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ORIGINAL ARTICLE

Immunoprofile of adenosquamous carcinoma in gastric cancer

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Abstract

Background: Gastric adenosquamous carcinoma (GASC) is a rare subtype of gastric cancer. Research on GASC treatment is limited, and its outcome is usually poor. We investigated the clinical features, immunoprofile of GASC, and determined the optimal treatment modality for these patients.

Methods: Patients with GASC from Taipei Veterans General Hospital were retrospectively reviewed. Clinical features and treatment outcomes were evaluated. Adequate samples were examined for surrogate biomarkers for immunotherapy by IHC staining.

Results: Total 14 (0.35%) GASC patients were found among 4034 gastric cancer patients. The median tumor size was 6.8 cm in 10 patients with stage III GASC, and all these patients underwent radical gastrectomy followed by adjuvant therapy. The median progression-free survival (PFS) and overall survival (OS) were 6.0 and 11.5 months, respectively. Two patients with stage IV GASC received frontline immunotherapy. Their median PFS and OS were 9.0 and 12.5 months. In immunoprofiling, 25.0% (n = 3), 75.0% (n = 9), and 33.3% (n = 4) of the samples had deficient mismatch repair (dMMR) protein, combined positive score (CPS) of ≥1, and CPS of ≥10, respectively. The univariate analysis revealed that programmed death-ligand 1 ≥5% (HR: 0.12; 95% CI: 0.01-0.97; $p = 0.047$) was significant associated with superior OS. One stage IV patient with CPS ≥10 and dMMR proteins received nivolumab monotherapy as frontline treatment that resulted 14-month PFS.

Conclusion: Patients with GASC are more likely to yield positive results for CPS and dMMR. Biomarkers should be examined, and immunotherapy can be considered as frontline systemic treatment.

Keywords: dMMR; Gastric adenosquamous cell carcinoma; Immune therapy; PDL1

1. INTRODUCTION

Gastric cancer is among the most common malignancies in Asia and the sixth leading cause of cancer-related deaths in Taiwan.[1] Adenocarcinoma accounts for approximately 90% of all gastric cancer cases. Gastric adenosquamous carcinoma (GASC) is a rare subtype accounting for <0.5% of cases.[2,3] Patients with GASC undergoing potentially curative surgery had shorter overall survival (OS) among gastric cancer.[4] Studies have determined the correlation among treatment outcomes, pathological features, and clinical parameters.[5–7] Determination of the proportion of the SCC component in tumors can have a prognostic value. However, optimal treatment modalities and predictive biomarkers remain unclear.

Immunohistochemical (IHC) staining is a practical and valuable method for identifying surrogate biomarkers for immunotherapy. Potential biomarkers include a combined positive score (CPS), deficient mismatch repair (dMMR) proteins, and Epstein–Barr virus-encoded small RNA (EBER).[8] High CPSs, dMMR protein detection, and EBER positivity in IHC staining may be associated with a more favorable response to immunotherapy.[9–11] A recent clinical trial demonstrated that patients with gastric adenocarcinoma who had high CPSs exhibited a more favorable treatment response.[12] However, the immunoprofile of

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patients with GASC remains unclear because of the small patient population. In addition, the treatment effect of immunotherapy on GASC remains unknown.

In this study, we investigated the immunoprofile of GASC and determined the optimal treatment modality for these patients by examining the long-term follow-up data and specimens of patients with gastric cancer from Taipei Veterans General Hospital.

2. METHODS

2.1. Study design and participants

In this retrospective study, we enrolled all patients with GASC who underwent treatment at Taipei Veterans General Hospital between January 1991 and June 2021. A total of 4034 patients who received a diagnosis of gastric cancer at Taipei Veterans General Hospital were identified. Among them, 14 (0.35%) patients received a diagnosis of GASC and 3 (0.07%) was diagnosed as gastric squamous cell carcinoma (SCC). Two of the 14 patients had limited clinical information and were not count in for analysis. This study was approved by the Institutional Review Board of Taipei Veterans General Hospital (2019-10-005AC) and followed the tenets of the Helsinki Declaration.

2.2. Investigation of potential biomarkers for immunotherapy

The 12 samples were subjected to IHC staining. The following markers were examined: p40, human epidermal growth factor receptor 2 (HER2), EBER, programmed death-ligand 1 (PD-L1) CPS, BRAF, and DNA mismatch repair proteins (eg, MLH1, MSH2, MSH6, and PMS2). The following primary antibody probes were utilized: p40 (BC28, Biocare, CA, USA), HER2 (A0485, DAKO, Carpinteria, CA, USA), EBER (PB0589; Biosystems, Muttenz, Switzerland), and BRAF (ab228461; Abcam, Cambridge, UK). DNA mismatch repair proteins were evaluated using the Ventana MMR RxDx Panel (Roche, Tucson, AZ, USA). PD-L1 expression was evaluated using the pharmDx immunohistochemistry assay (PD-L1 IHC 22C3; Agilent, Santa Clara, CA, USA) to determine the CPS. A CPS score of ≥1 was interpreted as positive PD-L1 expression. To compare immunoprofiles between different gastric cancer subtypes, previous studies including the same study group and providing details regarding high microsatellite instability (MSI-H) and PD-L1 expression were reviewed.[13,14] Fig. 1 illustrates the patient population and immunoprofile results.

2.3. Treatment, clinical response, and survival analysis

Surgery was performed on the basis of an experienced surgeon's judgment depending on the distance between the gastric cardia and tumor. Margins of 3 and 5 cm were maintained for a superficial and well-defined tumor and a poorly defined tumor, respectively. For distal gastric cancer, subtotal gastrectomy was performed. Total gastrectomy was performed for proximal gastric cancer on the basis of clinical evaluation. To facilitate curative resection, combined organ resection plus D2 dissection was the standard procedure, except in those for whom curative resection was not possible. Adjuvant and palliative chemotherapy regimens were chosen according to the physician's evaluation during the study period.

We collected information on patients' basic characteristics, namely age, sex, operation, specimen types, and treatment courses. The staging of gastric cancer was determined according to the eighth edition of the American Joint Committee on Cancer/Union for International Cancer Control tumor, node, and metastasis classification.[15] Location of tumor was according to the gastroscopy finding. Tumor size was measured through computed tomography or magnetic resonance imaging, with a follow-up interval of 3 months; this interval was adjusted if clinically indicated. The clinical response was evaluated on the basis of Response Evaluation Criteria in Solid Tumors, version 1.1. Progression-free survival (PFS) was defined as the duration from the initial treatment to disease progression. OS and treatment outcomes were recorded until the censor day, October 31, 2021. Univariate and multivariate Cox regression models were used to determine the prognostic relevance quantified as hazard ratios, with 95% confidence intervals. All the tests were analyzed using the Statistical Package for the Social Sciences (IBM SPSS version 22.0; International Business Machines Corp, NY, USA). The statistical significance was set at $p < 0.05$.

3. RESULTS

3.1. Patient characteristics

Table 1 summarizes the baseline characteristics of the 12 patients with GASC. The mean age of the patients was 64.5

Table 1

Patient characteristics of the twelve gastric adenosquamous carcinoma patients

No	Age/gender	Year of diagnosis	TNM	Stage	Location	Size (cm)	Operation	Frontline medical treatment	Progression site/duration (mo)	Outcome/ duration (mo)
1	47/Male	2000	T4aN3aM0	IIIC	NA	10.0	RTG	No	Brain/6	DOD/27
2	66/Male	2006	T4aN1M0	IIIA	NA	4.3	RTG	No	No/41	DO/41
3	78/Female	2006	T4aN3bM0	IIIC	NA	7.0	RTG	CCRT with cisplatin and irinotecan	No/1	DO/1
4	75/Female	2007	T4aN1M0	IIIA	Greater curvature	5.0	RTG	XELOX	Liver/4	DO/47
5	84/Male	2009	T4aN1M0	IIIA	Angularis	6.5	RSG	No	Liver/7	DO/8
6	57/Male	2011	T3N3bM0	IIIB	Body	7.0	RTG	PFL	Lymph node/6	DOD/14
7	73/Male	2012	T4aN3aM0	IIIC	Low body	11.0	RSG	Neoadjuvant ECF	Liver/2	DOD/2
8	56/Female	2013	T4bN3bM0	IIIC	Fundus	6.5	RTG	PFL	Liver/7	DOD/26
9	49/Male	2013	T4bN3bM0	IIIC	High body	11.0	RTG	CCRT with Ufur	Peritoneum/6	DOD/6
10	40/Male	2019	T4aN3bM0	IIIC	Body	6.5	RTG	TS-1	Peritoneum/8	DOD/9
11	70/Male	2020	T4bN3M1	IV	Antrum	6.8	No	Nivolumab	Primary tumor/14	Alive/19
12	79/Female	2021	T4bN2M1	IV	Pylorus	9.0	RSG	CCRT with nivolumab and Ufur	Lymph node/4	Alive/6

CCRT = concurrent chemoradiation; DO = died of other causes; DOD = died of disease; ECF = epirubicin combined with cisplatin and 5-FU/leucovorin; NA = not available; PFL = cisplatin combined with 5-FU/leucovorin; RTG = radical total gastrectomy; RSG = radical subtotal gastrectomy; XELOX = xeloda combined with oxaliplatin.

Table 2

Immunoprofile of the 12 gastric adenosquamous carcinoma patients

No.	Age/Gender	TNM	Stage	Specimens	HER2	EBER	CPS	dMMR	BRAF
1	47/Male	T4aN3aM0	IIIC	RTG	NA	–	1	No	NA
2	66/Male	T4aN1M0	IIIA	RTG	NA	–	5	No	NA
3	78/Female	T4aN3bM0	IIIC	RTG	NA	–	0	No	NA
4	75/Female	T4aN1M0	IIIA	RTG	NA	–	15	No	NA
5	84/Male	T4aN1M0	IIIA	RSG	NA	–	0	No	NA
6	57/Male	T3N3bM0	IIIB	RTG	1+	–	10	No	–
7	73/Male	T4aN3aM0	IIIC	RSG	1+	–	1	Yes	–
8	56/Female	T4bN3bM0	IIIC	RTG	1+	–	2	No	–
9	49/Male	T4bN3bM0	IIIC	RTG	1+	–	2	No	–
10	40/Male	T4aN3bM0	IIIC	RTG	–	–	0	No	–
11	70/Male	T4bN3M1	IV	Biopsy	–	–	10	Yes	–
12	79/Female	T4bN2M1	IV	RSG	2+	–	10	Yes	–

CPS = combined positive score; dMMR = deficient mismatch repair; EBER = Epstein–Barr virus-encoded RNA; HER2 = human epidermal growth factor receptor 2; NA = not available; RSG = radical subtotal gastrectomy; RTG = radical total gastrectomy.

years. Eight (66.7%) and four (33.3%) patients were men and women, respectively. Ten patients had stage III disease, and all of them underwent radical gastrectomy. Two patients had stage IV disease, and one of them underwent radical gastrectomy because no clinical metastatic lesion was noted before surgery.

For the 10 patients with stage III GASC who underwent radical gastrectomy, the median tumor size was 6.8 cm. PFS ranged from 1 to 41 months, and OS ranged from 1 to 47 months. The median PFS and OS were 6.0 and 11.5 months, respectively. Adjuvant chemotherapy included platinum, 5-fluouracil, and irinotecan. Radiotherapy was additionally administered to two patients (Nos. 3 and 9) with multiple lymph node involvement postsurgery. The primary recurrence site was the liver in four (33.3%) patients, the peritoneum in two (16.7%) patients, lymph nodes in one (8.3%) patient, and the brain in one (8.3%) patient. Recurrence was not noted in the other two (16.7%) patients who died of other causes.

Two patients (Nos. 11 and 12) were diagnosed as having stage IV disease. The tumor size of these two patients were 6.8 and 9.0 cm, respectively. Patient No. 11 was clinically diagnosed as having stage IV disease, and patient No. 12 was diagnosed as having stage IV disease postsurgery. Patient No. 11 received frontline nivolumab alone for metastasis. Patient No. 12 received nivolumab combined with concurrent chemoradiation therapy with tegafur/uracil for locoregional control enhancement. Peritoneal lymphadenopathy enlargement was noted in the fourth month during treatment, and the patient was alive for 6 months at the censor day.

3.2. Immunoprofile of GASC

Table 2 presents the IHC staining results of the 12 patients. All the patient showed p40 positive on SCC component. Both adenocarcinoma and SCC components fit the definition of adenosquamous carcinoma, wherein the SCC component consisted of ≥25% of all the tumor mass.[2] All the patients were negative for EBER. 7 patients with HER 2 staining all revealed negative. 3 patients had dMMR (3/12, 25.0%), 9 had a CPS of ≥1 (9/12, 75.0%), and 4 had a CPS of ≥10 (4/12, 33.3%).

The high microsatellite instability (MSI-H) proportion and PD-L1 expression for the gastric cancer cohort of Taipei Veterans General hospital were reported in the previous study. MSI-H was observed in 16.4% of gastric cancer cases, and PD-L1 expression was observed in 30.4% cases (Fig. 1).[13,14] The

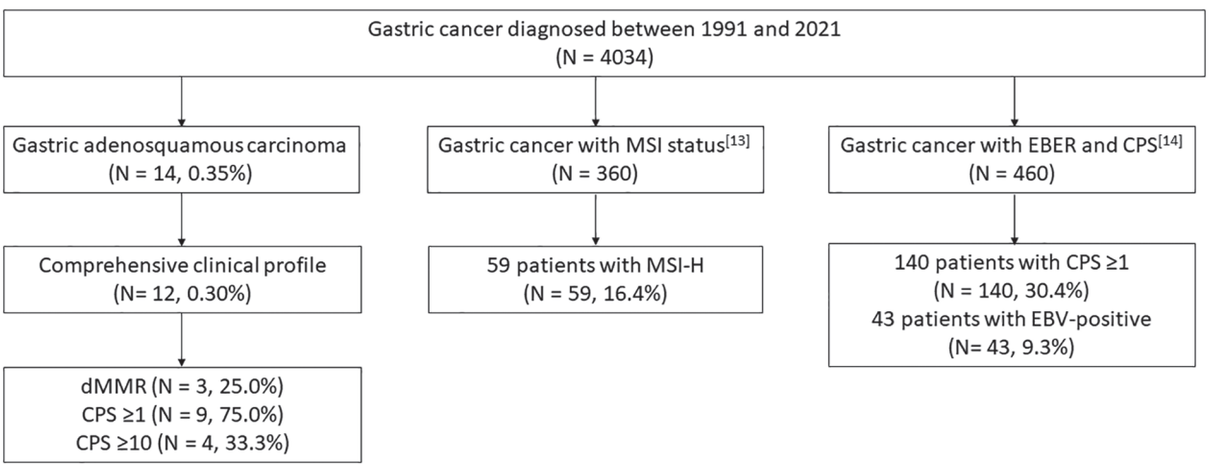

Fig. 1 Patients with gastric cancer who received treatment at Taipei Veterans General Hospital from 1991 to 2021. Previous studies of MSI-H and CPS were demonstrated.[13,14] CPS = combined positive score; dMMR= deficient mismatch repair; EBER= Epstein–Barr-encoded small RNA; IHC = immunohistochemistry; MSI-H= high microsatellite instability.

Table 3

Risk factor of mortality in 12 gastric adenosquamous carcinoma

	Univariate			Multivariable		
	HR	95% CI	*p*	HR	95% CI	*p*
Gender						
Male	Reference					
Female	0.53	(0.11-2.61)	0.436			
Age, y						
<65	Reference					
≥65	0.53	(0.13-2.25)	0.389			
Stage						
Limited	Reference					
Metastasis	0.04	(0.00-444.65)	0.493			
Size (cm)	1.32	(0.95-1.84)	0.095	1.06	(0.71-1.57)	0.778
CPS ≥1						
Negative	Reference					
Positive	0.17	(0.03-1.02)	0.052			
CPS ≥ 5						
Negative	Reference			Reference		
Positive	0.12	(0.01-0.97)	0.047*	0.14	(0.01-1.49)	0.103
CPS ≥ 10						
Negative	Reference					
Positive	0.19	(0.02-1.55)	0.122			
dMMR						
Negative	Reference					
Positive	0.83	(0.10-7.27)	0.870			

Cox proportional hazard regression.

CI = confidence interval; CPS = combined positive score; dMMR= deficient mismatch repair; HR = hazard ratio.

*$p < 0.05$,

result of this study showed that 75.0% of GASC patient had CPS ≥1. It was significantly higher than 30.4% (140/460) in our previous gastric adenocarcinoma cohort, analyzed by chi-square test ($p < 0.05$).[14]

3.3. Possible risk factors associated with inferior OS and PFS

Table 3 revealed Cox regression analysis of OS in GASC patients received tailored therapy. The univariate analysis revealed that CPS ≥5 (hazard ratio: 0.12; 95% confidence interval: 0.01-0.97; $p = 0.047$) was significant associated with superior OS. Multivariate analysis showed no independent factor for mortality. Cox regression analysis of PFS in GASC patients all showed negative result.

3.4. Case sharing

The histology and IHC staining results of patient No. 11 demonstrated a morphology of the adenosquamous carcinoma and p40 positivity in SCC component (Fig. 2). The glandular structure in adenocarcinoma component was hard to be identified due to the nature of poorly differentiation. Furthermore, PD-L1 expression and the loss of MLH1 and PMS2 were observed (Fig. 3).

The patient received radiotherapy for symptom relief and nivolumab monotherapy for metastasis. A partial response and PFS of 14 months were noted. We examined the findings of the patient's abdominal computed tomography performed 3 months later and observed good response of metastatic lymphadenopathy over the lesser gastric curvature (Fig. 4). After disease progression, 4 months of PFS was achieved using a combination of ipilimumab and nivolumab. Without any chemotherapy, the patient was free from progression for 18 months.

4. DISCUSSION

The incidence of GASC in our cohort (0.35%) is compatible with that reported in a previous study, which demonstrated an incidence of < 1%.[6] The nature of poor OS in GASC was validated by a previous study, which showed OS of GASC significantly worse than adenocarcinoma. Median overall survival time was 17 months for limited stage GASC received R0 resection.[16] Our study showed OS 11.5 months in stage III GASC received radical gastrectomy. For stage III gastric adenocarcinoma, the median OS was 30.7 months in real-world data.[17] It was difficult to make treatment-matched comparison of GASC and non-GASC in our cohort due to limited case numbers and heterogeneity of treatment. Only 2 GASC patients received immunotherapy.

However, the immunoprofile data of GASC were still waiting to be discovered. Only one case report presented a GASC case with a CPS of 10, without the loss of expression of DNA mismatch repair proteins.[18] Our study was the first to systemically evaluate CPS, EBER, and dMMR in the GASC population and proposed this phenomenon. Further validation may be required to make a solid conclusion.

In our previous studies, the incidence of MSI-H, Epstein–Barr virus-positive, and PD-L1 expression in all gastric cancers was 15.4%, 9.6%, and 30.4%.[13,14] This study demonstrated that 25.0% of the patients with GASC had dMMR, 75.0% had a CPS of ≥1, and 33.3% had a CPS of ≥10. In gastric adenocarcinoma, the proportion of PD-L1 expression was found to be approximately 15% in CheckMate 649 and ATTRACTION-4 studies.[12,19] The results of our study revealed that compared with other histology subtypes, GASC demonstrated a higher proportion of positive surrogate biomarkers on immunotherapy.[13,14]

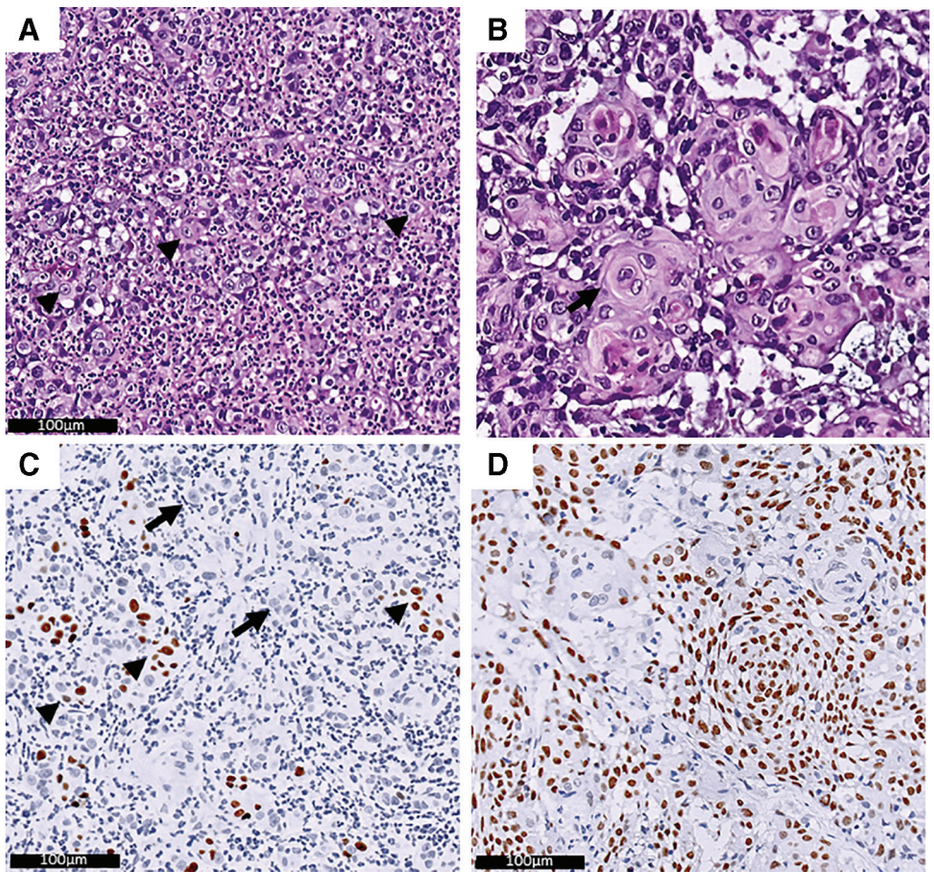

Fig. 2 Histological findings of the stomach biopsy of patient No. 11. A, Adenocarcinoma predominant area with scattered squamous carcinoma cells (arrowhead), (B) SCC predominant area with prominent keratinization (arrow), (C) negative p40 staining on adenocarcinoma component (arrow), and positive p40 staining on SCC component (arrowhead), (D) diffuse p40 staining on squamous cell carcinoma. SCC = squamous cell carcinoma.

The Cox regression revealed that CPS ≥5 was associated with better overall survival in univariate analysis. However, only two of these five patients received immunotherapy. The result should be interpretation very carefully and need further validation due to limited case numbers. Several outliers can significantly impact the result. The systemic treatment of these patient also owned high heterogeneity.

Because of the rarity of GASC, most of the GASC cases were treated as gastric adenocarcinoma. Radical surgical resection remained the curative management for local disease. No standard medical therapies have been established; however, adjuvant chemotherapy combined with radiotherapy may improve the survival of patients with GASC.[20] Traditional chemotherapy, including TS-1, did not result in a satisfactory treatment response in patients with GASC.[21] Anti-HER2 therapy may be beneficial in HER2-positive GASC; however, HER2 results were seldom positive.[22,23] Immunotherapy produced a durable response in selective gastric cancer cases.[24] Thus, immunotherapy may be a treatment of choice for patients with GASC, particularly for those with a high positivity rate of prediction markers in the tumor.

Our patient No.11 was diagnosed as having stage IV GASC with peritoneal seeding at the initial presentation. Because of old age and frail condition, chemotherapy was not feasible. Nivolumab monotherapy was used as the frontline treatment due to high CPS and dMMR. The patient exhibited a satisfactory treatment response. Furthermore, the administration of the combination of ipilimumab and nivolumab after disease progression resulted in 4 months of PFS.

This study has several limitations. The major limitation of this study is its small sample size owing to the rarity of the disease. Selection bias may exist. Second, most of the patients received their diagnoses before the era of immunotherapy and only two patients received immunotherapy. Thus, the efficacy of immunotherapy in GASC should be validated in future studies.

In conclusion, patients with GASC are more likely to have positive results for CPSs and dMMR. CPS, PD-L1 expression, dMMR proteins, and MSI should be examined as biomarkers to guide the use of immunotherapy. 2 GASC patient with positive immunoprofile got good response from immunotherapy. The treatment response of chemotherapy may be suboptimal, and immunotherapy may be considered as the frontline treatment.

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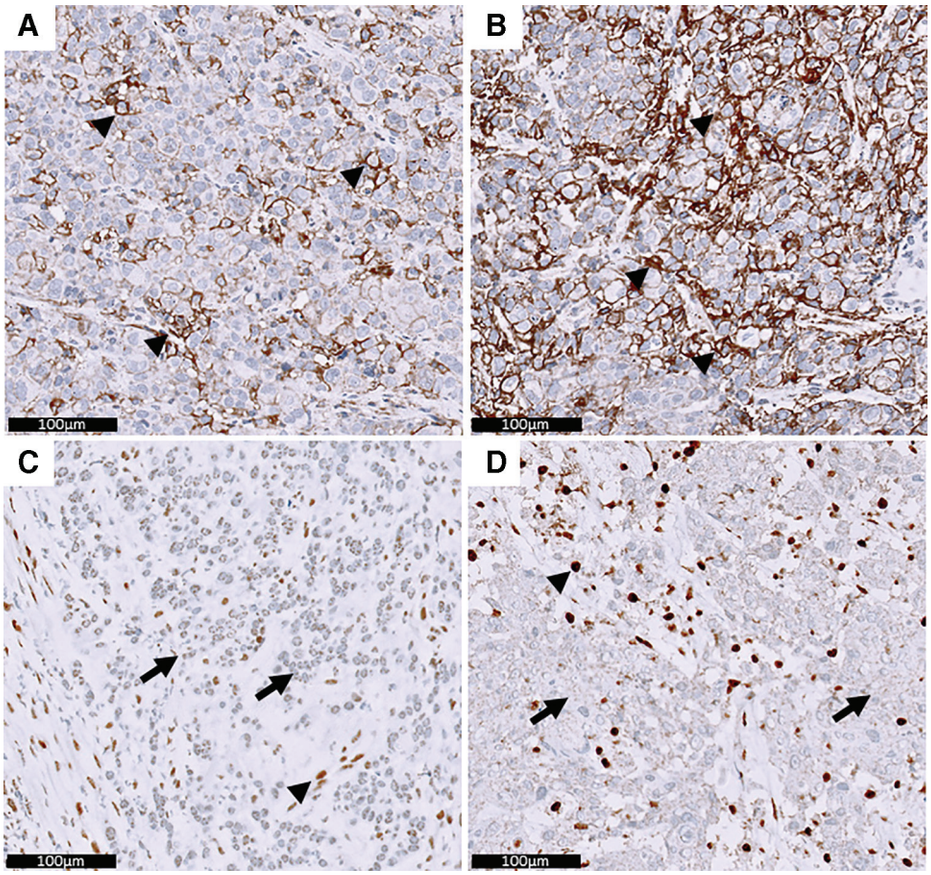

Fig. 3 Positive PD-L1 IHC staining and loss of nuclear positivity in the stomach biopsy of patient No. 11. A, PD-L1 in adenocarcinoma component (arrowhead), (B) PD-L1 in SCC component (arrowhead), (C) loss of nuclear positivity in MLH1 (arrow), and endothelial cells, inflammatory cells as internal control (arrowhead), (D) loss of nuclear positivity in PMS2 (arrow), and endothelial cells, inflammatory cells as internal control (arrowhead). IHC = immunohistochemistry; PD-L1 = programmed death-ligand 1; SCC = squamous cell carcinoma.

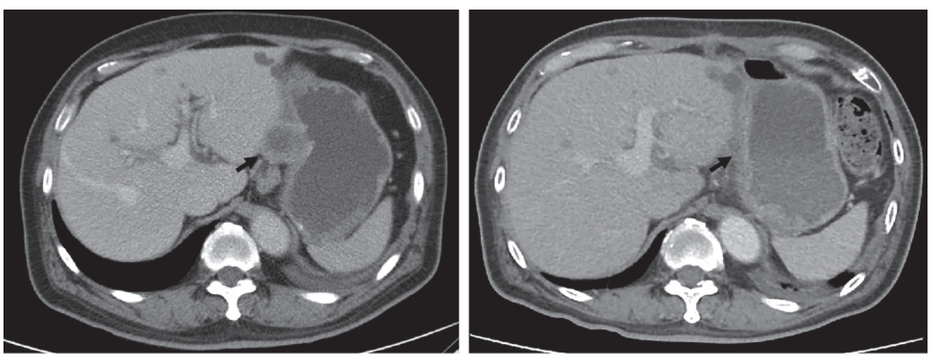

Fig. 4 Partial response with nivolumab monotherapy for 3 months in patient No. 11 observed on computed tomography. A, Initial regional metastatic lymphadenopathy over the lesser gastric curvature (arrow) and (B) regressive changes in previously noted enlarged lymph nodes at the lesser curvature of the stomach (arrow).

REFERENCES

1. Lin YT, Chiang CJ, Yang YW, Huang SP, You SL. Secular decreasing trends in gastric cancer incidence in Taiwan: a population-based cancer registry study. *World J Gastroenterol* 2021;**27**:5764–74.
2. Chen YY, Li AF, Huang KH, Lan YT, Chen MH, Chao Y, et al. Adenosquamous carcinoma of the stomach and review of the literature. *Pathol Oncol Res* 2015;**21**:547–51.
3. Machlowska J, Baj J, Sitarz M, Maciejewski R, Sitarz R. Gastric cancer: epidemiology, risk factors, classification, genomic characteristics and treatment strategies. *Int J Mol Sci* 2020;**21**:4012.
4. Li HS, Liu X, Zhang MY, Cheng K, Chen Y, Zhou YW, et al. Clinicopathologic characteristics, survival, and treatments for gastric adenosquamous carcinoma: a population-based study. *Curr Oncol* 2020;**27**:e527–36.
5. Chu YX, Gong HY, Hu QY, Song QB. Adenosquamous carcinoma may have an inferior prognosis to signet ring cell carcinoma in patients with stages I and II gastric cancer. *World J Gastrointest Oncol* 2020;**12**:101–12.
6. Akce M, Jiang R, Alese OB, Shaib WL, Wu C, Behera M, et al. Gastric squamous cell carcinoma and gastric adenosquamous carcinoma, clinical features and outcomes of rare clinical entities: a

National Cancer Database (NCDB) analysis. *J Gastrointest Oncol* 2019;**10**:85–94.
7. Li HS, Chen Y, Zhang MY, Cheng K, Zhou YW, Liu JY. Increased proportion of the squamous cell carcinoma component is associated with worse survival in resected gastric adenosquamous carcinoma: a STROBE compliant cohort study. *Medicine (Baltim)* 2020;**99**:e21980.
8. Di Pinto F, Armentano R, Arborea G, Schena N, Donghia R, Valentini AM. Are immunohistochemical markers useful in phenotypic gastric cancer classification? *Oncology (Huntingt)* 2020;**98**:566–74.
9. 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.
10. Cai L, Sun Y, Wang K, Guan W, Yue J, Li J, et al. The better survival of MSI subtype is associated with the oxidative stress related pathways in gastric cancer. *Front Oncol* 2020;**10**:1269.
11. Hagi T, Kurokawa Y, Kawabata R, Omori T, Matsuyama J, Fujitani K, et al. Multicentre biomarker cohort study on the efficacy of nivolumab treatment for gastric cancer. *Br J Cancer* 2020;**123**:965–72.
12. Janjigian YY, Shitara K, Moehler M, Garrido M, Salman P, Shen L, et al. First-line nivolumab plus chemotherapy versus chemotherapy alone for advanced gastric, gastro-oesophageal junction, and oesophageal adenocarcinoma (CheckMate 649): a randomised, open-label, phase 3 trial. *Lancet* 2021;**398**:27–40.
13. Fang WL, Chen MH, Huang KH, Chang SC, Lin CH, Chao Y, et al. The clinicopathological features and genetic mutations in gastric cancer patients according to EMAST and MSI atatus. *Cancers (Basel)* 2020;**12**:551.
14. Fang WL, Chen MH, Huang KH, Lin CH, Chao Y, Lo SS, et al. The clinicopathological features and genetic alterations in Epstein-Barr virus-associated gastric cancer patients after curative surgery. *Cancers (Basel)* 2020;**12**:1517.
15. O'Sullivan B, Brierley J, Byrd D, Bosman F, Kehoe S, Kossary C, et al. The TNM classification of malignant tumours-towards common understanding and reasonable expectations. *Lancet Oncol* 2017;**18**:849–51.
16. Feng F, Zheng G, Qi J, Xu G, Wang F, Wang Q, et al. Clinicopathological features and prognosis of gastric adenosquamous carcinoma. *Sci Rep* 2017;**7**:4597.
17. Chang SC, Liu KH, Hung CY, Tsai CY, Hsu JT, Yeh TS, et al. Adjuvant chemotherapy improves survival in stage III gastric cancer after D2 Surgery. *J Cancer* 2018;**9**:81–91.
18. Aly R, Gupta S, Gupta S, Nagra N, Goyal A, Singh B. Primary gastric adenosquamous carcinoma: a case report. *Gastroenterology Res* 2020;**13**:155–7.
19. Boku N, Ryu MH, Kato K, Chung HC, Minashi K, Lee KW, et al. Safety and efficacy of nivolumab in combination with S-1/capecitabine plus oxaliplatin in patients with previously untreated, unresectable, advanced, or recurrent gastric/gastroesophageal junction cancer: interim results of a randomized, phase II trial (ATTRACTION-4). *Ann Oncol* 2019;**30**:250–8.
20. Chen H, Shen C, Yin R, Yin Y, Chen J, Han L, et al. Clinicopathological characteristics, diagnosis, treatment, and outcomes of primary gastric adenosquamous carcinoma. *World J Surg Oncol* 2015;**13**:136.
21. Watanabe K, Achiwa K, Muto H, Arakawa N, Aoki K, Kubota M, et al. Different sensitivity to chemotherapy between adenocarcinoma and adenosquamous carcinoma in a case of synchronous multicentric gastric cancer. *Nihon Shokakibyo Gakkai Zasshi* 2012;**109**:408–17.
22. Kadowaki S, Yatabe Y, Nitta S, Ito Y, Muro K. Durable response of human epidermal growth factor receptor-2-positive gastric adenosquamous carcinoma to trastuzumab-based chemotherapy. *Case Rep Oncol* 2014;**7**:210–6.
23. Jin Z, Holubek M, Sukov WR, Sattler CA, Wiktor AE, Jenkins RB, et al. Identification of adenosquamous carcinoma as a rare aggressive HER2-negative subgroup of esophageal/gastroesophageal junction adenocarcinoma. *Am J Clin Oncol* 2019;**42**:190–5.
24. Yu HY, Li CP, Huang YH, Hsu SJ, Wang YP, Hsieh YC, et al. Microsatellite instability, Epstein-Barr virus, and programmed cell death ligand 1 as predictive markers for immunotherapy in gastric cancer. *Cancers (Basel)* 2022;**14**:218.