# Chapter 4
# Molecular Diagnosis and Targeted Therapy for Gastric Cancer

**Nobuhisa Matsuhashi, Kazuhiro Yoshida, Kazuya Yamaguchi, and Toshiyuki Tanahashi**

**Abstract**  Gastric cancer (GC) is a heterogeneous cancer with widely varied outcome and similar clinical and pathological features in Caucasians and Asians. The treatment results from Asian countries seem to be better than those of Western countries. There is an urgent need to clarify the differences in results between Eastern and Western countries and to determine the best management of patients with GC. Now, the molecular biology of cancer is gradually becoming clear. Molecular-targeted drugs are on the rise, and classifications corresponding to the biomarkers of these drugs are beginning to be used in clinical settings. In addition, huge quantities of genome information are gradually being analyzed in a short time with the appearance of next-generation sequencers that can identify gene variation and copy number abnormalities. Now, because a classification based on the characteristics of the genome level of GC has been reported, we review the latest information on GC.

**Keywords**  Molecular diagnosis • Targeted therapy • Gastric cancer

## 4.1  Introduction

Estimates of the worldwide incidence, mortality, and prevalence of 26 cancers in the year 2012 are now available in the GLOBOCAN series of the International Agency for Research on Cancer. The most commonly diagnosed cancers are lung (1.35 million), breast (1.15 million), and colorectal (1 million); the most

N. Matsuhashi • K. Yoshida (✉) • K. Yamaguchi • T. Tanahashi
Department of Surgical Oncology, Gifu University Graduate School of Medicine,
1-1 Yanagido, Gifu City 501-1194, Japan
e-mail: nobuhisa517@hotmail.com; kyoshida@gifu-u.ac.jp; kazuyay@gifu-u.ac.jp;
c2001049@yahoo.co.jp

common cause of cancer death is lung cancer (1.18 million deaths), and the second most common cause is gastric cancer (GC) (700,000 deaths) [1]. However, GC is the most common cancer in several areas of the world, most notably in Japan, Korea, and China. In Japan, the incidence of GC is almost ten times higher than that in the United States. In most areas in Japan, the incidence of GC in men is almost twice that of women [2]. GC, one of the most common human cancers, is a heterogeneous disease with different phenotypes and varying prognoses and responses to treatment. Among the various viewpoints, the newly developed concept of oncogene addiction provides a rationale for the use of targeted therapies.

### 4.1.1 Genetic and Molecular Alterations During Gastric Carcinogenesis Helicobacter pylori

Recent epidemiological studies have indicated that *Helicobacter pylori* plays a key role in the development of both intestinal-type and diffuse-type gastric carcinomas [3–5]. *H. pylori* is a gram-negative, spiral-shaped bacterium that infects the stomach of about half of the world's population. The acidic environment in the stomach usually prevents the survival of viruses, bacteria, and other microorganisms, but *H. pylori* has evolved to uniquely overcome this harsh environment. *H. pylori* secretes urease, a special enzyme that converts urea to ammonia to neutralize the acidity of the stomach, making the stomach a more hospitable place for *H. pylori*. With *H. pylori*'s ability to survive this harsh environment, the stomach provides it with a special living niche. Host inflammatory/immune cells that would normally recognize and attack invading bacteria are unable to cross from blood vessels through the stomach epithelial mucosa. Instead, the ineffective host cells continue to respond to the site of infection, where they die and release nutrients that feed the gastric pathogen. *H. pylori* infection is primarily acquired during childhood, and the transmission occurs through a fecal–oral or oral–oral mode, primarily within families. In the majority of cases, *H. pylori* infection is lifelong in the absence of eradication with antibiotics [6–8]. It is now well established that *H. pylori* infection predisposes individuals to gastric adenocarcinoma later in life [9, 10]. *H. pylori* has been classified by the International Agency for Research on Cancer (IARC) as a definite carcinogen to humans (group 1) [11]. *H. pylori* infection induces a chronic inflammation of the gastric mucosa that is intensified by the host inflammatory immune response with high levels of several cytokines. This chronic process leads, in a minority of infected individuals, to the development of GC through a series of intermediate progressive stages including mild and severe chronic gastritis; atrophic gastritis; gastric atrophy, characterized by hypochlorhydria; and intestinal metaplasia [4, 12]. Recent meta-analyses have estimated that *H. pylori* infection increases the risk of GC by two–threefold, with higher estimates for noncardiac GC [13–15].
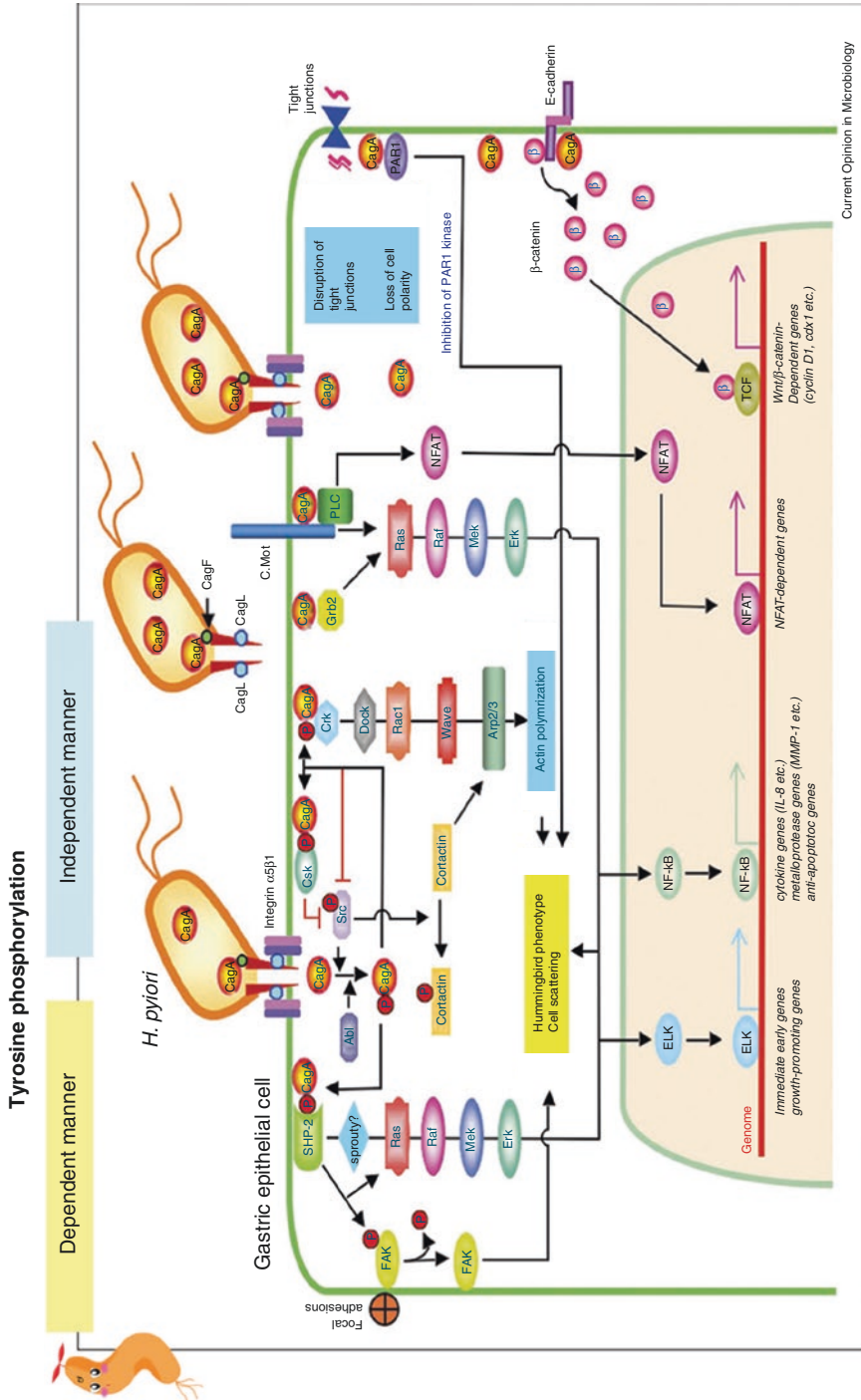
## 4.1.2   *Oncogenic Mechanisms of* H. pylori *CagA Protein*

The CagA gene, which encodes a 120–135 KDa immunodominant protein, CagA, is localized at one end of the Cag pathogenicity island (*ca*g PAI), a 40 kb DNA segment that is thought to be horizontally transferred into the *H. pylori* genome [16, 17]. *H. pylori* CagA is the first bacterial oncoprotein to be identified in relation to human cancer [18]. CagA is delivered into gastric epithelial cells through a bacterial type IV secretion system and localizes to the plasma membrane, where it undergoes tyrosine phosphorylation by host cell kinases. Infection with *cagA*-positive *H. pylori* strains has been associated with higher grades of gastric mucosal inflammation and severe atrophic gastritis and has been thought to play an important role in the development of gastric carcinoma. This link is further supported by the results of a combined analysis of 16 studies showing a twofold increase in the risk of GC associated with *cagA*-positive *H. pylori* compared to the risk of GC associated with *CagA*-negative *H. pylori* [19, 20].

It is well documented that chronic infection with *CagA*-positive *H. pylori* induces progressive histopathological changes in gastric mucosa that lead to intestinal-type gastric adenocarcinoma: superficial gastritis, atrophic gastritis, intestinal metaplasia, dysplasia, and adenocarcinoma [21–23] (Fig. 4.1). In addition, in terms of CagA polymorphisms, the vast majority of *H. pylori* isolates in East Asian countries bear East Asian CagA. In contrast, most if not all of the *H. pylori* cagA-positive strains isolated in non-East Asian countries carry Western CagA. A clear exception is Southeast Asia, where *H. pylori* strains carrying East Asian CagA and Western CagA coexist at various ratios in different areas and countries [23–25]. Chronic infection with cagA-positive strains of *H. pylori* hyperstimulates gastric epithelial turnover by constitutively exposing cells to oncogenic stress [26]. Long-term sustenance of such a situation substantially increases the chance of epithelial cells acquiring genetic and epigenetic defects in signaling pathways including those involved in senescence and apoptosis, the malfunctioning of which is an important hallmark of cancer [27] (Fig. 4.2).

## 4.1.3   *Claudin-18 Loss*

Claudin-18 has two alternative splicing forms, the lung and stomach types, which use a different first exon and the same exons 2–4; the two isoforms are regulated by different tissue-specific promotors. Because stomach-type claudin-18 is the predominant claudin expressed in stomach, it is expected to regulate the stomach-specific properties of the paracellular barrier, including resistance to $H^+$ leakage and/or pepsin, as implied by its overexpression in MDCK II cells [28, 29]. Epithelial cells adhere to each other to form cell sheets, and when the intercellular spaces between epithelial cells are sealed by tight junctions, the paracellular barrier function is established [30, 31, 32]. Sanada et al. [33] reported the downregulation of claudin-18

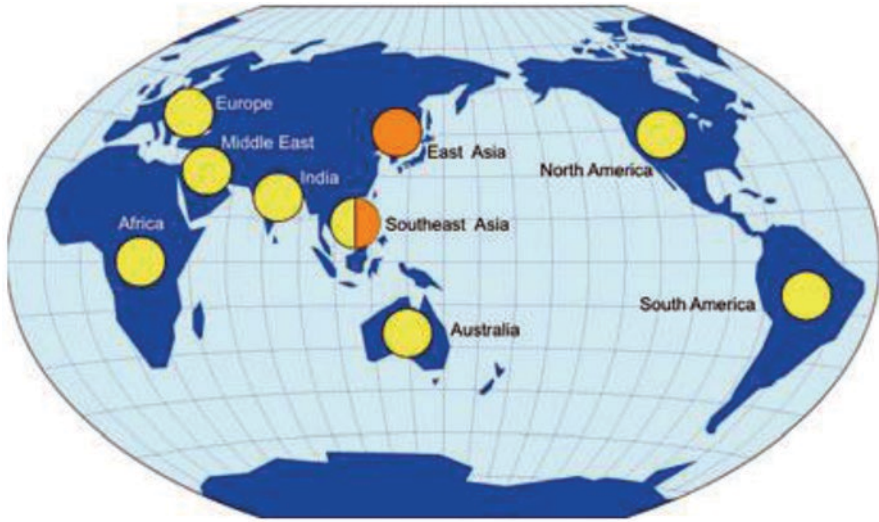**Fig. 4.1** Oncogenic mechanisms of *Helicobacter pylori* CagA protein. Source: Refs. [22, 23]

**Fig. 4.2** Global distribution of *Helicobacter pylori* Western CagA (*yellow*) and East Asian CagA (*orange*). Source: Ref. [23]

expression in human GC. Hayashi et al. [34] reported that that claudin-18-dependent formation of the paracellular barrier against H⁺ diffusion is likely to play a specific role in the prevention of gastritis. Because claudin-18 is the major tight junction (TJ) component of stomach epithelial cells, barrier dysfunction of the tight junctions is reduced in *H. pylori*-induced atrophic gastritis and intestinal metaplasia. According to this result, claudin-18 can lead to a precancerous condition [33, [34].

### *4.1.4 Overexpression of HER2, EGFR, and c-MET*

Recently, significant achievements have been made in the discovery and advancement of treatments for lung cancer due to the recognition of distinct molecular subtypes generally headlined by an actionable "driver mutation" such as rearrangement in anaplastic lymphoma kinase (ALK), c-ROS kinase (ROS1), or rearrangement during transformation (RET kinase) or amplification of *HER2* or *MET*, or mutations in epidermal growth factor receptor (EGFR), BRAF (V600E), and KRAS. Many of these same mutations have been described in GC [35] (Fig. 4.3).

HER2 overexpression has been observed in 9–38% of GC patients and occurs more frequently in gastroesophageal junction (GEJ) and intestinal-type tumors [36]. Treatment with the anti-HER2 monoclonal antibody trastuzumab has been proven to achieve improved survival in patients with HER2-positive advanced GC [37–39].
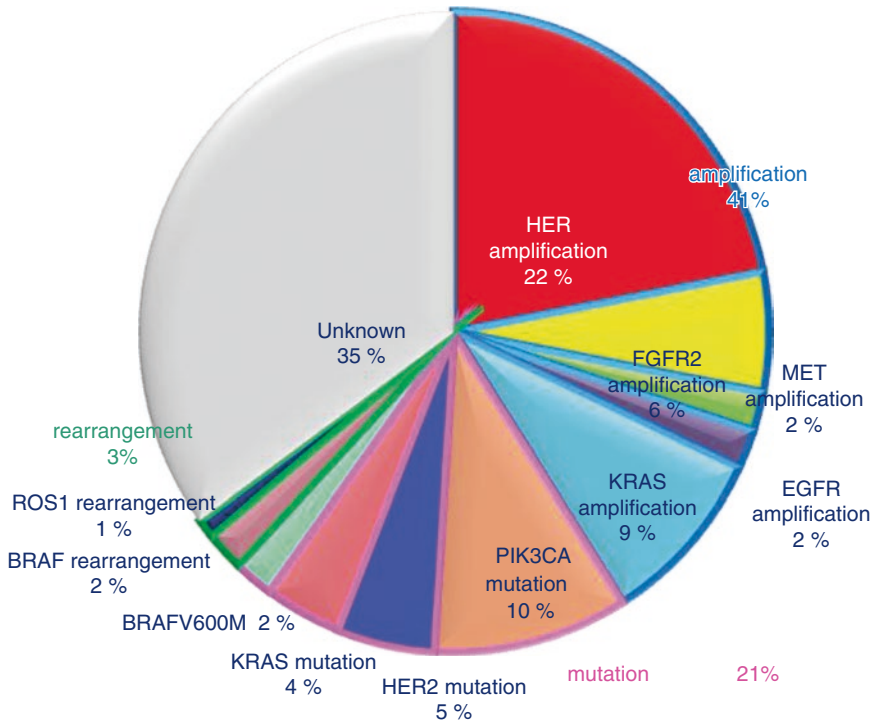
**Fig. 4.3** Proportion of potential driver mutations identified in gastric carcinoma. Source: Ref. (Lee J, Ou SH. Discov Med, 2013, 16:7–14)

The Trastuzumab for Gastric Cancer (ToGA) trial investigated the addition of trastuzumab to standard cisplatin and 5-fluorouracil-based chemotherapy to determine whether it would significantly improve the rate of overall survival (OS). Among 3665 patients with advanced GC or GEJ carcinoma who were successfully screened for HER2 overexpression, 810 (22.1%) were positive for HER2 overexpression. The rates of HER2 positivity were similar in Europe (23.6%) and Asia (23.5%) [40] and were higher in GEJ than GC (33.2% vs. 20.9%; $p < 0.001$) and in intestinal than diffuse/mixed cancer (32.2% vs. 6.1%/20.4%; $p < 0.001$) [40]. Of the patients enrolled in ToGA, 81.8% (478/584) had GC, whereas the rest had GEJ carcinoma. Most of the ToGA patients had intestinal histology (75.5%), with diffuse histology seen in 8.8% of the patients and mixed histology seen in the remaining 15.7% of patients. Slightly more than half of the enrolled patients (52.9%, 309/584) were Asians. Stratification was by performance status, stage, primary cancer site, and measurability of disease. The addition of trastuzumab to the chemotherapy regimen significantly improved the objective response rate to 47% as compared with 35% for chemotherapy alone (odds ratio [OR], 1.70; 95% confidence interval [CI], 1.22–2.38; $p = 0.0018$). Trastuzumab also significantly improved the rate of progression-free survival (PFS) from 5.5 months to 6.7 months (hazard ratio [HR] = 0.71; 95% CI, 0.59–0.85, $p = 0.0002$), and of most importance, the addition of trastuzumab

significantly improved OS from 11.1 months to 13.8 months (HR = 0.74; 95% CI, 0.60–0.91, $p$ = 0.0045). Post hoc subgroup analysis showed that OS had improved significantly in patients from Europe ($n$ = 190, HR = 0.63; 95% CI, 0.44–0.89) and Central/South America ($n$ = 52; HR = 0.44; 95% CI, 0.21–0.90) but that in Asian patients had not significantly improved ($n$ = 319; HR = 0.82; 95% CI, 0.61–1.11) [40]. To date, the only successful and approved targeted therapy for GC is trastuzumab in combination with chemotherapy in GC with HER2 overexpression [41].

The prognostic effect and clinicopathological features of EGFR and c-MET have also been studied. EGFR overexpression, which was observed in 27–44% of GC patients, has generally been reported to be a poor prognostic factor [42]. The correlation between EGFR status and clinicopathological characteristics has not been clearly elucidated. EGFR-positive status was reported to be frequently associated with the following factors: non-curatively treated GC [43], older age, moderately to poorly differentiated histological appearance, higher-stage disease [44], and recurrence after curative surgery and higher disease stages [45]. On the whole, c-MET overexpression, which was observed in 22–82% of GC patients, has also been reported to be associated with poor prognosis [42, 46, 47]; however, findings from a few other studies were contradictory. Recently, Fuse et al. [48] investigated co-overexpression of HER2, EGFR, and c-MET in patients with advanced GC who received standard chemotherapy and found that only c-MET was a significant and independent prognostic factor, which suggests that c-MET would be a good candidate for molecular-targeted agents [49] (Fig. 4.4). In the future, a new treatment strategy for patients simultaneously positive for EGFR or c-MET and HER2 is required.
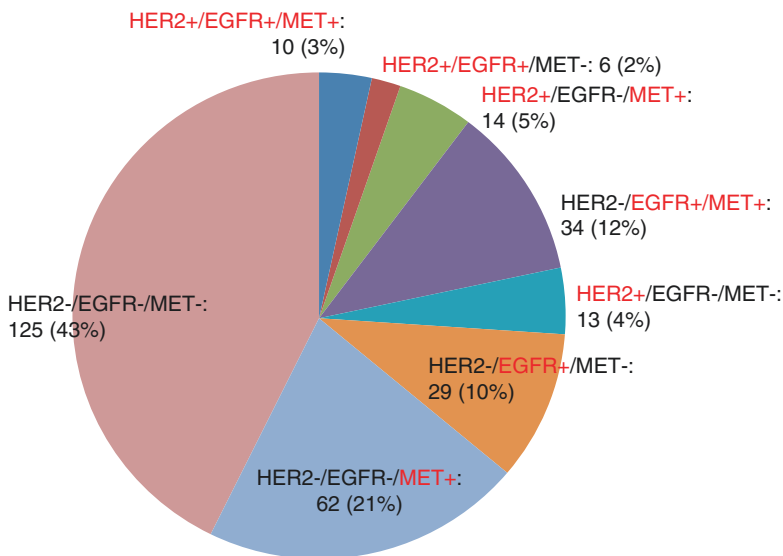


**Fig. 4.4** Co-overexpression status of human epidermal growth factor receptor 2 (HER2), epidermal growth factor receptor (EGFR), and c-MET. Source: Ref. [48]

### 4.1.5  VEGF-Directed Therapies

The VEGF family consists of five ligands [VEGFA, VEGFB, VEGFC, VEGFD, and placental growth factor (PlGF)] and three receptor tyrosine kinases [VEGF-R1, R2, and R3]. Of the VEGF receptors, VEGF-R2 expression is restricted to the vasculature and appears to play a key role in angiogenesis [49]. The failure of the AVAGAST trial was a setback for anti-angiogenic therapy for this disease [50]. Ramucirumab is a monoclonal antibody that binds to and prevents the activation of VEGF-R2. The recent REGARD trial, a randomized phase III trial of ramucirumab vs. placebo in patients with advanced, pretreated GC, met its primary endpoint of increased OS [51]. The subsequent RAINBOW trial pitting paclitaxel + ramucirumab against paclitaxel + placebo for advanced pretreated GC confirmed the survival advantage of this anti-angiogenic agent in GC [52]. Most (60%) of the patients were North American or European, and the remainder were Asian. Those patients treated with ramucirumab + paclitaxel experienced statistically significant and clinically meaningful improvement in OS compared with those treated with paclitaxel alone (9.63 m vs. 7.26 m, HR = 0.807; 95% CI, 0.678–0.962; $p = 0.0169$). Improvements of the response rate and PFS were also comparable in the study's experimental arm. A subgroup analysis showed that Asian patients did not obtain the same benefit of OS as the non-Asian patients did. Although the reasons for the discrepant outcome between Asian and Caucasian patients are unclear, one possibility is that Asian GC patients have a relatively less aggressive disease biology and often undergo third- and fourth-line therapies. The rates for these additional lines of therapy were 75% in Japanese patients but <40% in non-Asian patients. Compared with the Asian patients in this study, those from Europe or North America clearly derived more benefit from anti-angiogenic therapy [53].

### 4.1.6  Definition of Gastric and Intestinal Phenotypes of GC

GC, one of the most common human cancers, is a heterogeneous disease with different phenotypes and varying prognoses and responses to treatment. Therefore, subtype classification of GC is necessary to predict prognosis and decide on effective treatments. Histologically, GC demonstrates marked heterogeneity at both the architectural and cytologic levels, often with the coexistence of several histologic elements [54]. In Eastern and Western countries, the histologic classification of GC has largely been based on Lauren's criteria, in which intestinal-type and diffuse-type adenocarcinoma are the two major histologic subtypes, plus mixed type as an uncommon variant [55]. The relative frequencies are approximately 54% for intestinal type, 32% for diffuse type, and 15% for mixed type [55–57]. There are indications that the intestinal-type adenocarcinoma is more often associated with

intestinal metaplasia and *H. pylori* infection, whereas the diffuse-type gastric carcinoma is more often seen in families, females, and young individuals. Although the Lauren classification provides important information in clinical practice, it is not critical for predicting prognosis or determining treatment. Oue et al. [58] reported that GC can also be classified into a gastric or intestinal phenotype according to mucin expression. Accumulating evidence has indicated that gastric and intestinal phenotypes of GC have distinct clinical characteristics and exhibit specific genetic and epigenetic changes. Oue et al. [59] also focused on the clinical and molecular characteristics of the gastric and intestinal phenotypes of GC and reported that theTP53 mutation and allelic deletion of the APC gene are detected more frequently in the intestinal phenotype of GC. In contrast, CDH1 gene mutation is detected in differentiated-type GC showing a gastric phenotype [60]. Microsatellite instability (MSI) is detected more frequently in the gastric phenotype of GC [61]. Yasui et al. [56, 57] reported that GC cases showing CK7−/CK20+ were frequently found in the intestinal phenotype of GC, whereas GC cases showing CK7+/CK20- were commonly found in the gastric phenotype of GC. Nuclear β-catenin staining was frequently found in the intestinal phenotype of GC. However, expression of MMP7, laminin γ2, or HER2 was not correlated with gastric or intestinal phenotypes of GC [62, 63].

Whole genome or exon sequencing in GC has been performed, and mutation of the *RHOA* gene in undifferentiated-type GC has been reported [64] (Fig. 4.5). According to the COSMIC website (http://cancer.sanger.ac.uk), the most frequently mutated gene is *TP53* (32%), and the second most frequently mutated gene is *ARID1A* (14%). Frequencies of other gene mutations are approximately 10% or less [65]. Although the associations between mutation of these genes and gastric and intestinal phenotypes are unclear, driver gene mutation is a rare event, and it is difficult to plan an effective treatment according to such gene mutations. In contrast,
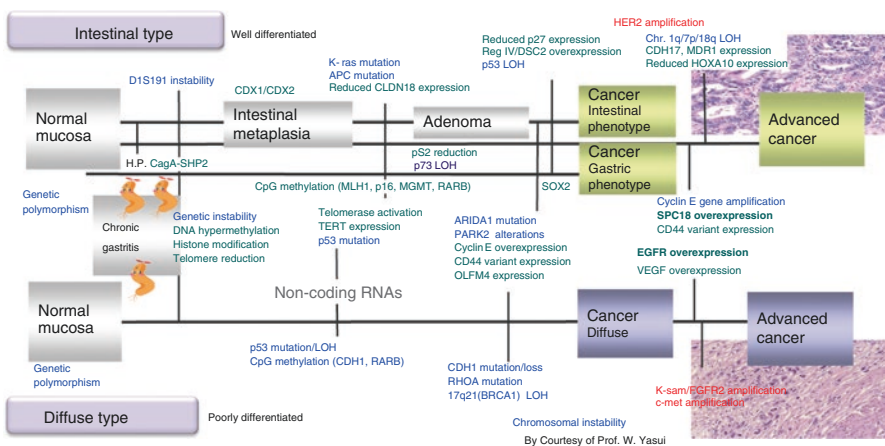


**Fig. 4.5** Definition of gastric and intestinal phenotypes of gastric cancer. Source: Ref. [60]

the Cancer Genome Atlas Network has reported that GC can be classified into four distinct molecular subtypes: GC positive for Epstein-Barr (EB) virus, microsatellite unstable GC, genomically stable GC, and GC with chromosomal instability [65]. As described above, MSI is detected more frequently in the gastric phenotype of GC. GC positive for EB virus is also frequently found in the gastric phenotype of GC [58]. However, the mucin phenotypes of genomically stable GC and GC with chromosomal instability remain unclear. In the future, classification of these subtypes may be used to provide personalized medicine.

### 4.1.7  Whole-Exome/Genome Sequencing Analyses of GC in Asia

GC is a leading cause of global cancer mortality, with high incidence rates in Asia and parts of Latin America [66]. Survival outcomes differ across geographical regions, with rates of 5-year OS being 10–15% in North America and 45–50% in East Asia [67–69]. These differences cannot be explained simply by improved early diagnosis in Asian countries as they persist even after stratifying for disease stage [70]. It has been suggested that these differences may reflect geographic variability in clinical practice. However, Asian patients treated in Western countries still exhibit superior outcomes compared with Caucasians, albeit worse outcomes than patients from Asian registries in their home countries [71, 72]. Lin et al. [1] assembled nine independent GC microarray cohorts comprising 1016 tumor gene expression profiles, six from Asian localities ($n = 890$) and three from outside Asia ($n = 126$). Except for tumor location, most of the clinicopathologic parameters, such as age, sex, and stage, were not significantly different between the Asian and non-Asian cohorts. However, there were significantly more cases of tumors in the upper third of the stomach in the non-Asian vs. Asian cohorts ($p = 0.04$). This study showed that for major cancer oncogenes such as KRAS, HER2, and FGFR2, somatic gene mutations and gene amplification rates are basically similar between Asian and non-Asian GCs. However, the association of GC with enrichment of tumor-infiltrating T cells and T-cell gene expression signatures, including CTLA-4 signaling, was stronger in non-Asian GCs [1]. In the future, differences in tumor immunity may contribute to geographical differences in clinical outcome and the design of future trials particularly in immuno-oncology.

### 4.1.8  Immune Checkpoint Blockade of PD-1/PD-L1

PD-1/PD-L1 blockade has recently been shown to be a promising treatment in a variety of tumor types [74–76]. Pembrolizumab is a humanized monoclonal anti-PD-1 antibody of the IgG4-kappa isotype that blocks the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Pembrolizumab is FDA approved for the

treatment of unresectable or metastatic melanoma and for PD-L1-positive metastatic non-small cell lung cancer [76–80]. Le et al. [81] reported that mismatch repair status predicted the clinical benefit of immune checkpoint blockade with pembrolizumab. In their cohort of patients with mismatch repair-deficient colorectal cancer (cohort A), median PFS and median OS were not reached. Contrastingly, in those with mismatch repair-proficient cancers (cohort B), median PFS was only 2.2 months (95% CI, 1.4–2.8), and median OS was 5.0 months (95% CI, 3.0 to not estimable). The median PFS in their cohort C (patients with mismatch repair-deficient noncolorectal cancer) was 5.4 months (95% CI, 3 to not estimable), and median OS was not reached. KEYNOTE-012 was a multicenter, open-label, phase 1b trial in which patients with advanced GC, urothelial cancer, triple-negative breast cancer, and head and neck cancer were treated. In their report, Seiwert et al. [80] described the results of the cohort with advanced GC, which comprised 39 patients (19 from East Asia and 20 from other areas in the world). Specimens from 24 patients with microsatellite instability were also analyzed. Four (17%) of these 24 patients had tumors with high microsatellite instability (two [8%] Asian patients and two [8%] from elsewhere) and the remaining 20 (83%) had tumors with microsatellite stability. Among all 32 patients with at least one post-baseline tumor assessment, 17 (53%) experienced a decrease in their target lesion size from baseline. A central review showed the median time to response to be 8 weeks. At the final analysis, four of the eight responders were alive, had no disease progression, and required no additional anticancer therapy. The median duration of response was 40 weeks, and decreased tumor burden was maintained over several assessments. One patient experienced 100% reduction in the target region but was not judged to have a complete response because of the subsequent development of new lesions. In the four patients with GC with high microsatellite instability, two experienced a partial response, but the disease progressed in the other two. From these results, mismatch repair status was unable to predict the clinical benefit of immune checkpoint blockade with pembrolizumab in GC [81]. Several ongoing studies are continuing to investigate the efficacy and safety of pembrolizumab in patients with advanced gastric or GEJ cancer. In view of the mechanism of action of pembrolizumab, the known expression of PD-L1 in GC and data from patients with non-small cell lung cancer suggest an improved response in patients whose tumors express PD-L1 [82] (Fig. 4.6a–c).

## 4.1.9 Conversion Therapy for Stage IV GC

### 4.1.9.1 Proposal of New Biological Categories of Classification

The strategy for treating stage IV GC remains controversial. Due to poor prognosis, the variance in physical status, and severe symptoms, it is important to determine the optimal strategy for treating each individual patient with stage IV disease. The survival efficacy of palliative gastrectomy by reductive gastrectomy for advanced
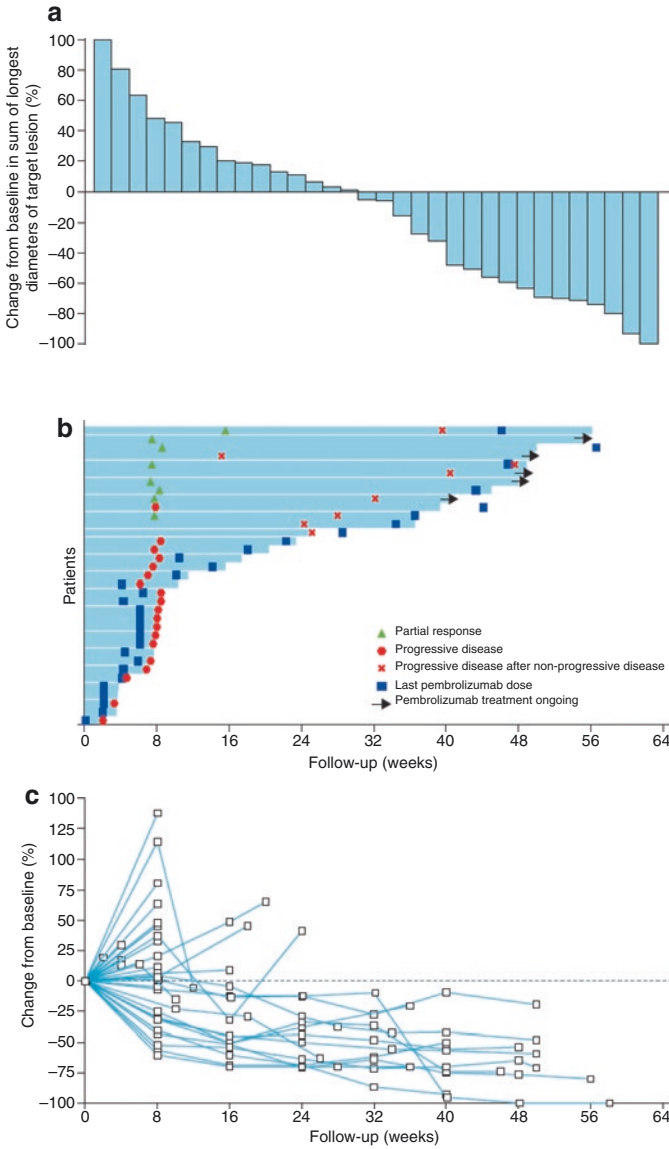
**Fig. 4.6 (a–c)** Assessment of the effect of immune factors on geographic locality-based and chemotherapy-based survival. Source: Ref. [82]

tumors has been denied in three Asian countries in the REGATTA trial [83]. However, the development of molecular technology and targeted therapies has drawn attention due to their potentially greater anticancer activity and fewer side effects than traditional chemotherapeutic agents. This suggests that the development of new cancer treatment strategies will require the discovery of more

candidates to target. For this reason, we reviewed the current status of GC to better understand the biology and indications of curative surgery as conversion therapy. We have proposed new categories for the classification of stage IV GC, taking into account the heterogeneous situation and treatment trends in general practice. In the new categories of classification, we have divided stage IV GC into two entities of macroscopic-positive and macroscopic-negative patients, who are further classified into four categories [84]. Patients without macroscopic peritoneal dissemination are classified into category 1 and category 2. The patients with potentially resectable metastasis are classified into category 1, whereas those with marginally resectable metastasis are classified into category 2. Patients with macroscopic peritoneal dissemination are classified into category 3 and category 4. The patients in category 3 are considered to be incurable and have unresectable metastases; however, resection may be performed to achieve local palliation. The patients in category 4 have non-curable metastases. It is essentially impossible to achieve a cure in any patient with peritoneal carcinomatosis from GC, irrespective of the extent of pretreatment or the ability to achieve an R0 resection. However, the survival outcomes differ according to the degree of disease progression and the extent of the disease, in addition to the response to therapy. Longer survival can be expected in patients in categories 1 and 2 who are treated with curative intent, whereas treatment of the patients in the other categories focuses on "care." The concept of conversion therapy or adjuvant surgery principally includes patients in category 2, some patients in category 3, and rarely patients in category 4 when operations are performed with the goal of achieving an R0 resection or a surgical cure [85] (Fig. 4.7). This suggests that the development of new cancer treatment strategies will require the discovery of more candidates to target. A retrospective cohort study is now being conducted in Asia through the
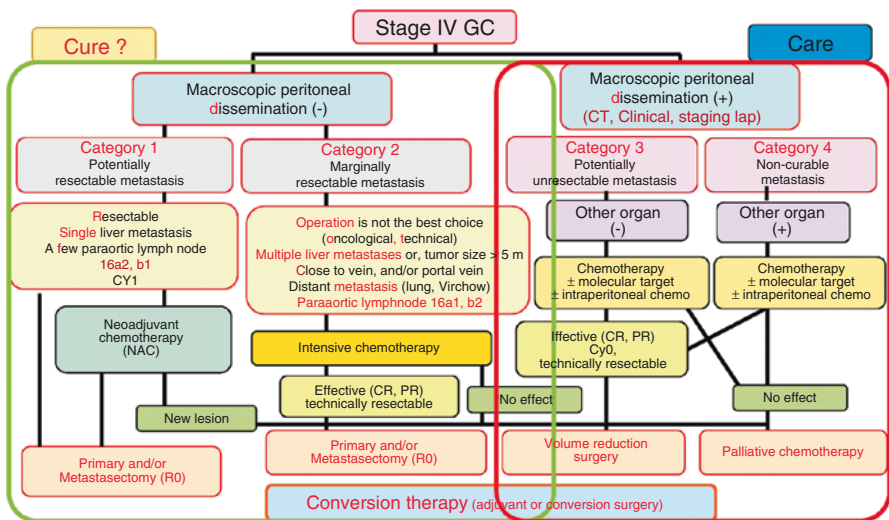


**Fig. 4.7** The new biological categories for the classification of stage IV gastric cancer. Source: Ref. [85]

Federation of Asian Clinical Oncology (FACO), which consists of the Japanese Society of Clinical Oncology (JSCO), the Korean Association of Clinical Oncology (KACO), and the Chinese Society of Clinical Oncology (CSCO), with the support of the Japanese Gastric Cancer Association (JGCA), the Korean Gastric Cancer Association (KGCA), and the Gastric Cancer Association of the Chinese Anti-cancer Association. Further analysis will prove to clarify the benefits of conversion therapy in the new strategic approach for stage IV GC.

In conclusion, the development of new DNA sequencing technologies, such as next-generation sequencing techniques, may dramatically increase the speed and reduce the cost of DNA sequencing, thus enabling more rapid and detailed analysis of gene amplifications and genetic alterations in GC. In turn, the development of more potent molecular diagnosis and targeted therapy for the treatment of GC will be expected.

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