Clinicopathological and Prognostic Significance of MSI Status and PD-L1 Expression in Turkish Patients with Gastric Cancer

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ABSTRACT

The aim of study was to evaluate the prognostic role of MSI status and PD-L1 expression in gastric cancer and the relationship of these parameters with clinicopathological features. Eighty-six gastric cancer patients who underwent surgical resection were analysed. MSI status and PD-L1 expression in tumour samples were evaluated by immunohistochemistry (IHC). PD-L1 IHC was scored using the combined positive score (CPS). Survival analysis was conducted using the Kaplan-Meier method. The rate of PD-L1 expression in tumour cells was 34.9% (n= 30), and the frequency of PD-L1 expression in immune cells with a CPS ≥ 1% was 57% (n= 49). MSI-high (MSI-H) was detected in 11.6% (n= 10) of cases and was more common among PD-L1–positive cases (p= 0.021). MSI-H status was significantly correlated with older age, larger tumours, positive PD-L1 expression, and the adenocarcinoma subtype. PD-L1 expression was associated with lymph node metastasis, the adenocarcinoma subtype, MSI, preoperative treatment and an improved response to preoperative chemotherapy. In our study, the impact of MSI status on survival was not demonstrated, but positive PD-L1 expression (≥ 1%) in tumour cells (15.7 vs. 53.4 months. p= 0.008)and in immune cells (20.4 vs. not reached (NR); p= 0.027) was associated with decreased overall survival. PD-L1 expression is related to a poor prognosis in patients with gastric cancer.

Keywords: Gastric cancer, MSI status, PD-L1 expression

INTRODUCTION

The programmed cell death-1 (PD-1)/programmed cell death-ligand 1 (PD-L1) pathway is important in the negative regulation of cell-mediated immune responses. Immunotherapy targeting the PD-1/PD-L1 axis has shown great promise in treating many types of cancer, and this therapeutic strategy represents a breakthrough in cancer treatment. Clinical data show that the blockade of PD-1 signalling significantly enhances antitumour immunity, produces durable clinical responses, and prolongs survival.[1] Currently, there are FDA-approved PD-L1 inhibitors for various malignancies. including melanoma, non–smallcell lung cancer (NSCLC), head and neck squamous cell cancer, classical Hodgkin lymphoma, urothelial carcinoma, hepatocellular carcinoma. Merkel cell carcinoma, renal cell carcinoma, and colorectal cancer.[2] In early clinical studies, anti-PD-1 therapies, including pembrolizumab[3-5], showed promising efficacy in metastatic gastric cancer (GC), and a recent phase III trial comparing nivolumab to best supportive care in the salvage setting demonstrated a survival benefit in GC.[6]

It is thus critically important to identify a molecular biomarker to predict the clinical response to anti-PD-1/PD-L1 immunotherapy. Additionally, data on the prevalence and prognostic value of PD-L1 expression in GC are limited and controversial.

Mismatch repair (MMR), an important DNA repair mechanism that ensures genomic integrity, is mediated by key proteins that heterodimerize and recognize and remove DNA errors. The loss of MMR proteins leads to an accumulation of DNA replication errors, a phenomenon known as microsatellite instability (MSI), and eventually to somatic mutations. Proteins encoded by some of these mutated genes are immunogenic and provoke an antitumour immune response by increasing immune cell infiltration and thereby improving sensitivity to immune checkpoint inhibitors (ICIs).[7] The response to ICIs has been shown to correlate with tumour mutation load (TML), deficient MMR (dMMR) and PD-L1 expression.[8] Recently, FDA approval was granted for the use of the checkpoint inhibitors pembrolizumab and nivolumab in selected gastrointestinal cancers. Pembrolizumab was approved for the treatment of metastatic nonhaematologic cancers characterized by MSI-high (MSI-H) status or dMMR and for PD-L1–positive advanced gastric or gastroesophageal junction (GEJ) adenocarcinomas.

Testing for biomarkers, including MSI and PD-L1, may therefore be necessary to broaden the identification of responders to ICIs and to achieve better patient stratification.[9] The aim of this study was to evaluate the prognostic role of MSI status and PD-L1 expression in surgically resected GC and to better understand the relationship between these biomarkers and clinicopathological features. To our knowledge, this study is the first to examine these biomarkers and relationships in a Turkish cohort with GC.

PATIENTS AND METHODS

Patients and Samples

This study included 86 patients with GC who underwent curative gastrectomy at Acibadem Atakent and Maslak Hospitals between February 2010 and January 2017. Clinicopathological factors, including demographics, tumour characteristics, and treatment outcomes, were identified retrospectively by reviewing electronic medical records. Tumour samples were evaluated for MSI and PD-L1 status by immunohistochemistry (IHC). This study was approved by the Institutional Review Board of Acibadem University.

Evaluation of PD-L1 Expression

PD-L1 IHC was scored using the combined positive score (CPS), which was calculated by dividing the number of PD-L1–positive cells, including tumour cells, lymphocytes, and macrophages, by the total number of viable tumour cells and multiplying the result by 100, as described previously.[4] Although the result of this calculation can exceed 100, the maximum CPS is defined as 100. The CPS was calculated by an experienced gastrointestinal system pathologist (S. E.) who was blinded to the clinical information. PD-L1 positivity was defined as an average CPS ≥ 1. All samples were confirmed to include at least 100 viable tumour cells, which is regarded as adequate for PD-L1 assessment.[10]

Statistical Analyses

Categorical variables are presented as numbers and percentages after comparison by the chi-square test or Fisher's exact test when appropriate. Continuous variables are expressed as the median and interquartile range after comparison by the Kruskal-Wallis test or Mann-Whitney U-test. We constructed overall survival (OS) curves using the Kaplan-Meier method, and we used the log-rank test to evaluate the statistical significance of differences and associations with each clinicopathological or molecular marker. The Cox proportional hazards regression model was used to assess the predictive effects of multiple covariates on OS simultaneously. A p-value of less than 0.05 was considered to indicate statistical significance.

RESULTS

Patients and Tumour Characteristics

The median patient age was 60 years (range, 30-85), and the study sample consisted of 55 men (64.0%) and 31 women (36%). Of the 86 GC

Table 1. Clinicopathological features of all patients

		n (86)	%
Age	Median	60	
	Range	30-85	
Sex	Male	55	64.0
	Female	31	36.0
HER2 Status	Negative	35	85.4
	Positive	6	14.6
MSI Status	p-MMR	76	88.4
	d-MMR	10	11.6
Presence of PD-L1	Tumor Cells	30	34.9
Expression	Immune Cells	49	57.0
	Combined Positive Score	49	57.0
Tumor Location	Cardia	16	18.6
	Corpus	34	39.5
	Antrum	28	32.6
	Diffuse	8	9.3
cT Status	T1-2	47	54.7
	T3-4	39	45.3
cN Status	Node negative	34	39.5
	Node positive	52	60.5
Perioperative	Yes	76	88.4
Treatment	No	5	5.8
Histological Subtype		39	45.3
Adenocarcinoma	Poorly cohesive carcinoma	30	34.9
	Mikst	16	18.6
	Other	1	1.2
Lymphovascular	Yes	55	64.0
Invasion	No	21	24.4
Lymphadenectomy	D0-1	10	11.6
	D2	76	88.4
Number of Removed	Mean	35	
Lymph Nodes	Median (Range)	32 (12-66)	
Number of Metastatic	Mean	8	
Lymph Nodes	Median (Range)	7 (0-46)	

MSI: Microsatellite Instability; p-MMR: Proficient MMR; d-MMR: Deficient MMR; CT: Chemotherapy

cases, 47 (54%) were diagnosed as early clinical T stage (T1-2), 39 (46%) as locally advanced T stage (T3-4), and nodal involvement was detected in 52 (60%) patients. At the time of this analysis, 28 patients (32.5%) had died of the disease. Most of the patients had adenocarcinoma (39, 45%) and poorly cohesive carcinoma (30, 34%). The rates of c-erbB-2 (Her-2) positivity, lymphovascular invasion (LVI) and perinodal invasion (PNI) were

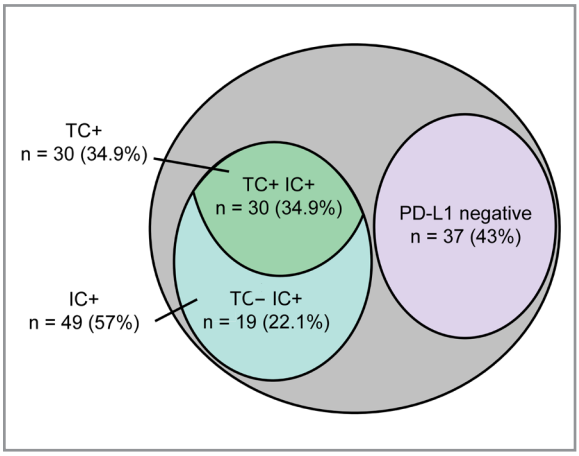

Figure 1. PD-L1 staining in all patients (n= 86)

15% (n= 6/41), 72% (55/76), and 60% (45/76), respectively. Most of the tumours were localized to the corpus (n= 34) and antrum (n= 28). In total, 76 (90%) patients received multimodal treatment. While 37 patients received neoadjuvant chemotherapy, 39 received adjuvant treatment (adjuvant chemoradiotherapy, 23 patients; adjuvant chemotherapy, 16 patients). A curative (R0) resection was achieved in 88% (76/86) of the patients. The frequency of lymph node metastasiswas 66% (n= 50/75). The median number of excised lymph nodes was 32 (range, 16-66), and the median number of metastasis-positive lymph nodes was 7 (range, 1-46) (Table 1).

Neoadjuvant chemotherapy was given to 37 patients. The pathologic response to neoadjuvant chemotherapy was evaluated, and a complete response was noted for 1 patient. Most patients who received neoadjuvant chemotherapy had an inadequate response to chemotherapy according to the Mandard TRG (tumour regression grade) system (70% of patients; 18/26 in grade 4-5) and the CAP (College of American Pathologists) TRG system (80% of patients; 20/25 patients in grade 2-3).

Association of PD-L1 Expression with Clinicopathological Features

The rate of PD-L1 expression in tumour cells was 34.9% (n= 30), and the frequency of PD-L1 expression in immune cells with a CPS ≥ 1% was 57% (n= 49) (Figure 1). There was no difference in the rate of PD-L1$^{Tc+/Ic+}$/CPS expression between

Table 2. PD-L1 expression of biopsy and resected samples

Patient Characteristics		Total n (%)	Resection n (%)	Endoscopic biopsy n (%)	Resection + biopsy n (%)	P
Presence of PD-L1 Expression	Tumor Cells	30 (34.9)	18 (60.0)	8 (26.7)	4 (13.3)	0.171
	Immune Cells	49 (57.0)	26 (53.1)	11 (22.4)	12 (24.5)	0.159
	Combined Positive Score	49 (57.0)	26 (53.1)	11 (22.4)	12 (24.5)	0.159

CPS: Combined Positive Score

biopsy and resected samples (Table 2). PD-L1 expression was higher in tumour and immune cells in the neoadjuvant chemotherapy group than in the primary surgery group (Table 3).

Table 4 shows the correlation of PD-L1 expression with clinicopathological findings. PD-L1 expression (≥ %1) was more frequently observed in the MSI-positive group (p^{Tc+}= 0.003; p^{Ic+}= 0.024), node-positive group (p^{Ic+}= 0.015), adenocarcinoma subtype group (p^{Tc+}= 0.038), non-poorly cohesive carcinoma subtype group (p^{Tc+}= 0.018), preoperative treatment group (p^{Tc+}= 0.018; p^{Ic}+= 0.008), and neoadjuvant treatment response group (p^{Tc+}= 0.012; p^{Ic+}= 0.013). Age, sex, tumour location, tumour size, cT-N and pT-N status, HER2 status, LVI and PNI demonstrated no significant correlation with PD-L1 IHC in either tumour cells or immune cells.

Positive PD-L1 expression based on a cutoff CPS of ≥ 5% was more frequently observed in MSI cases (p= 0.024), node-positive cases (p= 0.015), patients who received preoperative treatment (p= 0.008) and patients who showed a response to chemotherapy according to Mandard TRG classification (p= 0.012) (Table 4).

Positive PD-L1 expression based on a cut off CPS of ≥ 10% was more frequently observed in MSI cases (p= 0.006), the adenocarcinoma subtype group (p= 0.022), the non-poorly cohesive carcinoma subtype group (p= 0.029) and patients with a response to chemotherapy according to the Mandard and CAP TRG classification systems (p= 0.010 and p= 0.013, respectively) (Table 4).

Relationship of MSI Status with Clinicopathological Variables

MSI-H was detected in 11.6% of the cohort (n= 10). There was a significant association between MSI status and age (p= 0.011), tumour size (p= 0.020), positive PD-L1 expression (p= 0.021), the adenocarcinoma subtype (p= 0.003), and the non-poorly cohesive carcinoma subtype (p= 0.010). Sex, tumour location, cT-N and pT-N status, HER2 status, LVI, PNI, preoperative treatment and the chemotherapy response according to the Mandard and CAP TRG classification systems showed no significant correlation with MSI status.

In patients who received neoadjuvant therapy, PD-L1 expression was higher in both tumour cells (p=

Table 3. PD-L1 expression status in the patients between neoadjuvant chemotherapy group and primary surgery group

Patient Characteristics		Total n (%)	Neoadjuvant CT n (%)	Primary Surgery n (%)	P
Presence of PD-L1 Expression	TumorCells	30 (34.9)	18 (60.0)	12 (40.0)	0.018
	ImmuneCells	49 (57.0)	27 (55.1)	22 (45.9)	0.008
	Combined Positive Score	49 (57.0)	27 (55.1)	22 (45.9)	0.008
MSI Status	p-MMR	76 (88.4)	34 (44.7)	42 (55.3)	0.297
	d-MMR	10 (11.6)	3 (30.0)	7 (70.0)	

CPS: CombinedPositiveScore; MSI: MicrosatelliteInstability; p-MMR: Proficient MMR; d-MMR: Deficient MMR

Table 4. The relation between TC. IC and CPS PD-L1 positivity according to demographic distribution and clinicopathologic variables

Patient Characteristics		Tumor Cells (TC) Positive n= 30 (34.9%)	Negative n= 56 (65.1%)	P	Immune Cells (IC) / CPS (≥1) Positive n= 49 (57%)	Negative n= 37 (43%)	P
Age	< 60	12 (29.3)	29 (70.7)	0.207	21 (51.2)	20 (48.8)	0.209
	≥ 60	18 (40.0)	27 (60.0)		28 (62.2)	17 (37.8)	
Sex	Male	20 (36.4)	35 (63.6)	0.444	33 (60.0)	22 (40.0)	0.298
	Female	10 (32.3)	21 (67.7)		16 (51.6)	15 (48.4)	
TumorLocation	Cardia	7 (43.8)	9 (56.2)	0.461	9 (56.2)	7 (43.8)	0.531
	Corpus	13 (38.2)	21 (61.8)		22 (64.7)	12 (35.3)	
	Antrum	9 (32.1)	19 (67.9)		13 (46.4)	15 (54.6)	
	Diffuse	1 (12.5)	7 (87.5)		5 (62.5)	3 (37.5)	
cTStatus	T1-2	14 (29.8)	33 (70.2)	0.194	23 (48.9)	24 (51.1)	0.075
	T3-4	16 (41.0)	23 (59.0)		26 (66.7)	13 (33.3)	
cNStatus	Nodenegative	9 (26.5)	25 (73.5)	0.137	14 (41.2)	20 (58.8)	0.015
	Nodepositive	21 (40.4)	31 (59.6)		35 (67.3)	17 (32.7)	
HER2 Status	Negative	13 (37.1)	22 (62.9)	0.434	24 (68.6)	11 (31.4)	0.423
	Positive	3 (50.0)	3 (50.0)		5 (83.3)	1 (16.7)	
MSI Status	p-MMR	22 (28.9)	54 (71.1)	0.003	40 (52.6)	36 (47.4)	0.024
	d-MMR	8 (80.0)	2 (20.0)		9 (90.0)	1 (10.0)	
Perioperative	Yes	18 (48.6)	19 (51.4)	0.018	27 (73.0)	10 (27.0)	0.008
Treatment	No	12 (24.5)	37 (75.5)		22 (44.9)	27 (55.1)	
Histological	Adenocarcinoma	18 (46.2)	21 (53.8)	0.038	25 (64.1)	14 (35.9)	0.159
Subtype	Other	12 (25.5)	35 (74.5)		23 (51.1)	24 (48.9)	
Poorly cohesive	Yes	5 (16.7)	25 (83.3)	0.008	14 (46.7)	16 (53.3)	0.118
carcinoma	No	25 (44.6)	31 (55.4)		35 (62.5)	21 (37.5)	
Lymphovascular	Yes	19 (34.5)	36 (65.5)	0.573	33 (60.0)	22 (40.0)	0.237
Invasion	No	7 (33.3)	14 (66.7)		10 (47.6)	11 (52.4)	
Mandard-TRG	TRG 1-3	7 (87.5)	1 (12.5)	0.003	7 (87.5)	1 (12.5)	0.012
	TRG 4-5	7 (38.9)	11 (61.1)		14 (77.8)	4 (22.2)	
CAP-TRG	TRG 0-1	6 (100.0)	0 (0.0)	0.013	6 (100.0)	0 (0.0)	0.236
	TRG 2-3	8 (40.0)	12 (60.0)		15 (75.0)	5 (25.0)	

CPS: Combined Positive Score; MSI: Mikrosatellite Instability; p-MMR: Proficient MMR; d-MMR: Deficient MMR; TRG: Tumor Regression Grade

0.018) and immune cells (p= 0.008); however, there was no significant relationship with MSI status (Table 5).

Impact of PD-L1 Expression/MSI Status on OS

The median OS of patients with PD-L1^{Tc+} gastric carcinoma was shorter than that of patients with PD- L1^{Tc-}gastric carcinoma (15.7 months vs. 53.4 months, p= 0.008). The median OS was 20.4 months for patients with PD-L1^{Ic+} expression and was not reached for PD-L1Ic- patients (p= 0.027). Median OS were shorter among patients with a CPS ≥ 1% for PD-L1 (Figure 2). The survival rate was significantly worse among patients with positive PD-L1 expression based on a cut off CPS of ≥ 5% and ≥ 10%. Only 10 patients had a PD-L^{1C}-PS ≥ 50%, and the number of deaths was not sufficient for a survival analysis.

In the univariate analysis, the median OS was shorter among patients with clinical/radiological nodal positivity (p= 0.030), a more advanced T

Table 5. The relation between clinicopathological variables and microsatellite insitability status

Patient Characteristics		Total n (%)	p-MMR n (%)	d-MMR n (%)	P
Age	< 60	41 (47.7)	40 (97.6)	1 (2.4)	0.011
	≥ 60	45 (52.3)	36 (80.0)	9 (20.0)	
Sex	Male	55 (64.0)	48 (87.3)	7 (12.7)	0.481
	Female	31 (36.0)	28 (90.3)	3 (9.7)	
Tumor Location	Cardia	16 (18.6)	16 (100.0)	0 (0.0)	0.173
	Corpus	34 (39.5)	31 (91.2)	3 (8.8)	
	Antrum	28 (32.6)	22 (78.6)	6 (21.4)	
	Diffuse	8 (9.3)	7 (87.5)	1 (12.5)	
Tumor Size	≤ 6 cm	49 (63.6)	46 (93.8)	3 (6.2)	0.020
	> 6 cm	28 (36.4)	21 (75.0)	7 (25.0)	
cT Status	T1-2	47 (54.7)	42 (89.4)	5 (10.6)	0.506
	T3-4	39 (45.3)	34 (87.2)	5 (12.8)	
cN Status	Node negative	34 (39.5)	31 (91.2)	3 (8.8)	0.385
	Node positive	52 (60.5)	45 (86.5)	7 (13.5)	
HER2 Status	Negative	35 (85.4)	34 (97.1)	1 (2.9)	0.051
	Positive	6 (14.6)	4 (66.7)	2 (33.3)	
Presence of PD-L1	Tumor Cells	30 (34.9)	22 (73.3)	8 (26.7)	0.003
Expression	Immune Cells	49 (57.0)	40 (81.6)	9 (18.4)	0.021
	Combined Positive Score	49 (57.0)	40 (81.6)	9 (18.4)	0.021
PerioperativeTreatment	Yes	37 (43.0)	34 (91.9)	3 (8.1)	0.297
	No	49 (57.0)	42 (85.7)	7 (14.3)	
Histological Subtype	Adeno carcinoma	39 (45.3)	30 (76.9)	9 (23.1)	0.003
	Other	47 (54.7)	46 (97.9)	1 (2.1)	
Poorly cohesive	Yes	30 (34.9)	30 (100.0)	0 (0.0)	0.010
carcinoma	No	56 (65.1)	46 (82.1)	10 (17.9)	
Lymphovascular Invasion	Yes	55 (72.4)	46 (83.6)	9 (16.4)	0.171
	No	21 (27.6)	20 (95.2)	1 (4.8)	
Mandard-TRG	TRG 1-3	8 (30.7)	8 (100.0)	0 (0.0)	0.473
	TRG 4-5	18 (69.3)	15 (83.3)	3 (16.7)	
CAP-TRG	TRG 0-1	6 (23.1)	6 (100.0)	0 (0.0)	0.438
	TRG 2-3	20 (76.9)	17 (85.0)	3 (15.0)	

MSI: Mikrosatellite Instability; p-MMR: Proficient MMR; d-MMR: Deficient MMR; TRG: Tumor Regression Grade

stage (p= 0.001), and positive PD-L1 expression (p^{Tc+}= 0.008; p^{Ic+}= 0.027). There was no statistically significant relationship between OS and age, MSI status, HER2 status, tumour location, LVI or PNI. In the multivariate analysis, the most important independent predictors of OS were clinical nodal stage (HR: 5.13; p= 0.003) and positive PD-L1 expression in tumour cells (HR: 2.28; p= 0.047).

DISCUSSION

In this study on GC, PD-L1 expression was positive in more than one-third of tumour cells and in more than half of immune cells. MSI was found in 11.6% of the patients. PD-L1 positivity was significantly correlated with MSI. Age, tumour size, positive PD-L1 expression and histological subtype showed significant associations with MSI. PD-L1 expression was significantly higher in

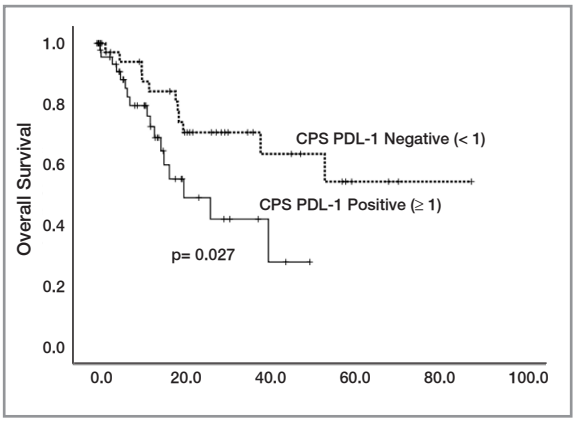

Figure 2. OS according to PD-L1 positivity (CPS ≥ 1%)

node-positive cases, the adenocarcinoma subtype, MSI cases, patients who received neoadjuvant therapy, and patients who showed a response to chemotherapy. PD-L1 expression in both tumour and immune cells was significantly associated with a shorter survival.

In this study, the rate of PD-L1 expression (≥ 1%) was 34.9% in tumour cells and 57% in immune cells. The reported positive rate and prognostic value of PD-L1 expression in GC are inconsistent in the literature. Several IHC studies on gastric carcinoma reported PD-L1 expression at rates from 5.1% to 65%[11-14], and expression was found to correlate to depth of invasion, lymph node metastasis, distant metastasis, and tumour size.[15] The diversity in positivity rates is thought to be due to factors such as heterogeneity in gastric carcinomas, the individualized tumour microenvironment, the use of different IHC antibodies, and the application of different cutoff values. Multiple studies have indicated that positive PD-L1 expression is associated with significantly worse OS[11,16,,17], but other studies did not confirm this finding.[12,18-20] In our study, positive PD-L1 expression was associated with shorter survival. A few recent meta-analyses showed a correlation between PD-L1 and prognosis in GC, demonstrating that PD-L1 overexpression is a poor prognostic factor in GC.[14,21-23]

In our study, node positivity, MSI, preoperative treatment, and chemotherapy response according to the Mandard and CAP TRG classification systems were associated with higher rates of PD-L1 expression. In studies evaluating the relationship between PD-L1 expression and clinicopathological features, patients with deep tumour infiltration, lymph node metastasis, and LVI were reported to have a higher incidence of PD-L1 expression.[22] Similarly, in another meta-analysis, patients with larger tumours and lymph node metastasis tended to have higher PD-L1 expression levels.[14] PD-L1 expression was found to correlate with the depth of invasion, lymph node metastasis, distant metastasis, and tumour size in a review of several studies.[15]

The frequency of MSI-H in GC ranges from 8.2% to 37%.[24] In our study, the rate of MSI in GC was 11.6%. MSI-H–positive tumours have increased PD-L1 expression, which has been shown to be a predictor of response to checkpoint blockade.[25] PD-L1 expression was found to be higher in patients with MSI, which can be attributed to the inflammatory microenvironment and immune response in MSI-H tumours. MSI in sporadic GC is mostly associated with the loss of MLH1/PMS2.

In our study, all patients with MSI showed a loss of nuclear MLH1/PMS2. Genetic mutations in MSH2 are often identified in Lynch syndrome. None of our patients had a familial history of cancer-related syndromes. Most studies on MSI status and PD-L1 expression have been from Asian countries; European data are limited, and no data have been reported in Turkey until now.

In our study, MSI-H status was significantly correlated with older age, increased tumour size, positive PD-L1 expression, and the adenocarcinoma subtype. One case of the medullary histological subtype showed MSI and PD-L1 positivity in both tumour and immune cells. MSI was more common in adenocarcinoma than in poorly cohesive carcinoma. No significant relationship was found between MSI status and tumour location, lymph node involvement, LVI, PNI, preoperative treatment or the response to chemotherapy. A recent meta-analysis showed that patients with GC and MSI-H tend to be older and female with distally located disease of the well-differentiated adenocarcinoma type that is diagnosed at a less advanced tumour stage.[24] The prognostic importance of MSI in GC is controversial. Certain studies support that MSI-H is associated with a good prognosis[26,27], while others report conflicting findings.[28,29] In this study, the

effect of MSI status on OS could not be demonstrated. A recent meta-analysis showed that MSI-H GC patients have an improved prognosis, accompanied by reduced risks of LN metastasis, tumour invasion and death.

Landmark analyses by The Cancer Genome Atlas (TCGA) proposed classifications based on comprehensive genomic profiling for 4 subtypes of GC: Epstein-Barr virus (EBV), MSI, genomically stable, and chromosomal instability.[31] EBV+ GC and MSI GC have abundant lymphocytic infiltration in the tumour stroma and thus can be classified as gastric carcinoma with prominent lymphoid stroma (medullary cancer). Furthermore, patients with EBV+ and MSI GC tend to show PD-L1 expression, indicating that these GC subtypes may be prime candidates for PD-L1-directed therapy.[23] MSI and PD-L1 expression have been shown to predict a stronger response to PD-1 inhibitors, as highlighted by the recent approvals of pembrolizumab in treatment-refractory solid tumours with MSI and in the third-line or greater treatment of PD-L1–positive advanced gastric/GEJ cancers. Pembrolizumab has been approved with a tissue-agnostic indication for treatment-refractory solid tumours that are MSI-H.

Immune parameters, including MSI status, tumour infiltrating lymphocytes (TILs), PD-L1 expression, and the TME immune profile, are among the potential predictive biomarkers for checkpoint inhibitors in advanced gastric/GEJ cancer. The application of biomarker-oriented immunotherapy increases the therapeutic efficacy, minimizes unnecessary exposure and reduces the financial burden on health systems. We anticipate that composite biomarkers will improve patient selection and potentially individualize treatment, although broader clinical implementation may be slow. The identification of more robust predictive biomarkers and the development of combination therapies incorporating ICIs represent necessary and ongoing areas of investigation to optimize this class of agents in gastric/GEJ cancer.

REFERENCES

1. Sharma P, Allison JP. The future of immune checkpoint therapy. Science 348: 56-61, 2015.
2. Wu X, Gu Z, Chen Y, et al. Application of PD-1 Blockade in Cancer Immunotherapy. Comput Struct Biotechnol J 17: 661-674, 2019.
3. Muro K, Chung HC, Shankaran V, et al. Pembrolizumab for patients with PD-L1-positive advanced gastric cancer (KEYNOTE-012): a multicentre. open-label. phase 1b trial. Lancet Oncol 17: 717-726, 2016.
4. Fuchs CS, Doi T, Jang RW, 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 4: e180013, 2018.
5. Joshi SS, Maron SB, Catenacci DV. Pembrolizumab for treatment of advanced gastric and gastroesophageal junction adenocarcinoma. Future Oncol 14: 417-430, 2018.
6. Kang YK, Boku N, Satoh T, 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, double-blind, placebo-controlled, phase 3 trial. Lancet 390: 2461-2471, 2017.
7. Le DT, Durham JN, Smith KN, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science 357: 409-413, 2017.
8. Mlecnik B, Bindea G, Angell HK, et al. Integrative analyses of colorectal cancer show Immunoscore is a stronger predictor of patient survival than microsatellite instability. Immunity 44: 698-711, 2016.
9. Salem ME, Puccini A, Grothey A, et al. Landscape of tumor mutation load, lismatch repair deficiency, and PD-L1 expression in a large patient cohort of gastrointestinal cancers. Mol Cancer Res 16: 805-812, 2018.
10. Kulangara K, Zhang N, Corigliano E, et al. Clinical utility of the combined positive score for programmed death ligand-1 expression and the approval of pembrolizumab for treatment of gastric cancer. Arch Pathol Lab Med 143: 330-337, 2019.
11. Wu C, Zhu Y, Jiang J, et al. Immunohistochemical localization of programmed death-1 ligand-1 (PD-L1) in gastric carcinoma and its clinical significance. Acta Histochem 108: 19-24, 2006.
12. Boger C, Behrens HM, Mathiak M, et al. PD-L1 is an independent prognostic predictor in gastric cancer of Western patients. Oncotarget 7: 24269-24283, 2016.
13. Li Z, Lai Y, Sun L, et al. PD-L1 expression is associated with massive lymphocyte infiltration and histology in gastric cancer. Hum Pathol 55: 182-189, 2016.
14. Zhang M, Dong Y, Liu H, et al. The clinicopathological and prognostic significance of PD-L1 expression in gastric cancer: a meta-analysis of 10 studies with 1.901 patients. Sci Rep 6: 37933, 2016.
15. Lin EM, Gong J, Klempner SJ, Chao J. Advances in immuno-oncology biomarkers for gastroesophageal cancer: Pro-

grammed death ligand 1, microsatellite instability, and beyond. World J Gastroenterol 24: 2686-2697, 2018.

16. Tamura T, Ohira M, Tanaka H, et al. Programmed Death-1 Ligand-1 (PDL1) expression is associated with the prognosis of patients with stage II/III gastric cancer. Anticancer Res 35: 5369-5376, 2015.
17. Zhang L, Qiu M, Jin Y, et al. Programmed cell death ligand 1 (PD-L1) expression on gastric cancer and its relationship with clinicopathologic factors. Int J Clin Exp Pathol 8: 11084-11091, 2015.
18. Huang B, Chen L, Bao C, et al. The expression status and prognostic significance of programmed cell death 1 ligand 1 in gastrointestinal tract cancer: a systematic review and meta-analysis. Onco Targets Ther 8: 2617-2625, 2015.
19. Dong M, Wang HY, Zhao XX, et al. Expression and prognostic roles of PIK3CA, JAK2, PD-L1, and PD-L2 in Epstein-Barr virus-associated gastric carcinoma. Hum Pathol 53: 25-34, 2016.
20. Wu Y, Cao D, Qu L, et al. PD-1 and PD-L1 co-expression predicts favorable prognosis in gastric cancer. Oncotarget 8: 64066-64082, 2017.
21. Wu P, Wu D, Li L, et al. PD-L1 and Survival in Solid Tumors: A Meta-Analysis. PLoS One 10: e0131403, 2015.
22. Gu L, Chen M, Guo D et al. PD-L1 and gastric cancer prognosis: A systematic review and meta-analysis. PLoS One 12: e0182692, 2017.
23. Qing Y, Li Q, Ren T, et al. Upregulation of PD-L1 and APE1 is associated with tumorigenesis and poor prognosis of gastric cancer. Drug Des Devel Ther 9: 901-909, 2015.
24. Zhu L, Li Z, Wang Y, et al. Microsatellite instability and survival in gastric cancer: A systematic review and meta-analysis. Mol Clin Oncol 3: 699-705, 2015.
25. Le DT, Uram JN, Wang H, et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. N Engl J Med 372: 2509-2520, 2015.
26. Fang WL, Chang SC, Lan YT, et al. Microsatellite instability is associated with a better prognosis for gastric cancer patients after curative surgery. World J Surg 36: 2131-2138, 2012.
27. An JY, Kim H, Cheong JH, et al. Microsatellite instability in sporadic gastric cancer: its prognostic role and guidance for 5-FU based chemotherapy after R0 resection. Int J Cancer 131: 505-511, 2012.
28. Wirtz HC, Muller W, Noguchi T, et al. Prognostic value and clinicopathological profile of microsatellite instability in gastric cancer. Clin Cancer Res 4: 1749-1754, 1998.
29. Oki E, Kakeji Y, Zhao Y, et al. Chemosensitivity and survival in gastric cancer patients with microsatellite instability. Ann Surg Oncol 16: 2510-2515, 2009.
30. Cancer Genome Atlas Research N. Comprehensive molecular characterization of gastric adenocarcinoma. Nature 513: 202-209. 2014.

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