

The dawn of precision medicine in diffuse-type gastric cancer

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Abstract: Gastric cancer (GC) is one of the most common malignancies worldwide. The histology- and morphology-based Lauren classification of GC has been widely used for over 50 years in clinical practice. The Lauren classification divides GC into intestinal and diffuse types, which have distinct etiology, molecular profiles, and clinicopathological features. Diffuse-type GC (DGC) accounts for approximately 30% of GCs. Tumor cells lack adhesion and infiltrate the stroma as single cells or small subgroups, leading to easy dissemination in the abdominal cavity. Clinically, DGC has aggressive traits with a high risk of recurrence and metastasis, which results in unfavorable prognosis. Although systemic chemotherapy is the main therapeutic approach for recurrent or metastatic GC patients, clinical benefits are limited for patients with DGC. Therefore, it is urgent to develop effective therapeutic strategies for DGC patients. Considerable research studies have characterized the molecular and genomic landscape of DGC, of which tight junction protein claudin-18 isoform 2 (CLDN18.2) and fibroblast growing factors receptor-2 isoform IIIb (FGFR2-IIIb) are the most attractive targets because of their close association with DGC. Recently, the impressive results of two phase II FAST and FIGHT trials demonstrate proof-of-concept, suggesting that anti-CLDN18.2 antibody (zolbetuximab) and FGFR2-IIIb antibody (bemarituzumab) are promising approaches for patients with CLDN18.2-positive and FGFR2-IIIb-positive GC, respectively. In this review, we summarize the clinicopathological features and molecular profiles of DGC and highlight a potential therapeutic target based on the findings of pivotal clinical trials.

Keywords: chemotherapy, claudin 18.2, diffuse-type, FGFR2, gastric cancer, Lauren classification

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Introduction

Gastric cancer (GC) remains a major clinical challenge. It is the fifth most common cancer and the third leading cause of cancer-related deaths worldwide.¹ The incidence of GC is high in East Asia, Eastern Europe, and South America. *Helicobacter pylori* infection is closely associated with the development of GC, and its eradication is effective in reducing GC incidence.² However, because of the lack of early clinical signs, GC is still frequently diagnosed at advanced stages that are not amenable to curative resection. For such patients, systemic chemotherapy is the main therapeutic option for prolonging survival and improving symptoms and quality of life.^{3,4}

The Lauren classification divides GC into intestinal and diffuse types based on cell histology and morphology and is clinically well used because of different phenotypes, responses to treatment, and prognoses.⁵ Diffuse-type GC (DGC) cells tend to scatter noncohesively into the stoma of the stomach and disseminate easily in the abdominal cavity.⁵ In addition, DGC cells have enhanced invasive abilities in the stomach wall and lymphatic vessel compared with intestinal-type GC (IGC) cells.⁶ Consequently, aggressive phenotypes of DGC result in poor survival outcomes *via* peritoneal dissemination or lymph node metastasis,^{6–8} and high recurrence frequency after curative surgery.⁹ DGC accounts for approximately 30%

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of GCs and is trending toward increasing prevalence.^{5,7} Eradication of *H. pylori* may induce an increased risk of developing DGC, in contrast to IGC.¹⁰ There is an urgent need to develop effective therapeutic strategies to overcome poor tumor cellularity in DGC.

Many molecular-targeted agents have failed to demonstrate significantly improved overall survival (OS) in clinical trials for patients with recurrent or metastatic GC, partially due to a lack of selective biomarkers and/or intratumoral heterogeneity. Currently, human epidermal growth factor receptor 2 (HER2),^{11,12} vascular endothelial growth factor receptor 2 (VEGFR2),^{13,14} and programmed death-1 (PD-1)^{15,16} are clinically validated targeted molecules in GC. However, these molecular-targeted agents may have limited clinical utility for patients with DGC because of the rare frequency of targeted molecule aberrations and weak efficacy. There is also less benefit from chemotherapy in DGC.¹⁷ An in-depth understanding of the complexity and diversity of molecular profiles will pave the way for establishing personalized molecular-targeted medicine for DGC patients.

Based on The Cancer Genome Atlas (TCGA) molecular classification, GC can be categorized into four subtypes: microsatellite instability (MSI), Epstein–Barr virus (EBV)-positive, chromosomal instability (CIN), and genomically stable (GS) tumors.¹⁸ GS tumors have frequent fusions of tight junction protein claudin-18 (CLDN18), and mutations of cadherin 1 (CDH1) or ras homolog family member A (RHOA), which mediates epithelial disintegration and diffuse-type phenotype.^{18–21} In addition, comprehensive molecular analyses demonstrate the aberration of fibroblast growing factor receptor-2 (FGFR2) as a critical molecule in DGC.¹⁸ Recently, promising results of anti-CLDN18 isoform 2 (CLND18.2) antibody, zolbetuximab, and anti-FGFR2 isoform IIIb (FGFR2-IIIb) antibody, bemarituzumab, were shown in phase II FAST²² and FIGHT²³ trials, respectively. Thus, CLDN18.2 and FGFR2-IIIb are relevant therapeutic targets and have attracted considerable attention as new hope for DGC patients.

In this review, we summarize the biology, molecular, and genetic landscape and clinical features of DGC, and highlight a potential therapeutic target based on the findings of pivotal clinical trials.

Clinicopathological and molecular features of diffuse-type gastric cancer

The histology- and morphology-based Lauren classification of GC has been widely used for 50 years.⁵ According to the Lauren classification, GC is mainly divided into two types (IGC and DGC), which have distinct etiology, clinicopathological features, and molecular profiles.

Clinicopathological features of diffuse-type gastric cancer

Clinicopathological features according to the Lauren classification are shown in Table 1. IGC is characterized by cohesive tumor cells with a glandular or intestinal structure, leading to an expanding growth pattern, while DGC is characterized by poorly cohesive tumor cells with few or no glandular structures, leading to a diffusely infiltrating growth pattern. DGC cells induce fibrosis and nestle within a rich fibrous stroma, which easily spreads not only along the stomach wall, but also in the upper layers of the stomach wall.^{24,25} In a meta-analysis of GC transcriptome data integrating 940 gastric transcriptomes, DGC showed higher stromal gene expression profiles associated with extracellular matrix biology and stromal cells than IGC, and DGC patients with high stromal profiles had more aggressive tumor biology and poor prognostic outcomes compared with those with low profiles.²⁶

DGC is more prevalent in younger, larger tumors that are infiltrating, proximal stomach predominant, advanced stage, advanced in depth of invasion, and poorly differentiated, scirrhous type stromal reactions, and lymphovascular invasions.^{7,27,41} Clinically, the two histology types of GC have different patterns of metastatic spread and prognostic outcomes. DGC tends toward peritoneal dissemination, lymph node metastasis, and direct extension into neighboring tissues, while IGC is associated with hematogenous metastasis including liver.^{6,9,38–40} DGC has a high risk of peritoneal recurrence, even after curative surgery.⁹ In a total of 20,218 patients from the Surveillance, Epidemiology, and End Results database, DGC patients had significantly less favorable cancer-specific survival compared with IGC patients, with a hazard ratio (HR) of 1.44 [95% confidence interval (CI), 1.38–1.50]. In a large cohort study of 3071 patients who underwent gastrectomy, DGC was an independent prognostic factor.⁷ A meta-analysis including 61,468 GC patients demonstrated similar unfavorable OS of patients with

Table 1. Clinicopathological features according to the Lauren classification.

Characteristics	Diffuse type	Intestinal type	Reference
Proportion	30%	70%	Lauren, ⁵ Chen <i>et al.</i> ⁷
Incidence trend	Increasing	Declining	Lauren, ⁵ Chen <i>et al.</i> ⁷
Environmental influence	Weak	Strong: <i>H. pylori</i> infection, gastric acid reflux, and lifestyle factors, such as salt intake, obesity, and smoking	Lordick and Janjigian ²⁷
Genetic factors	Strong: CDH1 germline mutations are associated with the development of hereditary diffuse gastric cancer.	Weak	Ajani <i>et al.</i> , ²⁴ Guilford <i>et al.</i> ²⁸
Age	Young	Elderly	Lee <i>et al.</i> ²⁹
Male/female ratio	1:1	2:1	Lee <i>et al.</i> ²⁹
Location of primary tumor	Proximal: Predominant in the middle third of the stomach	Distal: Predominant in the lower third of the stomach	Chen <i>et al.</i> , ⁷ Lee <i>et al.</i> ²⁹
Macroscopic growth pattern	Tumor spreads along the stomach wall	Tumor growth in the lumen	Lauren ⁵
WHO classification	Poorly cohesive	Papillary or tubular	Nagtegaal <i>et al.</i> ³⁰
Japanese classification	Poorly differentiated nonsolid adenocarcinoma or signet ring cell	Papillary adenocarcinoma or well to moderately differentiated tubular adenocarcinoma	Japanese Gastric Cancer Association ³¹
Carcinogenesis	Unknown: Development from morphologically normal gastric mucosa without atrophic gastritis	Well known: A sequence of multistep genetic and epigenetic alterations resulting from chronic inflammation	Chen <i>et al.</i> , ⁷ Ajani <i>et al.</i> , ²⁴ Cho <i>et al.</i> , ³² Uemura <i>et al.</i> ³³
Prognosis	Worse	Better	Zheng <i>et al.</i> , ⁶ Chen <i>et al.</i> , ⁷ Petrelli <i>et al.</i> ⁸
Glands	No: Lacked intercellular adhesion, with scattered cell morphology leading to the diffuse invasion growth pattern throughout the stroma	Yes: Glandular architecture leading to an expanding growth pattern	Lauren, ⁵ Ajani <i>et al.</i> ²⁴
Fibrosis	Marked: Tumor cell-induced rich fibrous stroma that reduces elasticity and provides a favorable mechano-environmental scaffold necessary for tumor progression	Not marked	Lauren, ⁵ Ajani <i>et al.</i> , ²⁴ Komuro <i>et al.</i> , ³⁴ Ng and Brugge ³⁵
Endocrine markers	Often: Neuroendocrine expression in PAS positive diffuse-type gastric cancer, especially marked in the signet ring cell subtype	Seldom	Bakkelund <i>et al.</i> , ³⁶ Waldum <i>et al.</i> ³⁷
TCGA molecular subtypes	GS	CIN	Cancer Genome Atlas Research Network ¹⁸
Metastasis	P, LYM	H	Zheng <i>et al.</i> , ⁶ Marrelli <i>et al.</i> , ⁹ Ajani <i>et al.</i> , ³⁸ Lee <i>et al.</i> , ³⁹ Wang <i>et al.</i> ⁴⁰

CDH1, cadherin 1; CIN, chromosomal instability; GS, genomically stable; H, hepatic metastasis; LYM, lymph node metastasis; P, peritoneal dissemination; PAS, Periodic Acid-Schiff; TCGA, The Cancer Genome Atlas; WHO, World Health Organization.

DGC *versus* IGC in all patients (HR, 1.23; 95% CI, 1.17–1.29) and in patients treated with adjuvant therapy only (HR, 1.27; 95% CI, 1.17–1.37).⁸ Collectively, DGC cells have aggressive traits and a high risk of recurrence and metastasis, which results in poor prognosis.

Molecular classification of diffuse-type gastric cancer

GC is a heterogeneous disease with diverse genetic and molecular levels, and four molecular subtypes (MSI, EVB, CIN, and GS tumors) were defined according to the results of comprehensive molecular analyses in the TCGA project.^{18,42} The GS subtype often shows a diffused cellular morphology due to the frequent loss of cell adhesion-related molecules *via* CLDN18-Rho GTPase-activating protein-6 or 26 (ARHGAP6, ARHGAP26) fusions (15% of GS subtype), mutations of CDH1 (37%), and RHOA (15%).^{18–21} In fact, DGC predominantly belongs to the GS subtype, and 73% of GS subtypes in the TCGA cohort were DGC.¹⁸ These genetic alterations are mutually exclusive, suggesting a contribution to carcinogenesis depending on cell adhesion-related signaling in DGC.

The Asian Cancer Research Group (ACRG) proposed four molecular subtypes according to the distinct molecular profiles and clinical outcomes using gene expression profiling, genome-wide copy number microarrays, targeted gene sequencing, and clinical information: MSI, microsatellite stable (MSS)/Tumor Protein P53 (TP53)-inactive, MSS/TP53-active, and MSS/epithelial-mesenchymal transition (EMT) tumors.⁴³ MSS/EMT tumors have similar features to the GS subtype in TCGA classification, associated with diffuse-type histology, lowest rate of mutations, early-onset, highest risk of recurrence, development of peritoneal carcinomatosis, and worst prognosis among the four ACRG subtypes. In fact, the majority (80%) of MSS/EMT tumors included DGC. However, there are substantial differences between the GS subtype in TCGA and MSS/EMT subtype in ACRG. Although 57% of DGCs were classified into the GS subtype in the TCGA cohort, only 27% of DGCs exhibited MSS/EMT subtype in the ACRG cohort, with distribution across all ACRG subtypes. In addition, mutations of CHD1 and RHOA were less common in the MSS/EMT subtype than in the GS subtype.

Lei *et al.* identified three subtypes with distinct biological properties and chemotherapy sensitivities

using a method of consensus hierarchical clustering with iterative feature selection: proliferative, metabolic, and mesenchymal tumors.⁴⁴ The mesenchymal subtype has features with cancer stem-like cells (CSCs) and is enriched in DGC. This subtype appears to have limited benefits from 5-fluorouracil (5-FU) chemotherapy. In a transcriptome analysis using the gene coexpression network, enriched mesenchymal stemness was a major driving force of DGC.⁴⁵

A growing number of studies have provided insight into the heterogeneity of DGC. A proteomic analysis showed three subtypes of DGC with distinct clinical outcomes based on the status of enriched immune response proteins and dysregulation in the cell cycle and EMT process.⁴⁶ A molecular signature of different prognostic subtypes of DGC was reported using RNA sequencing-based transcriptome data.⁴⁷ The intestinal-type-like subtype exhibited a signature associated with cell cycle or DNA repair, whereas the core diffuse-type subtype had poor prognosis and an enriched signature of EMT-associated functions, such as cell adhesion/migration and the TGF- β signaling pathway. In addition, DGC has a bimodal age distribution at diagnosis with early-onset and late-onset. Early-onset DGC has higher proportions of somatic mutations in CDH1 and TGF- β receptor 1 (TGFBR1) and lower proportions of RHOA mutations compared with late-onset DGC.⁴⁸

Collectively, although there is heterogeneity even among DGCs, most DGCs belong to the GS subtype in TCGA, MSS/EMT subtype in ACRG, and the mesenchymal subtype in Lei's classification.

Aberrant signaling pathway in diffuse-type gastric cancer

Typically, IGC develops in the background of atrophic gastritis and subsequent intestinal metaplasia through a sequence of complicated multi-step carcinogenesis.⁴⁹ Although the mechanism of developing DGC has not yet been fully understood, DGC may develop through a shorter, unidentified sequence of events from gastric epithelial cells.⁵⁰

A growing number of comprehensive genomic analyses of GC have shown that DGC exhibits different genetic and molecular profiles than IGC (Table 2 and Figure 1). In addition to mutations

Table 2. Molecular features according to the Lauren classification.

Molecule	Intestinal type	Diffuse type	Reference
HER2	Amp / OverExp (18–32%)	Amp / OverExp (0–7%)	Van Cutsem <i>et al.</i> , ⁵² Kurokawa <i>et al.</i> , ⁵³ Kataoka <i>et al.</i> , ⁵⁴ Kim <i>et al.</i> ⁵⁵
KRAS	Mt (5–12%)	Mt (1–5%)	Kakiuchi <i>et al.</i> , ²⁵ Hewitt <i>et al.</i> , ⁵⁶ Van Grieken <i>et al.</i> ⁵⁷
EGFR	Amp, OverExp (14–52%)	OverExp (4–30%)	Kim <i>et al.</i> , ⁵⁵ Park <i>et al.</i> , ⁵⁸ Nagatsuma <i>et al.</i> , ⁵⁹ Birkman <i>et al.</i> ⁶⁰
VEGF	OverExp (39–69%)	OverExp (16–43%)	Takahashi <i>et al.</i> , ⁶¹ Oh <i>et al.</i> ⁶²
mTOR	P-Exp (53–60%)	P-Exp (47–64%)	Lang <i>et al.</i> , ⁶³ Jung <i>et al.</i> ⁶⁴
HER3	OverExp (5–55%)	OverExp (26–61%)	Zhang <i>et al.</i> , ⁶⁵ Hayashi <i>et al.</i> ⁶⁶
MET	OverExp (10–35%), Amp (0–2%)	OverExp (8–40%), Amp (1–15%)	Kim <i>et al.</i> , ⁵⁵ Nagatsuma <i>et al.</i> , ⁵⁹ Peng <i>et al.</i> , ⁶⁷ Kawakami <i>et al.</i> , ⁶⁸ Lennerz <i>et al.</i> ⁶⁹
FGFR2	Amp (3–4%), OverExp (2–29%)	Amp (8–11%), OverExp (5–33%)	Ahn <i>et al.</i> , ⁷⁰ Park <i>et al.</i> , ⁷¹ Minashi <i>et al.</i> ⁷²
CDH1	LoEX, Mt (2%)	LoEX, Mt (33–56%), Meth (56%)	Kakiuchi <i>et al.</i> , ²⁵ Birkman <i>et al.</i> , ⁶⁰ Machado <i>et al.</i> ⁷³
RhoA	Mt (0%)	Mt (15–25%)	Cancer Genome Atlas Research Network, ¹⁸ Kakiuchi <i>et al.</i> , ²⁵ Wang <i>et al.</i> ⁷⁴
ARID1A	Mt (12–16%)	Mt (9–15%)	Kakiuchi <i>et al.</i> , ²⁵ Wu <i>et al.</i> , ⁷⁵ Garcia-Pelaez <i>et al.</i> ⁷⁶
Claudin 18.2	OverExp (15–46%)	OverExp (28–75%)	Sahin <i>et al.</i> , ⁷⁷ Dottermusch <i>et al.</i> , ⁷⁸ Pellino <i>et al.</i> , ⁷⁹ Coati <i>et al.</i> ⁸⁰
MSI-H /dMMR	4–10%	0–4%	Kim <i>et al.</i> , ⁵⁵ Birkman <i>et al.</i> , ⁶⁰ Zhang <i>et al.</i> ⁸¹
EBV	1–9%	0–4%	Kim <i>et al.</i> , ⁵⁵ Birkman <i>et al.</i> , ⁶⁰ Zhang <i>et al.</i> ⁸¹
PD-L1*	OverExp (28–65%)	OverExp (19–54%)	Fukamachi <i>et al.</i> , ⁸² Liu <i>et al.</i> , ⁸³ Gu <i>et al.</i> ⁸⁴

Amp, amplification; CDH1, cadherin 1; dMMR, deficient mismatch repair; EBV, Epstein-Barr virus; EGFR, endothelial growth factor receptor; FGFR2, fibroblast growing factors receptor-2; HER3, human epidermal growth factor receptor 3; LoEX: low expression; Meth: methylation; MSI-H, microsatellite instability-high; Mt: mutation; mTOR, mammalian target of the rapamycin; OverExp: overexpression; PD-1, programmed death-1; P-Exp: phospho-expression; VEGF, vascular endothelial growth factor.

*The varied percentage of the PD-L1 positive expression was due to the different definitions of PD-L1 positivity and the cutoff values.

of genes associated with cell adhesion, cytoskeleton, and cell motility, including CDH1, RHOA, and Rho-associated coiled-coil-containing protein kinase (ROCK),^{18–21,51} multiplex profiling of peritoneal metastases from GC showed that DGC had high rates of heterozygous deletion of chromosome 3p, which encompasses multiple tumor

suppressor genes, and duplication of 20q, which encompasses oncogenes.⁴⁰ Furthermore, DGC has frequent dysregulation of signaling pathways associated with hallmarks of cancer, including G2/M cell cycle checkpoint, mitotic spindle assembly, MYC, and inflammatory response. In a comparative study between signet ring cell

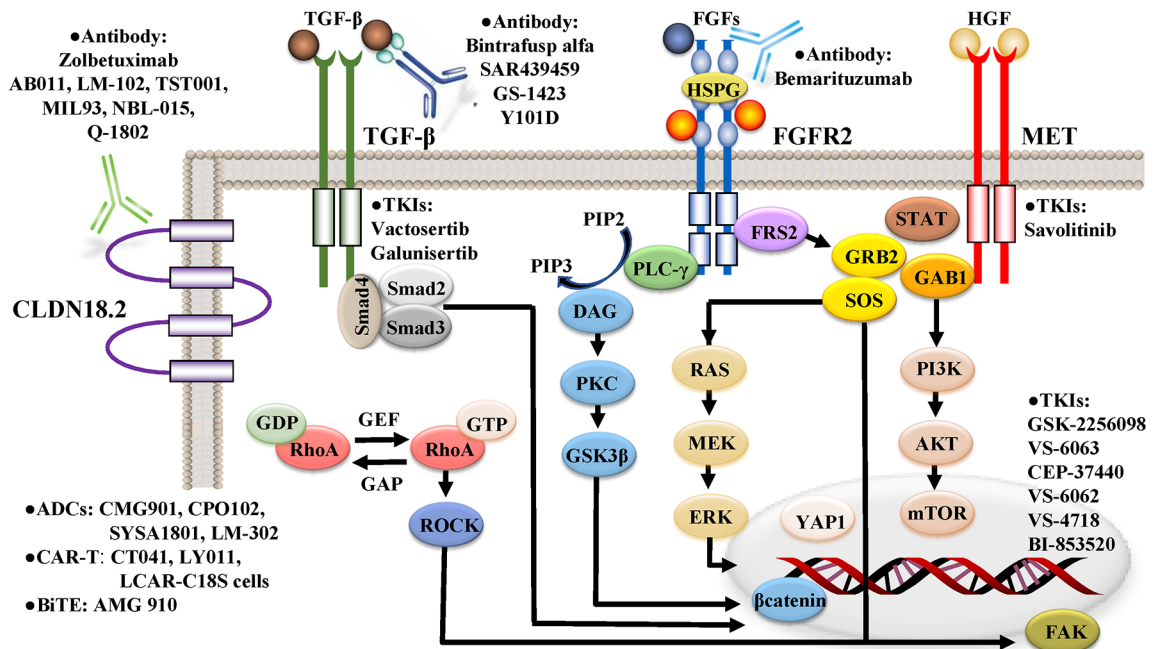


Figure 1. Pivotal signaling pathways, including CLDN18.2, TGF- β , FGFR2, and MET, in DGC.

MET and FGFR2 are members of the RTK family. Binding the ligands, such as hepatocyte growth factor (HGF) and fibroblast growth factors (FGFs), to the RTK receptor leads to cytoplasmic tyrosine phosphorylation, resulting in the activation of MAPK and PI3 K-AKT signaling *via* the recruitment of various effector molecules, such as growth factor receptor-bound 2 (GRB2), GRB2-associated binding protein 1 (GAB1) son of sevenless (SOS), and phospholipase C- γ (PLC- γ). FGFR2 activates not only MAPK and PI3 K-AKT signaling *via* FGFR substrate 2 (FRS2) as an adaptor protein, but also FRS2-independent cascades, including protein kinase C (PKC)-glycogen synthase kinase 3 β (GSK3 β)- β -catenin axis, Janus kinase (JAK)-signal transducer and activator of transcription (STAT) axis, and c-Jun-Yes-associated protein1 (YAP1) axis. Activated ras homolog family member A (RhoA) interacts with downstream effectors, including Rho-associated, coiled-coil-containing protein kinase (ROCK), and focal adhesion kinase (FAK). CLDN18.2 may interact with the RhoA signaling pathway. YAP1 is activated by RhoA signaling, in addition to the Hippo and FGFR2 signaling pathways. The canonical TGF- β signaling pathway involves ligand-dependent assembly of a heteromeric receptor complex and subsequent accumulation of Smad proteins as transcriptional regulators in the nucleus. The mammalian target of the rapamycin (mTOR) pathway is frequently dysregulated in DGC *via* overexpression of phosphorylated mTOR and PI3 K-AKT signaling activated by PTEN loss and RTKs, such as MET and FGFR2. ADCs, antibody-drug conjugates; BiTE, bispecific T cell engager; CAR-T, chimeric antigen receptor T; DAG, diacylglycerol; GAP, GTPase-activating protein; GEF, guanine nucleotide exchange factor; HSPG, heparan sulfate proteoglycan; IP3, inositol-1,4,5-trisphosphate; PIP2, phosphatidylinositol-4,5-bisphosphate; TKIs, tyrosine kinase inhibitors.

phenotype and nonsignet ring cell phenotype among DGCs, the nonsignet ring cell phenotype had more frequent mutations of TP53, BRAF, phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha (PIK3CA), SMAD Family Member 4 (SMAD4), and RHOA, supporting heterogeneity of DGC.³⁰

CDH1 is a tumor suppressor gene that codes for E-cadherin, which is a calcium-dependent transmembrane glycoprotein that forms the adherens junction *via* the extracellular domains, connects to the cytoskeleton *via* the cytoplasmic domains, and mediates cellular signaling.⁸⁵ Loss of its function confers diffused cellular morphology, migratory and invasive abilities, metastasis, and the EMT process.^{85,86} In DGC, E-cadherin

expression is predominantly downregulated *via* somatic mutations or promoter hypermethylation of CDH1,^{18,19,40} and the loss of its expression is associated with an increased risk of early invasion and metastasis.⁸⁷ CDH1 germline mutations have been identified in patients with hereditary DGC,²⁸ and abnormal expression of E-cadherin occurs even in early gastric carcinomas,⁸⁸ suggesting that loss of CDH1 function is acquired early in the pathogenesis of DGC.

RHOA mutations predominantly occur in DGC, ranging from 15% to 25%,^{18,25,74} and the mutant RHOA acts as a gain-of-function.²⁵ The GS subtype exhibits fusions of ARHGAP6/26, which regulates RhoA activity as GTPase-activating proteins.^{18,20,21} Approximately 30% of GS subtypes

have aberrant alterations in the components of the Rho signaling pathway. Activated RhoA signaling pathway regulates the actin cytoskeleton, cell migration, cytokinesis, the cell cycle, resistance to anoikis, and CSC phenotype with invasive ability and chemoresistance.^{25,74,89,90} In a mouse model, RHOA Y42 C, the most common RHOA hotspot mutation in DGC, coupled with loss of CDH1, induces metastatic DGC *via* actin cytoskeletal rearrangements and activation of Yes-associated protein1 (YAP1), phosphoinositide 3-kinase (PI3 K)-AKT, and β -catenin signaling pathways.⁹⁰ In 288 GC patients who underwent curative surgery, increased RHOA activity was an independent prognostic factor of OS in DGC patients, but not in IGC patients.⁸⁹ Thus, the aberrant activity of the RHOA signaling pathway has been implicated in the development and aggressive phenotype of DGC.

MET is a member of the RTK family and plays a key role in tumor cell proliferation, migration, invasion, survival, angiogenesis, and metastasis.⁹¹ In a subset of GC, the MET signaling pathway is activated *via* the overexpression and gene amplification of MET.⁹² The positivity rate of MET overexpression varies from 6% to 39% of GC,^{93,67} and MET gene amplification has been found in 2% to 7% of GC,^{67,68,94,95} which confers an unfavorable prognosis.⁹⁶ MET amplification is predominantly enriched in DGC compared with IGC,^{67,68,96,97} and is observed in both primary GC tissues and their corresponding cancer cells from malignant ascites. Thus, MET-amplified GC cells may have intrinsic potential for peritoneal dissemination, which is a major metastatic pattern of DGC.^{38,39}

TGF- β is a multifunctional cytokine associated with tumor progression.⁹⁸ Approximately half of the GS subtype showed activation of the TGF- β signaling pathway in the TCGA cohort.⁹⁹ DGC is characterized by a rich fibrous stroma³⁴ and exhibits a high stromal super module expression with elevated levels of the TGF- β pathway in a comprehensive genomic meta-analysis using a network-modeling approach.²⁶ TGF- β ligands secreted by tumor cells alter normal fibroblasts to a myofibroblast-like phenotype, promoting tumor growth, vascularization, and metastasis.¹⁰⁰ Furthermore, activation of the TGF- β pathway has been observed in cancer-associated fibroblasts (CAFs) isolated from human DGCs, and the highly motile CAFs induced DGC with

invasive abilities into the extracellular matrix and lymphatic vessels.¹⁰¹ Thus, TGF- β signaling may act as a key indicator of a protumorigenic stromal environment in DGC.⁹⁸

Biological features of diffuse-type gastric cancer

DGC is often classified into mesenchymal subtype with features of CSC in Lei's classification,⁴⁴ MSS/EMT subtype in ACRG cohort,⁴³ and GS subtype characterized by activation of RHOA, TGF- β , and Wnt pathways in TCGA classification,^{18,99} which have been implicated in the induction and maintenance of EMT and CSC phenotypes.^{89,102,103} These molecular subtypes support the association of DGC with EMT and CSC phenotypes. EMT is a cellular process of redifferentiation of epithelial cells into mesenchymal ones, which is implicated in embryogenesis, wound healing, carcinogenesis, and cancer progression.¹⁰³ In a clustering analysis of the TCGA cohort using an EMT gene set, the cluster with active EMT had an enriched TGF- β signaling pathway and MET amplification, which consisted of a high proportion (59%) of DGCs.¹⁰⁴ Importantly, EMT programs facilitate gastric CSC generation and expansion.^{105,106} CSCs are a relatively rare population of cancer cells that contribute to the driving force of tumorigenesis and metastasis due to their cancer stemness properties, including sphere formation, self-renewal, invasion, differentiation, antiapoptosis, immune evasion, and drug resistance.^{102,107,108} Thus, aggressive phenotypes of DGC, including chemoresistance, invasive and metastatic abilities, and poor survival outcomes, may be partially explained by the biological features of EMT and CSC phenotypes.

YAP1 is a downstream transcription coactivator of the Hippo signaling pathway, which acts not only as a prominent molecule for tumorigenesis, but also as an oncogenic driver in GC.^{109–113} YAP1 plays a crucial role in CSC expansion and properties in various tumor types, including GC.^{107,114,115} In fact, genetic knockdown of YAP1 has been found to suppress gastric CSC traits, tumorigenesis, and peritoneal metastasis *in vitro* and *in vivo*.¹¹⁶ The Hippo pathway was also activated in the cluster with enriched EMT-related signatures in a comprehensive multiomic analysis of malignant ascites samples and their corresponding GC cell lines.¹⁰⁴ Thus, YAP1 is a promising target for gastric CSCs.

Treatment of patients with diffuse-type gastric cancer

Systemic chemotherapy includes three types of treatment: (1) cytotoxic agents, including fluoropyrimidines, platinum compounds, taxanes, trifluridine/tipiracil, and irinotecan; (2) immune checkpoint inhibitors (ICIs) targeting PD-1; and (3) molecular-targeted agents for HER2 or VEGFR2. Although there are different clinicopathological and molecular features in DGC and IGC, the same approach of treatment with systemic chemotherapy is used, regardless of histology types. In this section, the clinical efficacy of individual chemotherapy agents is described according to histology types.

Cytotoxic chemotherapy

The impact of cytotoxic chemotherapy on DGC has been evaluated in a few clinical trials and several exploratory analyses (Table 3). Generally, DGC is associated with low responsiveness to cytotoxic chemotherapy and chemoradiation, but the mechanisms of resistance are not yet understood. The adenosine triphosphate (ATP)-binding cassette (ABC) transporters confer multidrug resistance to tumor cells through an increased efflux of chemotherapy agents, and overexpression of ABC transporters has been reported predominantly in CSCs.¹¹⁷ In a large-scale meta-analysis of GC transcriptome data, the negative association between stroma and cell proliferation in DGC relevant to a rich fibrous stroma may contribute to resistance to chemotherapy targeting active dividing cells. In addition, a high proportion of intratumoral stroma may directly inhibit the effects of chemotherapy by reducing drug delivery to tumor cells¹¹⁸ and protecting cells against chemotherapy-induced apoptosis.¹¹⁹

Palliative chemotherapy. Combination of fluoropyrimidine with platinum is a standard first-line backbone regimen in GC, regardless of histology type.^{3,4} In real-world data, including 1303 patients who received chemotherapy, from the AGAMENON national registry research group, DGC patients showed decreased overall response rate (ORR) and increased mortality.¹³³ In a meta-analysis of 33 studies comprising 10,246 patients (4888 patients for IGC and 5358 patients for DGC) treated with chemotherapy, chemotherapy showed significantly improved OS in IGC patients compared with DGC patients (HR, 0.76; 95% CI, 0.71–0.82; $p < 0.001$).¹⁷ Similar results were observed, even when subgroup analyses were

performed according to the regimen of first-line chemotherapy. Among platinum compounds, oxaliplatin-based chemotherapy may be more efficacious than cisplatin-based chemotherapy for DGC patients.^{130,131}

In a second-line setting, including 2311 patients in the AGAMENON group, DGC was also more refractory to chemotherapy than IGC.¹³⁴ In a salvage-line setting, a phase III TAGS trial evaluated the efficacy of perioperative trifluridine/tipiracil *versus* the best supportive care in 507 patients with refractory GC treatment and demonstrated more improved survival outcomes in IGC compared with DGC.¹³⁵ Collectively, DGC is associated with a poor prognosis and less sensitivity to chemotherapy.

Adjuvant/perioperative chemotherapy. In a large cohort study of 1290 locally advanced GC patients who underwent either primary surgery or preoperative chemotherapy followed by surgery, preoperative chemotherapy resulted in a deterioration of survival outcomes compared with primary surgery in DGC patients, in contrast to IGC patients, who had improved survival rates with preoperative chemotherapy.¹³⁶ Similarly, another large cohort study that assessed the survival impact of perioperative chemotherapy *versus* primary surgery in 924 patients with signet ring cell GC showed the detrimental effect of perioperative chemotherapy.¹³⁷ Thus, inherent chemoresistance may cause DGC patients to miss a chance of curative resection by delaying surgery through preoperative chemotherapy, especially in patients with signet ring cell GC. A phase II/III FREGAT trial (PRODIGE-19-FFCD1103-ADCI002), which compared perioperative chemotherapy (epirubicin, cisplatin, and 5-FU) with primary surgery followed by adjuvant chemotherapy, was conducted to elucidate whether primary surgery was a potential option for patients with resectable signet ring cell GC.¹³⁸ In phase II, the 2-year OS rates as a primary endpoint were met (60% in primary surgery and 54% in perioperative chemotherapy), and curative resection rates were 88% and 78%, respectively.¹³⁹ Currently, phase III is ongoing.

Immune therapy

Antitumor immune escape is often promoted by inhibitory immune checkpoint molecules, such as PD-1 and its ligand 1 (PD-L1), during the cancer-immunity cycle process.¹⁴⁰ PD-1 receptors on T cells bind to PD-L1, and the activated PD-1/

Table 3. Results of phase III trials of cytotoxic agents according to histology types.

Trials	Line	Phase	Treatment	No. of DGC (%)	HR (95% CI) of OS in DGC	HR (95% CI) of OS in IGC	Reference
FLOT4-AIO	Peri	III	Peri FLOT <i>versus</i> ECF	191 (26.7)	0.85 (N/A)	0.75 (N/A)	Al-Batran <i>et al.</i> ¹²⁰
PRODIGY	Peri/adj	III	Pre DOS and adj S-1 <i>versus</i> Adj S-1	189 (63.0)	0.81 (0.48–1.37)*	0.38 (0.16–0.88)*	Kang <i>et al.</i> ¹²¹
RESOLVE	Peri/adj	III	Peri SOX <i>versus</i> Adj CAPOX	344 (50.4)	0.70 (0.50–0.96)*	0.90 (0.64–1.29)*	Zhang <i>et al.</i> ¹²²
RESOLVE	Adj	III	SOX <i>versus</i> CAPOX	344 (50.2)	0.82 (0.59–1.12)*	0.89 (0.64–1.25)*	Zhang <i>et al.</i> ¹²²
ACTS-GC	Adj	III	S-1 <i>versus</i> Surgery alone	608 (59.0)	0.67 (0.51–0.89)	0.67 (0.48–0.94)	Sakuramoto <i>et al.</i> ¹²³
JACCRO GC-07	Adj	III	DTX plus S-1 <i>versus</i> S-1	544 (59.6)	0.79 (0.61–1.02)	0.62 (0.40–0.94)	Yoshida <i>et al.</i> ¹²⁴
ARTIST	Adj	III	XP + RT <i>versus</i> XP	274 (59.8)	0.83 (0.54–1.26)	0.44 (0.23–0.85)	Park <i>et al.</i> ¹²⁵
ARTIST-2	Adj	III	S-1/SOX <i>versus</i> SOX + RT	260 (61.2)	1.00 (0.65–1.54)	1.32 (0.69–2.50)	Park <i>et al.</i> ¹²⁶
ARTIST-2	Adj	III	SOX/SOX + RT <i>versus</i> S-1	260 (61.2)	0.81 (0.54–1.24)	0.45 (0.25–0.80)	Park <i>et al.</i> ¹²⁶
SPIRITS	1st	III	SP <i>versus</i> S-1 alone	192 (64.6)	0.79 (0.59–1.06)	0.76 (0.50–1.14)	Koizumi <i>et al.</i> ¹²⁷
FLAGS	1st	III	SP <i>versus</i> FP	590 (57.3)	0.83 (0.70–0.99)	N/A	Ajani <i>et al.</i> ¹²⁸
DIGEST	1st	III	SP <i>versus</i> FP	361 (100)	0.99 (0.76–1.28)	–	Ajani <i>et al.</i> ³⁸
JCOG1013	1st	III	DCS <i>versus</i> CS (SP)	241 (65.1)	0.98 (0.81–1.18)	1.06 (0.81–1.39)	Yamada <i>et al.</i> ¹²⁹
G-SOX	1st	III	SOX <i>versus</i> SP	351 (54.8)	0.85 (0.67–1.07)	1.09 (0.83–1.42)	Yamada <i>et al.</i> ¹³⁰
SOX-GC	1st	III	SOX <i>versus</i> SP	362 (65.6)	0.71 (0.57–0.90)	–	Xu <i>et al.</i> ¹³¹
TAGS	3rd	III	TAS102 <i>versus</i> BSC	74 (29.5)	0.69 (0.36–1.31)	0.58 (0.39–0.87)	Taberbero <i>et al.</i> ¹³²

Adj, adjuvant chemotherapy; BSC, best supportive care; CAPOX, capecitabine + oxaliplatin; CI, confidence interval; DCS, docetaxel + cisplatin + S-1; DGC, diffuse-type gastric cancer; DOS, docetaxel + oxaliplatin + S-1; DTX, docetaxel; ECF, epirubicin + cisplatin + fluorouracil; FLOT, fluorouracil + leucovorin + oxaliplatin + docetaxel; FP, 5-fluorouracil + cisplatin; HR, hazard ratio; IGC, intestinal-type gastric cancer; N/A: not assessment; OS, overall survival; Peri, perioperative chemotherapy; Pre, preoperative chemotherapy; SOX, S-1 + oxaliplatin; SP (CS), S-1 + cisplatin; TAS102, Trifluridine/tipiracil; XP, capecitabine + cisplatin.

*Disease-free survival (DFS).

PD-L1 signaling axis induces immune tolerance in the tumor microenvironment by disrupting the functioning of both cytotoxic and effector T cells.^{141,142} Currently, ICIs targeting PD-1/PD-L1 have dramatically changed therapeutic paradigms because of the durable clinical response in GC. However, the exploratory analyses of clinical trials suggest a poor response to treatment with ICIs in DGC (Table 4).

From the antitumor immunogenic perspective, according to the molecular subtypes, DGC has several negative factors of sensitivity to ICIs. GS

tumors exhibit lower PD-L1 expression, a lower tumor mutation burden that confers production of immunogenic neoantigens, and a lesser degree of immune cell signaling pathway as a tumor immunogenicity compared with MSI and EBV tumors,¹⁸ leading to antitumor immune tolerance and evasion. In fact, DGC exhibits frequent heterozygous deletion of chromosome 9p24 involving PD-L1,⁴⁰ and consequently, low frequency of PD-L1 expression.^{157–159} Expression of major histocompatibility complex (MHC) class II is also low in DGC, which impairs antigen presentation.¹⁶⁰ In addition, DGC is associated with a mesenchymal-like

Table 4. Results of phase II/III trials of molecular-targeted agents according to histology types.

Trial	Line	Phase	Target	Agent	Treatment	No. of DGC (%)	HR (95% CI) of OS in DGC	HR (95% CI) of OS in IGC	Reference
ToGA	1st	III	HER2	Tmab	XP or FP ± Tmab	51 (8.8)	1.07 (0.56–2.05)	0.69 (0.54–0.88)	Bang <i>et al.</i> ¹¹
TRIO-013 /LOGIC	1st	III	HER2	Lapa	CAPOX ± Lapa	19 (3.9)	0.64 (0.25–1.65)	0.93 (0.75–1.17)	Hecht <i>et al.</i> ¹⁴³
JACCOB	1st	III	HER2	Pertu	XP/FP plus Tmab ± Pertu	39 (5.0)	0.82(0.40–1.69)	0.85 (0.71–1.02)	Taberero <i>et al.</i> ¹³²
GATSBY	2nd	III	HER2	TDM-1	PTX or DTX ± TDM-1	55 (28.7)	0.91(0.43–1.92)	1.24 (0.77–2.00)	Thuss-Patience <i>et al.</i> ¹⁴⁴
DESTINY G01	3rd	II	HER2	T-DXd	PTX or CPT-11 versus T-DXd	46 (26.6)	0.38 (0.17–0.86)	0.65 (0.39–1.07)	Shitara <i>et al.</i> ¹²
EXPAND	1st	III	EGFR	Cetu	XP ± Cetu	170 (32.0)	1.44(1.01–2.03)	0.90 (0.69–1.16)	Lordick <i>et al.</i> ¹⁴⁵
RILOMET-1	1st	III	MET	Rilo	EOX ± Rilo	129 (41.0)	1.24(0.83–1.84)	0.88 (0.58–1.33)	Catenacci <i>et al.</i> ⁹⁴
METGastric	1st	III	MET	Onar	FOLFOX ± Onar	131 (49.8)	0.82(0.37–1.81)	1.23 (0.56–2.72)	Shah <i>et al.</i> ¹⁴⁶
GRANITE-1	2nd	III	mTOR	Evero	placebo versus Evero	130 (49.6)	0.95(0.62–1.47)	1.02 (0.67–1.55)	Ohtsu <i>et al.</i> ¹⁴⁷
GOLD	2nd	III	PARP	Olap	Placebo versus Olap	230 (51.5)	0.80 (0.60–1.07)	0.87 (0.62–1.20)	Bang <i>et al.</i> ¹⁴⁸
RAINFALL	1st	III	VEGFR2	Ram	XP ± Ram	271 (42.3)	1.15 (0.87–1.51)	0.80 (0.58–1.12)	Fuchs <i>et al.</i> ¹⁴⁹
RAINBOW	2nd	III	VEGFR2	Ram	PTX ± Ram	248 (37.3)	0.86 (0.64–1.15)	0.71 (0.53–0.93)	Wilke <i>et al.</i> ¹⁴
REGARD	2nd	III	VEGFR2	Ram	Placebo versus Ram	140 (39.4)	0.56 (0.37–0.86)	1.01 (0.58–1.75)	Fuchs <i>et al.</i> ¹³
AVAGAST	1st	III	VEGFA	Bev	XP/FP ± Bev	382 (34.3)	0.83 (0.65–1.06)	N/A	Shah <i>et al.</i> ¹⁵⁰ Ohtsu <i>et al.</i> ¹⁵¹
CheckMate 649*	1st	III	PD-1	Nivo	CAPOX or FOLFOX ± Nivo	281 (17.8)	0.96 (N/A)	0.76 (N/A)	Janjigian <i>et al.</i> ¹⁵² Moehler <i>et al.</i> ¹⁵³
ATTRACTION-4	1st	III	PD-1	Nivo	SOX or CAPOX ± Nivo	368 (53.8)	0.99 (N/A)	0.87 (N/A)	Boku <i>et al.</i> ¹⁵⁴
KEYNOTE-062	1st	III	PD-1	Pembro	FP or XP ± Pembro	195 (43.9)	0.59 (0.43–0.82)	0.94 (0.69–1.27)	Shitara <i>et al.</i> ¹⁵⁵
KEYNOTE-062	1st	III	PD-1	Pembro	Pembro versus FP or XP	200 (45.0)	0.73 (0.53–0.99)	0.92 (0.67–1.25)	Shitara <i>et al.</i> ¹⁵⁵
KEYNOTE-061	2nd	III	PD-1	Pembro	PTX versus Pembro	91 (53.5)	0.88 (0.54–1.45)	0.66 (0.40–1.11)	Shitara <i>et al.</i> ¹⁵⁶
ATTRACTION-2	3rd	III	PD-1	Nivo	Placebo versus Nivo	169 (46.1)	0.82 (0.57–1.17)	0.59 (0.41–0.85)	Kang <i>et al.</i> ¹⁵
FAST	1st	II	Claudin 18.2	Zolbe	EOX ± Zolbe	72 (49.3)	0.44 (0.26–0.74)	0.73 (0.41–1.30)	Sahin <i>et al.</i> ²²
FIGHT	1st	II	FGFR2-IIIb	Bema	FOLFOX ± Bema	N/A	N/A	N/A	Wainberg <i>et al.</i> ²³

Bema, Bemarituzumab; Bev, Bevacizumab; CAPOX, capecitabine + oxaliplatin; Cetu, Cetuximab; CI, confidence interval; CPT-11, irinotecan; DGC, diffuse-type gastric cancer; DTX, docetaxel; EGFR, endothelial growth factor receptor; EOX, epirubicin + oxaliplatin + capecitabine; Evero, Everolimus; FGFR2, fibroblast growth factors receptor-2; FOLFOX, fluorouracil + leucovorin + oxaliplatin; FP, 5-fluorouracil + cisplatin; HER2, human epidermal growth factor receptor 2; HR, hazard ratio; IGC, intestinal-type gastric cancer; Lap, Lapatinib; mTOR, mammalian target of the rapamycin; N/A, not assessment; Nivo, Nivolumab; Olap, Olaparib; Onar, Onartuzumab; OS, overall survival; PAMP, poly ADP-ribose polymerase; PD-1, programmed death-1; Pembro, Pembrolizumab; Pertu, Pertuzumab; PTX, paclitaxel; Ram, Ramucirumab; Rilo, Rilutumab; SOX, S-1 + oxaliplatin; Tmab, Trastuzumab; TDM-1, Trastuzumab emtansine; T-DXd, Trastuzumab deruxtecan; VEGFA, vascular endothelial growth factor A; VEGFR2, vascular endothelial growth factor receptor 2; XP, Capecitabine + cisplatin; Zolbe, Zolbetuximab.

*Analysis of population of signet ring cell carcinoma.

phenotype,^{43–45} resulting in immune exhaustion due to the high expression of immune checkpoint T-cell immunoglobulin mucin receptor 3 (TIM3), its ligand galectin-9, another immune checkpoint V-domain Ig suppressor of T-cell activation (VISTA), and TGF- β 1.⁴⁰ In the tumor microenvironment in DGC, there are low levels of tumor-infiltrating lymphocytes (TILs)¹⁵⁹ and frequent dysfunction of intratumoral CD8+ T cells.¹⁶¹ Recently, multiplex profiling of the immune and stromal cell composition from peritoneal metastasis tissues has shown a distinct tumor microenvironment with lower levels of cytotoxic lymphocytes, monocytes, natural killer (NK) cells, and myeloid dendritic cells in DGC compared with IGC.⁴⁰ As other key components of the tumor microenvironment in DGC, abundant M2 tumor-associated macrophages (TAMs) play a pivotal role in promoting immunosuppressive signals.^{46,162} Collectively, DGC may be a ‘cold tumor’ with low TILs and low PD-L1 expression,¹⁴² and the immunologically ignorant phenotype may have a poor response to ICIs. Therefore, novel treatment strategies to turn immunologically ‘cold’ tumors with poor immune activation into ‘hot’ tumors with strong immune infiltration are needed.

Molecular-targeted therapy

Based on the comprehensive molecular profiling of GC, many molecular-targeted agents have been developed. Unfortunately, most have failed to demonstrate treatment efficacy regardless of histology type in clinical trials (Table 4). Currently, clinically validated targeted molecules are HER2 and VEGFR2 in GC.

HER2 monoclonal antibody trastuzumab, in combination with chemotherapy, is now a preferred first-line treatment regimen for patients with HER2-positive GC.^{3,4} However, in a phase III ToGA trial, the survival benefit appeared to be limited in DGC patients (HR, 1.07; 95% CI, 0.56–2.05) in contrast to IGC patients (HR, 0.69; 95% CI, 0.54–0.88).¹¹ Recently, trastuzumab deruxtecan, an antibody-drug conjugate consisting of an anti-HER2 and a cytotoxic topoisomerase I inhibitor, showed remarkable improvement in ORR and OS compared with the physician’s choice of standard chemotherapy in a phase II DESTINY-Gastric01 trial.¹² A greater benefit for OS was found in DGC (HR, 0.38; 95% CI, 0.17–0.86) than in IGC (HR, 0.65; 95% CI, 0.39–1.07), along with that of ORR in DGC (66.7% *versus* 0%) compared with IGC (47.8%

versus 21.6%). Trastuzumab deruxtecan may be effective even for DGC, which is characterized by a scattered growth pattern⁵ and a high incidence of intratumoral HER2 heterogeneity,¹⁶³ *via* a potent bystander effect due to a highly membrane-permeable payload.¹⁶⁴ However, the rate of HER2 positivity is rare in DGC, and the positive rates were 6.1% and 31.8% in DGC and IGC, respectively,⁵² suggesting the limited benefits of HER2-targeted therapy in DGC patients.

Angiogenesis contributes to the progression of gastric tumorigenesis and metastasis by providing nutrition, growth factors, and an oxygen supply.¹⁶⁵ VEGF and its receptor VEGFR are one of the molecules responsible for angiogenesis, and functional genomic analysis has shown notably high expression of angiogenesis-related genes in DGC.⁴⁵ However, the dependency on angiogenesis is likely to be higher in IGC than in DGC.¹⁶⁶ Anti-VEGFR2 monoclonal antibody ramucirumab is the first molecular-targeted agent with survival benefits as a monotherapy in GC, as demonstrated in a phase III REGARD trial of ramucirumab *versus* placebo as a second or latter line in 355 GC patients.¹³ Although the OS benefit of ramucirumab was better in DGC patients than in IGC patients, the PFS benefit was similar between DGC (HR, 0.49; 95% CI, 0.32–0.75) and IGC patients (HR, 0.46; 95% CI, 0.27–0.78). In a phase III RAINBOW trial that established ramucirumab plus paclitaxel over paclitaxel alone as a standard second-line regimen,¹⁴ the addition of ramucirumab showed less benefit in DGC than IGC in terms of PFS (HR, 0.70; 95% CI, 0.52–0.93 *versus* HR, 0.53; 95% CI, 0.41–0.69) and OS (HR, 0.86; 95% CI, 0.64–1.15 *versus* HR, 0.71; 95% CI, 0.53–0.93). Similar findings were found in a phase III RAINFALL trial, which assessed whether the addition of ramucirumab to first-line chemotherapy prolonged OS in 645 patients.¹⁴⁹ These preclinical and clinical data suggest a lower efficacy of ramucirumab in DGC patients compared with IGC patients.

MET gene amplification is associated with DGC, and several MET inhibitors have been investigated in clinical trials. Onartuzumab is a fully humanized, monovalent monoclonal antibody. A randomized phase III METGastric trial of onartuzumab *versus* placebo in combination with first-line chemotherapy in MET expression-positive GC patients, assessed by immunohistochemistry (IHC), was conducted.¹⁴⁶ Enrollment was stopped early due to a lack of efficacy, and the

Table 5. Results of phase II FAST and FIGHT trials.

Phase	FAST ²²				FIGHT ^{23,168}			
	II		II		II		II	
Inclusion	CLDN18.2 positive		≥70% tumor cells with CLDN18.2		FGFR2b positive		≥10% tumor cells with FGFR2b overexpression	
Treatment	E0X	E0X + Zolbe	E0X	E0X + Zolbe	mFOLFOX + placebo	mFOLFOX + Bema	mFOLFOX + placebo	mFOLFOX + Bema
Number of patients	84	77	59	57	78	77	52	44
ORR (%)	25.0	39.0	-	-	33.0	44.0	-	-
DCR (%)	76.2	83.1	-	-	96.6	96.2	-	-
DOR (months)	5.4	8.2	-	-	7.1	12.2	-	-
PFS								
Median (months)	5.3	7.5	5.7	9.0	7.4	9.5	7.3	14.1
HR (95% CI)	0.44 (0.29–0.67)		0.38 (0.23–0.62)		0.68 (0.44–1.04)		0.44 (0.25–0.77)	
OS								
Median (months)	8.3	13.0	8.9	16.5	13.5	19.2	11.1	NR
HR (95% CI)	0.55 (0.39–0.77)		0.50 (0.33–0.74)		0.60 (0.38–0.94)		0.41 (0.22–0.79)	

Bema, bemarituzumab; CI, confidence interval; CLDN18.2, claudin-18 isoform 2; DCR, disease control rate; DOR, duration of response; E0X, epirubicin plus oxaliplatin plus capecitabine; FGFR2b, fibroblast growing factor receptor-2b; FOLFOX, fluorouracil + leucovorin + oxaliplatin; HR, hazard ratio; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; Zolbe, zolbetuximab.

clinical benefit was not observed even in a subset with an MET intensity of 2+ /3+. However, DGC showed a favorable HR of OS (HR, 0.82) compared with that of IGC (HR, 1.23). Rilotumumab is a fully human immunoglobulin (Ig) G2 monoclonal antibody. In a phase III RILOMET-1 trial of first-line chemotherapy plus rilotumumab *versus* chemotherapy plus placebo in MET expression-positive GC patients,⁹⁴ the addition of rilotumumab in combination with chemotherapy showed no effective clinical outcomes. Both the HR of OS and PFS showed poor trends in DGC compared with IGC. The main issues are coamplification of other RTKs such as HER2 and EGFR in MET-amplified GC, and heterogeneous MET amplification status between primary and metastatic lesions, leading to possible treatment resistance to MET inhibitors.¹⁶⁷

Promising targetable molecules in diffuse-type gastric cancer

Recently, two phase II trials demonstrated promising results of anti-CLND18.2 antibody (zolbetuximab)

in the FAST trial²² and anti-FGFR2-IIIb antibody (bemarituzumab) in FIGHT²³ (Table 5). Thus, CLDN18.2 and FGFR2-IIIb will be relevant therapeutic targets for DGC patients. This section focuses on CLND18.2 and FGFR2 in GC from both basic and preclinical viewpoints (Figure 1).

Claudin 18.2 inhibitor

CLDNs are structural components of tight junction strands in the paracellular region that play critical roles in barrier function, permeability, paracellular transport, cell polarity, and signal transduction.¹⁶⁹ CLDN18 forms a paracellular barrier in the normal stomach, and its deficiency causes atrophic gastritis.¹⁷⁰ Two major CLDN18 isoforms, CLDN18.1 and CLDN18.2, are expressed almost exclusively in normal lungs and stomachs, respectively.⁷⁷ In the normal stomach, the expression of CLDN18.2 is strictly confined to differentiated epithelial cells in the gastric mucosa as a highly selective gastric lineage molecule. In GC, CLDN18.2 expression remains in primary and metastatic sites, and the high expression levels of

CLDN18.2 (staining intensity of $\geq 2+$ in $\geq 60\%$ of tumor cells by IHC) were more frequently observed in DGC (75%) compared with IGC (46%). CLDN18-ARHGAP fusions are also predominantly detected in DGC.²⁰ In normal tissue, epitopes within the tight junction are generally inaccessible to intravenous antibodies. However, epitopes of CLDN18.2 may be exposed on the cell surface, possibly due to perturbations in cell polarity through malignant transformation and cell–cell detachment in DGC, leading to accessible drug targets.⁷⁷

Zolbetuximab (IMAB362) is a first-in-class chimeric IgG1 monoclonal anti-CLDN18.2 antibody. The two isoforms of CLDN18 are different in the N-terminal 69 amino acids, and the differences of amino acids in the first extracellular loop are only eight of the 51 amino acids. Nevertheless, zolbetuximab selectively binds to CLDN18.2, but not CLDN18.1, indicating lower cross-reactivity of zolbetuximab with CLDN18.1. In pre-clinical studies, treatment with zolbetuximab resulted in an antitumor effect by activating antibody-dependent cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) *via* immune effector stimulation.¹⁷¹ Furthermore, zolbetuximab acts as a synergistic agent in combination with cytotoxic agents and enhances T-cell infiltration into the tumor microenvironment as an immunomodulator.¹⁷¹

A phase II FAST trial was conducted to assess the clinical benefit of adding zolbetuximab to first-line epirubicin, oxaliplatin, and capecitabine (EOX) chemotherapy in recurrence or metastatic patients with CLDN18.2-positive GC, defined as moderate-to-strong CLDN18.2 expression in $\geq 40\%$ of tumor cells by IHC (Table 5).²² A total of 334 (48.7%) of the 686 patients assessed by IHC had positive CLDN18.2 expression. Finally, 161 patients were randomly assigned to EOX ($n=84$) and EOX plus zolbetuximab ($n=77$). The primary endpoint, PFS, was met (HR, 0.44; 95% CI, 0.29–0.67; $p < 0.001$), and median OS as the secondary endpoint was 8.3 months for EOX and 13.0 months for EOX plus zolbetuximab (HR, 0.55; 95% CI, 0.39–0.77; $p < 0.001$). In terms of adverse events (AEs), nausea (81.8%) and vomiting (67.5%) were the most common, possibly due to the targeted effect on the normal stomach. Subgroup analyses of PFS and OS were performed according to the histology types, in which DGC was included in 49.3% of a total population with known histology (Table 4). The PFS benefit

of zolbetuximab was higher for DGC (HR, 0.29; 95% CI, 0.14–0.59) than for IGC (HR, 0.61; 95% CI, 0.31–1.21). Addition of zolbetuximab also resulted in improved OS for DGC (HR, 0.44; 95% CI, 0.26–0.74) compared with IGC (HR, 0.73; 95% CI, 0.41–1.30). The survival benefits were more remarkable for patients with $\geq 70\%$ of tumor cells positive for CLDN18.2, especially in DGC, with HRs of 0.28 for PFS (95% CI, 0.13–0.60) and 0.33 for OS (95% CI, 0.17–0.63) in DGC patients *versus* HRs of 0.55 for PFS (95% CI, 0.22–1.36) and 0.87 for OS (95% CI, 0.41–1.88) in IGC patients.²² Thus, DGC frequently exhibits CLDN18.2-positive expression, and zolbetuximab in combination with first-line chemotherapy will be a promising treatment approach for patients with CLDN18.2-positive DGC. Currently, two global phase III trials, SPOTLIGHT (NCT03504397) and GLOW (NCT03653507), are ongoing to compare the treatment efficacy of first-line chemotherapy plus zolbetuximab with chemotherapy plus a placebo in recurrent or metastatic CLDN18.2-positive GC.

Considering the high frequency of CLDN18.2 overexpression in primary and metastatic tumors but its restricted expression in short-lived differentiated epithelial cells of gastric mucosa,⁷⁷ CLDN18.2 could be a promising molecule not only for a monoclonal antibody but also for an antigen targeted by antibody-drug conjugates (ADCs), bispecific T-cell engager (BiTE), and chimeric antigen receptor (CAR) T cells. ADCs comprise a cytotoxic payload conjugated by a linker to a monoclonal antibody against tumor-specific surface molecules, thereby enabling efficient drug delivery to tumor cells with minimum systemic exposure and off-target toxicity.¹⁷² The redirection of T cells against tumors using CAR T cells or BiTEs has been demonstrated as a promising strategy for cancer treatment by activating T cells to kill tumor cells.^{173,174} Currently, several early phase trials of agents targeting CLDN18.2 are ongoing in CLDN18.2-positive tumors: (1) monoclonal antibodies including AB011 (NCT04400383), LM-102 (NCT05008445), TST001 (NCT04495296), MIL93 (NCT04671875), and NBL-015 (NCT05153096); (2) bispecific antibody targeting both CLDN18.2 and PD-L1 (Q-1802, NCT04856150); (3) anti-CLDN18.2 ADCs including CMG901 (NCT04805307), CPO102 (NCT05043987), SYSA1801 (NCT05009966), and LM-302 (NCT05161390); (4) BiTE targeting both

CLDN18.2 and CD3 (AMG910, NCT04260191); and (v) CAR T cells including CAR-CLDN18.2 T cells (NCT03874897), CT041 (NCT04404595), LY011 (NCT04966143), and LCAR-C18 S cells (NCT04467853).

FGFR2-IIIb inhibitor

The FGFR family consists of four highly conserved receptors (FGFR1, FGFR2, FGFR3, and FGFR4). The FGFR pathway is cancer-specifically dysregulated by their overexpression and genetic alterations in various tumor types, driving cancer development and progression.^{175,176} In GC, FGFR2 amplification is the most frequent genetic alteration among FGFR family members, ranging from 3% to 15% of GC, which confers an unfavorable prognosis.^{70,109,177–179} FGFR2 amplification is predominantly enriched in the GS molecular subtype in TCGA¹⁸ or MSS/EMT subtype in ACRG,⁴³ indicating a key alteration in DGC.^{70,178} DGC also exhibits frequent FGFR2 overexpression with prognostic relevance, but IGC does not.^{70,180} FGFR2 isoforms (FGFR2-IIIb and IIIc) are determined by alternative splicing of a ternary extracellular immunoglobulin domain III, of which FGFR2-IIIb is predominantly overexpressed in GC, especially in DGC.^{70,180,181} In addition, FGFR ligands are overproduced in DGC cells and CAFs, leading to ligand-dependent FGFR2 activation through the paracrine and autocrine loops.^{182,183} Detailed information about the FGFR2 signaling pathway in GC has been reviewed elsewhere.¹⁷⁷ Preclinical studies have shown the antitumor efficacy of FGFR2 inhibitors in FGFR2-amplified DGC models.¹⁸⁴ Thus, the FGFR signaling pathway has attracted considerable attention as a targetable molecule, especially in DGC with FGFR2 aberrations.

A major area of drug development targeting FGFR is small molecule tyrosine kinase inhibitors (TKIs), and the therapeutic efficacy of FGFR-selective TKIs has been demonstrated for cholangiocarcinoma with FGFR2 fusions or rearrangements¹⁸⁵ and urothelial carcinoma with FGFR2/3 fusions or FGFR3 mutations.¹⁸⁶ Although several trials of FGFR-TKIs have been conducted in a subset of GC patients with FGFR aberrations, they have failed to demonstrate their clinical benefit.^{177,187} High-level clonal FGFR2 amplification may be an important predictive biomarker for selecting patients who would benefit from FGFR-TKIs.¹⁸⁸

Anti-FGFR2 monoclonal antibodies competitively bind to the extracellular domain of FGFR2 and block the activation of FGFR2 signaling, which has preclinical antitumor effects and less toxicity.¹⁸⁹ Bemarituzumab (FPA144) is a first-in-class humanized IgG1 monoclonal FGFR2-IIIb isoform-selective antibody glycoengineered for enhanced ADCC activity.¹⁹⁰ A randomized, double-blind, placebo-controlled phase II FIGHT trial was conducted to evaluate the clinical benefits of adding bemarituzumab to first-line modified oxaliplatin, 5-FU, and leucovorin (mFOLFOX6) chemotherapy in patients with FGFR2b-positive GC, defined as FGFR2-IIIb overexpression using IHC or FGFR2 gene amplification determined by circulating tumor DNA (ctDNA) (Table 5).²³ Of 910 patients who underwent prescreening, 275 (30.2%) were FGFR2b positive. Finally, 155 patients were randomly treated with bemarituzumab ($n=77$) or a placebo ($n=78$) in combination with mFOLFOX6. The primary endpoint, PFS, was met, with an improvement in median PFS of 9.5 months for bemarituzumab *versus* 7.4 months for the placebo (HR, 0.68; 95% CI, 0.44–1.04; $p=.073$). After an updated follow-up period of a median of 12.5 months, treatment with bemarituzumab resulted in prolonged OS, with a median OS of 19.2 months for bemarituzumab *versus* 13.5 months for placebo (HR, 0.60; 95% CI, 0.38–0.94).¹⁶⁸ The survival benefits increased with more homogeneous FGFR2-IIIb overexpression, with HRs of 0.44 for PFS (95% CI, 0.25–0.77) and 0.41 for OS (95% CI, 0.22–0.79) in GC patients with FGFR2-IIIb overexpression in $\geq 10\%$ of tumor cells. Stomatitis (31.6% *versus* 13.0%) and corneal AEs (67.1% *versus* 10.4%) were more common in bemarituzumab than the placebo. It remains unclear whether bemarituzumab has better treatment efficacy for patients with FGFR2-IIIb overexpressing DGC than IGC. However, considering the association of DGC with FGFR2-IIIb overexpression and FGFR2 amplification, bemarituzumab will be the main therapeutic pillar for patients with FGFR2-IIIb overexpressing DGC. A phase III study is warranted to confirm these results in a larger population of FGFR2b-positive GC.

Other potent molecular-targeted inhibitors

Although CDH1 is frequently mutated in DGC,^{18,19,40} it is not a conventional druggable molecule because of its function as a tumor

suppressor gene and its loss of expression. Recently, synthetic lethality has been attracting attention as having the potential to target tumor cells that carry CDH1 mutations. The integrated genetic and drug screens, using breast tumor cells with CRISPR/Cas9-engineered CDH1 mutations or with homozygous deletion of CDH1, identified synthetic lethality between E-cadherin and the ROS proto-oncogene 1, receptor tyrosine kinase (ROS1).^{191,192} Because E-cadherin deficient tumor cells are dependent on ROS1, which is likely related to cytokinesis, ROS1 inhibitor crizotinib elicited synthetic lethality in the E-cadherin deficient GC cell models.¹⁹¹ These preclinical findings provide the rationale for assessing the efficacy of ROS1 inhibitors. There is an ongoing phase II trial of crizotinib in E-cadherin negative DGC or CDH1 mutated solid tumors (NCT03620643).

The AT-rich interaction domain 1A (ARID1A), a subunit of the switch/sucrose nonfermentable (SWI/SNF) chromatin remodeling complex, is one of the most commonly mutated genes across various tumor types. ARID1A mutations compromise diverse gene programs and cellular processes through the dysregulation of transcription, DNA repair, and chromatin segregation, thereby promoting tumorigenesis.^{193,194} In GC, ARID1A mutations are prevalent in the GS subtypes next to the EVB subtypes.¹⁸ DGC are classified into early-onset and late-onset cancers, and ARID1A mutations were observed in 15–18% of early-onset DGC.⁴⁸ Several preclinical studies have reported crucial targets that induce synthetic lethality with ARID1A deficiency, such as enhancer of zeste homolog 2 (EZH2) histone methyltransferase that is a catalytic subunit of the polycomb repressive complex 2 (PRC2) that antagonizes the function of ARID1A,¹⁹⁵ BIRC5/Survivin that is a transcriptional regulatory module induced by ARID1A mutation,¹⁹⁴ and the glutamate-cysteine ligase synthetase catalytic subunit that is a rate-limiting enzyme for antioxidant glutathione synthesis.¹⁹⁶ Thus, the inhibition of molecules that create therapeutic vulnerability of the ARID1A-deficient tumor cells may be of clinical importance. In addition, ARID1A deficiency impairs the mismatch repair (MMR) system that plays a key role in correcting DNA replication errors, thereby resulting in an increased mutation burden, MSI-H genomic signature, TILs, and PD-L1 expression.¹⁹⁷ In xenograft models, ARID1A-deficient tumors, but not ARID1A-wild-type, were regressed by treatment with

ICI.¹⁹⁷ GC patients with ARID1A mutation are likely to benefit from treatment with ICI.

Recurrent mutations of RHOA are a hallmark in DGC development.^{18,25,74,90} The genetic knock-down of RHOA repressed spheroid formation as a CSC phenotype and sensitized diffuse-type CSC cells to 5-FU and cisplatin chemotherapy.⁸⁹ RHOA inhibition also decreased the expression of EMT-related molecules.¹⁹⁸ In addition, ROCK is a downstream effector of RHOA, and treatment with the ROCK inhibitor (HA-1077) resulted in tumor regression in a transgenic GC mouse model.¹⁹⁹ As RHOA mutations promoted the activation and dependency of focal adhesion kinase (FAK), the small molecule inhibitors of FAK, including GSK-2256098, VS-6063, CEP-37440, VS-6062, VS-4718, and BI-853520, are considered to be a promising cancer therapy in RHOA mutant GC.²⁰⁰ Furthermore, FAK may also serve as a target even for DGC without RHOA mutation.⁹⁰ These preclinical findings support the clinical utility of targeting the RHOA signaling pathway.

DGC are characterized by a rich fibrous stroma, which has been associated with overexpression in TGF- β signaling.³⁴ As TGF- β signaling contributes to tumor progression, metastasis, and drug resistance,⁹⁸ the TGF- β pathway has been pharmacologically targeted using monoclonal antibodies (SAR439459), small molecule inhibitors (vactosertib and galunisertib), ligand traps, and vaccines.²⁰¹ Based on a key immunosuppressive role of TGF- β signaling in the tumor microenvironment by restricting T-cell penetration in tumors,²⁰² a phase I trial of bintrapfusp alfa (M7824), a bifunctional fusion protein composed of a human anti-PD-L1 IgG1 monoclonal antibody fused with the extracellular domain of TGF- β receptor II, was conducted in patients with heavily pretreated GC. The ORR was 16%, and median duration of response was 8.7 months.²⁰³ Furthermore, a rationale for dual blockade of TGF- β signaling and immune checkpoint molecules has been assessed in early phase trials using treatment with TGF- β inhibitor plus ICI (NCT03192345, NCT03724851), a bispecific antibody targeting both TGF- β and PD-L1 (Y101D, NCT05028556), and anti-CD73/TGF- β trap bifunctional antibody (GS-1423, NCT 03954704).

Previous phase III trials have highlighted that biomarker selection for the specific molecular

alteration is mandatory to enrich the efficacy of MET inhibitors.^{94,146} Savolitinib is a reversible MET-selective TKI,²⁰⁴ and it has showed promising efficacy for MET-amplified GC patients assigned by targeted next-generation sequencing (NGS) using tissue DNA in a prospective biomarker-driven VIKTORY trial.⁹⁵ The ORR was 50% (10 in 20 GC patients) despite the second-line setting. Importantly, in the VIKTORY trial, the treatment efficacy was greater in GC patients with gene amplification detected by ctDNA than in tissue DNA.⁹⁵ Thus, the assessment of ctDNA is likely a strict approach to predicting treatment response to molecular-targeted agents by identifying high-level and clonal amplified tumors among GCs with intratumoral heterogeneity. A multicenter phase II trial to evaluate the efficacy and safety of savolitinib in MET-amplified GC patients is ongoing (NCT04923932).

Conclusion

DGC is associated with less chemosensitivity and an unfavorable prognosis through EMT and CSC phenotypes. There are no established therapeutic agents for DGC, so the development of novel treatment strategies for DGC is the most urgent need. The impressive results of two phase II trials demonstrate proof-of-concept, suggesting that anti-CLDN18.2 antibodies (zolbetuximab) and FGFR2-IIIb antibodies (bemarituzumab) are promising approaches for patients with CLDN18.2-positive and FGFR2b-positive GC, respectively. A new era of precision medicine for patients with DGC is dawning.

Author contributions

Akira Ooki: Conceptualization; Investigation; Writing – original draft.

Kensei Yamaguchi: Conceptualization; Writing – review & editing.

Conflict of interest statement

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