The impact of programmed death-ligand 1 expression on mismatch repair deficiency and Epstein-Barr virus status on survival outcomes in patients with stage II/III gastric cancer after surgery

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The impact of programmed death-ligand 1 expression on mismatch repair deficiency and Epstein-Barr virus status on survival outcomes in patients with stage II/III gastric cancer after surgery

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Short running head: Biomarkers of stage II/III gastric caner

Abstract

Background

The aim of this study was to investigate the prognostic impact of mismatch repair (MMR) status, programmed death-ligand 1 (PD-L1) expression and Epstein-Barr virus (EBV) status in stage II/III gastric cancer after surgery.

Methods

This study included 679 patients diagnosed with pathological stage II/III gastric cancer who underwent curative gastrectomy followed by adjuvant chemotherapy or observation between 2007 and 2015. Clinical outcomes were retrospectively reviewed and compared with stratification by adjuvant chemotherapy and other clinicopathological factors.

Results

Patients were divided into adjuvant chemotherapy (AC; $n = 484$) or surgery alone (SA; $n = 195$) groups and further stratified by MMR and EBV status: MMR-deficient (DMMR) and MMR-proficient (PMMR) groups. Comparing the AC-DMMR group vs the AC-PMMR group, 5-year overall survival was 92.0% vs 74.0% (log-rank $P < 0.01$), respectively. Comparing the SA-DMMR group vs the SA-PMMR group, 5-year overall survival was 71.1% vs 73.7% (log-rank $P = 0.89$), respectively. DMMR (hazard ratio,

0.25, 95% confidence interval, 0.07–0.81) was identified as an independent prognostic factor in the AC group but not in the SA group. In the subgroup analysis, PD-L1-negative patients in the EBV-positive patients or in the DMMR group had a poor prognosis in both the AC and SA groups. The prognosis of the PMMR and EBV-negative patients was similar regardless of PD-L1 expression.

Conclusions

DMMR was associated with a favorable prognosis in stage II/III gastric cancer after surgery and adjuvant therapy. PD-L1 expression may affect the prognosis of DMMR and EBV-positive gastric cancer.

Keywords: gastric cancer, microsatellite instability, programmed cell death-ligand 1, Epstein-Barr virus, adjuvant chemotherapy

Synopsis

DMMR was associated with a favorable prognosis in stage II/III gastric cancer after surgery and adjuvant therapy. PD-L1 expression may affect the prognosis of DMMR and EBV-positive gastric cancer.

Introduction

The Cancer Genome Atlas project reported that gastric cancer can be divided into four subtypes: Epstein-Barr virus (EBV), microsatellite instability (MSI), genomically stable, and chromosomal instability. Previous studies suggested that high MSI (MSI-H) status is observed in 7%–22% of patients with gastric cancer. [1 - 3] MSI-H status is caused by mismatch repair deficiency (DMMR), which results from either germline somatic mutations or epimutation in mismatch repair (*MMR*) genes and is closely associated with tumor mutation burden. Therefore, immune checkpoint inhibitors (ICIs) are considered highly effective in these patients. Furthermore, the therapeutic effect of chemotherapy for patients with MSI-H is reported to be poor in advanced gastric cancers. [4 - 7] Additionally, adjuvant chemotherapy is standard treatment for stage II/III gastric cancer in East Asia. [8 - 10] In Europe, perioperative chemotherapy is the standard treatment for resectable gastroesophageal cancer. [11] Previous reports on colon cancer suggested that fluorouracil-based adjuvant chemotherapy does not improve MSI-H patients' survival outcomes. Therefore, measurement of MSI is recommended for stage II colorectal cancer. [12 - 15] Similar findings have been reported for adjuvant chemotherapy in MSI-H gastric cancers. [2, 3] However, information regarding the

clinicopathological features of MSI status in patients with stage II/III gastric cancer undergoing S-1-based adjuvant chemotherapy is limited.

Programmed death-ligand 1 (PD-L1) and EBV are predictive biomarkers for ICI efficacy. [16 - 18] Antigen escape and overexpression of immune checkpoint proteins observed in gastric cancer are the basis of immunotherapy antibody targeting programmed death-1 (PD-1) and PD-L1. [19, 20] Moreover, MSI and EBV status were associated with higher PD-L1 expression, and the combination of MSI and PD-L1 was a reported predictive factor for prognosis. [21, 22] However, the association between PD-L1 expression and prognosis in gastric cancer is still controversial.

In this study, we investigated the impact of MMR status, and PD-L1 expression for each MMR status or EBV status, on survival outcomes in patients with stage II/III gastric cancer after surgery.

Methods

Patients and Methods

This was a single-institutional, retrospective, case-control study. We retrospectively reviewed the clinical records of 679 patients who underwent gastrectomy with R0 resection for stage II/III gastric cancer between 2007 and 2015, using prospectively

collected data from an in-house database at the National Cancer Center Hospital East in Kashiwa, Japan. Patients who received neoadjuvant chemotherapy were excluded. To construct the tissue microarrays (TMAs), two representative tumor cores, obtained from the infiltrated area of the tumor, were formalin-fixed and embedded in paraffin. Serial sections were cut at 4-μm intervals and used for immunohistochemistry (IHC) and in situ hybridization. All tissue cores were evaluated by a pathologist. Tumor staging was performed in accordance with the UICC 8th TNM classification, [23] and the numbering of lymph node stations was performed in accordance with the classification of the Japanese Gastric Cancer Association (3rd English version). [24] This study was approved by the institutional review board of the National Cancer Center, Japan (IRB file No. 2017-416, approval date: 2 March 2018).

Immunohistochemistry

The following primary antibodies were used for IHC: anti-PD-L1 (22C3) rabbit monoclonal antibody (PD-L1 IHC 22C3 pharmDx; Agilent Technologies, Carpinteria, CA, USA), anti-mutLhomolog 1 (MLH1; ES05) monoclonal antibody, anti-mutShomolog 2 (MSH2; FE11) monoclonal antibody, anti-postmeiotic segregation

increased 2 (PMS2; EP51) monoclonal antibody, and anti-mutShomolog 6 (MSH6; EP49) monoclonal antibody (Dako, Copenhagen, Denmark) on the Dako autostainer.

Evaluation of PD-L1 expression

Combined positive score (CPS) was determined by the number of PD-L1-positive cells, including tumor cells, lymphocytes, and macrophages, divided by the total number of viable tumor cells. For cases that had two cores that exhibited different PD-L1 expression scores, the highest score was selected.

Evaluation of MMR status

Expression of MLH1, PSM2, MSH2, or MSH6 was determined in the tumor cell nucleus. The absence of expression of any of the following: MLH1, PSM2, MSH2, or MSH6 was considered DMMR, whereas tumors expressing all markers were considered mismatch repair proficient (PMMR).

EBV in situ hybridization

EBV-encoded RNA was analyzed using ISH with an INFORM EBER probe (Ventana, Tucson, AZ, USA). EBER-ISH was performed with an iViewBlue detection kit (Ventana) using the BenchMark ULTRA staining system (Ventana).

Statistical analysis

All statistical analyses were performed using R version 3.6.1 (www.r-project.org). Fisher's exact test and the Mann–Whitney *U* test were used for the statistical analyses. All *P* values less than 0.05 were considered statistically significant. Survival curves were constructed using the Kaplan–Meier method, and the log-rank test was used to assess survival differences. Data were censored on 23 September 2021.

Results

Baseline characteristics and survival outcomes of the patients

First, we divided the patients into either the adjuvant chemotherapy (AC) group (*n* = 484) or the surgery alone (SA) group (*n* = 195) because S-1-based adjuvant chemotherapy has been the standard treatment for patients with stage II/III gastric cancer in Japan since 2007. Four hundred and eighty-four patients (71.2%) received adjuvant chemotherapy, which included S-1 monotherapy (96.5%). The patients'

demographic information is summarized in Table 1. The AC group comprised patients who were eligible for standard treatment. The SA group comprised patients who were not eligible for standard treatment mainly owing to older age, the presence of pathological stage II cancer, or patients had no wish to receive adjuvant chemotherapy. The SA group had a significantly higher proportion of PD-L1 expression, and a lower proportion of DMMR-positive patients than those in the AC group. The ratio of deaths due to other diseases to the total number of deaths was 50.8% (30/59) in the SA group and 7.9% (11/138) in the AC group. Second, the AC and SA groups were further divided into two groups according to MMR status (AC-DMMR group [n = 41] and AC-PMMR group [n = 443], respectively, and SA-DMMR group [n = 30] and SA-PMMR group [n = 165], respectively), and the demographic information of the AC group is shown in Table 2. The AC-DMMR group was older and had a significantly higher proportion of PD-L1 expression compared with the AC-PMMR group. Comparing the AC-DMMR vs the AC-PMMR group, the 5-year overall survival rate was 92.0% vs 74.0% (log-rank $P < 0.01$), respectively, and the relapse-free survival rate was 90.0% vs 62.7% (log-rank $P < 0.01$), respectively (Fig. 1).

The demographic information of the SA-DMMR group (n = 30) and SA-PMMR group (n = 165) is shown in Table 3. The SA-DMMR group was older and had a significantly

higher proportion of PD-L1 expression compared with the SA-PMMR group.

Comparing the SA-DMMR vs the SA-PMMR group, the 5-year overall survival rate was 71.1% vs 73.7% (log-rank $P = 0.89$), respectively, and the relapse-free survival rate was 68.2% vs 67.9% (log-rank $P = 0.95$), respectively (Fig. 1).

Five-year overall survival (Fig. 2) and 5-year relapse-free survival (Supplemental Fig. 1) were stratified for stage II and stage III cancer in the AC group and the SA group. Comparing the AC-DMMR vs the AC-PMMR group, the 5-year overall survival rate was 100.0% vs 87.8% (log-rank $P < 0.01$), respectively, for stage II and 83.8% vs 65.8% (log-rank $P = 0.08$), respectively, for stage III. Comparing the SA-DMMR vs the SA-PMMR group, the 5-year overall survival rate was 84.4% vs 81.0% (log-rank $P = 0.72$), respectively, for stage II and 41.7% vs 50.1% (log-rank $P = 0.41$), respectively, for stage III. Of the four patients in the stage III AC-DMMR group with recurrence, two patients were pN3 and one patient was pT4b. Of the five patients in the stage III SA-DMMR group with recurrence, two patients were pN3 and two patients were pT4b.

The 5-year overall survival rates based on PD-L1 expression for each MMR status or EBV status are shown in Fig. 3, and 5-year relapse-free survival rates are shown in Supplemental Fig. 2. The patients' demographic information is summarized in Supplemental Tables 1 and 2. In the subgroup analysis, PD-L1-negative patients in the

DMMR or EBV-positive groups had a poorer prognosis than that of the PD-L1-positive patients in the same groups. In particular, in the EBV-positive group, the prognosis of PD-L1-negative cases was significantly poorer than that of the PD-L1-positive cases in the AC group (55.6% vs 92.9%, respectively; log-rank $P = 0.04$) and the SA group (0% vs 77.8%, respectively; log-rank $P < 0.01$).

Multivariate analysis

The results of the multivariate analysis for overall survival in the AC and SA groups are shown in Table 4. In the AC group, DMMR status (hazard ratio: 0.25, 95% confidence interval: 0.07–0.81) was identified as an independent prognostic factor. However, PD-L1-positive status (CPS ≥ 1; hazard ratio: 0.81, 95% confidence interval: 0.53–1.23) and EBV-positive status (hazard ratio: 0.63, 95% confidence interval: 0.25–1.58) were not independent prognostic factors. Whereas, in the SA group, DMMR status (hazard ratio: 1.39, 95% confidence interval: 0.61–3.18), PD-L1-positive status (CPS ≥ 1; hazard ratio: 0.66, 95% confidence interval: 0.36–1.24), and EBV-positive status (hazard ratio: 2.15, 95% confidence interval: 0.68–6.80) were not identified as independent prognostic factors.

Discussion

In this study, we investigated the MMR status, PD-L1 expression, and EBV status of 679 patients in our institution who underwent mainly S-1-based adjuvant chemotherapy as the standard treatment for stage II/III gastric cancer. Most of the patients who did not receive adjuvant chemotherapy were elderly, were diagnosed with stage II gastric cancer, or had no wish to receive adjuvant chemotherapy. Previous reports indicated that fluorouracil-based adjuvant chemotherapy is not effective for MSI-H colon cancer and gastric cancer. [12 – 15] In colon cancer, a previous report confirmed an overall survival benefit of oxaliplatin as adjuvant chemotherapy for stage III colon cancer, whereas another report showed no improvement in prognosis with oxaliplatin. [25, 26] In a post hoc analysis of the CLASSIC trial data, adjuvant chemotherapy was not effective for MSI-H stage II/III gastric cancer. Additionally, in an exploratory analysis of the MAGIC trial data, perioperative chemotherapy was associated with a negative prognostic effect in patients with MSI-H-resectable gastroesophageal cancer. [2, 3] In our study, it was difficult to verify the effect of adjuvant chemotherapy owing to the large selection bias in the AC and SA groups. However, DMMR gastric cancer was associated with a better prognosis than that with PMMR gastric cancer in patients who

received S-1-based adjuvant chemotherapy. This result is consistent with that of a previous report of a subanalysis of the CLASSIC trial data. [3]

In the AC group, DMMR was identified as a prognostic factor, whereas in the SA group, DMMR was not a prognostic factor in patients with stage II/III gastric cancer. PD-L1 expression and EBV status were not identified as prognostic factors in the AC and SA groups. A systematic review reported that the prognosis of DMMR gastric cancer was better than that of PMMR gastric cancer with a high proportion of lymph node metastasis in stage III/IV gastric cancer. In contrast, DMMR and PMMR had a similar prognosis in patients with stage II gastric cancer and a low proportion of lymph node metastasis. [27] Therefore, the 5-year overall survival rate was stratified for each stage in the AC and SA groups, in this study. In the AC group, the DMMR group had better survival than that in the PMMR group in both stage II and stage III gastric cancer. The 5-year overall survival of the SA-DMMR group and the SA-PMMR group were almost the same in stage II patients. In contrast, although there was no statistical difference, the 5-year overall survival rate of the SA-DMMR group was lower than that in the SA-PMMR group in stage III patients. The reason may be, at least in part, that the SA group was older than the AC group because postoperative adjuvant chemotherapy was not performed for elderly patients. Hence, the ratio of deaths due to other diseases

to the total number of deaths was higher in the SA group than that in the AC group. In the SA group, the rate of death due to other diseases was higher than that in the AC group, and age was also considered one of the causes of the poor prognosis in the SA-DMMR group. We also further evaluated recurrent cases in the stage III AC-DMMR group and SA-DMMR group. Most cancers were stage pT4b or patients had multiple lymph node metastases. Even in the DMMR gastric cancer group, stage pT4b and multiple lymph node metastasis were considered risk factors for recurrence.

The association between PD-L1 expression and prognosis in gastric cancer is controversial. It has also been reported that EBV and MSI-H gastric cancers have a higher proportion of immune infiltration and that MSI-H is associated with a favorable prognosis in metastatic gastric cancer patients. [28, 29] However, in the subgroup analysis in the present study, the prognosis of PD-L1-negative patients in the DMMR-positive or EBV-positive groups was poor in both the AC and SA groups (Fig. 3). Patients with PD-L1-negative status may have a poor prognosis owing to a poor immune response even for DMMR-positive and EBV-positive cases.

There are some limitations to the current study. First, this was a retrospective study performed in a single institution. There was a large selection bias for patients receiving adjuvant chemotherapy, and our results should be validated using larger multicenter

datasets. Second, a major limitation of this study is that we investigated only a small portion of the total tumor volume using TMAs. Third, we determined the cut-off value for PD-L1 positivity based on findings in previous reports; however, it was unclear whether this cut-off value could be applied to assess the prognostic importance of PD-L1 expression. Studies using multicenter datasets with larger samples are warranted to reach definitive conclusions.

Conclusions

PD-L1 expression may affect DMMR- and EBV-positive gastric cancer, and further treatment should be considered in addition to S-1 based adjuvant chemotherapy.

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Conflict of interest

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Table 1 Patient characteristics according to the treatment strategy

		AC (*n* = 484)	SA (*n* = 195)	*P*-value
Sex	Male	324 (66.9%)	132 (67.7%)	0.92
	Female	160 (33.1%)	63 (32.3%)	
Age, years		66 (30–84)†	71 (30–90)†	< 0.01
ECOG–PS	0	452 (93.4%)	175 (89.7%)	0.04
	1	32 (6.6%)	18 (9.2%)	
	2	0 (0%)	2 (1.0%)	
Histological type	Intestinal	251 (51.9%)	114 (58.5%)	0.12
	Diffuse	233 (48.1%)	81 (41.5%)	
Approach	Open	366 (75.6%)	148 (75.9%)	1
	Laparoscopic	118 (24.4%)	17 (24.1%)	
Surgical procedure	Distal gastrectomy	268 (55.4%)	124 (63.6%)	0.05
	Proximal gastrectomy	1 (3.1%)	8 (4.1%)	
	Total gastrectomy	200 (41.3%)	62 (31.8%)	
	other	1 (0.2%)	1 (0.5%)	
Pathological T factor	1	16 (3.3%)	12 (6.2%)	< 0.01
	2	74 (15.3%)	20 (10.3%)	
	3	165 (34.1%)	119 (61.0%)	
	4a	209 (43.2%)	41 (21.0%)	
	4b	20 (4.1%)	3 (1.5%)	
Pathological N factor	0	61 (12.6%)	93 (47.7%)	< 0.01
	1	133 (27.5%)	38 (19.5%)	

	2	141 (29.1%)	44 (22.6%)	
	3a	102 (21.1%)	17 (8.7%)	
	3b	47 (9.7%)	3 (1.5%)	
Pathological stage	IIA	73 (15.1%)	105 (53.8%)	< 0.01
	IIB	112 (23.1%)	36 (18.5%)	
	IIC	0 (0%)	1 (0.5%)	
	IIIA	159 (32.9%)	35 (17.9%)	
	IIIB	92 (19.0%)	16 (8.2%)	
	IIIC	48 (9.9%)	2 (1.0%)	
MMR status	DMMR	41 (15.4%)	30 (8.5%)	0.01
	PMMR	443 (84.6%)	165 (91.5%)	
PD-L1 expression	Positive	144 (29.8%)	85 (43.6%)	<0.01
	Negative	340 (70.2%)	110 (56.4%)	
EBV status	Positive	24 (5.0%)	11 (5.6%)	0.70
	Negative	460 (95.0%)	184 (94.4%)	

AC, adjuvant chemotherapy; DMMR, mismatch repair-deficient; ECOG-PS, Eastern Cooperative Oncology Group-Performance Status; EBV, Epstein-Barr virus; MMR, mismatch repair; PD-L1, programmed death-ligand 1; PMMR, mismatch repair-proficient; SA, surgery alone

[†]Median (range).

Table 2 Patient characteristics according to MMR status in the AC group

		PMMR (*n* = 443)	DMMR (*n* = 41)	*P*-value
Sex	Male	300 (67.7%)	24 (58.5%)	0.23
	Female	143 (32.3%)	17 (41.5%)	
Age, years		64 (30–82)†	71 (39-84)†	< 0.01
ECOG–PS	0	417 (94.1%)	35 (85.4%)	0.04
	1	26 (5.9%)	6 (14.6%)	
Histological type	Intestinal	229 (51.7%)	22 (53.7%)	0.87
	Diffuse	214 (48.3%)	19 (46.3%)	
Approach	Open	330 (74.5%)	36 (87.8%)	0.05
	Laparoscopic	113 (25.5%)	5 (12.2%)	
Surgical procedure	Distal gastrectomy	245 (55.3%)	23 (56.1%)	0.11
	Proximal gastrectomy	15 (3.4%)	0 (0%)	
	Total gastrectomy	183 (41.3%)	17 (41.5%)	
	other	0 (0%)	1 (2.4%)	
Pathological T factor	1	16 (3.6%)	0 (0%)	< 0.01
	2	69 (15.6%)	5 (12.2%)	
	3	147 (33.2%)	18 (43.9%)	
	4a	197 (44.5%)	12 (29.3%)	
	4b	14 (3.2%)	6 (14.6%)	
Pathological N factor	0	52 (11.7%)	9 (22.0%)	< 0.01
	1	120 (27.1%)	13 (31.7%)	
	2	129 (29.1%)	12 (29.3%)	

	3a	97 (21.9%)	5 (12.2%)	
	3b	45 (10.2%)	2 (4.9%)	
Pathological stage	IIA	67 (15.1%)	6 (14.6%)	0.57
	IIB	98 (22.1%)	14 (34.1%)	
	IIIA	148 (33.4%)	11 (26.8%)	
	IIIB	85 (19.2%)	7 (17.1%)	
	IIIC	45 (10.2%)	3 (7.3%)	
PD-L1 expression	Positive	111 (25.1%)	33 (80.5%)	<0.01
	Negative	332 (74.9%)	8 (19.5%)	
EBV status	Positive	24 (5.4%)	0 (0%)	0.24
	Negative	419 (94.6%)	41 (100%)	

AC, adjuvant chemotherapy; DMMR, mismatch repair-deficient; ECOG-PS, Eastern Cooperative Oncology Group-Performance Status; EBV, Epstein-Barr virus; PD-L1, programmed death-ligand 1; PMMR, mismatch repair-proficient

[†]Median (range).

Table 3

Patient characteristics according to MMR status in the SA group

		PMMR (*n* = 165)	DMMR (*n* = 30)	*P*-value
Sex	Male	119 (72.1%)	13 (43.3%)	< 0.01
	Female	46 (27.9%)	17 (56.7%)	
Age, years		70 (30–90)†	74.5 (50–86)†	< 0.01
ECOG–PS	0	150 (90.9%)	25 (83.3%)	0.20
	1	14 (8.5%)	4 (13.3%)	
	2	1 (0.6%)	1 (3.3%)	
Histological type	Intestinal	94 (57.0%)	20 (66.7%)	0.42
	Diffuse	71 (43.0%)	10 (33.3%)	
Approach	Open	122 (73.9%)	26 (86.7%)	0.42
	Laparoscopic	43 (26.1%)	4 (13.3%)	
Surgical procedure	Distal gastrectomy	102 (61.8%)	22 (73.3%)	0.50
	Proximal gastrectomy	8 (4.8%)	0 (0%)	
	Total gastrectomy	54 (32.7%)	8 (26.7%)	
	other	1 (0.6%)	0 (0%)	
Pathological T factor	1	10 (6.1%)	2 (6.7%)	0.19
	2	16 (9.7%)	4 (13.3%)	
	3	102 (61.8%)	17 (56.7%)	
	4a	36 (21.8%)	5 (16.7%)	
	4b	1 (0.6%)	2 (6.7%)	
Pathological N factor	0	81 (49.1%)	12 (40.0%)	0.20

	1	33 (20.0%)	5 (16.7%)	
	2	33 (20.0%)	11 (36.7%)	
	3a	16 (9.7%)	1 (3.3%)	
	3b	2 (1.2%)	1 (3.3%)	
Pathological stage	IIA	91 (55.2%)	14 (46.7%)	0.57
	IIB	30 (18.2%)	6 (20.0%)	
	IIC	1 (0.6%)	0 (0%)	
	IIIA	28 (17.0%)	7 (23.3%)	
	IIIB	14 (8.5%)	2 (6.7%)	
	IIIC	1 (0.6%)	1 (3.3%)	
PD-L1 expression	Positive	61 (37.0%)	24 (80.0%)	<0.01
	Negative	104 (63.0%)	6 (20.0%)	
EBV status	Positive	11 (6.7%)	0 (0%)	0.22
	Negative	154 (93.3%)	30 (100%)	

DMMR, mismatch repair-deficient; ECOG-PS, Eastern Cooperative Oncology Group-Performance Status; EBV, Epstein-Barr virus; PD-L1, programmed death-ligand 1; PMMR, mismatch repair-proficient; SA, surgery alone

[†]Median (range).

Table 4

(A) Results of the multivariate analysis of overall survival in the AC group

		Multivariate	
		Hazard ratio [95% confidence interval]	*P*–value
Sex	Female	1	0.83
	Male	0.96 [0.66–1.38]	
Age, years	< 66	1	0.63
	≥ 66	1.08 [0.76–1.53]	
Histological type	Intestinal	1	0.12
	Diffuse	1.30 [0.92–1.85]	
Pathological stage	II	1	< 0.01
	III	2.14 [1.44–3.18]	
PD-L1 expression	Negative	1	0.33
	Positive	0.81 [0.53–1.23]	
MMR status	PMMR	1	0.02
	DMMR	0.25 [0.07–0.81]	
EBV status	Negative	1	0.32
	Positive	0.63 [0.25-1.58]	

AC, adjuvant chemotherapy; DMMR, mismatch repair-deficient; EBV, Epstein-Barr virus; MMR, mismatch repair; PD-L1, programmed death-ligand 1; PMMR, mismatch repair-proficient

(B) Results of the multivariate analysis of overall survival in the SA group

		Multivariate	
		Hazard ratio [95% confidence interval]	*P*–value
Sex	Female	1	0.32
	Male	1.38 [0.72–2.62]	
Age, years	< 71	1	0.68
	≥ 71	0.87 [0.46–1.65]	
Histological type	Intestinal	1	0.80
	Diffuse	0.92 [0.50–1.68]	
Pathological stage	II	1	< 0.01
	III	4.02 [2.29–7.07]	
PD-L1 expression	Negative	1	0.20
	Positive	0.66 [0.36–1.24]	
MMR status	PMMR	1	0.42
	DMMR	1.39 [0.61–3.18]	
EBV status	Negative	1	0.18
	Positive	2.15 [0.68-6.80]	

DMMR, mismatch repair-deficient; EBV, Epstein-Barr virus; MMR, mismatch repair; PD-L1, programmed death-ligand 1; PMMR, mismatch repair-proficient; SA, surgery alone

Supplemental Table 1

(A) Patient characteristics according to MMR status based on PD-L1 expression in the AC group

		PMMR (*n* = 443)			DMMR (*n* = 41)		
		PD-L1 Positive (*n* = 111)	PD-L1 Negative (*n* = 332)	*P*-value	PD-L1 Positive (*n* = 33)	PD-L1 Negative (*n* = 8)	*P*-value
Sex	Male	87 (78.4%)	213 (64.2%)	< 0.01	18 (54.5%)	6 (75.0%)	0.43
	Female	24 (21.6%)	119 (35.8%)		15 (45.5%)	2 (25.0%)	
Age, years		65 (44-81)	63 (30-82)	0.05	68 (39–84)†	71 (55-77)†	0.74
ECOG–PS	0	104 (93.7%)	313 (94.3%)	0.81	28 (84.8%)	7 (87.5%)	1
	1	7 (6.3%)	19 (5.7%)		5 (15.2%)	1 (12.5%)	
Histological type	Intestinal	64 (57.7%)	165 (49.7%)	0.15	16 (48.5%)	6 (75.0%)	0.24
	Diffuse	47 (42.3%)	167 (50.3%)		17 (51.5%)	2 (25.0%)	
Approach	Open	85 (76.6%)	245 (73.8%)	0.61	29 (87.9%)	7 (87.5%)	1
	Laparoscopic	26 (23.4%)	87 (26.2%)		4 (12.1%)	1 (12.5%)	
Surgical procedure	Distal gastrectomy	56 (50.5%)	189 (56.9%)	0.07	19 (57.6%)	4 (50.0%)	0.76
	Proximal gastrectomy	1 (0.9%)	14 (4.2%)		0 (0%)	0 (0%)	
	Total gastrectomy	54 (48.6%)	129 (38.9%)		13 (39.4%)	4 (50.0%)	
	other	0	0		1 (3.0%)	0 (0%)	
Pathological T factor	1	3 (2.7%)	13 (3.9%)	0.30	0 (0%)	0 (%)	0.69
	2	20 (18.0%)	49 (14.8%)		4 (12.1%)	1 (12.5%)	
	3	42 (37.8%)	105 (31.6%)		13 (39.4%)	5 (62.5%)	
	4a	41 (36.9%)	156 (47.0%)		11 (33.3%)	1 (12.5%)	
	4b	5 (4.5%)	9 (2.7%)		5 (15.2%)	1 (12.5%)	
Pathological N factor	0	19 (17.1%)	33 (9.9%)	0.02	8 (24.2%)	1 (12.5%)	0.45

	1	22 (19.8%)	98 (29.5%)		9 (27.3%)	4 (50.0%)	
	2	32 (28.8%)	97 (29.2%)		10 (30.3%)	2 (25.0%)	
	3a	31 (27.9%)	66 (19.9%)		5 (15.2%)	0 (0%)	
	3b	7 (6.3%)	38 (11.4%)		1 (3.0%)	1 (12.5%)	
Pathological stage	IIA	20 (18.0%)	46 (13.9%)	0.48	4 (12.1%)	2 (25.0%)	0.72
	IIB	23 (20.7%)	78 (23.5%)		11 (33.3%)	3 (37.5%)	
	IIIA	35 (31.5%)	109 (32.8%)		10 (30.3%)	1 (12.5%)	
	IIIB	25 (22.5%)	61 (18.4%)		6 (18.2%)	1 (12.5%)	
	IIIC	8 (7.2%)	38 (11.4%)		1 (6.1%)	1 (12.5%)	
EBV status	Positive	15 (13.5%)	9 (2.7%)	< 0.01	0 (0%)	0 (0%)	1
	Negative	96 (86.5%)	323 (97.3%)		33 (100%)	8 (100%)	

AC, adjuvant chemotherapy; DMMR, mismatch repair-deficient; ECOG-PS, Eastern Cooperative Oncology Group-Performance Status; EBV, Epstein–Barr virus; PD-L1, programmed death-ligand 1; PMMR, mismatch repair-proficient

[†]Median (range).

(B) Patient characteristics according to EBV status based on PD-L1 expression in the AC group

		EBV Positive (*n* = 24)			EBV Negative (*n* = 460)		
		PD-L1 Positive (*n* = 15)	PD-L1 Negative (*n* = 9)	*P*-value	PD-L1 Positive (*n* = 129)	PD-L1 Negative (*n* = 331)	*P*-value
Sex	Male	13 (86.7%)	7 (77.8%)	0.61	92 (71.3%)	212 (64.0%)	0.15
	Female	2 (13.3%)	2 (22.2%)		37 (28.7%)	119 (36.0%)	
Age, years		65 (48-79)	59 (42-68)	0.19	66 (39–84)†	64 (30-82)†	< 0.01
ECOG–PS	0	15 (100%)	8 (88.9%)	0.37	117 (90.7%)	312 (94.3%)	0.21
	1	0 (0%)	1 (11.1%)		12 (9.3%)	19 (5.7%)	
Histological type	Intestinal	4 (26.7%)	2 (22.2%)	1	76 (58.9%)	169 (51.1%)	0.14
	Diffuse	11 (73.3%)	7 (77.8%)		53 (41.1%)	162 (48.9%)	
Approach	Open	13 (86.7%)	7 (77.8%)	0.61	101 (78.3%)	245 (74.0%)	0.40
	Laparoscopic	2 (13.3%)	2 (22.2%)		28 (21.7%)	86 (26.0%)	
Surgical procedure	Distal gastrectomy	4 (26.7%)	2 (22.2%)	0.58	71 (55.0%)	191 (57.7%)	0.08
	Proximal gastrectomy	0 (0%)	1 (11.1%)		1 (0.8%)	13 (3.9%)	
	Total gastrectomy	11 (73.3%)	6 (66.7%)		56 (43.4%)	127 (38.4%)	
	other	0 (0%)	0 (0%)		1 (0.8%)	0 (0%)	
Pathological T factor	1	1 (6.7%)	0 (0%)	0.46	2 (1.6%)	13 (3.9%)	0.07
	2	2 (13.3%)	1 (11.1%)		22 (17.1%)	49 (14.8%)	
	3	6 (40.0%)	1 (11.1%)		49 (38.0%)	109 (32.9%)	
	4a	5 (33.3%)	6 (66.7%)		47 (36.4%)	151 (45.6%)	
	4b	1 (6.7%)	1 (11.1%)		9 (7.0%)	9 (2.7%)	
Pathological N factor	0	3 (20.0%)	1 (11.1%)	0.69	24 (18.6%)	33 (10.0%)	< 0.01
	1	5 (33.3%)	2 (22.2%)		26 (20.2%)	100 (30.2%)	

	2	2 (13.3%)	1 (11.1%)		40 (31.0%)	98 (29.6%)	
	3a	4 (26.7%)	2 (22.2%)		32 (24.8%)	64 (19.3%)	
	3b	1 (6.7%)	3 (33.3%)		7 (5.4%)	36 (10.9%)	
Pathological stage	IIA	3 (20.0%)	1 (11.1%)	0.38	21 (16.3%)	47 (14.2%)	0.60
	IIB	3 (20.0%)	0 (0%)		31 (24.0%)	81 (24.5%)	
	IIIA	6 (40.0%)	3 (33.3%)		39 (30.2%)	107 (32.3%)	
	IIIB	2 (13.3%)	2 (22.2%)		29 (22.5%)	60 (18.1%)	
	IIIC	1 (6.7%)	3 (33.3%)		9 (7.0%)	36 (10.9%)	
MMR status	DMMR	0 (0%)	0 (0%)	1	33 (25.6%)	8 (2.4%)	< 0.01
	PMMR	15 (100%)	9 (100%)		96 (74.4%)	323 (97.6%)	

AC, adjuvant chemotherapy; DMMR, mismatch repair-deficient; ECOG-PS, Eastern Cooperative Oncology Group-Performance Status; EBV, Epstein–Barr virus; PD-L1, programmed death-ligand 1; PMMR, mismatch repair-proficient

[†]Median (range).

Supplemental Table 2

(A) Patient characteristics according to MMR status based on PD-L1 expression in the SA group

		PMMR (n = 165)			DMMR (n = 30)		
		PD-L1 Positive (n = 61)	PD-L1 Negative (n = 104)	P-value	PD-L1 Positive (n = 24)	PD-L1 Negative (n = 6)	P-value
Sex	Male	43 (70.5%)	76 (73.1%)	0.72	10 (41.7%)	3 (50.0%)	1
	Female	18 (29.5%)	28 (26.9%)		14 (58.3%)	3 (50.0%)	
Age, years		72 (43-90)	69 (30-86)	0.02	77.5 (54–86)†	69.5 (50-77)†	< 0.01
ECOG–PS	0	57 (93.4%)	93 (89.4%)	0.73	19 (79.2%)	6 (100.0%)	0.64
	1	4 (6.6%)	10 (9.6%)		4 (16.7%)	0 (0%)	
	2	0 (0%)	1 (1.0%)		1 (4.2%)	0 (0%)	
Histological type	Intestinal	40 (65.6%)	54 (51.9%)	0.10	15 (62.5%)	5 (83.3%)	0.63
	Diffuse	21 (34.4%)	50 (48.1%)		9 (37.5%)	1 (16.7%)	
Approach	Open	46 (75.4%)	76 (73.1%)	0.85	22 (91.7%)	4 (66.7%)	0.16
	Laparoscopic	15 (24.6%)	28 (26.9%)		2 (8.3%)	2 (33.3%)	
Surgical procedure	Distal gastrectomy	36 (59.0%)	66 (63.5%)	0.24	18 (75.0%)	4 (66.7%)	0.64
	Proximal gastrectomy	1 (1.6%)	7 (6.7%		0 (0%)	0 (0%)	
	Total gastrectomy	24 (39.3%)	30 (28.8%)		6 (25.0%)	2 (33.3%)	
	other	0 (0%)	1 (1.0%		0 (0%)	0 (0%)	
Pathological T factor	1	4 (6.6%)	6 (5.8%)	0.69	2 (8.3%)	0 (0%)	0.86
	2	6 (9.8%)	10 (9.6%)		3 (12.5%)	1 (16.7%)	
	3	41 (67.2%)	61 (58.7%)		14 (58.3%)	3 (50.0%)	
	4a	10 (16.4%)	26 (25.0%)		3 (12.5%)	2 (33.3%)	
	4b	0 (0%)	1 (1.0%)		2 (8.3%)	0 (0%)	

Pathological N factor	0	32 (52.5%)	49 (47.1%)	0.76	9 (37.5%)	2 (33.3%)	0.13
	1	12 (19.7%)	21 (20.2%)		5 (20.8%)	1 (16.7%)	
	2	10 (16.4%)	23 (22.1%)		10 (41.7%)	1 (16.7%)	
	3a	7 (11.5%)	9 (8.7%)		0 (0%)	1 (16.7%)	
	3b	0 (0%)	2 (1.9%		0 (0%)	1 (16.7%)	
Pathological stage	IIA	35 (57.4%)	56 (53.8%)	0.53	12 (50.0%)	2 (33.3%)	0.13
	IIB	14 (23.0%)	17 (16.3%)		4 (16.7%)	2 (33.3%)	
	IIIA	7 (11.5%)	20 (19.2%)		6 (25.0%)	0 (0%)	
	IIIB	5 (8.2%)	9 (8.7%)		2 (8.3%)	1 (16.7%)	
	IIIC	0 (0%)	2 (1.9%)		0 (0%)	1 (16.7%)	
EBV status	Positive	9 (14.8%)	2 (1.9%)	< 0.01	0 (0%)	0 (0%)	1
	Negative	52 (85.2%)	102 (98.1%)		24 (100%)	6 (100%)	

DMMR, mismatch repair-deficient; ECOG-PS, Eastern Cooperative Oncology Group-Performance Status; EBV, Epstein–Barr virus; PD-L1, programmed death-ligand 1; PMMR, mismatch repair-proficient; SA, surgery alone

[†]Median (range).

(B) Patient characteristics according to EBV status based on PD-L1 expression in the SA group

		EBV Positive (n = 11)			EBV Negative (n = 184)		
		PD-L1 Positive (n = 9)	PD-L1 Negative (n = 2)	P-value	PD-L1 Positive (n = 76)	PD-L1 Negative (n = 108)	P-value
Sex	Male	7 (77.8%)	2 (100.0%)	1	46 (60.5%)	77 (71.3%)	0.15
	Female	2 (22.2%)	0 (0%)		30 (39.5%)	31 (28.7%)	
Age, years		60 (44-80)	69 (63-75)	0.32	75 (43–90)†	69 (30-86)†	< 0.01
ECOG–PS	0	8 (88.9%)	2 (100%)	1	68 (89.5%)	97 (89.8%)	1
	1	1 (11.1%)	0 (0%)		7 (9.2%%)	10 (9.3%)	
	2	0 (0%)	0 (0%)		1 (1.3%)	1 (0.9%)	
Histological type	Intestinal	3 (33.3%)	1 (50.0%)	1	52 (68.4%)	58 (53.7%)	0.04
	Diffuse	6 (66.7%)	1 (50.0%)		24 (31.6%)	50 (46.3%)	
Approach	Open	6 (66.7%)	2 (100%)	1	62 (81.6%)	78 (72.2%)	0.16
	Laparoscopic	3 (33.3%)	0 (0%		14 (18.4%)	30 (27.8%)	
Surgical procedure	Distal gastrectomy	3 (33.3%)	0 (0%)	0.23	51 (67.1%)	70 (64.8%)	0.43
	Proximal gastrectomy	0 (0%)	1 (50.0%)		1 (1.3%)	6 (5.6%)	
	Total gastrectomy	6 (66.7%)	1 (50.0%)		24 (31.6%)	31 (28.7%)	
	other	0 (0%)	0 (0%)		0 (0%)	1 (0.9%)	
Pathological T factor	1	0 (0%)	0 (0%)	0.18	6 (7.9%)	6 (5.6%)	0.61
	2	0 (0%)	0 (0%)		9 (11.8%)	11 (10.2%)	
	3	9 (100%)	1 (50%)		46 (60.5%)	63 (58.3%)	
	4a	0 (0%)	1 (50%)		13 (17.1%)	27 (25.0%)	
	4b	0 (0%)	0 (0%)		2 (2.6%)	1 (0.9%)	
Pathological N factor	0	8 (88.9%)	1 (50%)	0.34	33 (43.4%)	50 (46.3%)	0.72

	1	1 (11.1%)	0 (0%)		16 (21.1%)	22 (20.4%)	
	2	0 (0%)	0 (0%)		20 (26.3%)	24 (22.2%)	
	3a	0 (0%)	1 (50%)		7 (9.2%)	9 (8.3%)	
	3b	0 (0%)	0 (0%)		0 (0%)	3 (2.8%)	
Pathological stage	IIA	8 (88.9%)	1 (50%)	0.34	39 (51.3%)	57 (52.8%)	0.69
	IIB	1 (11.1%)	0 (0%)		17 (22.4%)	19 (17.6%)	
	IIIA	0 (0%)	0 (0%)		13 (17.1%)	20 (18.5%)	
	IIIB	0 (0%)	1 (50%)		7 (9.2%)	9 (8.3%)	
	IIIC	0 (0%)	0 (0%)		0 (0%)	3 (2.8%)	
MMR status	DMMR	0 (0%)	0 (0%)	1	24 (31.6%)	6 (5.6%)	< 0.01
	PMMR	9 (100%)	2 (100%)		52 (68.4%)	102 (94.4%)	

DMMR, mismatch repair-deficient; ECOG-PS, Eastern Cooperative Oncology Group-Performance Status; EBV, Epstein–Barr virus; PD-L1, programmed death-ligand 1; PMMR, mismatch repair-proficient; SA, surgery alone

[†]Median (range).

Figure Legends

Fig. 1 Five-year overall survival (A) and relapse-free survival (B) of the AC-DMMR group and AC-PMMR group. Five-year overall survival (C) and relapse-free survival (D) of the SA-DMMR group and SA-PMMR group.

AC, adjuvant chemotherapy; DMMR, mismatch repair-deficient; OS, overall survival; PMMR, mismatch repair-proficient; RFS, relapse-free survival; SA, surgery alone

Fig. 2 Five-year overall survival of the AC-DMMR group and AC-PMMR group in stage II/III gastric cancer, and 5-year overall survival of the SA-DMMR group and SA-PMMR group in stage II/III gastric cancer.

AC, adjuvant chemotherapy; DMMR, mismatch repair-deficient; OS, overall survival; PMMR, mismatch repair-proficient; SA, surgery alone

Fig. 3 Five-year overall survival of the patients based on PD-L1 expression for each MMR status or EBV status in the AC group and SA group.

AC, adjuvant chemotherapy; DMMR, mismatch repair-deficient; EBV, Epstein-Barr virus; MMR, mismatch repair; OS, overall survival; PD-L1, programmed death-ligand 1; PMMR, mismatch repair-proficient; SA, surgery alone

Supplemental Fig. 1 Five-year relapse-free survival of the AC-DMMR group and AC-PMMR group in stage II/III gastric cancer, and 5-year overall survival of the SA-DMMR group and SA-PMMR group in stage II/III gastric cancer.

AC, adjuvant chemotherapy; DMMR, mismatch repair-deficient; OS, overall survival; PMMR, mismatch repair-proficient; SA, surgery alone

Supplemental Fig. 2 Five-year relapse-free survival of the patients based on PD-L1 expression for each MMR status or EBV status in the AC group and SA group.

AC, adjuvant chemotherapy; DMMR, mismatch repair-deficient; EBV, Epstein–Barr virus; MMR, mismatch repair; OS, overall survival; PD-L1, programmed death-ligand 1; PMMR, mismatch repair-proficient; SA, surgery alone

Fig. 1

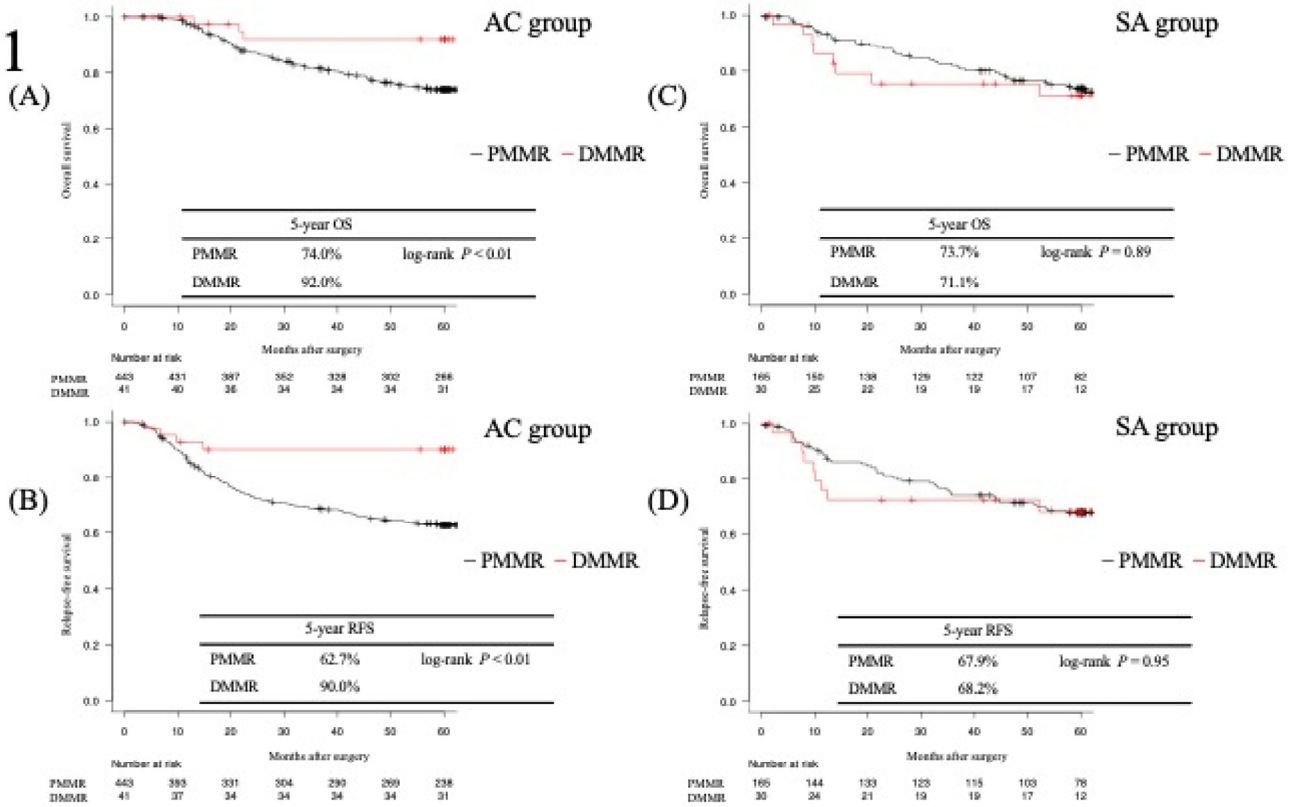

(A)
AC group
PMMR DMMR
Overall survival
5-year OS
PMMR 74.0% log-rank P < 0.01
DMMR 92.0%
Months after surgery
Number at risk
PMMR 443 431 387 352 328 302 268
DMMR 41 40 36 34 34 34 31
(B)
AC group
PMMR DMMR
Relapse-free survival
5-year RFS
PMMR 62.7% log-rank P < 0.01
DMMR 90.0%
Months after surgery
Number at risk
PMMR 443 393 331 304 290 269 238
DMMR 41 37 34 34 34 34 31
(C)
SA group
PMMR DMMR
Overall survival
5-year OS
PMMR 73.7% log-rank P = 0.89
DMMR 71.1%
Months after surgery
Number at risk
PMMR 165 150 138 129 122 107 82
DMMR 30 25 22 19 19 17 12
(D)
SA group
PMMR DMMR
Relapse-free survival
5-year RFS
PMMR 67.9% log-rank P = 0.95
DMMR 68.2%
Months after surgery
Number at risk
PMMR 165 144 133 123 115 103 78
DMMR 30 24 21 19 19 17 12

Fig. 2

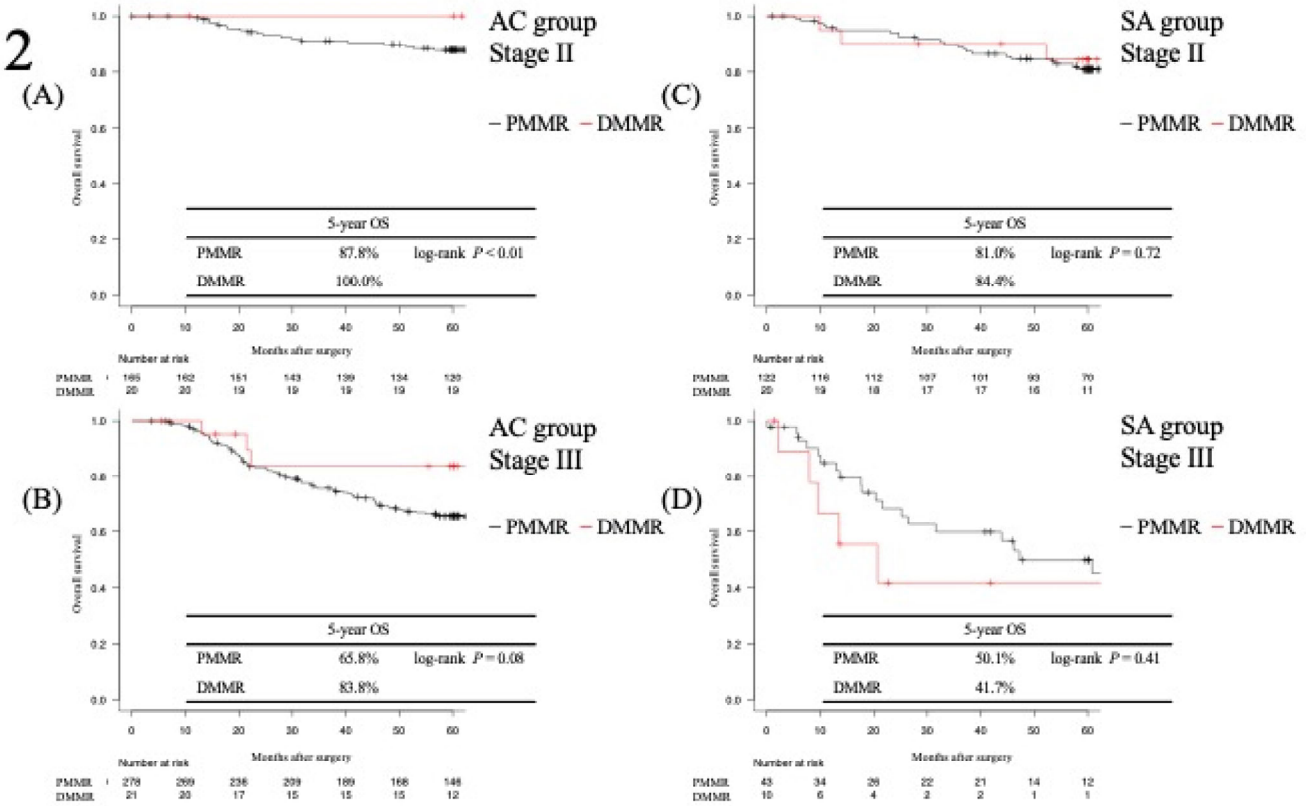
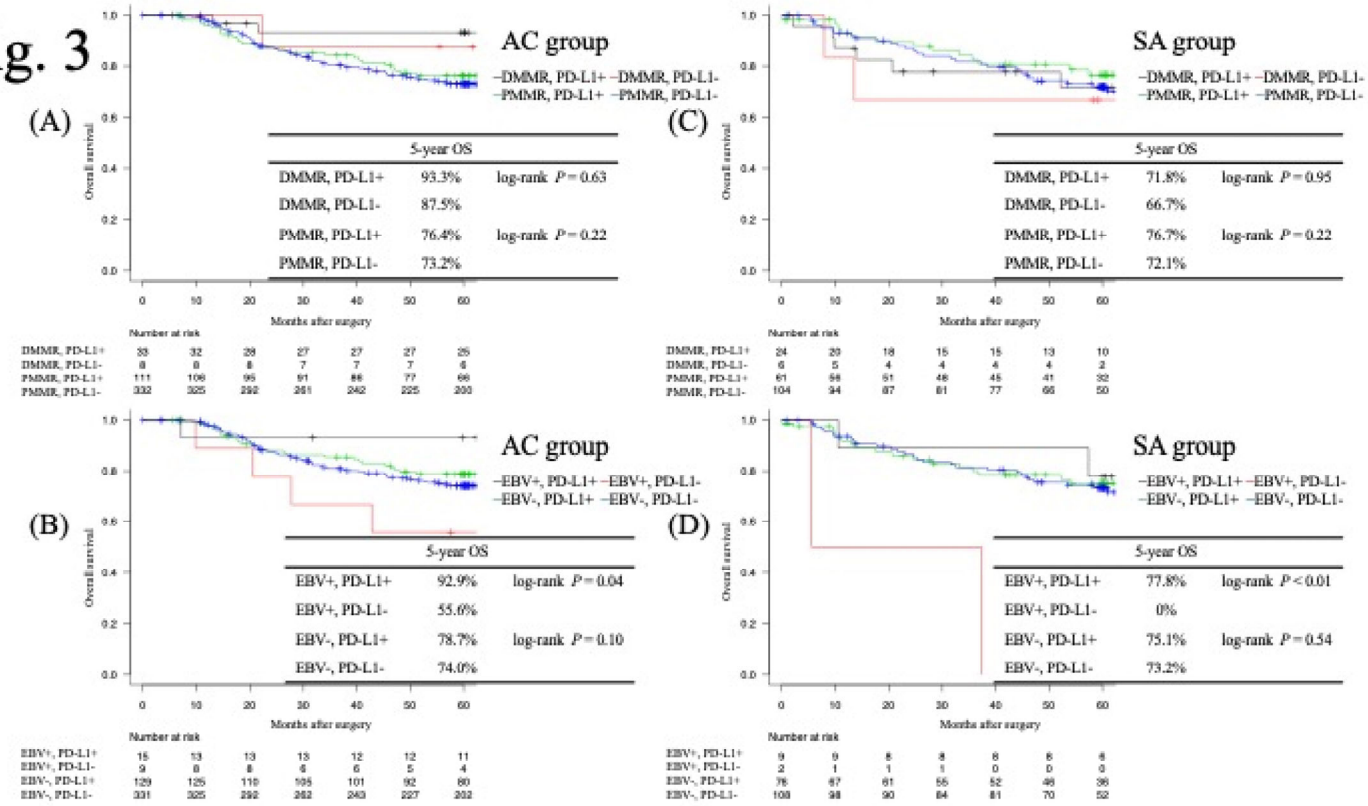
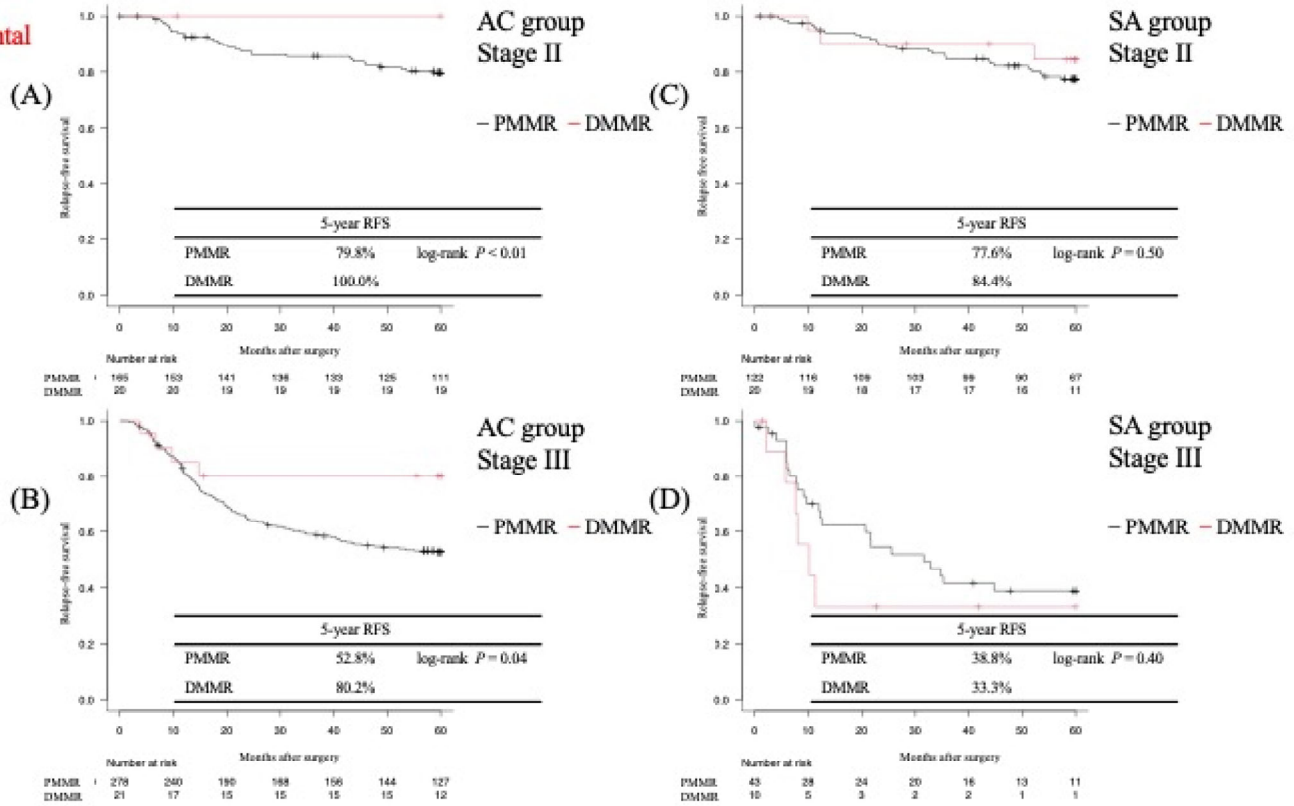

(A)
AC group
Stage II
– PMMR – DMMR
Overall survival
5-year OS
PMMR 87.8% log-rank P < 0.01
DMMR 100.0%
Months after surgery
Number at risk
PMMR 165 162 151 143 139 134 120
DMMR 20 20 19 19 19 19 19
(C)
SA group
Stage II
– PMMR – DMMR
Overall survival
5-year OS
PMMR 81.0% log-rank P = 0.72
DMMR 84.4%
Months after surgery
Number at risk
PMMR 122 116 112 107 101 93 70
DMMR 20 19 18 17 17 16 11
(B)
AC group
Stage III
– PMMR – DMMR
Overall survival
5-year OS
PMMR 65.8% log-rank P = 0.08
DMMR 83.8%
Months after surgery
Number at risk
PMMR 278 269 236 209 189 168 148
DMMR 21 20 17 15 15 15 12
(D)
SA group
Stage III
– PMMR – DMMR
Overall survival
5-year OS
PMMR 50.1% log-rank P = 0.41
DMMR 41.7%
Months after surgery
Number at risk
PMMR 43 34 28 22 21 14 12
DMMR 10 6 4 2 2 1 1

Fig. 3


(A)
AC group
–DMMR, PD-L1+ –DMMR, PD-L1-
–PMMR, PD-L1+ –PMMR, PD-L1-
Overall survival
Months after surgery
5-year OS
DMMR, PD-L1+ 93.3% log-rank P = 0.63
DMMR, PD-L1- 87.5%
PMMR, PD-L1+ 76.4% log-rank P = 0.22
PMMR, PD-L1- 73.2%
Number at risk
DMMR, PD-L1+ 33 32 28 27 27 27 25
DMMR, PD-L1- 8 8 8 7 7 7 6
PMMR, PD-L1+ 111 106 95 91 86 77 66
PMMR, PD-L1- 332 325 292 261 242 225 203
(C)
SA group
–DMMR, PD-L1+ –DMMR, PD-L1-
–PMMR, PD-L1+ –PMMR, PD-L1-
Overall survival
Months after surgery
5-year OS
DMMR, PD-L1+ 71.8% log-rank P = 0.95
DMMR, PD-L1- 66.7%
PMMR, PD-L1+ 76.7% log-rank P = 0.22
PMMR, PD-L1- 72.1%
Number at risk
DMMR, PD-L1+ 24 20 18 15 15 13 10
DMMR, PD-L1- 6 5 4 4 4 4 2
PMMR, PD-L1+ 61 58 51 48 45 41 32
PMMR, PD-L1- 104 94 87 81 77 68 50
(B)
AC group
–EBV+, PD-L1+ –EBV+, PD-L1-
–EBV-, PD-L1+ –EBV-, PD-L1-
Overall survival
Months after surgery
5-year OS
EBV+, PD-L1+ 92.9% log-rank P = 0.04
EBV+, PD-L1- 55.6%
EBV-, PD-L1+ 78.7% log-rank P = 0.10
EBV-, PD-L1- 74.0%
Number at risk
EBV+, PD-L1+ 15 13 13 13 12 12 11
EBV+, PD-L1- 9 8 8 6 6 5 4
EBV-, PD-L1+ 129 125 110 105 101 92 80
EBV-, PD-L1- 331 325 292 262 243 227 202
(D)
SA group
–EBV+, PD-L1+ –EBV+, PD-L1-
–EBV-, PD-L1+ –EBV-, PD-L1-
Overall survival
Months after surgery
5-year OS
EBV+, PD-L1+ 77.8% log-rank P < 0.01
EBV+, PD-L1- 0%
EBV-, PD-L1+ 75.1% log-rank P = 0.54
EBV-, PD-L1- 73.2%
Number at risk
EBV+, PD-L1+ 9 9 8 8 8 8 8
EBV+, PD-L1- 2 1 1 1 0 0 0
EBV-, PD-L1+ 76 67 61 55 52 46 36
EBV-, PD-L1- 108 98 90 84 81 70 52

Supplemental
Fig. 1



Supplemental Fig. 2