The Impact of Molecular Subtype on Efficacy of Chemotherapy and Checkpoint Inhibition in Advanced Gastric Cancer

Yohei Kubota[1,2], Akihito Kawazoe[1], Akinori Sasaki[1], Saori Mishima[1], Kentaro Sawada[1], Yoshiaki Nakamura[1], Daisuke Kotani[1], Yasutoshi Kuboki[1], Hiroya Taniguchi[1], Takashi Kojima[1], Toshihiko Doi[1], Takayuki Yoshino[1], Genichiro Ishii[2,3], Takeshi Kuwata[3], and Kohei Shitara[1]

ABSTRACT

Purpose: We evaluated the association between molecular subtypes of advanced gastric cancer (AGC) and the efficacy of standard chemotherapy or immune checkpoint inhibitors.

Experimental Design: Patients with AGC who received systemic chemotherapy from October 2015 to July 2018 with available molecular features were analyzed. We investigated the efficacy of standard first- (fluoropyrimidine + platinum ± trastuzumab) and second-line (taxanes ± ramucirumab) chemotherapy, and subsequent anti–PD-1 therapy in patients with four molecular subtypes: MMR-D (mismatch repair deficient), EBV$^+$, HER2$^+$, and all negative.

Results: 410 patients were analyzed: MMR-D 5.9%, EBV$^+$ 4.1%, HER2$^+$ 13.7%, and all negative 76.3%. In 285 patients who received standard first-line chemotherapy, the median progression-free survival (PFS) times were 4.2, 6.0, 7.5, and 7.6 months and the objective response rates (ORR) were 31%, 62%, 60%, and 49% in MMR-D, EBV$^+$, HER2$^+$, and all-negative subtypes, respectively. Multivariate analysis showed shorter PFS in MMR-D versus all-negative patients [HR, 1.97; 95% CIs, 1.09–3.53; $P = 0.022$]. In second-line setting, there were no significant differences in efficacy. In 110 patients who received anti–PD-1 therapy, median PFS times were 13.0, 3.7, 1.6, and 1.9 months and the ORRs were 58%, 33%, 7%, and 13%, respectively. Twelve patients with MMR-D received subsequent anti–PD-1 therapy and showed longer PFS compared with that in 10 (83%) patients who received earlier-line chemotherapy.

Conclusions: MMR-D might result in shorter PFS with first-line chemotherapy for AGC. Subsequent anti–PD-1 therapy achieved higher ORR and longer PFS than prior chemotherapy in most patients with MMR-D, supporting the earlier use of immune checkpoint inhibitors.

Introduction

Gastric cancer is the fifth most common type of cancer and the third leading cause of death from cancer globally (1). A combination of fluoropyrimidines and a platinum agent (with trastuzumab for HER2-positive cases) as first-line therapy is the standard treatment in patients with advanced gastric cancer (AGC; refs. 2–6). Second-line therapy includes taxane agents with or without ramucirumab or irinotecan (7–12). In third-line or later therapy, two anti–PD-1 inhibitors have been approved for AGC: pembrolizumab by the U.S. Food and Drug Administration (FDA) for PD-L1–positive tumors, and nivolumab in Asian countries, regardless of PD-L1 status (13, 14). Despite the recent increase in treatment options, the median overall survival time of patients with AGC treated with a combination and sequential regimen involving these agents was less than 15 months.

The molecular characterization of gastric cancer has recently been rapidly evolving. As shown in The Cancer Genome Atlas and The Asian Cancer Research Group (15, 16), the microsatellite instability–high (MSI-H) subtype exhibits a greater number of mutations in multiple genes and hypermethylation compared with other subtypes, which contributes to the enhanced expression of neoantigens and subsequent high infiltration of CD8$^+$ T cells. Indeed, the FDA-approved pembrolizumab for patients with MSI-H/mismatch repair-deficient (MMR-D) solid tumors including AGC based on the durable response shown in several trials (14, 17–19). Also, Epstein–Barr virus-(EBV)-positive status has been reported to be associated with a high amplification rate of the *CD274* gene (which encodes PD-L1) and the *PDCD1LG2* gene (which encodes PD-L2; refs. 15, 16), as well as high expression of these immune checkpoints, which might lead to favorable outcomes for AGC following the use of immune checkpoint inhibitors (20). However, little is known about the association between molecular subtypes and the efficacy of standard chemotherapy or the relative efficacy of immune checkpoint inhibitors for AGC. Therefore, we investigated the efficacy of standard first- and second-line chemotherapy for various clinical molecular subtypes: MMR-D, EBV$^+$, HER2$^+$, and others (all negative). The impact of these subtypes on the efficacy of subsequent anti–PD-1 therapy was also analyzed in this study.

Materials and Methods

Patients

We performed a single-institute study to evaluate the efficacy of standard first- (fluoropyrimidine + platinum ± trastuzumab) and

[1]Department of Gastroenterology and Gastrointestinal Oncology, National Cancer Center Hospital East, Chiba, Japan. [2]Courses of Advanced Clinical Research of Cancer, Juntendo University Graduate School of Medicine, Tokyo, Japan. [3]Department of Pathology and Clinical Laboratories, National Cancer Center Hospital East, Chiba, Japan.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Y. Kubota and A. Kawazoe contributed equally to this article.

Corresponding Author: Kohei Shitara, Department of Gastroenterology and Gastrointestinal Oncology, National Cancer Center Hospital East, 6-5-1 Kashiwanoha, Kashiwa, Chiba 277-8577, Japan. Phone: 814-7133-1111; Fax: 814-7134-6865; E-mail: kshitara@east.ncc.go.jp

Clin Cancer Res 2020;26:3784–90

doi: 10.1158/1078-0432.CCR-20-0075

Translational Relevance

Despite the recent increase in treatment options, the prognosis of patients with advanced gastric cancer (AGC) remains poor. Recently, the molecular characterization of gastric cancer has been rapidly evolving, but little is known about the association between molecular subtypes and the efficacy of standard chemotherapy or the relative efficacy of immune checkpoint inhibitors for AGC. The aim of the present study was to analyze the efficacy of first- and second-line chemotherapy, and subsequent anti–PD-1 therapy on the molecular subtypes (MMR-D, EBV$^+$, HER2$^+$, and all-negative) of AGC. This article provides important data on the impact of these subtypes on responses to first- or second-line standard chemotherapy and the relative efficacy of subsequent anti–PD-1 therapy for AGC, which data may support treatment optimization according to the molecular subtypes.

second-line (taxanes ± ramucirumab) chemotherapy, and subsequent anti–PD-1 therapy in patients with AGC of four clinical molecular subtypes: MMR-D, EBV$^+$, HER2$^+$, and others (all negative). The eligibility criteria were as follows: (i) an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0–2; (ii) histologically proven, unresectable, locally advanced, or metastatic gastric adenocarcinoma; (iii) adequate bone marrow, hepatic, and renal function; (iv) received systemic chemotherapy from October 2015 to July 2018; and (v) with available molecular features (MMR/EBV/HER2 tests were all required, while PD-L1 expression or gene alterations were not). Patients with recurrence within 6 months of adjuvant chemotherapy were excluded from first-line analysis. All patients provided written informed consent for the biomarker analysis. The study protocol was approved by the Institutional Review Board at the National Cancer Center Japan. This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki.

Molecular characteristics

Molecular characteristics, such as the status of HER2, PD-L1, mismatch repair (MMR), and EBV, were analyzed using formalin-fixed paraffin-embedded tissue specimens from archival tissue samples. As described previously (21), IHC using a monoclonal anti-HER2 antibody [PATHWAY HER2 (4B5), Ventana] and FISH using the PathVysion HER-2 Probe Kit (Abbott Laboratories) were performed to assess HER2 status. HER2 positivity was defined as an IHC of 3+ or an IHC of 2+ and a positive FISH result according to College of American Pathologists/American Society of Clinical Oncology criteria (22). PD-L1 expression was assessed by IHC using an anti-PD-L1 rabbit mAb (Clone SP142 or SP263, Ventana) and measured using the combined positive score (CPS), defined as the number of PD-L1–positive cells (tumor cells, lymphocytes, and macrophages) as a proportion of the total number of tumor cells multiplied by 100. MMR status was assessed by IHC using mAbs for anti-mutL homolog 1 (MLH1, ES05), anti-mutS homolog 2 (MSH2, FE11), anti-postmeiotic segregation increased 2 (PMS2, EP51), and anti-mutS homolog 6 (MSH6, EP49; Agilent Technologies); tumors that lacked MLH1, MSH2, PMS2, or MSH6 expression were considered MMR-deficient (MMR-D), whereas those that maintained expression of MLH1, MSH2, PMS2, and MSH6 were considered MMR-proficient (MMR-P). Chromogenic *in situ* hybridization for EBV-encoded RNA (EBER) using fluorescein-labeled oligonucleotide probes (INFORM EBER Probe, Ventana) was performed to evaluate EBV status (23). All specimens in this study were reviewed by T. Kuwata.

Genomic alterations were analyzed using Oncomine Comprehensive Assay version 3 or Oncomine Cancer Research Panel (Thermo Fisher Scientific). TMB was defined as the number of nonsynonymous mutations, including indels, per megabase (mt/Mb) of genome examined in tumor tissue.

Outcomes and statistical analysis

We evaluated the objective response rate (ORR), the disease control rate (DCR), and progression-free survival (PFS). Tumor response was assessed in patients with measurable lesions using the Response Evaluation Criteria in Solid Tumors version 1.1. The ORR was defined as the proportion of patients with the best overall response of complete response (CR) or partial response (PR). The DCR was defined as the proportion of patients with the best overall response of CR, PR, or stable disease. The PFS was defined as the interval from the start of treatment until disease progression or death from any cause or the last follow-up visit and estimated by the Kaplan–Meier method. The χ^2 test or Fisher exact test was used to compare baseline characteristics and response rates. PFS was also compared between each molecular subtype using the Cox proportional hazards model and presented as an HR with 95% confidence intervals (CIs). Confounders in the multivariate analyzes of PFS were prespecified according to previously reported prognostic factors in AGC receiving standard chemotherapy (24–28); ECOG PS (1–2 vs 0), liver metastasis (yes vs no), peritoneal metastasis (yes vs no), number of metastatic sites (2 or more vs 1), prior gastrectomy (no vs yes), ALP [≥ upper limit normal (ULN) vs < ULN], histologic type (diffuse vs intestinal), and a measurable lesion (yes vs no). Statistical analyzes were performed using SPSS Statistics software V22 (IBM). All tests were two-sided; $P < 0.05$ was considered to indicate statistical significance.

Results

Patient characteristics

A total of 410 patients who received systemic chemotherapy from October 2015 to July 2018 were enrolled in this study. The proportions of patients who went on to second-line and third line treatment were 94% and 56%, respectively. All 410 specimens were collected from primary tumor samples before chemotherapy; 338 were biopsy specimens, and 72 were surgical specimens. All 410 patients were tested for MMR/EBV/HER2. Furthermore, results of PD-L1 expression and genomic alterations were available in 375 and 259 patients, respectively. PD-L1 expression and genomic alterations were analyzed using same tumor blocks. PD-L1 expression was assessed by IHC using mainly SP263 (82%) and partially SP142 (18%). Of the 410 patients in the overall population, MMR-D, EBV$^+$, HER2$^+$, and all-negative subtypes were identified in 24 (5.9%), 17 (4.1%), 56 (13.7%), and 313 (76.3%) patients, respectively, with no overlapping among molecular subtypes. Patient characteristics are shown in **Table 1**. In patients with MMR-D, the frequency of lymph node metastasis, CPS ≥10, TMB ≥10, *PIK3CA* mutation, and *KRAS* mutation was significantly higher compared with their frequency in all-negative subtype patients. One patient with MMR-D with appendix cancer had lack of MSH2 and MSH6 expressions and underwent germline testing for MMR genes, resulting in a diagnosis as lynch syndrome with MMR gene mutation. All other 23 patients with MMR-D with lack of MLH1 and PMS2 expression did not undergo germline testing. In EBV$^+$ patients, the frequency of *PIK3CA* mutation, CPS ≥1, and CPS ≥10 was higher

Table 1. Patient characteristics.

		MMR-D $N = 24$ (5.9%)	EBV$^+$ $N = 17$ (4.1%)	HER2$^+$ $N = 56$ (13.7%)	All-negative $N = 313$ (76.3%)
Age	Median (range)	69 (45–83)	62 (40–74)[a]	69 (38–83)	67 (23–89)
Gender	Male	19 (79%)	14 (82%)	40 (71%)	208 (67%)
Primary site	Gastric	24 (100%)	17 (100%)	50 (89%)	281 (90%)
	Gastroesophageal	0 (0%)	0 (0%)	6 (11%)	32 (10%)
Histologic type	Intestinal	17 (71%)	8 (47%)	44 (79%)[a]	164 (52%)
	Diffuse	7 (29%)	9 (53%)	12 (21%)[a]	149 (48%)
Site of metastasis	Liver	3 (13%)	3 (18%)	21 (38%)	82 (26%)
	Lung	0 (0%)	2 (12%)	11 (20%)[a]	19 (6%)
	Peritoneal	9 (38%)	7 (41%)	13 (23%)[a]	144 (46%)
	Lymph node	22 (92%)[a]	11 (65%)	47 (84%)[a]	200 (64%)
PD-L1	CPS ≥1	20/22 (91%)	14/14 (100%)	41/50 (82%)	207/289 (72%)
	CPS ≥10	15/22 (68%)[a]	7/14 (50%)	17/50 (34%)	71/289 (25%)
TMB	≥10 (mt/Mb)	13/14 (93%)[a]	4/10 (40%)	16/32 (50%)	111/189 (59%)
Gene alterations[b]	*TP53* mt	5/15 (33%)	3/12 (25%)	26/37 (70%)[a]	90/195 (46%)
	PIK3CA mt	9/15 (60%)[a]	5/12 (42%)	1/37 (3%)	16/195 (8%)
	KRAS mt	11/15 (73%)[a]	0/12 (0%)	0/37 (0%)	11/195 (6%)
	ERBB2 amp	0/15 (0%)	0/12 (0%)	22/37 (59%)[a]	1/195 (0.5%)
	CCNE1 amp	0/15 (0%)	1/12 (8%)	7/37 (19%)	16/195 (8%)

Abbreviations: amp, amplification; mt, mutation.
[a]$P < 0.05$ (using the χ^2 test and compared with the all-negative group).
[b]The top-five most frequent gene alterations.

compared with their frequency in all-negative subtype patients, although there were no statistically significant differences.

Therapeutic effect of standard first- or second-line chemotherapy

Among 410 patients, 285 patients received standard first-line chemotherapy and 252 patients received standard second-line chemotherapy (**Fig. 1**): Among 24 patients with MMR-D, 16 patients received standard first-line chemotherapy (the other eight patients received nonstandard chemotherapy) and 11 patients (including two patients who received nonstandard first-line chemotherapy) received standard second-line chemotherapy; Among 17 EBV$^+$ patients, nine patients received standard first-line chemotherapy (the other eight patients received nonstandard chemotherapy) and 11 patients (including five patients who received nonstandard first-line chemotherapy) received standard second-line chemotherapy. In 243 patients with measurable disease who received standard first-line chemotherapy, the ORRs were 31%, 62%, 60%, and 49% in MMR-D, EBV$^+$, HER2$^+$, and all-negative subtypes, respectively (**Table 2A**). The median PFS times with first-line chemotherapy were 4.2, 6.0, 7.5, and 7.6 months in MMR-D, EBV$^+$, HER2$^+$, and all-negative subtypes, respectively (**Fig. 2A**). PFS in patients with MMR-D tended to be shorter compared with that in all-negative patients, with an HR of 1.64 (95% CI, 0.95–2.84; $P = 0.075$).

This difference was found to be statistically significant by multivariate analysis after adjustment for confounding factors (HR, 1.97; 95% CIs, 1.09–3.53; $P = 0.022$), suggesting shorter PFS following first-line chemotherapy in patients with MMR-D (**Table 3**). No other differences between the subgroups were statistically significant.

In 238 patients with measurable disease who received standard second-line chemotherapy, the ORRs were 30%, 40%, 22%, and 27% in MMR-D, EBV$^+$, HER2$^+$, and all-negative subtypes, respectively (**Table 2B**). The median PFS times following second-line chemotherapy were 3.4, 6.6, 3.7, and 3.9 months in MMR-D, EBV$^+$, HER2$^+$, and

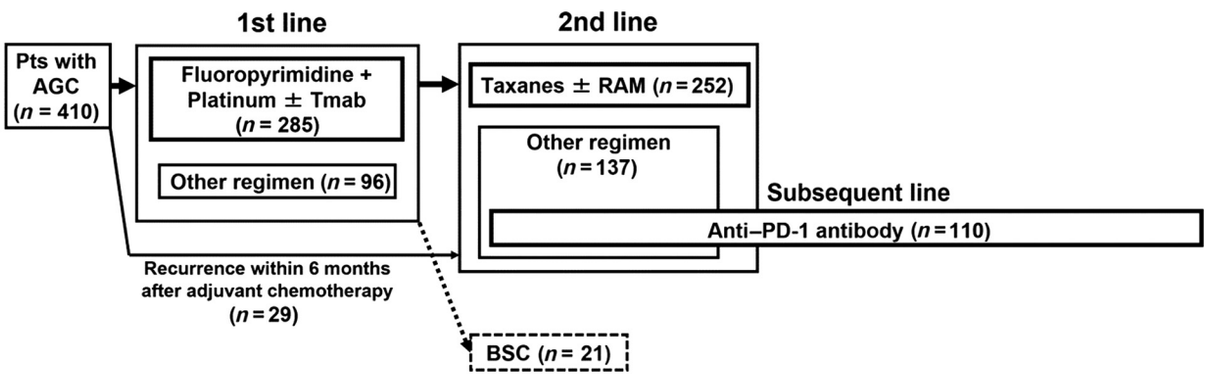

Figure 1.
CONSORT diagram. Among 410 patients, 285 patients received standard first-line chemotherapy, 252 patients, and 110 patients received subsequent anti–PD-1 therapy including second-line setting.

Table 2. The ORR with each type of chemotherapy.

A. The ORR with first-line chemotherapy

	MMR-D $N = 16$	EBV$^+$ $N = 9$	HER2$^+$ $N = 52$	All-negative $N = 208$	*P* (MMR-D vs all-negative)
Measurable lesion +	13	8	50	171	
CR	0	0	0	2	
PR	4	5	30	82	
SD	4	1	16	60	
PD	4	2	2	20	
NE	1	0	2	7	
ORR (%)	31	62	60	49	0.256
DCR (%)	62	75	92	84	0.053

B. The ORR with second-line chemotherapy

	$N = 11$	$N = 11$	$N = 42$	$N = 188$	(MMR-D vs all-negative)
Measurable lesion +	10	10	41	174	
CR	0	0	0	0	
PR	3	4	9	47	
SD	4	3	20	78	
PD	2	2	10	33	
NE	1	1	2	10	
ORR (%)	30	40	22	27	0.715
DCR (%)	70	70	71	72	1.000

C. The ORR with subsequent anti–PD-1 therapy

	$N = 12$	$N = 6$	$N = 14$	$N = 78$	(MMR-D vs all-negative)
Measurable lesion +	12	6	14	78	
CR	1	0	0	0	
PR	6	2	1	10	
SD	4	3	3	20	
PD	0	1	9	44	
NE	1	0	1	4	
ORR (%)	58	33	7	13	0.001
DCR (%)	92	83	29	38	0.0009

Abbreviations: CR, complete response; DCR, disease control rate (CR, PR, or SD); NE, not evaluated; PD, progressive disease; PR, partial response; SD, stable disease.

all-negative subtypes, respectively (**Fig. 2B**). There were no significant differences in efficacy seen among the four subtypes following second-line chemotherapy.

Therapeutic effect of subsequent anti–PD-1 therapy

Among 410 patients, 110 patients received subsequent anti–PD-1 antibodies (**Fig. 1**): Among 24 patients with MMR-D, 12 patients received subsequent anti–PD-1 therapy (four patients in the second line and eight patients in the third or later line); Among 17 EBV$^+$ patients, six patients received subsequent anti–PD-1 therapy (two patients in the second line and four patients in the third or later line). In 110 patients with measurable disease receiving subsequent anti–PD-1 antibodies, the ORRs were 58%, 33%, 7%, and 13% in MMR-D, EBV$^+$, HER2$^+$, and all-negative subtypes, respectively ($P = 0.001$, MMR-D vs all negative; **Table 2C**). The median PFS times were 13.0, 3.7, 1.6, and 1.9 months in MMR-D, EBV$^+$, HER2$^+$, and all-negative subtypes, respectively (**Fig. 2C**). Patients with MMR-D had a significantly longer PFS following anti–PD-1 therapy compared with the PFS in all-negative patients (HR, 0.14; 95% CI: 0.06–0.36; $P < 0.001$). The patient diagnosed as Lynch syndrome showed durable objective response to anti–PD-1 therapy (shown in **Fig. 3** as patients no. 6). Patients with EBV$^+$ subtypes also tended to show longer PFS compared with PFS in all-negative patients (HR, 0.48; 95% CI, 0.22–1.05; $P = 0.064$). Among patients receiving subsequent anti–PD-1 therapy, the percentage of patients who showed longer PFS following anti–PD-1 therapy compared with earlier-line chemotherapy were 83%, 33%, 14%, and 26% in MMR-D, EBV$^+$, HER2$^+$, and all-negative subtypes, respectively (Swimmer plot of PFS times with each line chemotherapy in 12 patients with MMR-D who received subsequent ant-PD-1 therapy is shown in **Fig. 3**).

Comparisons of clinical and molecular features between responders (CR or PR) and nonresponders (SD or PD) in each subtype with anti–PD-1 therapy were also available in Supplementary Table S1. In all-negative subtype, higher ORRs were observed in patients with PS 0 than in those with PS ≥1 (25% vs 6%) and in patients with TMB ≥10 than in those with TMB <10 (17% vs 9%), although these were not statistically significant (Supplementary Table S1D).

Discussion

We investigated the efficacy of systemic chemotherapy in patients with AGC according to their different clinical molecular subtypes. To

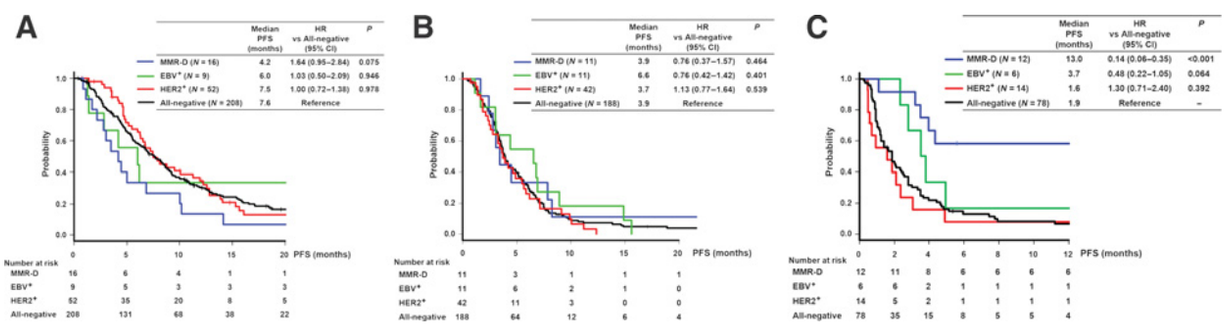

Figure 2.
Progression-free survival (PFS) with each line of chemotherapy. **A,** PFS with first-line chemotherapy. **B,** PFS with second-line chemotherapy. **C,** PFS with subsequent anti–PD-1 therapy.

Table 3. Subgroup analysis of PFS with first-line chemotherapy in MMR-D and all-negative patients ($N = 224$).

		Univariate analysis		Multivariate analysis	
	N	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
PS 1, 2 (vs PS 0)	54 (170)	1.58 (1.11–2.25)	0.011	1.39 (1.95–2.05)	0.088
Liver metastasis + (vs −)	60 (164)	1.54 (1.12–2.12)	0.008	1.12 (0.73–1.70)	0.597
Peritoneal metastasis + (vs −)	107 (117)	0.89 (0.67–1.19)	0.433	1.02 (0.71–1.49)	0.877
No. of metastatic sites ≥2 (vs 1)	112 (112)	1.85 (1.37–2.49)	<0.001	1.47 (1.03–2.09)	0.031
Gastrectomy: no (vs yes)	52 (172)	1.41 (0.99–1.20)	0.057	1.21 (0.81–1.79)	0.340
ALP ≧ULN[a] (vs < ULN)	62 (162)	1.28 (0.93–1.76)	0.130	1.12 (0.79–1.58)	0.517
Diffuse (vs intestinal)	102 (122)	0.98 (0.73–1.32)	0.935	1.03 (0.74–1.42)	0.847
Measurable lesion + (vs −)	184 (40)	2.13 (1.42–3.20)	<0.001	1.79 (1.12–2.88)	0.014
MMR-D (vs all-negative)	16 (208)	1.64 (0.95–2.84)	0.075	1.97 (1.09–3.53)	0.022

[a]ULN of ALP: 322 IU/L.

our knowledge, this is the first report to provide detailed information on the impact of these subtypes on responses to first- or second-line standard chemotherapy and the relative efficacy of subsequent anti–PD-1 therapy for AGC.

In our patient cohort, the MMR-D subtype was associated with significantly shorter PFS and a lower ORR following first-line chemotherapy compared with other subtypes. This observation is consistent with a small earlier study that demonstrated shorter PFS following first-line chemotherapy for AGC patients with MSI-H compared with those with non-MSI-H tumors (29). Also, a recent meta-analysis of four randomized trials (MAGIC, CLASSIC, ARTIST, and ITACA-S) showed that patients with MSI-H locally advanced gastroesophageal cancer did not exhibit any survival benefit from perioperative cytotoxic chemotherapy (30). Several preclinical studies using cancer cell line models demonstrated that a loss of MMR results in an inability to detect DNA damage and activate apoptosis of tumor cells, leading to resistance to DNA-damaging cytotoxic agents, such as fluoropyrimidine and platinum compounds (31–34). However, a phase III study, KEYNOTE-062, which compared the efficacy of cytotoxic agents with that of pembrolizumab monotherapy in patients with untreated AGC showed that the ORRs to first-line standard chemotherapy in MSI-H AGC were almost equal to those of the whole population (35). Thus, further analysis regarding the impact of MSI/MMR status on the efficacy of combinations of fluoropyrimidines and a platinum agent is needed. In a second-line setting, we found that there were no significant differences in efficacy among the clinical molecular subtypes. Taxanes exert antitumor effects by impairing microtubule function and inhibiting cell division, and its process is not associated with MMR function, which might be one of the reasons for the almost equal efficacy of second-line chemotherapy for MMR-D AGC and the other subtypes.

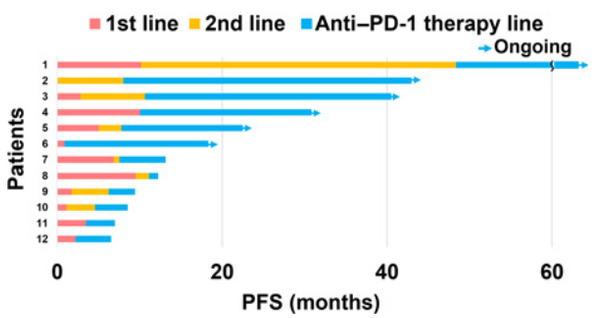

Figure 3.
Swimmer plot of PFS times with each line chemotherapy in 12 patients with MMR-D who received subsequent anti–PD-1 therapy.

Consistent with previous reports (19, 20, 29), subsequent anti–PD-1 therapy was more effective in patients with AGC with MMR-D. Importantly, most patients with MMR-D showed an objective response and longer PFS compared with earlier-line chemotherapy. These results support the earlier use of immune checkpoint inhibitors for AGC patients with MSI-H/MMR-D.

In a recent phase II study of pembrolizumab, extremely high ORRs (100%) were reported in 6 patients with EBV^+ AGC (20), while one of four patients (25%) with EBV^+ AGC achieved a PR in a phase Ib/II study of toripalimab (a PD-1 antibody; ref. 36). All responders with EBV^+ in these studies showed PD-L1–positive status. In our study, two (one with CPS ≥10 and one with unknown CPS status) of six patients (33%) with EBV^+ achieved a PR. Among four patients with CPS evaluation, one of four patients (25%) with CPS ≥ 1 achieved a PR, while one of two patients (50%) with CPS ≥ 10 did. The impact of EBV status as well as PD-L1 expression on the efficacy of immune checkpoint inhibitors should be evaluated in a larger cohort.

Although an exploratory subgroup analysis of a phase III trial, ATTRACTION-2, showed that nivolumab improved clinical outcomes compared with placebo regardless of prior trastuzumab use in patients with AGC (37), HER2 alterations in gastric cancer were associated with decreased immunogenicity in terms of immune-related gene mRNA expression, immune infiltrates, and neoantigen level (38). Indeed, patients with $HER2^+$ AGC in our study had shorter PFS and a lower ORR with anti–PD-1 therapy, which warrants further evaluation in a larger cohort.

There were some limitations inherent in this study. First, this was a single-institution study with a limited sample size. Owing to the small number of patients with MMR-D- and EBV-positive status, we could not evaluate the exact impact of these molecular factors on the efficacy of standard chemotherapy, which warrants further evaluation with a larger cohort. Second, timing of radiographic evaluation was not specified prospectively due to retrospective nature of the study. Third, PD-L1 expression or gene alterations were not analyzed in all patients who received systemic chemotherapy. Fourth, we used SP263 (82%) or SP142 (18%) assay for PD-L1 assessment rather than 22C3 assay. A previous study showed that a higher proportion of PD-L1+ status (≥1% in tumor cells or immune cells) was observed in patients with lung cancer with SP263 than in those with 22C3 (39). This difference in staining might be

associated with the relatively higher PD-L1 rate in this study. Finally, overall survival was not evaluated in this study as it was affected by sequential therapies.

In conclusion, MMR-D might result in poor clinical outcomes with first-line chemotherapy for patients with AGC compared with other subtypes. However, subsequent anti–PD-1 therapy achieved favorable outcomes compared with outcomes following prior chemotherapy in most patients with MMR-D, supporting the earlier use of immune checkpoint inhibitors.

Disclosure of Potential Conflicts of Interest

D. Kotani reports receiving speakers bureau honoraria from Takeda, MerckBiopharma, Chugai, Lilly, Taiho, and Sysmex. Y. Kuboki reports receiving speakers bureau honoraria from MSD and Ono. H. Taniguchi reports receiving speakers bureau honoraria from Ono Pharma, MSD, and Taiho. T. Kojima is an employee/paid consultant for Bristol-Myers Squibb, and reports receiving commercial research grants from Chugai Pharma, MSD, Astellas Amgen BioPharama, Shionogi, and Ono Pharmaceutical. T. Yoshino reports receiving other commercial research support from Novartis Pharma K.K., MSD.K.K., Sumitomo Dainippon Pharma Co., Ltd., Chugai Pharmaceutical CO., LTD., Sanofi K.K, Daiichi Sankyo Company, Limited, PAREXEL International Inc., Ono Pharmaceutical CO., LTD., GlaxoSmithKline K.K., and Boehringer Ingelheim Japan, Inc. T. Kuwata reports receiving commercial research grants from Ono and Daiichi-Sankyo, and speakers bureau honoraria from Chugai, Taiho, and MSD. K. Shitara is an employee/paid consultant for Astellas Pharma, Lilly, Bristol-Myers Squibb, Takeda, Pfizer, Ono Pharmaceutical, Taiho, MSD, Novartis, Abbvie, and GlaxoSmithKline, reports receiving commercial research grants from Lilly, Ono Pharmaceutical, Dainippon Sumitomo Pharma, MSD, Daiichi Sankyo, Taiho Pharmaceutical, Chugai Pharma, Astellas Pharma, and Medi Science, and holds ownership interest (including patents) in Novartis, Abbvie, and Yakult. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: Y. Kubota, A. Kawazoe, T. Yoshino, K. Shitara
Development of methodology: Y. Kubota, A. Kawazoe, T. Yoshino, K. Shitara
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): Y. Kubota, S. Mishima, Y. Kuboki, H. Taniguchi, T. Kojima, T. Yoshino, T. Kuwata, K. Shitara
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): Y. Kubota, A. Kawazoe, A. Sasaki, Y. Nakamura, H. Taniguchi, T. Doi, T. Yoshino, K. Shitara
Writing, review, and/or revision of the manuscript: Y. Kubota, A. Kawazoe, A. Sasaki, K. Sawada, Y. Nakamura, D. Kotani, Y. Kuboki, H. Taniguchi, T. Kojima, T. Yoshino, G. Ishii, K. Shitara
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): Y. Kubota, A. Sasaki, S. Mishima, K. Shitara
Study supervision: T. Doi, K. Shitara

Acknowledgments

This study was supported by a research funding from National Cancer Center Hospital East.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received January 8, 2020; revised February 19, 2020; accepted March 5, 2020; published first March 10, 2020.

References

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015;136:E359–86.
2. National Comprehensive Cancer Network I. NCCN clinical practice guidelines in oncology. Gastric cancer (version 5.2017); 2017. Available from: https://www.nccn.org/professionals/physician_gls/default.aspx.
3. Van Laethem JL, Carneiro F, Ducreux M, Messman H, Lordick F, Ilson DH, et al. The multidisciplinary management of gastro-oesophageal junction tumours. European Society of Digestive Oncology (ESDO): expert discussion and report from the 16th ESMO World Congress on Gastrointestinal Cancer, Barcelona. Dig Liver Dis 2016;48:1283–9. Available from: https://www.dldjournalonline.com/article/S1590-8658(16)30689-2/fulltext.
4. Smyth EC, Verheij M, Allum W, Cunningham D, Cervantes A, Arnold D. Gastric cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol 2016;27:v38–49. Available from: https://www.esmo.org/guidelines/gastrointestinal-cancers/gastric-cancer.
5. Japanese Gastric Cancer Association. Japanese gastric cancer treatment guidelines 2018 (version 5). Available from: https://link.springer.com/article/10.1007/s10120-020-01042-y.
6. Bang YJ, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, et al. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomized controlled trial. Lancet 2010;376:687–97.
7. Ford HE, Marshall A, Bridgewater JA, Janowitz T, Coxon FY, Wadsley J, et al. Docetaxel versus active symptom control for refractory oesophagogastric adenocarcinoma (COUGAR-02): an open-label, phase 3 randomised controlled trial. Lancet Oncol 2014;15:78–86.
8. Thuss-Patience PC, Kretzschmar A, Bichev D, Deist T, Hinke A, Breithaupt K, et al. Survival advantage for irinotecan versus best supportive care as second-line chemotherapy in gastric cancer—a randomized phase III study of the Arbeitsgemeinschaft Internistische Onkologie (AIO). Eur J Cancer 2011;47:2306–14.
9. Kang JH, Lee SI, Lim do H, Park KW, Oh SY, Kwon HC. Salvage chemotherapy for pretreated gastric cancer: a randomized phase III trial comparing chemotherapy plus best supportive care with best supportive care alone. J Clin Oncol 2012;30:1513–8.
10. Hironaka S, Ueda S, Yasui H, Nishina T, Tsuda M, Tsumura T, et al. Randomized, open-label, phase III study comparing irinotecan with paclitaxel in patients with advanced gastric cancer without severe peritoneal metastasis after failure of prior combination chemotherapy using fluoropyrimidine plus platinum: WJOG 4007 trial. J Clin Oncol 2013;31:4438–44.
11. Fuchs CS, Tomasek J, Yong CJ, Dumitru F, Passalacqua R, Goswami C, et al. Ramucirumab monotherapy for previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (REGARD): an international, randomised, multicentre, placebo-controlled, phase 3 trial. Lancet 2014;383:31–9.
12. Wilke H, Muro K, Van Cutsem E, Oh SC, Bodoky G, Shimada Y, et al. Ramucirumab plus paclitaxel versus placebo plus paclitaxel in patients with previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (RAINBOW): a double-blind, randomised phase 3 trial. Lancet Oncol 2014;15:1224–35.
13. Kang YK, Boku N, Satoh T, Ryu M-H, Chao Y, Kato K, et al. Nivolumab in patients with advanced gastric or gastro-oesophageal junction cancer refractory to, or intolerant of, at least two previous chemotherapy regimens (ONO-4538-12, ATTRACTION-2): a randomised, doubleblind, placebo-controlled, phase 3 trial. Lancet 2017;390:2461–71.
14. Fuchs CS, Doi T, Jang RW, Muro K, Satoh T, Machado M, et al. Safety and efficacy of pembrolizumab monotherapy in patients with previously treated advanced gastric and gastroesophageal junction cancer: phase 2 clinical KEYNOTE-059 trial. JAMA Oncol 2018;4:e180013.
15. Cancer Genome Atlas Research Network. Comprehensive molecular characterization of gastric adenocarcinoma. Nature 2014;513:202–9.
16. Cristescu R, Lee J, Nebozhyn M, Kim KM, Ting JC, Wong SS, et al. Molecular analysis of gastric cancer identifies subtypes associated with distinct clinical outcomes. Nat Med 2015;21:449–56.
17. Diaz LA Jr, Marabelle A, Delord JP, Shapira-Frommer R, Geva R, Peled N, et al. Pembrolizumab therapy for microsatellite instability high (MSI-H) colorectal cancer (CRC) and non-CRC. J Clin Oncol 2017;35:3071.
18. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med 2015;372:2509–20.
19. Le DT, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science 2017;357:409–13.

20. Kim ST, Cristescu R, Bass AJ, Kim KM, Odegaard JI, Kim K, et al. Comprehensive molecular characterization of clinical responses to PD-1 inhibition in metastatic gastric cancer. Nat Med 2018;24:1449–58.
21. Mishima S, Kawazoe A, Nakamura Y, Sasaki A, Kotani D, Kuboki Y, et al. Clinicopathological and molecular features of responders to nivolumab for patients with advanced gastric cancer. J Immunother Cancer 2019;7:24.
22. Wolff AC, Hammond MEH, Hicks DG, Dowsett M, McShane LM, Allison KH, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American pathologists clinical practice guideline update. J Clin Oncol 2013;31:3997–4013.
23. Kawazoe A, Shitara K, Kuboki Y, Bando H, Kojima T, Yoshino T, et al. Clinicopathological features of 22C3 PD-L1 expression with mismatch repair, Epstein-Barr virus status, and cancer genome alterations in metastatic gastric cancer. Gastric Cancer 2019;22:69–76.
24. Chau I, Norman AR, Cunningham D, Waters JS, Oates J, Ross PJ, et al. Multivariate prognostic factor analysis in locally advanced and metastatic esophago-gastric cancer–pooled analysis from three multicenter, randomized, controlled trials using individual patient data. J Clin Oncol 2004;22: 2395–403.
25. Lee J, Lim T, Uhm JE, Park KW, Park SH, Lee SC, et al. Prognostic model to predict survival following first-line chemotherapy in patients with metastatic gastric adenocarcinoma. Ann Oncol 2007;18:886–91.
26. Takahari D, Boku N, Mizusawa J, Takashima A, Yamada Y, Yoshino T, et al. Determination of prognostic factors in Japanese patients with advanced gastric cancer using the data from a randomized controlled trial, Japan clinical oncology group 9912. Oncologist. 2014;194:358–66.
27. Yamada Y, Higuchi K, Nishikawa K, Gotoh M, Fuse N, Sugimoto N, et al. Phase III study comparing oxaliplatin plus S-1 with cisplatin plus S-1 in chemotherapy-naïve patients with advanced gastric cancer. Ann Oncol 2015; 26:141–148.
28. Paoletti X, Oba K, Bang YJ, Bleiberg H, Boku N, Bouché O, et al. Progression-free survival as a surrogate for overall survival in advanced/recurrent gastric cancer trials: a meta-analysis. J Natl Cancer Inst 2013;105:1667–70.
29. Janjigian YY, Sanchez-Vega F, Jonsson P, Chatila WK, Hechtman JF, Ku GY, et al. Genetic predictors of response to systemic therapy in esophagogastric cancer. Cancer Discov 2018;8:49–58.
30. Pietrantonio F, Miceli R, Raimondi A, Kim YW, Kang WK, Langley RE, et al. Individual patient data meta-analysis of the value of microsatellite instability as a biomarker in gastric cancer. J Clin Oncol 2019;37:3392–40.
31. Fink D, Aebi S, Howell SB. The role of DNA mismatch repair in drug resistance. Clin Cancer Res 1998;4:1–6.
32. Meyers M, Wagner MW, Mazurek A, Schmutte C, Fishel R, Boothman DA. DNA mismatch repair-dependent response to fluoropyrimidine-generated damage. J Biol Chem 2005;280:5516–26.
33. Meyers M, Wagner MW, Hwang HS, Kinsella TJ, Boothman DA. Role of the hMLH1 DNA mismatch repair protein in fluoropyrimidine-mediated cell death and cell cycle responses. Cancer Res 2001;61:5193–201.
34. Tajima A, Hess MT, Cabrera BL, Kolodner RD, Carethers JM. The mismatch repair complex hMutS alpha recognizes 5-fluorouracil-modified DNA: implications for chemosensitivity and resistance. Gastroenterology 2004;127: 1678–84.
35. Tabernero J, Van Cutsem E, Bang YJ, Fuchs CS, Wyrwicz L, Lee KW, et al. Pembrolizumab with or without chemotherapy versus chemotherapy for advanced gastric or gastroesophageal junction (G/GEJ) adenocarcinoma: the phase III KEYNOTE-062 study. J Clin Oncol 2019;37:18s (suppl; abstr LBA4007).
36. Wang F, Wei1 XL, Wang FH, Xu N, Shen L, Dai GH, et al. Safety, efficacy and tumor mutational burden as a biomarker of overall survival benefit in chemo-refractory gastric cancer treated with toripalimab, a PD-1 antibody in phase Ib/II clinical trial NCT02915432. Ann Oncol 2019;30:1479–86.
37. Satoh T, Kang YK, Chao Y, Ryu MH, Kato K, Chung HC, et al. Exploratory subgroup analysis of patients with prior trastuzumab use in the ATTRACTION-2 trial: a randomized phase III clinical trial investigating the efficacy and safety of nivolumab in patients with advanced gastric/gastroesophageal junction cancer. Gastric Cancer 2019;23:143–153.
38. Wei B, Xing X, Li Z, Han Q, Hao Y, Zhou J, et al. Tumor mutation burden and immunogenicity in gastric cancer with HER2 alterations. J Clin Oncol 2017;37: 4024.
39. Hendry S, Byrne DJ, Wright GM, Young RJ, Sturrock S, Cooper WA, et al. Comparison of four PD-L1 immunohistochemical assays in lung cancer. J Thorac Oncol 2018;13:367–376.