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Running title: PD-1 inhibitors combined with apatinib

Efficacy and prognostic factors of PD-1 inhibitors combined with apatinib in advanced diffuse gastric cancer

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Refractory diffuse gastric cancer (DGC) is rising in incidence and has a bad prognosis. Individuals who are administered second-line or subsequent therapies frequently exhibit diminished physical fitness, rendering them inappropriate for intensive therapy. Despite this, PD-1 inhibitors and anti-angiogenesis drug apatinib have demonstrated efficacy in advanced gastric cancer. This study aimed to evaluate the effectiveness, prognostic factors, and safety of PD-1 inhibitors in combination with apatinib in advanced DGC. The present study is a retrospective analysis of 34 patients with advanced DGC treated with apatinib combined with PD-1 inhibitors in the Affiliated Cancer Hospital of Zhengzhou University from 2019 to 2022. Apatinib 250 mg was administered to patients once a day. The median progression-free survival (mPFS) and the median overall survival (mOS) were estimated using Kaplan-Meier curves, whereas objective response rate (ORR), disease control rate (DCR), prognostic variables, and adverse events were among the other outcomes. Data from 34 patients were collected, and the ORR was 5.9% (2 out of 34), while the DCR was 55.9% (19 out of 34). The mPFS was 2.5 months (95% CI: 1.9-3.0), while the mOS was 6.8 months (95% CI: 3.7-9.9). Log-rank univariate analysis indicated that the mOS of patients with carcinoembryonic antigen (CEA) levels < 4.7 ng/ml (11.3 months, 95% CI: 7.1-15.5) was significantly different from those with levels ≥ 4.7 ng/ml (2.7 months, 95% CI: 0.0-6.1) ($p = 0.008$). A notable disparity in mOS and mPFS was observed between patients with CA125 < 35 U/ml (7.7 months, 95% CI: 3.6-11.9) and those with CA125 ≥ 35 U/ml (2.5 months, 95% CI: 1.9-3.0) ($p = 0.003$), as well as between patients with lactate dehydrogenase (LDH) < 245 U/L (11.3 months, 95% CI: 7.2-15.5) and those with LDH ≥ 245 U/L (2.2 months, 95% CI: 1.5-2.9) ($p = 0.007$), and between patients with PLTs < 350×10^9/L (7.5 months, 95% CI: 6.4-8.7) compared to those with PLTs ≥ 350×10^9/L (1.7 months, 95% CI: 0.0-3.9) ($p = 0.001$). Multivariate Cox regression analysis indicated that CA125, LDH, and PLT levels were independent prognostic variables. The occurrence of grade 3 or 4 treatment-related adverse events was 17.6% (6/34). The study suggests that the integration of PD-1

inhibitors and apatinib in second-line and subsequent therapies demonstrated promising efficacy and acceptable safety in advanced DGC patients. The concentrations of CA125, LDH, and PLTs may serve as prognostic indicators for DGC.

Key words: diffuse gastric cancer (DGC); programmed cell death protein 1 (PD-1) inhibitors; apatinib; effectiveness; prognosis; tumor markers

Gastric cancer is among the most prevalent malignant neoplasms globally. GLOBOCAN 2022 reports 968,350 new gastric cancer cases and 659,853 deaths globally in 2022, positioning it 5th in incidence rate (4.9%) and 5th in fatality rate (6.8%). In 2022, China reported 358,672 cases of stomach cancer and 260,372 deaths, ranking fifth in incidence rate (7.4%) and third in mortality rate (10.1%) [1, 2]. The overall situation of gastric cancer prevention and control remains quite severe in China. In 1965, Lauren first categorized gastric cancer into intestinal and diffuse kinds, a system currently referred to as Lauren's classification. This classification disclosed substantial disparities in cancer incidence, epidemiology, risk factors, biological behavior, molecular attributes, prognosis, and potential therapeutic responses. DGC exhibit diffuse growth, lack cellular cohesion, typically do not develop glandular structures, and are primarily characterized as poorly differentiated adenocarcinomas or indolent cell carcinomas with a high malignancy potential [3, 4]. Despite a general decline in gastric cancer incidence in recent decades, the occurrence of DGC has risen, comprising approximately 30-45% of all gastric cancers [5, 6]. DGC, characterized by the poorest prognosis among gastric cancer subtypes, presents at a younger age, exhibits a lower 5-year survival rate, and demonstrates a higher recurrence rate, with peritoneal metastasis exceeding 80% compared to intestinal and mixed gastric cancer [7-9].

Apatinib is a small molecule tyrosine kinase inhibitor (TKI) with anti-angiogenic properties that predominantly targets the vascular endothelial growth factor receptor-2 (VEGFR2), inducing apoptosis and inhibiting tumor development [10]. Phase II and Phase III clinical trials have conclusively established the significant survival advantage of apatinib in progressive or advanced gastric cancer [11, 12]. Consequently, the National Medical Products Administration (NMPA) in China approved it for the treatment of advanced stomach cancer or gastroesophageal junction adenocarcinoma in 2014 [13]. DGC is less reliant on blood vessels compared to intestinal gastric cancer, so the efficacy of anti-angiogenic agents alone is constrained. Tumor immunotherapy is a prominent area of research in cancer treatment that aims to restore the body's natural anti-tumor immune response by activating immune cells and enhancing their capabilities, thereby facilitating the destruction of tumor cells by

immune cells. Inhibitors of programmed cell death protein 1 (PD-1) and its ligand (PD-L1) are among the most prevalent tumor immunotherapies, capable of reinstating T cell activity, augmenting the body's immune response, and facilitating the immune system's recognition and destruction of cancer cells, with numerous malignancies deriving substantial benefits from their application [14, 15]. DGC possesses a distinctive tumor microenvironment (TME), characterized by extracellular matrix remodeling that leads to a dense tumor stroma, angiogenesis, and diminished tumor antigen expression due to genomic stability, manifesting a "cold tumor" phenotype with poor efficacy of immunotherapy alone [16, 17]. Targeted combination immunotherapy has demonstrated the ability to activate immune cells, thereby stimulating novel antigens and enhancing their immunogenicity for synergistic anti-tumor actions [18, 19]. To date, however, few pertinent clinical trials have specifically included diffuse gastric cancer, despite an increasing prevalence of such patients in clinical practice, and there have been limited real-world retrospective investigations described. This study was a real-world study (RWS) aimed at assessing the efficacy and prognostic determinants of PD-1 inhibitors in conjunction with antiangiogenic targeted therapies in DGC, thereby offering substantial clinical evidence for the management of patients with this refractory variant of gastric cancer.

Patients and methods

Subjects. Participants were patients with advanced DGC who received PD-1 inhibitors in conjunction with apatinib from January 2019 to January 2022 at the Affiliated Cancer Hospital of Zhengzhou University, as identified through the Linkdoc database. Inclusion criteria: a) histologically or cytologically verified metastatic DGC; b) absence of secondary primary tumor; c) age ≥ 18 years; d) no evident contraindications for treatment; e. treated with PD-1 inhibitors in conjunction with apatinib for a minimum of 2 cycles; f) comprehensive clinicopathological data. Exclusion criteria: a) Patients staged by Lauren with non-DGC; b) Patients with DGC classified as TNM stage I, II, or III; c) Patients exhibiting significant abnormalities in routine blood tests, liver and renal function, coagulation function, or possessing contraindications to therapy. The Ethics Committee of the Affiliated Cancer Hospital of Zhengzhou University approved this study (2021-KY-0192). The trial was monitored via telephone every three months utilizing Linkdoc doctor software until the patients' demise, with the final follow-up occurring on December 31, 2022. Linkdoc is our hospital's patient follow-up information system, which records the general condition, disease changes, adverse reactions, and other information of patients during each follow-up visit.

Study design

Administration method. Patients administered 250 mg of apatinib orally once daily. Patients received intravenous treatment with commercially available immune checkpoint inhibitor anti-PD-1 monoclonal antibodies on Day 1, in conjunction with apatinib. The suggested dosage for each anti-PD-1 antibody is as follows: camrelizumab 200 mg biweekly, nivolumab 240 mg biweekly, pembrolizumab 200 mg every three weeks, sintilimab 200 mg every three weeks, tislelizumab 200 mg every three weeks, and toripalimab 240 mg every three weeks.

Efficacy evaluation. Data about general clinical characteristics, pathological aspects, genotyping, treatment-related information, and patient survival status were gathered. The efficacy assessment was conducted in accordance with the Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST v1.1), examined every 2 or 3 cycles of immunotherapy, or sooner if there were more pronounced indications of potential disease progression. The primary outcome was overall survival (OS), defined as the duration from the initiation of treatment with a PD-1 inhibitor combined with apatinib to death from any cause. Additional outcomes encompassed progression-free survival (PFS, delineated as the duration from the initiation of combination therapy to tumor progression or mortality), disease control rate (DCR, characterized as the percentage of patients attaining complete response (CR), partial response (PR), and stable disease (SD) among all evaluable patients), and objective response rate (ORR, defined as the proportion of patients achieving CR and PR in relation to treatment among all evaluable patients).

Factors related to efficacy and prognosis. The clinical data presented were analyzed using log-rank univariate and Cox regression multivariate methods in this study. The variables of gender, age, primary lesion resection status, liver metastasis, lung metastasis, peritoneal metastasis, bone metastasis, distant lymph node metastasis, number of metastatic sites, type of immunotherapy, combination therapy approaches, duration of immunotherapy, expression levels of HER-2 and PD-L1, and concentrations of CEA, CA199, CA125, albumin, lactate dehydrogenase (LDH), and platelet (PLT) were evaluated to assess efficacy and prognostic determinants.

Adverse reaction evaluation. Adverse responses following therapy delivery were evaluated in accordance with the National Cancer Institute Common Terminology Criteria for Adverse Events (NCICTCAE) version 5.0 and documented at routine follow-up visits. Adverse reactions were categorized into five levels based on their severity. 1) Level 1: mild, asymptomatic or mild, identified clinically or diagnosed without necessitating treatment; 2) Level 2: moderate, necessitating minor,

localized or non-invasive treatment, limiting labor-related activities of daily life relative to age; 3) Level 3: severe or medically significant but not immediately life-threatening, resulting in hospitalization or extended stay, disability, and restricted personal daily activities; 4) Level 4: life-threatening, necessitating emergency treatment; Level 5: Mortality associated with adverse effects. Adverse events were observed continuously throughout the trial duration and documented during each follow-up interval. This study categorized events in accordance with NCICTCAE v5.0 and meticulously observed any occurrences at or above level 3 throughout the research procedure, implementing necessary dosage modifications or treatment cessation as required.

Statistical analysis. The analysis utilized SPSS version 26.0 software. Non-normally distributed variables were expressed as medians, but categorical data were denoted by the count of instances (%). The impact of various determinants on survival was assessed by constructing survival curves utilizing the Kaplan-Meier method and the Log-rank test. COX regression analysis was employed for multivariate analysis to ascertain independent prognostic markers associated with the prognosis of patients with advanced DGC. The significance level was α=0.05. A p-value of less than 0.05 was deemed statistically significant.

Results

Patient's clinicopathologic characteristics. This study included 34 eligible patients (Table 1), of whom 20 (58.8%) were male and 18 (52.9%) were under 60 years of age. A total of 15 patients (41.1%) had excision of the main lesion. Eleven (32.4%) presented with liver metastases; eight (23.5%) with lung metastases; sixteen (47.1%) with peritoneal metastases; seven (20.6%) with bone metastases; thirty-one (91.2%) with distant lymph node metastases; twenty-four (70.6%) with fewer than three metastatic sites. Twenty-one patients (61.8%) received camrelizumab combination therapy, two patients (5.9%) received pembrolizumab combination therapy, five patients (14.7%) received sintilimab combination therapy, four patients (11.7%) received toripalimab combination therapy, and two patients (5.9%) received tislelizumab combination therapy. Additionally, twelve patients (35.3%) were treated with a PD-1 inhibitor combined with apatinib as the second-line regimen, while twenty-two patients (64.7%) received it as subsequent therapy. Nine individuals (26.5%) underwent PD-1 inhibitor cross-line treatment, while twenty-five individuals (73.5%) received non-cross-line treatment.

Note: Abbreviations: mDGC, metastatic diffuse gastric cancer; PD-L1, programmed cell death ligand 1; CEA, carcino-embryonic antigen; CA199, carbohydrate antigen 199; CA125, carbohydrate antigen 125;

LDH, lactate dehydrogenase; PLT, platelet.

Twenty-eight patients (82.4%) were classified as human epidermal growth factor receptor 2-negative (HER2-), fourteen (41.2%) as PD-L1 positive, seventeen (50.0%) as CEA ≥ 4.7 ng/ml, eighteen (52.9%) as CA199 ≥ 27 U/ml, fourteen (41.2%) as CA125 ≥ 35 U/ml, fifteen (44.1%) as LDH ≥245 U/l, ten (29.4%) as albumin < 40 g/l, and three (8.8%) as PLT ≥ 350 × 10^9/l.

Effectiveness analysis. The mPFS of 34 patients was 2.5 months (95% confidence interval: 1.9-3.0), and the mOS was 6.8 months (95% confidence interval: 3.7-9.9) (Figure 1). Among patients administered the PD-1 inhibitor in conjunction with apatinib, two patients (5.9%) exhibited PR, 17 patients (50.0%) had SD, 15 patients (44.1%) experienced PD, and no patients achieved CR. The ORR was 5.9% (2 out of 34), and the DCR was 55.9% (19 out of 34).

Log-rank univariate analysis indicated no statistically significant differences in mPFS or mOS concerning gender, age, primary site resection, metastatic site, type of immunotherapy, treatment lines, cross-line PD-1 inhibitor usage, HER-2 expression, PD-L1 expression, CA199 levels, and albumin levels. Patients with CEA levels < 4.7 ng/ml exhibited a statistically significant median overall survival (mOS) of 11.3 months (95% CI: 7.1-15.5) compared to those with CEA levels ≥ 4.7 ng/ml, who had a mOS of 2.7 months (95% CI: 0.0-6.1) (p=0.008). Patients with CA125 levels < 35 U/ml exhibited a statistically significant median overall survival (mOS) of 7.7 months (95% CI: 3.6-11.9) compared to the CA125 > 35 U/ml cohort, which had a mOS of 2.5 months (95% CI: 1.9-3.0) (p=0.003). Additionally, there was a significant mPFS difference, with 3.7 months for the lower CA125 group against 1.8 months for the higher CA125 group (p=0.011). Patients with LDH levels below 245 U/l exhibited a statistically significant mOS of 11.3 months (95% CI: 7.2-15.5) compared to those with LDH levels of 245 U/L or more, who had a mOS of 2.2 months (95% CI: 1.5-2.9) (p=0.007). Additionally, there was a significant mPFS difference, with 3.8 months for the lower LDH group against 2.2 months for the higher LDH group (P=0.004). A statistically significant difference in mOS was observed between the group with platelet counts (PLTs) < 350 × 10^9/l (7.5 months, 95% CI: 6.4-8.7) and the group with PLTs > 350 × 10^9/l (1.7 months, 95% CI: 0.0-3.9) (p=0.001). Additionally, a statistically significant difference in mPFS was noted (2.5 months vs. 1.7 months, p=0.015) (Figure 2).

A univariate analysis was conducted on clinical characteristics that may affect the patients' PFS and OS, and the results showed that the level of CEA, CA125, PLT, LDH of patients, and the presence of combined bone metastases may affect the patients' PFS or OS. These clinical characteristics were also

subjected to multivariate COX regression analysis, showing that the level of CA125, PLT, and LDH were independent prognostic factors for OS (Table 2).

Adverse reactions. Twenty-three individuals experienced at least one adverse reaction, predominantly comprising hypertension (29.4%), hypothyroidism (17.6%), anorexia (23.5%), fatigue (26.5%), hand-foot syndrome (20.6%), elevated transaminases (14.7%), proteinuria (17.6%), pneumonia (8.8%), skin rash (11.8%), oral mucositis (8.8%), and capillary hyperplasia (23.5%). The majority of patients experienced grade 1-2 adverse reactions, with only 6 cases (17.6%) exhibiting grade $\geq$ 3 adverse reactions (Table 3). The combined therapy was generally well tolerated.

Discussion

Among the four principal molecular subtypes of gastric cancer identified in a 2014 Nature publication, the genomically stable subtype is designated as DGC [20]. This subtype is characterized by gene silencing, little somatic copy number change, and the activation of angiogenic pathways, classifying it as a non-immunogenic tumor [21]. A study conducted by the Asian Cancer Research Group (ACRG) categorized gastric cancer into four subtypes based on gene expression data, each linked to distinct clinical outcomes. Among these, DGC with microsatellite stability and epithelial to mesenchymal transition (MSS/EMT) exhibited the most unfavorable prognosis [22]. This unfavorable prognosis is linked to its distinctive tumor microenvironment. DGC is distinguished by pronounced mesenchymal fibrous hyperplasia and a thick tumor stromal architecture that impedes the migration of immune cells into the tumor tissue, resulting in diminished proliferation of immune T cells. Abnormal angiogenesis within the tumor microenvironment facilitates the recruitment and proliferation of immunosuppressive cells, including regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs), while enhancing the expression of M2-like macrophages, which subsequently inhibit effector T cells, resulting in localized immunosuppression [22, 23]. The clinical stage and prevalent target mutations of gastric cancer are the primary determinants of therapeutic options, and there are no established criteria or guidelines for selecting treatment based on histology staging. Wang et al. performed a meta-analysis of 33 trials and discovered that patients with intestinal gastric cancer (IGC) exhibited significantly superior overall survival (OS) compared to those with DGC using analogous chemotherapy regimens [23]. Results from ONO-4538, a phase III clinical trial of advanced refractory gastric cancer, indicated that patients receiving PD-1 inhibitors experienced prolonged overall survival compared to the placebo group; however, subgroup analysis revealed no statistically significant advantage in DGC [24]. A

comparative evaluation of PD-1 inhibitors and paclitaxel for advanced gastric cancer or gastroesophageal junction cancer, after disease progression after first-line chemotherapy, revealed no statistically significant improvement in overall survival for patients with diffuse gastric cancer in a subgroup analysis [25]. Consequently, standard radiation, chemotherapy, targeted therapy, and immunotherapy alone is ineffective in DGC.

Research indicates that anti-angiogenic medicines can regulate blood vessels and reconfigure the immunosuppressive tumor milieu into an immune-supportive one, thereby augmenting the efficacy of immunotherapy [26, 27]. In recent years, small-molecule tyrosine kinase inhibitors (TKIs) targeting the angiogenic signaling pathway have garnered significant interest from clinical researchers, with apatinib being the first small-molecule TKI demonstrated to be safe and efficacious for advanced gastric cancer [28]. Xu et al. undertook an open-label trial of the anti-PD-1 antibody SHR-1210 in conjunction with apatinib for the treatment of gastric cancer, advanced hepatocellular carcinoma, or esophagogastric junction cancer [19] Thirty-nine reviewed patients exhibited an ORR of 30.8% (95% confidence interval: 17.0% to 47.6%). The trial established that the ideal dosage of apatinib is 250 mg when administered in conjunction, and that this combination is safe and manageable regarding medication toxicity. There are relatively few studies examining the combination of apatinib with immunotherapy in post-line DGC. The availability of the apatinib and PD-1 inhibitors combination in real-world clinical settings in China positions us favorably to undertake this investigation. The PD-1 inhibitor in conjunction with apatinib in this study demonstrated a mOS of 6.8 months, an ORR of 5.9%, and a DCR of 55.9%. These results are inferior to those reported by Xu et al. However, it is crucial to note that this study targets advanced DGC, which has the most unfavorable prognosis among gastric cancers [19]. Patients undergoing second-line and subsequent treatments typically exhibit diminished physical fitness and tolerance compared to those receiving first-line therapy, rendering them unsuitable for aggressive treatment regimens. No standardized global treatment protocol exists for these patients. This trial has yielded preliminary results and safety with a combination of targeted treatment and immunotherapy, warranting additional prospective investigation.

Prior research has established that tumor markers serve as pertinent indicators for assessing the prognosis of gastric cancer and hold significant importance in its early detection and prognosis [29]. LDH is a crucial enzyme in glycolysis, and its elevation can facilitate tumor cells in acquiring energy for proliferation and division via anaerobic metabolism. Numerous studies indicate that an elevated level of LDH correlates with numerous malignant tumors, including gastric, renal, and colorectal

cancers, and plays a significant role in prognostic prediction for patients [30, 31]. Increased PLTs have been shown to facilitate tumor infiltration and metastasis by inducing immune evasion of tumor cells and enhancing the adherence of tumor cells to vascular endothelial cells, hence boosting hematogenous metastasis. Platelet levels are inversely associated with patient prognosis. Consequently, elevated PLT levels serve as an independent prognostic indicator for individuals with advanced gastric cancer [32, 33]. Our study data indicated that tumor markers, such as CEA, CA125, LDH, and PLT levels, were independent prognostic variables for patients with advanced DGC, corroborating findings from prior research. Individuals exhibiting normal levels of tumor markers CEA, CA125, PLT, and LDH prior to immunotherapy demonstrated superior prognosis and extended PFS and OS compared to those with elevated levels. Peripheral blood can be utilized to measure those laboratory markers, facilitating testing. During treatment, these laboratory markers can be utilized to initially assess patient prognosis, enabling the formulation of tailored treatment programs to extend survival and enhance therapeutic outcomes.

In conclusion, PD-1 inhibitors combined with apatinib as second-line and subsequent therapy have demonstrated initial efficacy and acceptable tolerability in advanced DGC. For patients with a poor prognosis, compromised physical state, and ineligibility for intense therapy, a chemotherapy-free approach is a more suitable treatment selection.CA125, PLTs, and LDH serve as independent prognostic indicators in DGC, with elevated pre-treatment serum levels correlating with poorer prognosis. Nonetheless, there remains an absence of precise predictive markers for the success of immunotherapy in DGC. To enhance the accessibility of immunotherapy for patients with DGC, it is imperative to investigate novel biomarkers, which will guide our future development and initiatives. This is a retrospective observational study and possesses specific limitations. Firstly, the sample size is limited, potentially introducing statistical bias; secondly, four of the five anti-PD-1 inhibitors are accessible in China but not in other nations; and thirdly, there is an absence of fundamental studies to investigate molecular indicators for a more thorough screening of the precise beneficiary population. Consequently, an increase in sample size or a prospective design is required to advance the study.

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298
299

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Figure Legends

Figure 1. Kaplan-Meier survival curves. A) mPFS of 34 evaluable patients. B) mOS of the whole cohort.

Figure 2. Kaplan-Meier survival curve in patients. A) Kaplan-Meier survival curve in patients with CA125 < 35 U/l vs. CA125 ≥35 U/l (PFS). B) Kaplan-Meier survival curve in patients with CA125 < 35 U/l vs. CA125 ≥ 35 U/l (OS). C) Kaplan-Meier survival curve in patients with LDH < 245 U/l vs. LDH ≥ 245 U/l (PFS). D) Kaplan-Meier survival curve in patients with LDH < 245 U/l vs. LDH ≥ 245 U/l (OS). E) Kaplan-Meier survival curve in patients with PLT < 350×10^9/l vs. ≥ 350×10^9/l (PFS). F) Kaplan-Meier survival curve in patients with PLT < 350×10^9/l vs. ≥ 350×10^9/l (OS).

418 **Table 1.** Baseline demographic and clinical characteristics of 34 DGC patients.

Characteristics	Patients N (%)
Age (year)	
< 60	18 (52.9%)
≥ 60	16 (47.1%)
Gender	
Male	20 (58.8%)
Female	14 (41.2%)
Whether the primary tumor is resected	
Resected	15 (41.1%)
Not resected	19 (58.9%)
Type of metastasis	
Liver metastasis	
With liver metastasis	11 (32.4%)
Without liver metastasis	23 (67.6%)
Lung metastasis	
With lung metastasis	8 (23.5%)
Without lung metastasis	26 (76.5%)
Peritoneum metastasis	
With peritoneum metastasis	16 (47.1%)
Without peritoneum metastasis	18 (52.9%)
Bone metastasis	
With bone metastasis	7 (20.6%)
Without bone metastasis	27 (79.4%)
Distant lymph nodes metastasis	
With distant lymph nodes metastasis	31 (91.2%)
Without distant lymph nodes metastasis	3 (8.8%)
Number of metastatic sites	
< 3	24 (70.6)
≥ 3	10 (20.4)
Immunization agents	
Camrelizumab	21 (61.8%)
Pembrolizumab	2 (5.9%)
Sintilimab	5 (14.7%)
Toripalimab	4 (11.7%)
Tislelizumab	2 (5.9%)
Lines of treatment	
Second-line treatment	12 (35.3%)
Third- or later- lines	22 (64.7%)
Whether PD-1 inhibitor was cross line	
Cross line	9 (26.5%)
Not cross line	25 (73.5%)
HER-2 expression level	
HER-2 (-)	28 (82.4%)
HER-2 (+)	3 (8.8%)

Unknown	3 (8.8%)
PD-L1 expression level	
PD-L1 CPS < 1	12 (35.3%)
PD-L1 CPS ≥ 1	14 (41.2%)
PD-L1 CPS unknown	8 (23.5%)
CEA	
< 4.7 ng/ml	17 (50.0%)
≥ 4.7 ng/ml	17 (50.0%)
CA199	
< 27 U/ml	16 (47.1%)
≥ 27 U/ml	18 (52.9%)
CA125	
< 35 U/ml	20 (58.8%)
≥35 U/ml	14 (41.2%)
LDH	
< 245 U/l	19 (55.9%)
≥ 245 U/l	15 (44.1%)
Albumin level	
< 40 g/l	10 (29.4%)
≥ 40 g/l	24 (70.6%)
PLT	
< 350×10^9/l	31 (91.2%
≥ 350×10^9/l	3 (8.8%)

Note: Abbreviations: mDGC-metastatic diffuse gastric cancer; PD-L1-programmed cell death ligand 1; CEA-carcino-embryonic antigen; CA199-carbohydrate antigen 199; CA125-carbohydrate antigen 125; LDH-lactate dehydrogenase; PLT-platelet

422 **Table 2.** Univariable and multivariable analysis COX regression models of OS for all patients.

	Univariable		Multivariable	
	HR (95%CI)	p-value	HR (95%CI)	p-value
Age	1.202 (0.784, 1.842)	0.399		
Gender	0.638 (0.274, 1.483)	0.296		
whether the primary lesion was resected	1.236 (0.508, 3.007)	0.640		
liver metastasis	0.756 (0.447, 1.277)	0.295		
lung metastasis	1.275 (0.760, 2.140)	0.358		
peritoneal metastasis	1.330 (0.848, 2.086)	0.215		
bone metastasis	1.566 (0.981, 2.502)	0.060		
distant lymph node metastasis	0.932 (0.502, 1.731)	0.823		
number of metastatic sites	0.655 (0.420, 1.022)	0.062		
Immunization agents	0.267 (0.049, 1.453)	0.332		
lines of treatment	0.647 (0.302, 1.387)	0.263		
whether PD-1 inhibitor was cross line	1.882 (0.833, 4.248)	0.128		
HER-2	1.459 (0.331, 6.439)	0.618		
PD-L1	1.346 (0.467, 3.880)	0.582		
CEA	0.515 (0.315, 0.844)	0.008*	0.318 (0.095, 1.069)	0.064
CA199	0.632 (0.391, 1.021)	0.061		
CA125	0.513 (0.328, 0.803)	0.003*	0.224 (0.076, 0.660)	0.007*
LDH	0.201 (0.062, 0.648)	0.007*	0.160 (0.050, 0.512)	0.002*
albumin	1.420 (0.882, 2.284)	0.149		
PLT	0.253 (0.112, 0.569)	0.001*	0.136 (0.022, 0.847)	0.033*

423 Note: *$p < 0.05$

424 **Table 3.** Summary of adverse reactions of 34 DGC patients.

Adverse reactions	Grade 1-2 n (%)	Grade 3-4 n (%)	Total
Hypertension	8 (23.5)	2 (5.8)	10 (29.4)
Hypothyroidism	6 (17.6)	0 (0.0)	6 (17.6)
Anorexia	8 (23.5)	0 (0.0)	8 (23.5)
Fatigue	9 (26.5)	0 (0.0)	9 (26.5)
Hand foot syndrome	5 (14.7)	2 (5.8)	7 (20.6)
Elevated transaminase	5 (14.7)	0 (0.0)	5 (14.7)
Proteinuria	6 (17.6)	0 (0.0)	6 (17.6)
Pneumonia	3 (8.8)	0 (0.0)	3 (8.8)
Rash	3 (8.8)	1 (2.9)	4 (11.8)
Oral mucositis	3 (8.8)	0 (0.0)	3 (8.8)
Hemangioma	7 (20.6)	1 (2.9)	8 (23.5)

425

Fig. 1 Download full resolution image

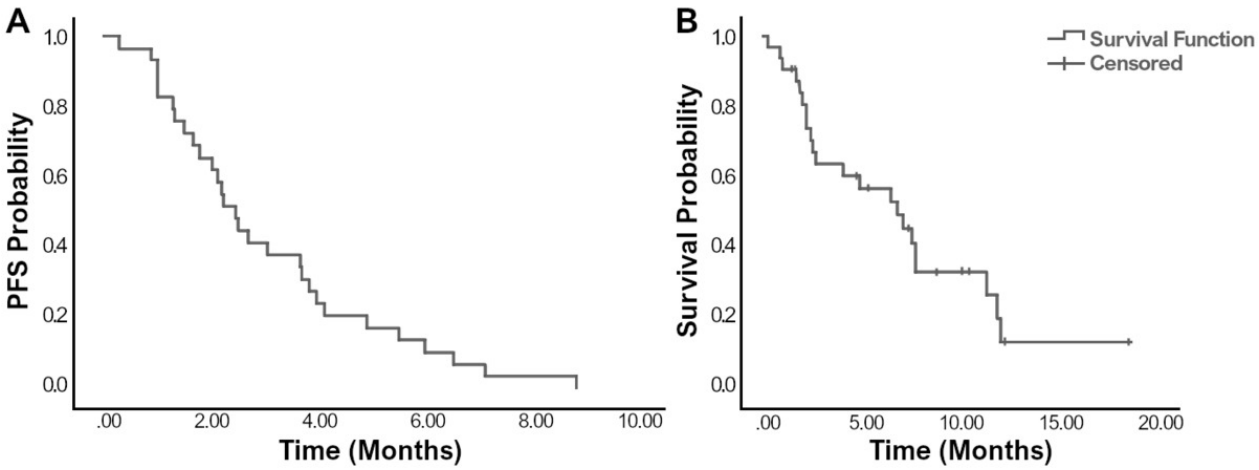

Fig. 2 Download full resolution image