGAP26* fusion with *TP53* loss. There are several promising therapeutic options outside of the currently approved chemotherapy and immunotherapy regimens that target the focal adhesion kinase and Hippo pathways. Created with BioRender.com. *FGFR2* fibroblast growth factor receptor 2, *DGC* diffuse-type gastric cancer

3.2 Mechanism of Tumor Initiation and Development

The underlying mechanisms of tumor initiation in most cases of HDGC involve inactivation of wild-type *CDH1* through promoter hypermethylation, somatic mutation of *CDH1*, or loss of heterozygosity, which results in loss of cellular polarity [59]. The precursor lesions that develop are intramucosal signet ring carcinomas and are confined to the lamina propria. There is enrichment of these precursor lesions in the distal third of stomach and body-antral transition zone, suggesting a potential epigenetic relationship [60]. *CDH1* loss alone is not sufficient to develop advanced DGC and requires downstream events such as chronic inflammation from infection or other environmental factors [59, 61]. EMT may mediate progression from early to advanced HDGC [62]. There may also be newly acquired mutations in *TP53* or *RHOA*, but the overall mechanism of disease progression is still not yet well understood [59].

3.3 Clinical Management

Genetic testing is recommended by criteria published from the 2020 International Gastric Cancer Linkage Consortium (IGCLC) (Table 2). Given the high lifetime risk of developing DGC, prophylactic total gastrectomy (PTG) is recommended in patients who have pathogenic or likely pathogenic *CDH1* mutation and family history of a first- or second-degree relative with DGC [63]. This surgery is recommended between 20 and 30 years of age given the possibility of early-onset disease [64]. While this surgery can be lifesaving, there may be significant postoperative

Table 2 2020 International Gastric Cancer Linkage Consortium genetic testing guidelines [63]

Individual criteria	Family criteria (first- or second-degree blood relatives)
DGC < 50 years of age	≥ 2 cases of gastric cancer in the family regardless of age, with at least one DGC
DGC at any age in individuals of Māori ethnicity	≥ 1 case of DGC at any age and ≥ 1 case of lobular breast cancer < 70 years of age in different family members
DGC at any age in individuals with a personal or family history (first-degree) of cleft lip/cleft palate	≥ 2 cases of lobular breast cancer in family members < 50 years of age
History of DGC and lobular breast cancer, both diagnosed < 70 years of age	
Bilateral lobular breast cancer, diagnosed < 70 years of age	
Gastric in situ signet ring cells and/or pagetoid spread of signet ring cells in individuals < 50 years of age	

DGC diffuse-type gastric cancer

morbidity and several long-term adverse effects, including weight loss, malabsorption, nutritional deficiencies, and osteoporosis [63, 65, 66]. Given the significant morbidities, there is controversy about PTG in certain contexts [67, 68].

Endoscopic surveillance is recommended for patients who qualify for PTG and decline, or other criteria per the IGCLC algorithm [63]. This surveillance can be done according to the Cambridge or Bethesda protocols, which involve random biopsies of all areas of the stomach [69, 70]. One concern with endoscopic surveillance is the variable behavior of early precursor lesions detected on and the lesions can progress significantly between surveillance endoscopies. However, a recent large prospective cohort study found that patients who declined PTG and underwent endoscopic surveillance had low rates of incident tumors, suggesting that this strategy was an acceptable alternative [71]. Nonetheless, there are currently no other preventative therapies for this patient population, which represents a large unmet need.

4 Current Systemic Treatments in Gastric Cancer

The following sections detail the currently recommended frontline and mechanistically unique later-line therapies for gastric cancer per National Comprehensive Cancer Network (NCCN) guidelines [72]. Several phase III clinical trials leading to the approval of these treatments are highlighted. When available, prespecified subgroup survival analysis by histology is included for these key clinical trials (Table 2). These subgroup analyses are likely not powered to provide definitive evidence of a benefit or lack of benefit in a particular histology but can provide some insight within these landmark clinical trials (Table 3).

4.1 Chemotherapy

Systemic chemotherapy with cytotoxic agents with fluoropyrimidine and platinum agents is the backbone of treatment for gastric cancer in the localized and advanced setting [73]. A meta-analysis of 33 studies with neoadjuvant chemotherapy, adjuvant chemotherapy, or palliative chemotherapy in gastric cancer found improved overall survival in IGC versus DGC in all three types of treatment [74]. This study included commonly used therapies, including platinum and fluoropyrimidine-based, irinotecan, and taxane-based chemotherapy regimens.

The current standard-of-care treatment for locally advanced, resectable gastric or gastroesophageal adenocarcinoma is perioperative chemotherapy with fluorouracil, leucovorin, oxaliplatin, and docetaxel (FLOT) per the FLOT4 trial [75]. This phase II/III trial compared perioperative FLOT (four cycles of FLOT preoperatively and four cycles postoperatively) with perioperative epirubicin, cisplatin, and capecitabine/fluorouracil (ECF) [three cycles of ECF preoperatively and three cycles postoperatively], which was the prior standard of care. There were significantly more pathologic complete responses with FLOT after the neoadjuvant portion of therapy in the IGC cohort compared with the DGC cohort [76]. The final trial results found improved overall survival with FLOT, with the greatest survival benefit in IGC [75].

Trifluridine/tipiracil (TAS-102) is an oral cytotoxic chemotherapy that incorporates into DNA to inhibit DNA synthesis [77]. The phase III TAGS trial compared trifluridine/tipiracil versus best supportive care for treatment after at least two prior chemotherapy treatments in patients with metastatic gastric or gastroesophageal adenocarcinoma. There was improved overall survival with the benefit seen primarily in IGC, with a hazard ratio (HR) of 0.58 (95% confidence interval [CI] 0.39–0.87) compared with DGC (HR 0.69, 95% CI 0.36–1.31) [78].

Table 3 Results of key clinical trials with histology subgroup analysis

Trial name	Mechanism	Line of therapy	Phase	Treatment versus control	Patients with DGC in the treatment arm (%)	HR (95% CI) of overall survival in DGC patients in the treatment arm	HR (95% CI) of overall survival in IGC patients in the treatment arm	References
FLOT4	Cytotoxic	Perioperative	III	FLOT versus ECF	96 (27)	0.852 (NA)	0.746 (NA)	Al-Batran et al. [75]
TAGS	Cytotoxic	Third	III	Trifluridine/tipiracil versus best supportive care	53 (16)	0.69 (0.36–1.31)	0.58 (0.39–0.87)	Shitara et al. [78]
REGARD	VEGFR	Second	III	Ramucirumab versus placebo	96 (40)	0.560 (0.366–0.857)	1.009 (0.583–1.745)	Fuchs et al. [85]
RAINBOW	VEGFR	Second	III	Ramucirumab + paclitaxel versus paclitaxel	115 (35)	0.856 (0.641–1.146)	0.705 (0.534–0.932)	Wilke et al. [86]
TOGA	HER2	First	III	Trastuzumab + capecitabine/cisplatin or fluorouracil/cisplatin versus capecitabine/cisplatin or fluorouracil/cisplatin	26 (9)	1.07 (0.56–2.05)	0.69 (0.54–0.88)	Bang et al. [91]
DESTINY-Gastric01	HER2	Third	II	Trastuzumab deruxtecan versus physician's choice of chemotherapy	28 (22)	0.38 (0.17–0.86)	0.65 (0.39–1.07)	Shitara et al. [93]
CheckMate 649	Immune checkpoint inhibitor	First	III	Nivolumab + FOLFOX or CAPOX versus FOLFOX or CAPOX	137 (29)	0.73 (0.60–0.90)	0.91 (0.74–1.10)	Janjigian et al. [96]
KEYNOTE-859	Immune checkpoint inhibitor	First	III	Pembrolizumab + fluorouracil/cisplatin or CAPOX versus fluorouracil/cisplatin or CAPOX	284 (36)	0.76 (0.64–0.90)	0.69 (0.55–0.87)	Rha et al. [97]
SPOTLIGHT	CLDN18.2	First	III	Zolbetuximab + mFOLFOX6 versus mFOLXOX6	82 (29)	0.77 (0.53–1.11)	0.55 (0.36–0.85)	Shitara et al. [117]
GLOW	CLDN18.2	First	III	Zolbetuximab + CAPOX versus CAPOX	187 (37)	0.726 (0.493–1.069)	0.702 (0.403–1.222)	Shah et al. [118]

DGC diffuse-type gastric cancer, *HR* hazard ratio, *CI* confidence interval, *FLOT* fluorouracil, leucovorin, oxaliplatin, and docetaxel, *ECF* epirubicin, cisplatin, and capecitabine/fluorouracil, *VEGFR* vascular endothelial growth factor receptor, *HER2* human epidermal growth factor receptor 2, *FOLFOX* folinic acid, fluorouracil, and oxaliplatin, *CAPOX* capecitabine and oxaliplatin, *CLDN18.2* Claudin 18.2, *mFOLFOX6* modified folinic acid, fluorouracil, and oxaliplatin, *NA* not available, *IGC* intestinal-type gastric cancer.

Overall, there appears to be decreased benefit with chemotherapy in DGC across a variety of clinical settings.

4.2 Vascular Endothelial Growth Factor Receptor-Directed Therapy

Angiogenesis is a key regulator of growth and metastasis for several malignancies, including gastric cancer [79]. Vascular endothelial growth factor (VEGF) and VEGF receptors (VEGFR) are key mediators of angiogenesis and have been subject to considerable therapeutic development [80]. Elevated VEGF and VEGFR levels have been linked to worse prognosis in gastric cancer [81–83]. Ramucirumab is a monoclonal antibody that binds to VEGFR-2 and blocks VEGF ligands from activating this pathway [84]. The phase III REGARD trial compared ramucirumab versus placebo for advanced gastric or gastroesophageal adenocarcinoma after progression on first-line chemotherapy [85]. There was marginal, although statistically significant, improved overall survival with ramucirumab, with a median overall survival of 5.2 months compared with 3.8 months for placebo. In further subgroup analysis by histology, the survival benefit was limited to DGC (HR 0.560, 95% CI 0.366–0.857) compared with IGC (HR 1.009, 95% CI 0.583–1.745).

The phase III RAINBOW trial compared ramucirumab and paclitaxel versus paclitaxel alone for advanced gastric or gastroesophageal adenocarcinoma after progression on first-line chemotherapy [86]. There was improved overall survival with ramucirumab and paclitaxel, with a median overall survival of 9.6 months compared with paclitaxel alone, with a median overall survival of 7.4 months. In further subgroup analysis by histology, the survival benefit was limited to IGC (HR 0.705, 95% CI 0.534–0.932) compared with DGC (HR 0.856, 95% CI 0.641–1.145). One potential reason for benefit limited to IGC is decreased prevalence of VEGF-A amplification in DGC [87]. Based on the results of the RAINBOW and REGARD trials, the combination of ramucirumab and paclitaxel is a preferred second-line treatment rather than ramucirumab monotherapy [72].

4.3 Human Epidermal Growth Factor Receptor 2-Directed Therapy

The human epidermal growth factor receptor 2 (HER2) pathway is implicated in cell proliferation, migration, and differentiation, and is overexpressed in several malignancies including gastric cancer [88, 89]. Trastuzumab is a monoclonal antibody that can target HER2, induce antibody-dependent cellular cytotoxicity (ADCC), and inhibit HER2 signaling [90].

The phase III Trastuzumab for Gastric Cancer (TOGA) trial compared trastuzumab plus chemotherapy versus chemotherapy alone for first-line treatment of advanced HER2-positive gastric or gastroesophageal adenocarcinoma [91]. HER2-positivity was defined as 3+ on immunohistochemistry (IHC) or fluorescence in-situ hybridization positive. There was improved overall survival benefit with trastuzumab, but in further subgroup analysis by histology, the overall survival benefit was limited to the IGC cohort alone (HR 0.69, 95% CI 0.54–0.88) compared with DGC (HR 1.07, 95% CI 0.56–2.05).

Trastuzumab deruxtecan is an antibody drug conjugate (ADC) with an anti-HER2 antibody and cytotoxic topoisomerase I inhibitor [92]. The phase II DESTINY-Gastric01 trial compared trastuzumab deruxtecan versus chemotherapy in HER2-positive gastric or gastroesophageal adenocarcinoma who had progressed on at least two prior therapies, including trastuzumab [93]. There was improved overall survival benefit with trastuzumab deruxtecan, but in further subgroup analysis by histology, that overall survival benefit was limited to the DGC cohort (HR 0.38, 95% CI 0.17–0.86) compared with IGC (HR 0.65, 95% CI 0.39–1.07).

HER2-directed therapies are thus quite valuable in the treatment of gastric cancer when biomarker positive; however, the number of DGC patients who may benefit from these therapies is likely lower due to decreased prevalence of *HER2* positivity and amplification [91, 94].

4.4 Immune Checkpoint Inhibitors

Immune checkpoint inhibitors, e.g. programmed cell death protein 1 (PD-1) inhibitors such as nivolumab and pembrolizumab, activate the immune system as an anti-tumor therapy and have demonstrated significant benefit in multiple malignancies, including gastric cancer [95].

The phase III CheckMate 649 trial compared nivolumab plus chemotherapy versus chemotherapy alone as the first-line treatment for advanced HER-2-negative gastric, gastroesophageal, and esophageal adenocarcinoma [96]. Overall survival benefit was seen with the addition of nivolumab, particularly in the primary endpoint population of programmed cell death ligand-1 (PD-L1) combined positive score (CPS) ≥ 5. Subgroup analysis by histology found overall survival benefit in IGC (HR 0.73, 95% CI 0.60–0.90), but not in DGC (HR 0.91, 95% CI 0.74–1.10). However, this difference in overall survival benefit by histology was not seen when stratified by PD-L1 CPS ≥5 with IGC (HR 0.69, 95% CI 0.53–0.90) and DGC (HR 0.73, 95% CI 0.56–0.97). Notably, the prevalence of PD-L1 CPS ≥ 5 was decreased in DGC (278/527 patients, 52.7%) compared with IGC (347/539 patients, 64.4%).

The phase III KEYNOTE-859 trial compared pembrolizumab plus chemotherapy versus chemotherapy alone as first-line therapy for advanced HER2-negative gastric or gastroesophageal adenocarcinoma [97]. However, survival benefit was only seen in patients with PD-L1 CPS ≥ 1 and

the greatest benefit was seen in those with PD-L1 CPS ≥ 10. Subgroup analysis of this trial by histology found benefit in both IGC (HR 0.81, 95% CI 0.67–0.98) and DGC (HR 0.76, 95% CI 0.64–0.90). In patients with PD-L1 CPS ≥10, further subgroup analysis by histology found overall survival benefit for DGC (HR 0.57, 95% CI 0.42–0.78) but not for IGC (HR 0.77, 95% CI 0.56–1.01). However, the prevalence of PD-L1 CPS ≥10 was lower in DGC (191/619, 30.8 %) compared with IGC (210/557, 37.7%). The phase III KEYNOTE-811 trial compared pembrolizumab in combination with trastuzumab and chemotherapy versus trastuzumab and chemotherapy alone as first-line therapy for advanced HER2-positive gastric or gastroesophageal adenocarcinoma [98]. There was improved progression-free survival in the pembrolizumab group and survival benefit was seen in IGC (HR 0.73, 95% CI 0.57–0.92) but not in DGC (HR 0.71, 95% CI 0.47–1.07).

Given the therapeutic implications shown with PD-L1 CPS ≥ 1, other studies have retrospectively analyzed various cohorts, including the ACRG cohort for PD-L1 positivity, as defined by PD-L1 CPS ≥ 1 [99]. There was a numerically higher percentage of PD-L1 positivity in IGC, at 64.7%, compared with DGC at 54.2%, although not statistically significant per the Chi-square test, with a *p* value of 0.1624. Another large gastric cancer cohort study identified higher rates of PD-L1 positivity in tumor cells in IGC, at 29.2%, compared with DGC at 6.2% (Chi-square test with a *p* value of <0.001), but PD-L1 positivity in immune cells in IGC, at 37.5%, compared with DGC at 30.3% (Chi-square test with a *p* value of 0.089).

MSI-high (MSI-H) status is a key marker that also predicts response to immune checkpoint inhibitors [100]. Pembrolizumab monotherapy has pan-tumor US Food and Drug Administration (FDA) approval for the treatment of advanced MSI-H cancers in the second-line setting per the phase II KEYNOTE-158 trial [100, 101]. Within this trial, patients with MSI-H advanced gastric cancer had an overall response rate of 31%, with a complete response rate of 9.5% and partial response rate of 21.4%; however, there was decreased prevalence of MSI-H in DGC compared with IGC [102].

Thus, for patients with DGC, there are several indicators of decreased predicted benefit from currently approved immune checkpoint inhibitors.

5 Emerging Treatments for Diffuse-Type Gastric Cancer

The following section highlights the most promising proteins and pathways that are targetable. While certain targeted therapies against certain receptors such as VEGFR and HER2 have demonstrated success in gastric cancer, there have been several unsuccessful efforts targeting other pathways. These targets include mammalian target of rapamycin (mTOR) with everolimus, epidermal growth factor receptor (EGFR) with cetuximab, and receptor for hepatocyte growth factor (MET) with rilotumumab and onartuzumab [103–106]. Nonetheless, several successful recent clinical trials have generated optimism for some of these more novel targets.

5.1 Claudin 18.2

CLDN18 is a tight junction protein in epithelial cells that plays a key role in maintaining barrier function and cell polarity [107]. CLDN18.2 is a splice isoform of CLDN18 that is transiently expressed in short-lived gastric epithelial cells, and, during malignant transformation of gastric cancer, has loss of cell polarity resulting in CLDN18.2 exposure and accessibility to antibodies [108]. CLDN18.2 expression is likely only a byproduct of malignant transformation in certain gastric cancers rather than a cause of its pathogenesis, which is distinct from CLDN18.2/ARHGAP fusions. Several studies have identified higher rates of CLDN18.2 expression in DGC compared with IGC [109–114].

Zolbetuximab is a first-in-class chimeric immunoglobulin (Ig) G1 monoclonal antibody that targets and binds to CLDN18.2 to induce ADCC and complement-dependent cytotoxicity [115]. Per the phase II FAST study, clinical benefit of first-line zolbetuximab with chemotherapy for advanced gastric/gastroesophageal adenocarcinoma is only seen with higher CLDN18.2 expression, particularly in patients with moderate-to-strong CLDN18.2 expression in ≥ 70% of tumor cells [116]. Subsequent phase III trials that studied zolbetuximab with chemotherapy for advanced HER2-negative gastric/gastroesophageal adenocarcinoma utilized a higher CLDN18.2 expression cut-off of ≥ 75% of tumor cells with moderate-to-strong CLDN18 membranous staining [117, 118].

The phase III SPOTLIGHT trial compared zolbetuximab plus modified folinic acid, fluorouracil, and oxaliplatin (mFOLFOX6) versus mFOLFOX6 alone for CLDN18.2-positive, HER2-negative advanced gastric/gastroesophageal adenocarcinoma [117]. There was improved overall and progression free-survival with the zolbetuximab combination. Subgroup analysis of this trial by histology found overall benefit in IGC (HR 0.55, 95% CI 0.36–0.85), but not DGC (HR 0.77, 95% CI 0.653–1.11). There was a higher proportion of DGC patients in this trial (199/565, 35.2%) compared with IGC (136/565, 24.1%). The phase III GLOW trial compared zolbetuximab plus capecitabine and oxaliplatin (CAPOX) versus CAPOX alone for first-line treatment of CLDN18.2-positive, HER2-negative advanced gastric or gastroesophageal adenocarcinoma [118]. There was improved overall and progression-free survival with the zolbetuximab combination. Subgroup analysis of this trial

by histology found a trend towards overall survival in IGC (HR 0.702, 95% CI 0.403–1.222) and DGC (HR 0.726, 95% CI 0.493–1.069). There was a higher proportion of DGC patients in this trial (187/507, 36.9%) compared with IGC (77/507, 15.2%). Treatment-related adverse effects attributed to zolbetuximab included nausea, vomiting, and decreased appetite.

These studies have generated considerable excitement for the development of further CLDN18.2-directed therapies, particularly those that may be efficacious at lower CLDN18.2 expression levels given the high threshold needed for benefit with zolbetuximab. There is ongoing development of therapeutics that utilize CLDN18.2 through a variety of drug mechanisms, including ADCs, chimeric antigen receptor (CAR) T cells, and bispecific antibodies (Fig. 3). Some of these therapeutics may take advantage of the bystander effect, which can result in killing of non-CLDN18.2-expressing tumor cells [119–121].

ADCs utilize a monoclonal antibody linked to a cytotoxic payload to deliver more targeted and cytotoxic therapy to specific cells of interest. There are numerous ADCs that target CLDN18.2 with a monomethyl auristatin E (MMAE) cytotoxic payload, including SYSA1801, CMG901, and LM-302 [120, 122, 123]. These have shown promising results in their early-phase trials with manageable toxicity. The dose expansion elements of these trials included patients with CLDN18.2 expression of at least 40% with ≥ 2+ on IHC. Several other ADCs are in development as well as early-phase trials.

CAR T cells are engineered T cells that express receptors that bind to antigens of interest. CAR T-cell therapies have greatly improved treatment of hematologic malignancies but have shown limited benefit in solid tumors thus far [124, 125]. CT041 (satricabtagene autoleucel) is a CAR T-cell therapy with a humanized anti-CLDN18.2 single-chain variable fragment, a CD8α hinge region,

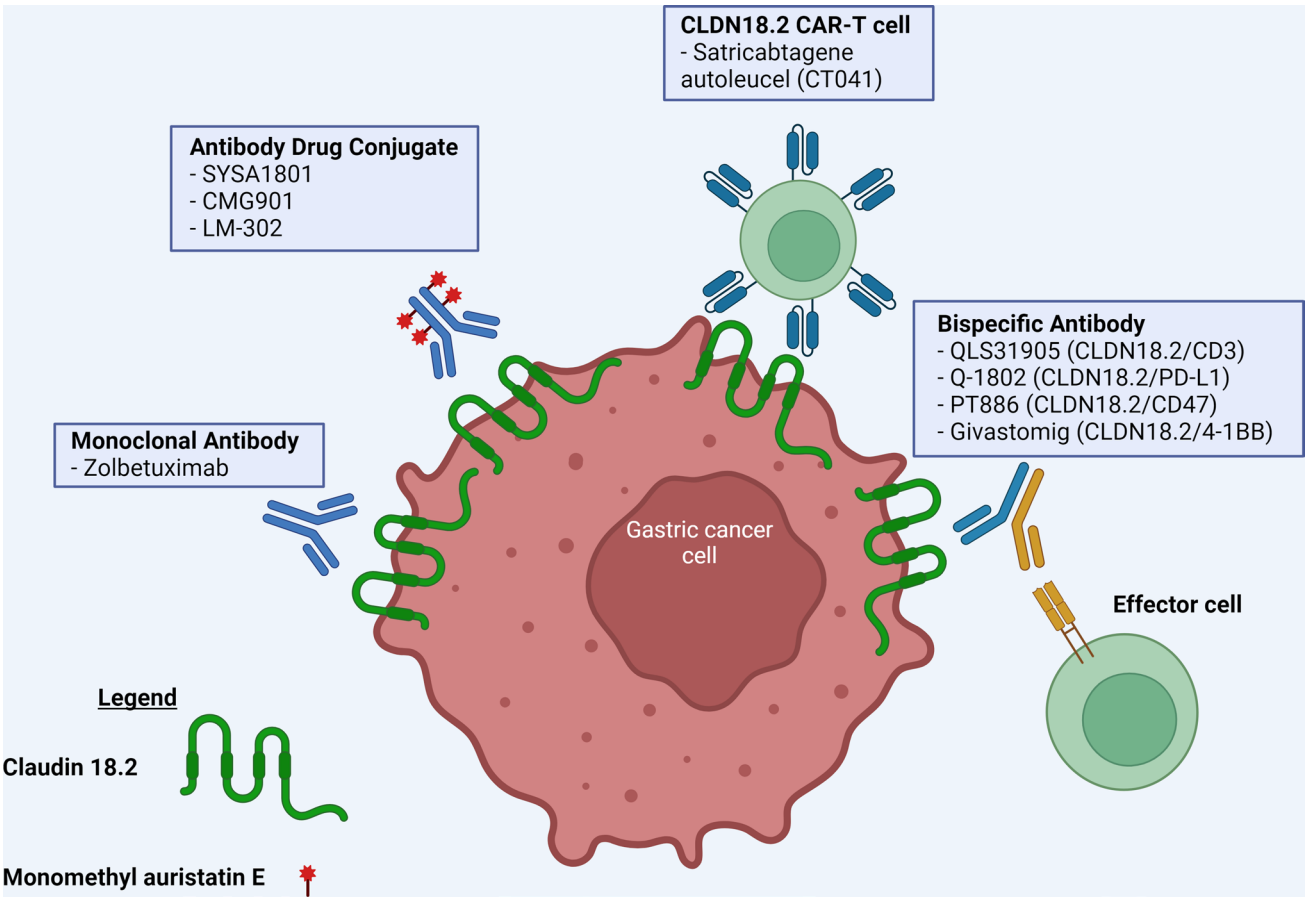

Fig. 3 CLDN18.2-directed therapies. There are several therapeutics in clinical development that target CLDN18.2 and that take advantage of preferential expression of CLDN18.2 in gastric cancer. These mechanisms include monoclonal antibody binding leading to antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity, antibody drug conjugates with monoclonal antibody delivery of a cytotoxic payload, CAR T-cell therapies, and bispecific antibodies that bind to CLDN18.2 and other ligands that recruit immune effector cells. Antibody drug conjugates, CAR-T cells, and bispecific antibodies have the potential to be more effective at lower CLDN18.2 expression given bystander effect. Created with BioRender.com. *CLDN18.2* Claudin 18.2, *CAR* chimeric antigen receptor

a CD28 co-stimulatory domain, and a CD3ζ signaling domain [126]. Interim results in an ongoing phase I trial utilizing CT041 in China found an overall response rate of 57.1% and 6-month overall survival rate of 81.2% in heavily treated patients with CLDN18.2-positive gastric cancer with high representation of DGC [127]. 48.6% of patients had low or medium CLDN18.2 expression, which was defined as < 40% or intensity of 1+ with any percentage on IHC, and 40–69% with intensity of ≥ 2+ on IHC, respectively. In a phase Ib/IIb study in the United States, CT041 had an overall response rate of 42.9% and median duration of response of 6.9 months in advanced CLDN18.2-positive gastric cancer [128]. Other CAR T-cell therapies are in development with differing features, such as in their co-stimulatory domain.

Bispecific antibodies are engineered to bind two distinct epitopes between two different cells in close proximity or on the membrane of the same cell [129]. This binding can result in either simultaneous or sequential pathway activation. There are numerous potential combinations to mediate anti-tumor efficacy in gastric cancer. Bispecific T-cell engagers are bispecific antibodies that link an antibody that targets a tumor-specific antigen to an antibody that activates T cells in proximity to target epitope. QLS31905 is a CLDN18.2/CD3 bispecific antibody that functions to recruit CD-3 on the surface of T cells to CDN18.2 and has undergone phase I testing for heavily pretreated advanced CLDN18.2-positive tumors defined as in ≥ 1% CLDN18.2 [130]. In patients who received 200 µg/kg or higher weekly, there was a high rate of stable disease in gastric cancer, particularly in patients with intermediate or high CLDN18.2 expression. Q-1802 is a CLDN18.2/PD-L1 bispecific antibody that functions to recruit PD-L1-expressing cells such as T cells, macrophages, and natural killer cells, in addition to mediating ADCC and antibody-dependent cell-mediated phagocytosis (ADCP) [131]. Interim results of a phase I study testing Q-1802 in advanced gastrointestinal malignancies led to two partial responses and four stable diseases out of nine CLDN18.2-positive patients [132]. PT886 is a CLDN18.2/CD47 bispecific antibody that binds to CD47 to increase macrophage-mediated phagocytotic activity and utilize IgG1 to enhance ADCC and ADCP [133, 134]. There is an ongoing phase I dose escalation and expansion trial with PT886 for CLDN18.2-positive advanced gastric, gastroesophageal, and pancreatic adenocarcinoma [134]. Givastomig is a CLDN18.2/4-1BB bispecific antibody that binds to CD137 to induce tumor necrosis factor (TNF)-α-mediated CD8+ T-cell stimulation, natural killer cell proliferation, and interferon-γ production, and does so in a CLDN18.2-dependent manner [135]. Givastomig has demonstrated preliminary efficacy in a phase I dose escalation trial with CLDN18.2-positive cancers, with four partial responses and two stable diseases in 18 gastric cancer patients [136].

These therapeutics represent a fraction of the ongoing development of CLDN18.2-directed therapeutics. Given the elevated proportion of CLDN18.2 expression in DGC, CLDN18.2-directed therapies are highly promising to provide clinical benefit for this population.

5.2 Fibroblast Growth Factor Receptor 2

The fibroblast growth factor receptor (FGFR) pathway stimulates cell proliferation, angiogenesis, and transformation in several malignancies [137]. This pathway is mediated by four transmembrane tyrosine kinase receptors (FGFR1-4). The FGFR2 pathway is altered in a subset of gastric cancer through *FGFR2* gene amplification, *FGFR2* mutations, *FGFR2* fusions, and overexpression of the FGFR2b splice variant [138, 139]. There is downstream activation of the mitogen-activated protein kinase (MAPK) and PI3K pathways (Fig. 2) [140]. *FGFR2* gene amplifications are present in approximately 4–9% of gastric cancers, and FGFR2b overexpression is present in approximately 30% of gastric cancers [141–144]. These FGFR2 alterations are disproportionately elevated in DGC and are associated with worse clinical outcomes [141, 145, 146].

Bemarituzumab is an afucosylated monoclonal antibody that targets the FGFR2b receptor to block FGFR2b signaling and activate FcγRIIIa/CD16a to enhance ADCC [147, 148]. The phase II FIGHT trial compared bemarituzumab with chemotherapy (mFOLFOX6) versus mFOLFOX6 alone in advanced HER2-negative, FGFR2b-selected gastric or gastroesophageal adenocarcinoma [144]. In this trial, FGFR2b overexpression was defined by FGFR2b overexpression on IHC (≥ 2+ staining in more than 0% of tumor cells) or *FGFR2* gene amplification by circulating tumor DNA. In the final clinical analysis of this trial, there was a trend toward improved progression-free and overall survival with bemarituzumab in the overall cohort [149]. In a prespecified subgroup analysis for patients with FGFR2b overexpression in ≥ 10% of tumor cells, there was statistically improved progression-free and overall survival, with a notable median survival of 24.7 months (95% CI 14.2–30.1 months) compared with 11.1 months (95% CI 8.4–13.8 months). There was no subgroup analysis by histology. A larger follow-up phase III trial is being conducted and will only include patients with FGFR2b overexpression in ≥ 10% on IHC (NCT05052801) [150].

There are also several clinical trials utilizing tyrosine kinase inhibitors to target *FGFR2* alterations in patients with gastric cancer, including futibatinib (NCT04604132), erdafitinib (NCT02699606), infigratinib (NCT05019794), and derazantinib (NCT04604132). The full results of these trials have not yet been published.

Overall, these findings underscore the potential of targeting *FGFR2* alterations as a promising therapeutic strategy in

DGC, with ongoing clinical trials expected to further clarify their efficacy.

5.3 Focal Adhesion Kinase Pathway

Focal adhesion kinase (FAK) is a non-receptor tyrosine kinase and kinase-independent scaffold involved in regulation of cell migration, growth factor signaling, and cell survival [151, 152]. Aberrant FAK signaling is present in numerous malignancies, including gastric cancer, and is linked with cell proliferation, invasion, metastasis, mechanotransduction, EMT, immunosuppressive microenvironment, and drug resistance [152, 153]. Phosphorylated FAK, a marker of FAK signaling, is a negative prognostic marker in localized gastric cancer and this is estimated to be present in ~30–40% of DGCs [154, 155]. Evaluation of surgical samples in one study found greater phosphorylated FAK in DGC samples compared with IGC samples [46].

One preclinical model of DGC with *CDH1* loss and *RHOA* Y42C mutation found increased FAK activation and dependency which further induced the PI3K/AKT and YAP/TAZ pathways (Fig. 2) [46]. FAK inhibition monotherapy with defactinib or PF-573228 decreased in vitro and in vivo growth. Further preclinical studies with this DGC model found a synergistic effect of FAK inhibition with combination palbociclib (cyclin-dependent kinase 4/6 inhibitor) or VS-6766 (rapidly accelerated fibrosarcoma [RAF]/MAPK kinase [MEK] inhibitor; now known as avutometinib) [156]. The choice of these combination regimens is based on prior synergistic evaluation and previously identified adaptive resistance mechanisms identified with FAK inhibition monotherapy in other disease models [157–160]. Likewise, MEK inhibition can lead to an adaptive resistance mechanism of FAK signaling [161].

Numerous FAK inhibitors have been tested in clinical trials, including defactinib, ifebemtinib (IN10018), GSK2256098, conteltinib (CT-707), and APG-2449 [162]. Mechanistically, these FAK inhibitors are adenosine 5′triphosphate (ATP)-competitive inhibitors. Defactinib is an ATP-competitive, reversible inhibitor of FAK and proline-rich tyrosine kinase-2 (Pyk2) [163]. There has been modest efficacy with FAK inhibitor monotherapy in clinical trials, likely due to intrinsic or acquired drug resistance [153, 163–165]. Avutometinib is a first-in-class RAF/MEK clamp inhibitor that blocks MEK and the compensatory reactivation mechanism of MEK by upstream RAF [166, 167]. The defactinib and avutometinib combination is being explored in a variety of other solid malignancies, including non-small cell lung cancer (NCT04620330), thyroid cancer (NCT06007924), mesonephric gynecologic cancer (NCT05787561), pancreatic cancer (NCT05669482), and low-grade serous ovarian cancer (NCT04625270). There has been promising clinical efficacy in phase Ib/II clinical trials in recurrent low-grade serous ovarian cancer with defactinib and avutometinib alone, and in pancreatic cancer with defactinib and avutometinib in combination with gemcitabine and nab-paclitaxel [168, 169]. The combination is overall well tolerated, but grade 3 or higher adverse effects include elevated creatine phosphokinase, fatigue, and rash. A clinical trial of defactinib and avutometinib for the treatment of metastatic DGC after progression on first-line systemic treatment has recently opened for enrollment and is the first clinical trial to specifically enroll individuals based on DGC histology (NCT06487221). Given the preclinical data and overall well-tolerated adverse effect profile in other clinical trials, this combination may be promising for DGC.

Another preclinical model of DGC driven by *CLDN18-ARHGAP26* fusion with *TP53* mutation found activation of RHOA and downstream activation of the FAK and YAP/TEAD pathway (Fig. 2) [54]. There was benefit with FAK inhibition monotherapy with defactinib or PF-573228. Combination treatment with defactinib and the TEAD inhibitor VT103 showed synergistic benefit halting in vitro and in vivo growth.

Thus, combination therapy with FAK inhibition and targeting other compensatory mechanisms of resistance may be a promising therapeutic strategy for patients with DGC, particularly those with the unique driver mutations in *RHOA* Y42C and *CLDN18-ARHGAP26* fusion.

5.4 Hippo Signaling Pathway

The Hippo signaling pathway is a key regulator of organ size and tissue homeostasis, and its dysregulation is linked to the development of several malignancies, including gastric cancer [170]. Activation of the Hippo pathway results in inhibiting its terminal effectors are Yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ) through kinase-mediated phosphorylation cascade [171]. Upstream inactivation of the regulators in the Hippo pathway leads to YAP/TAZ nuclear localization and binding to TEAD 1–4 to mediate several pathways key to tumorigenesis, proliferation, and survival.

Overexpression of YAP/TAZ is linked to increased metastasis, progression, and worse prognosis in gastric cancer [172, 173]. Likewise, inactivation of upstream negative regulators of YAP/TAZ, such as large tumor suppressor kinase 1 (LATS1) or mammalian STE20-like protein kinase 1/2 (MST1/2), is linked to similarly poor clinical outcomes [174, 175]. TEAD alterations are present in 14% of gastric cancers in TCGA database, and overexpression of TEAD1–4 is correlated with poor clinical outcomes in gastric cancer and is linked to mesenchymal-subtype gastric cancer [27, 154, 176].

The Hippo pathway can be targeted pharmacologically in gastric cancer through numerous mechanisms, including

targeting the YAP/TAZ-TEAD complex, inhibiting YAP/TAZ nuclear localization, targeting upstream regulatory pathways, and inhibiting TEAD palmitoylation or protein–protein interaction.

Verteporfin (VP) is a benzoporphyrin derivative that can inhibit the YAP/TAZ-TEAD complex and reduce gastric cancer growth in vitro and in vivo by suppressing FAT atypical cadherin 1 (FAT1) and targeting gastric cancer stem cells [173, 177, 178]. CA-3 is a YAP inhibitor that can modulate YAP/TEAD transcriptional activity and reduce YAP expression [179]. Mimetic agents to target transcriptional regulators such as vestigial-like family member 4 (VGLL4) have also shown promising efficacy [180]. VGLL4 competes with YAP to bind to TEAD via the Tondu (TDU domain) and functions as a YAP antagonist. Super-TDU is a VGLL4-mimicking peptide that reduces gastric cancer growth in vitro and in vivo. Striatin-3 (STRN3) is a regulatory subunit of protein phosphatase 2A (PP2A) that recruits MST1/2 and leads to dephosphorylation, which causes downstream YAP activation [175]. STRN3-derived Hippo activating peptide (SHAP) is a selective peptide inhibitor that can disrupt STRN3-PP2A interactions and prevent YAP/TAZ nuclear localization to inhibit gastric cancer growth *in vivo*. Manipulation of the actin cytoskeleton and mechanotransduction system may result in aberrant YAP/TAZ signaling [181–183]. In the previously mentioned preclinical model of DGC with *CDH1* loss and *RHOA* Y42C mutation, RHOA-mediated FAK activation induced YAP/TAZ pathway activation, and there was benefit with FAK inhibition [46].

Given that TEAD1–4 require auto-palmitoylation at a conserved cysteine to become functional, there are numerous inhibitors designed to covalently and noncovalently block TEAD auto-palmitoylation [184, 185]. Covalent inhibitors include TED-347 (TEAD4–YAP interaction), DC-TEADin02 (TEAD4-selective), and K-975 (pan-TEAD) [186–188]. Non-covalent inhibitors include VT103 (TEAD1-selective) [189]. These TEAD inhibitors and other therapeutics targeting other aspects of the Hippo pathway have shown efficacy in several preclinical models of DGC.

The preclinical model of DGC with *CLDN18-ARHGAP26* fusion and *TP53* loss found activation of RHOA and downstream activation of the FAK and YAP/TEAD pathway (Fig. 2) [54]. There was benefit with FAK inhibition monotherapy with defactinib or PF-573228. Combination treatment with defactinib and the TEAD1 inhibitor VT103 showed synergistic benefit in halting in vitro and in vivo growth. The previously mentioned MesGC consensus phenotype was proposed to be a unifying phenotype for DGC based on four large datasets, and TEAD1 was identified as a master regulator of oncogenic transcription programs [27]. Inhibition of YAP/TEAD with VP, CA3, or TED-347 significantly reduced in vitro growth of MesGC cell lines.

Tanaka et al. analyzed multiomic data from 98 gastric cancers with malignant ascites, a condition strongly associated with DGC [190]. Through hierarchical clustering of gene expression profiles, two distinct clusters were identified with EMT and non-EMT, both of which had a high number of genetic alterations in the MAPK pathway. In the EMT group, there was overexpression of YAP, TAZ, TEAD1, TEAD2, and TEAD4. K-975 was utilized in EMT cell lines and in vivo models [188]. There was significant tumor growth suppression in vitro proportional to the expression level of TEAD1. Given the high incidence of MAPK genetic alterations and prior evidence that YAP/TAZ–TEAD activation was a resistance mechanism to MEK inhibition, combination treatment with MEK1/2 inhibition with binimetinib and K-975 was performed in EMT gastric cancer cell lines and showed synergistic effect in cell lines with high TEAD1 expression [191, 192].

Most therapeutics targeting various aspects of the Hippo pathway have not yet reached clinical trials. There is an ongoing phase I/II clinical trial of liposomal VP in recurrent high-grade EGFR-mutated glioblastoma (NCT04590664). ION537 is an antisense oligonucleotide targeting YAP mRNA and has been evaluated in a phase I clinical trial for advanced solid tumors (NCT04659096) [193]. The current TEAD inhibitors in clinical trials include IAG933 (NCT04857372), IK-930 (NCT05228015), and VT3989 (NCT04665206). IAG933 is a pan-TEAD inhibitor that directly targets the YAP-TEAD protein–protein interaction by sterically hindering several coactivators [194–196]. Preclinical studies have shown benefit combining IAG933 with RTK inhibitors in a variety of in vitro models across various malignancies, including gastric carcinoma [196]. IAG933 is currently being studied in a phase I clinical trial in patients with advanced mesothelioma, hemangioendothelioma, neurofibromatosis 2/LATS1/LATS2 mutations, or functional YAP/TAZ fusions (NCT04857372). IK-930 is a TEAD1 inhibitor that targets palmitoylation and is in a clinical trial with advanced malignancies with neurofibromatosis 2 mutations or YAP/TAZ fusions, malignant pleural mesothelioma, or epithelioid hemangioendothelioma [197]. VT3989 is a TEAD inhibitor that targets palmitoylation and has shown promising phase I clinical trial data in malignant mesothelioma and other tumors with neurofibromatosis 2 mutations or YAP/TAZ gene alterations [198]. There was clinical activity with a 57% clinical benefit response rate (stable disease or partial response), adverse effect profile with reversible albuminuria, proteinuria and peripheral edema, and no dose-limiting toxicity. Clinical response for potential patients with gastric cancer enrolled in these trials has not yet been reported.

Clinically, drugs targeting the Hippo pathway are currently in the early stages of development, but there remains significant promise given the key role YAP/TEAD activation plays in gastric cancer and in several particularly aggressive preclinical models of DGC.

6 Conclusion

DGC is a clinically aggressive subtype of gastric cancer with inferior response to many of the currently approved clinical treatments compared with the IGC subtype. There are concerning rising rates of DGC in younger populations, females, and minorities. Multiomic studies have better characterized gastric adenocarcinoma into molecular subtypes and have identified unique molecular alterations for DGC, notably with *CDH1* and *RHOA* mutations and *CLDN18-ARHGAP26* fusions. Preclinical models of DGC generated through these alterations have provided additional insight into key mechanisms of pathogenesis and signaling pathway abnormalities and identified potential therapeutic vulnerabilities through targeting of the FAK and Hippo pathways. The first clinical trial to target DGC with FAK inhibition along with MAPK inhibition is ongoing. We are optimistic for other similar agents targeting these pathways to enter the clinical trial space.

There is considerable excitement for CLDN18.2-based and FGFR2b-directed therapeutics due to recent promising clinical trials showing clinical benefit in both DGC and IGC. CLDN18.2-based therapeutics are particularly versatile and can be utilized through several therapeutic mechanisms, including ADCs, bispecific antibodies, and CAR T-cells, which may enable greater effectiveness at lower levels of CLDN18.2 positivity. These therapeutics are in various stages of preclinical and clinical development and may potentially be more effective for DGC patients given higher rates of biomarker positivity. These preclinical and clinical advancements represent a promising future for the treatment of DGC.

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Consent to participate Not applicable.

Consent for publication All authors consent to the publication of this article.

Availability of data and material No datasets were generated or analyzed during this study.

Code availability Not applicable.

Authors' contributions LWW and RHM proposed the review topic. LWW wrote the original draft of this article. LF provided representative images of diffuse-type and intestinal-type gastric cancer. All authors have reviewed, revised, and provided critical feedback on the manuscript, and provided final approval of the manuscript.

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