Baseline lesion number as an efficacy predictive and independent prognostic factor and its joint utility with TMB for PD-1 inhibitor treatment in advanced gastric cancer

Ther Adv Med Oncol

2021, Vol. 13: 1–14

DOI: 10.1177/1758835921988996

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Abstract

Background: We previously reported tumor mutation burden (TMB) as a potential prognostic factor for patients with advanced gastric cancer (AGC) receiving immunotherapy. We aimed to comprehensively understand the impact of tumor burden and TMB on efficacy and prognosis in immunotherapy-treated AGC patients.

Methods: A total of 58 patients with refractory AGC receiving PD-1 inhibitor monotherapy from a phase Ib/II clinical trial (ClinicalTrials.gov identifier: NCT02915432) were retrospectively included. Univariate and multivariate logistical regression analyses and the Cox proportional hazards model were performed for prognostic value of baseline factors. Factors reflecting baseline tumor burden, including baseline lesion number (BLN), the maximum tumor size (MTS) and the sum of target lesion size (SLS) were analyzed. The objective response rate (ORR) and disease control rate (DCR) were compared by Chi-square test.

Results: In univariate analysis, high BLN was associated with poor median progression-free survival (mPFS) [1.7 months *versus* 3.4 months; hazard ratio (HR), 2.696, $p < 0.05$] and median overall survival (mOS) (3.2 months *versus* 7.6 months; HR, 1.997, $p < 0.05$), while high TMB was a positive prognostic factor. In multivariable analysis, both BLN and TMB were independent prognostic factors for mOS (BLN: HR, 2.782, $p < 0.05$; TMB: HR, 0.288, $p < 0.05$), while MTS or SLS had no association with survival. Better ORR and DCR were observed in the low BLN group (15.4% *versus* 5.3%, $p > 0.05$; 86.96% *versus* 54.29%, $p < 0.05$). When combining BLN and TMB, the best efficacy and survival were observed in the $BLN^{low}TMB^{high}$ group (ORR: 37.5%, DCR: 62.5%, mPFS and mOS: not reached). The worst efficacy and survival were shown in the $BNL^{high}TMB^{low}$ group [ORR: 0% (0/15); DCR: 13.3%; mPFS: 1.7 months; mOS: 2.7 months (all $p < 0.05$)].

Conclusions: BLN, rather than factors regarding baseline tumor size, is perhaps a potential predictor for benefit from immunotherapy and its combination with TMB could further risk-stratify patients with AGC receiving immunotherapy.

Keywords: baseline lesion number, gastric cancer, PD-1 inhibitor, prognostic factor, tumor mutation burden

Received: 26 July 2020; revised manuscript accepted: 22 December 2020.

Introduction

Gastric cancer (GC) is the fifth most common cancer and the third leading cause of cancer-related death worldwide.[1] Most patients with GC present with advanced or metastatic disease.[2,3] Systemic cytotoxic chemotherapy and molecular targeted therapy are current main options for those patients. However, therapeutic options are lacking for patients whose disease progresses after two or more lines of systemic therapy.[4,5] New

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treatment options based on a fundamental understanding of GC biology are needed.

Programmed death protein-1 (PD-1) inhibitor nivolumab and pembrolizumab have been approved for the third-line treatment of metastatic GC with a low response rate of about 12%.[6,7] There is an urgent need to identify effective and reliable biomarkers and clinical indictors to guide patient selection.

Recent studies found that microsatellite instability (MSI) status, Epstein–Barr virus (EBV) infection, programmed death ligand 1 (PD-L1) expression and tumor mutation burden (TMB) could be potential biomarkers associated with efficacy of PD-1 inhibitors.[8–16] Patients with MSI-H or EBV infection achieve better efficacy from immunotherapy. However, the proportion of them is quite slim, which brings benefit to only a small population.[9,15] Some researchers showed that PD-L1 expression was a potential predictive factor.[14,15] However, the ORR of immunotherapy in PD-L1-positive AGC was slightly improved, at only about 20%.[17] On 16 June 2020, the US Food and Drug Administration approved pembrolizumab monotherapy for patients with unresectable or metastatic solid tumors that have high TMB (tissue TMB $\geqslant$ 10 mutations/Mb) and fail prior therapy. Two clinical studies also showed TMB's predictive value for immunotherapy in AGC,[15,18,19] including our previous phase Ib/II clinical trial of toripalimab monotherapy (ClinicalTrials.gov identifier: NCT02915432) for treatment of refractory AGC.[19] With a cut-off value of 12 mutations/Mb, better ORR (33.3% *versus* 7.1%, $p = 0.02$) and mOS (14.6 *versus* 4.0 months, $p = 0.04$) were achieved in the high TMB group.[19] TMB seems to be not enough for selection of patients potentially benefiting from immunotherapy in AGC. Furthermore, several limitations also exist in TMB: TMB could not completely represent the antigen load; it is plagued to measure TMB accurately for various technical and biological pitfalls; it is expensive to test TMB.[11] By contrast, some clinical indicators could be conveniently used to risk-stratify patients and help decision making for immunotherapy in patients with cancer.

Tumor burden, a familiar clinical factor, refers to the number of cancer cells, the size of tumor, and the amount of cancer in the body according to the National Institutes of Health. While it is basically impossible to measure the number of tumor cells in patients with cancer, some indicators regarding tumor burden may be used as surrogates, especially tumor size and lesion number. Several studies showed that tumor size could be an effective predictor of PD-1 inhibitors in melanoma, head and neck cancer and lung cancer.[20–25] But in their study, other indicators reflecting tumor burden have been barely investigated, which impedes fully understanding prognostic value of tumor burden and hinder its application into clinical practice.[20–25] So far, the association between tumor burden and efficacy for immunotherapy in patients with AGC has not been reported.

Hence, we aimed to get a comprehensive understanding of the efficacy predictive and prognostic value of tumor burden in patients with AGC treated with PD-1 inhibitors using the data of a previously reported phase Ib/II clinical trial of toripalimab, a humanized IgG4 monoclonal antibody against PD-1 (ClinicalTrials.gov identifier: NCT02915432).[19] We assessed the value of baseline lesion number (BLN), the maximum tumor size (MTS), the sum of target lesion size (SLS) and their potential to jointly predict clinical outcomes with TMB for immunotherapy in AGC.

Patients and methods

Patients

We retrospectively analyzed the data of 58 chemorefractory patients with AGC with the monotherapy of toripalimab (JS001) (3 mg/kg, d1, Q2W) in a multicenter phase Ib/II study. All patients were pathologically confirmed to have advanced adenocarcinoma of the stomach or gastro-esophageal junction. Response was assessed according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 by independent radiological review committee before treatment, once every 8 weeks in the first year and then once every 12 weeks from the second year until disease progression. All patients had Eastern Cooperative Oncology Group performance status of 0 or 1, adequate organ and bone marrow function at enrollment of the clinical trial. None of the patients received any form of immunotherapy previously. We acquired the informed consent in written format. Our study was based on the data from a clinical trial approved by the institutional ethics committee of Sun Yat-sen University Cancer Center (approval number: A2016-046) and conducted in accordance with the Declaration of Helsinki.

Evaluation of tumor burden

We measured baseline clinical tumor burden based on computed tomography scan before treatment. Three indicators, BLN, MTS and SLS were used to evaluate the tumor burden. BLN was defined as the total number of both target lesions and non-target lesions, MTS referred to the largest size of single evaluable lesions, and SLS was the sum of the size of target lesions (the longest diameter for solid lesions and the size of the short axis of lymph nodes).

Assessment of molecular biomarkers

PD-L1 expression was assessed by immunohistochemistry (IHC) staining with an anti-human PD-L1 monoclonal antibody (SP142, VENTANA, USA).[12] PD-L1-positive status was defined as membrane staining of any intensity in $\geqslant$1% of tumor cells or tumor-infiltrating immune cells. The EBV DNA copy number was assessed by unique reads detected in pre-treatment archived tumor tissues. TMB was detected by whole exome sequencing with IDT xGen Exome Research Panel version 1.0 on tumor tissues and matched peripheral blood mononuclear cell samples. TMB was determined by analyzing somatic mutations, including coding base substitution and INDELs per Mb. The top 20% of the TMB (12 mutations/Mb) in this research was selected as the cut-off value and defined as TMB^{high}. Patients with TMB $<$ 12 mutations/Mb were defined as TMB^{low}.

Statistical analysis

All quantitative data were analyzed with R (version 3.6.1). A two-tailed p-value $<$ 0.05 was considered statistically significant. The efficacy, including ORR and disease control rate (DCR) were calculated by Chi-square test. Progression-free survival (PFS) was defined as the time from first dosing to first recorded progression of disease or death due to any reasons, whichever came first. Overall survival (OS) was calculated as the time interval between first dose of toripalimab and death due to any reasons. Univariate and multivariate logistical regression analyses were performed for the prognostic value for PFS and OS. Survival curves were plotted by the Kaplan–Meier method and compared with a log-rank test. The Cox proportional hazards model was used to calculate hazard ratio (HR) and 95% confidence interval (CI).

Results

Patient characteristics

All 58 eligible patients treated with toripalimab monotherapy from clinical trial NCT02915432 were retrospectively included for analyses in this study. Table 1 shows patient demographics and clinical characteristics. The median age was 60 years (range from 52 to 66 years) and 70.7% patients were male. About half of patients experienced at least three lines of prior therapy. Baseline elevated lactate dehydrogenase (LDH) was detected in 18 (31.0%) patients. The most common site of metastasis was lymph nodes accounting for 56.9%. The number of patients developed peritoneal metastasis was 17 (29.3%), the same as patients with liver metastasis. Based on the minimum p-value method for PFS and OS, we determined 5, 40 mm and 100 mm as cut-off value of BLN, MTS and SLS, respectively. Overall, 19 (32.8%) patients had BLN $>$5, the same as patients with SLS $>$100 mm, and 26 (44.8%) patients developed MTS $>$44 mm. A total of 47 patients (81%) had negative PD-L1 and 42 patients (72.4%) had TMB $<$12 mutations/Mb.

Univariate and multivariate analysis of baseline factors associated with mPFS and mOS

For all patients, the mPFS was 1.9 months. Several factors were included for univariate analysis and the results are shown in Table 2. Among them, high BLN was associated with poor mPFS [HR, 2.696; CI, (1.461–4.977); $p < 0.05$]. The improved mPFS was shown in the low BLN group [3.4 months *versus* 1.7 months, $p < 0.001$, Figure 1(a)]. No significant difference was shown in the other two factors concerning tumor burden including MTS and SLS. We defined TMB $>$ 12 (mutations/Mb) as high TMB group in consistent with our previous report, which showed a trend of better mPFS than low TMB group [HR, 0.450; CI, (0.196–1.034); $p > 0.05$]. The multivariate analysis of mPFS could not be performed because only one factor, BLN, was statistically significant by univariate analysis.

The mOS was 4.8 months for all patients. Table 3 presents the detailed results of univariate and multivariate analysis. Several baseline clinical factors were associated with poor mOS including at least three lines of prior therapy compared with 1–2 lines [HR, 1.501; CI, (1.092–2.062);

Table 1. Patient demographics and clinical characteristics.

Characteristics	No. of patients (%)
Total number	58 (100)
Sex	
Male	41 (70.7)
Female	17 (29.3)
Age, years, median (range)	60 (52–66)
Lines of prior therapy	
1–2	28 (48.3)
⩾3	30 (51.7)
Baseline LDH	
Elevated	18 (31.0)
Normal	38 (65.5)
Not available	2 (3.5)
Site of metastasis	
Lymph node	33 (56.9)
Peritoneal	17 (29.3)
Liver	17 (29.3)
Lung	9 (15.5)
Other	9 (15.5)
BLN	
1–5	39 (67.2)
>5	19 (32.8)
SLS (mm)	
<100	39 (67.24)
⩾100	19 (32.76)
MTS (mm)	
<40	32 (55.17)
⩾40	26 (44.83)
Previous gastrectomy	
Yes	25 (43.10)
No	33 (56.90)
Post-trial treatment	
Yes	22 (37.93)
No	36 (62.07)
PD-L1 results[a]	
Positive	8 (13.8)
Negative	47 (81.0)
Not available	3 (5.2)
TMB mutations/Mb	
TMB < 12	42 (72.4)
TMB ⩾ 12	12 (20.7)
Not available	4 (6.9)
EBV	
Positive	4 (6.9)
Negative	51 (87.9)
Not available	3 (5.2)
MSI status	
MSI-H	1 (1.7)
MSS	54 (93.1)
Not available	3 (5.2)

[a]Positive defined as ⩾1% of tumor cells or immune cells by SP142 IHC staining.
BLN, the baseline lesion number; EBV, Epstein–Barr virus; LDH, lactate dehydrogenase; MSI, microsatellite instability; MSI-H, MSI-high; MSS, microsatellite stability; MTS, the maximum tumor size; PD-L1, programmed cell death ligand 1; SLS, sum of the target lesions' longest diameters; TMB, tumor mutational burden.

$p < 0.05$], high SLS compared with low SLS [HR, 1.978; CI, (1.049–3.729); $p < 0.05$], high MTS compared with low MTS [HR, 2.211; CI, (1.189–4.110); $p < 0.05$], high BLN compared low BLN [HR, 1.997; CI, (1.061–3.761); $p < 0.05$], peritoneal metastasis compared non-peritoneal metastasis [HR, 2.173; CI, (1.113–4.243), $p < 0.05$]. Improved mOS was shown in high TMB group [HR, 0.407; CI, (0.169–0.981); $p < 0.05$].

Table 2. Univariate analysis of progression-free survival.

Factors	Number of patients (%)	Univariate analysis HR (95% CI)	*p*-value
Sex		1.767 (0.959–3.254)	0.08
Male	41 (70.70)		
Female	17 (29.30)		
Age		0.860 (0.489–1.513)	0.60
<60	29 (50.00)		
⩾60	29 (50.00)		
Line of prior therapy		1.195 (0.901–1.586)	0.20
1–2	28 (48.28)		
⩾3	30 (51.72)		
SLS (mm)		1.402 (0.775–2.536)	0.30
<100	39 (67.24)		
⩾100	19 (32.76)		
MTS (mm)		1.455 (0.828–2.557)	0.20
<40	32 (55.17)		
⩾40	26 (44.83)		
Previous gastrectomy		1.222 (0.872–1.522)	0.31
No	33 (56.90)		
Yes	25 (43.10)		
Baseline LDH		1.397 (0.790–2.471)	0.30
Normal	38 (67.86)		
Elevated	18 (31.03)		
BLN		2.696 (1.461–4.977)	0.01
⩽5	39 (67.24)		
>5	19 (32.76)		
Lung metastasis		2.149 (0.965–4.784)	0.08
No	49 (84.48)		
Yes	9 (15.52)		
Liver metastasis		1.629 (0.898–2.955)	0.10
No	41 (70.69)		
Yes	17 (29.31)		
Peritoneal metastasis		1.487 (0.793–2.790)	0.20
No	41 (70.69)		
Yes	17 (29.31)		

(Continued)

Table 2. (Continued)

Factors	Number of patients (%)	Univariate analysis HR (95% CI)	*p*-value
PD-L1 status[a]		0.454 (0.178–1.159)	0.07
Positive	8 (14.55)		
Negative	47 (81.03)		
TMB (mutations/Mb)		0.450 (0.196–1.034)	0.06
⩽12	42 (77.78)		
>12	12 (22.22)		

[a]Positive defined as ⩾1% of tumor cells or immune cells by SP142 IHC staining.
The multivariate analysis of progression-free survival could not be performed because only one factor, BLN, is statistically significant by univariate analysis.
BLN, the baseline number of metastasis lesion; CI, confidence interval; HR, hazard ratio; LDH, lactate dehydrogenase; MTS, the maximum tumor size; PD-L1, programmed cell death ligand 1; SLS, sum of the target lesions' longest diameters; TMB, tumor mutational burden.

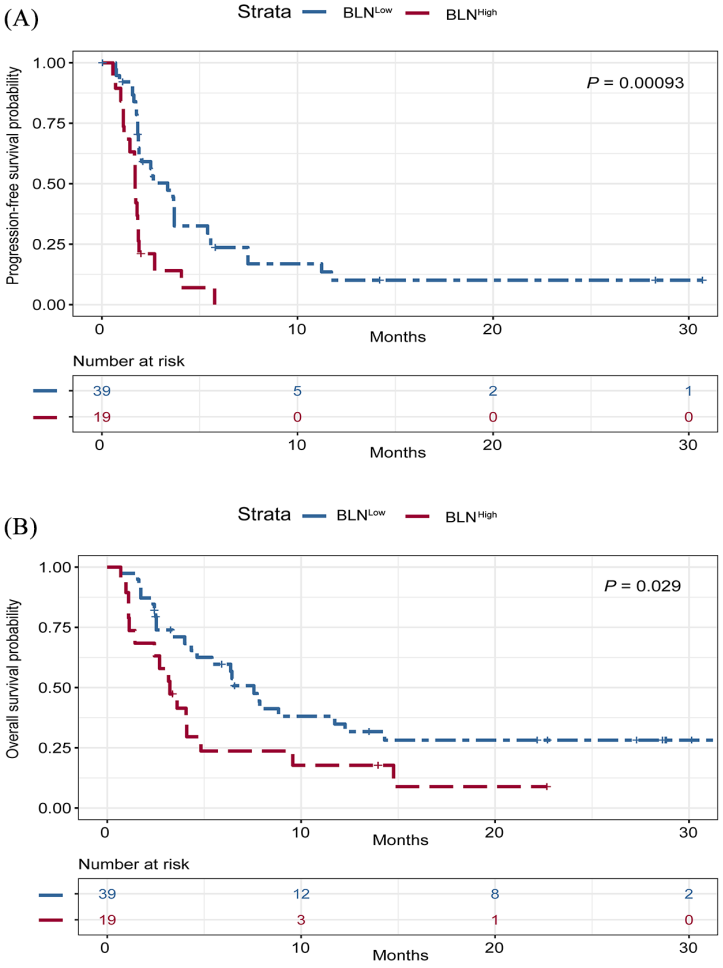

Figure 1. Kaplan–Meier plots of progression-free survival and overall survival stratified by BLN (BLN ⩽ 5/ BLN > 5) in treatment-refractory advanced gastric cancer patients receiving PD-1 inhibitor. (a) Progression-free survival; (b) Overall survival.
Patients with lower BLN (BLN ⩽ 5) had significantly superior median progression-free survival (3.4 months *versus* 1.7 months, $p < 0.001$) and median overall survival (7.6 months *versus* 3.2 months, $p < 0.05$).
BLN, baseline lesion number; PD-1, programmed cell death-1.

Table 3. Univariate analysis and multivariate analysis of overall survival.

Factors	Number of patients (%)	Univariate analysis HR (95% CI)	*p*-value	Multivariate analysis HR (95% CI)	*p*-value
Sex		1.525 (0.786–2.957)	0.20		
Male	41 (70.7)				
Female	17 (29.3)				
Age		0.950 (0.514–1.755)	0.90		
<60	29 (50.0)				
⩾60	29 (50.0)				
Line of prior therapy		1.501 (1.092–2.062)	0.01	1.796 (1.260–2.560)	0.01
1–2	28 (48.28)				
⩾3	30 (51.72)				
SLS (mm)		1.978 (1.049–3.729)	0.04	0.745 (0.287–1.932)	0.54
<100	39 (67.24)				
⩾100	19 (32.76)				
MTS (mm)		2.211 (1.189–4.110)	0.01	1.867 (0.721–4.832)	0.20
<40	32 (55.17)				
⩾40	26 (44.83)				
Previous gastrectomy		1.211 (0.835–1.622)	0.39		
No	33 (56.90)				
Yes	25 (43.10)				
Post-trial treatment		0.789 (0.623–1.001)	0.06		
No	36 (62.07)				
Yes	22 (37.93)				
Baseline LDH		1.826 (0.985–3.386)	0.05		
Normal	38 (67.86)				
Elevated	18 (31.03)				
BLN		1.997 (1.061–3.761)	0.03	2.782 (1.324–5.847)	0.01
⩽5	39 (67.24)				
>5	19 (32.76)				
Lung metastasis		1.387 (0.828–2.323)	0.20		
No	49 (84.48)				
Yes	9 (15.52)				

(Continued)

Table 3. (Continued)

Factors	Number of patients (%)	Univariate analysis HR (95% CI)	*p*-value	Multivariate analysis HR (95% CI)	*p*-value
Liver metastasis		1.502 (0.786–2.870)	0.20		
No	41 (70.69)				
Yes	17 (29.31)				
Peritoneal metastasis		2.173 (1.113–4.243)	0.03	2.162 (0.989–4.726)	0.05
No	41 (70.69)				
Yes	17 (29.31)				
PD-L1 status[a]		0.637 (0.226–1.799)	0.40		
Positive	8 (14.55)				
Negative	47 (81.03)				
TMB (muts/Mb)		0.407 (0.169–0.981)	0.03	0.288 (0.103–0.798)	0.02
⩽12	42 (77.78)				
>12	12 (22.22)				

[a]Positive defined as ⩾1% of tumor cells or immune cells by SP142 IHC staining.
BLN, the baseline number of metastasis lesion; CI, confidence interval; HR, hazard ratio; LDH, lactate dehydrogenase; MTS, the maximum tumor size; PD-L1, programmed cell death ligand 1; SLS, sum of the target lesions' longest diameters; TMB, tumor mutational burden.

Of 58 patients, we performed the multivariate analysis in 54 patients after removing 4 with missing data of TMB. Among the six factors associated with mOS in the univariate model, three indicators remained independently associated with mOS in the multivariate model: lines of prior therapy [HR, 1.796; CI, (1.260–2.560); $p < 0.05$], BLN [HR, 2.782; CI, (1.324–5.847); $p < 0.05$], and TMB [HR, 0.288; CI, (0.103–0.798); $p < 0.05$]. The Kaplan–Meier curves for mOS in low BLN group and high BLN group are shown in Figure 1(b) (7.6 months *versus* 3.2 months, $p < 0.05$).

The analysis of anti-tumor efficacy association of BLN

Seven patients achieved partial response (PR) and 20 patients (34.5%) had progressive disease (PD). The ORR and DCR of all patients were 12.1% and 39.7%, respectively. Table 4 shows the association of BLN with anti-tumor response. Patients in high BLN group had a remarkably poor response, with only one (5.3%) patient achieving PR while nine (47.4%) patients developed PD in this group. The response difference between the low BLN and high BLN group achieved statistical significance ($p < 0.05$). Better ORR and DCR were observed in the low BLN group (15.4% *versus* 5.3%, $p > 0.05$; 51.3% *versus* 15.8%, $p < 0.05$).

The combinatorial predictive value of BLN and TMB

As BLN is an independent factor for prognosis, we assessed its correlation with other baseline factors (Supplementary Table 1). No correlation was shown between BLN and TMB or other factors. Because BLN and TMB were two independent prognostic factors in our study, to further risk-stratify patients, we divided 54 patients (four patients lacked results of TMB) into three subgroups: $BLN^{low}TMB^{high}$ [eight patients (14.8%)], $BLN^{high}TMB^{high}$ or $BLN^{low}TMB^{low}$ [31 patients (53.4%)] and $BLN^{high}TMB^{low}$ [15 patients (25.9%)]. The ORR was 37.5% in $BLN^{low}TMB^{high}$ group, 12.9% in the $BLN^{high}TMB^{high}$ or $BLN^{low}TMB^{low}$ group and 0% in the $BLN^{high}TMB^{low}$ ($p < 0.05$, Table 5). The best and worst DCR were shown in the $BLN^{low}TMB^{high}$ group and $BLN^{high}TMB^{low}$ group, respectively (62.5% *versus* 13.3%, $p < 0.05$, Table 5). Improved mPFS was achieved in the

Table 4. The analysis between BLN and response.

	ORR				DCR		
	No. of patients	No. of responses	%	*p*-value	No. of responses	%	*p*-value
Subgroups				0.410			0.001
BLN < 5	39	6	15.4		20	51.3	
BLN ⩾ 5	19	1	5.3		3	15.8	

BLN, the baseline lesion number; DCR, disease control rate; ORR, objective response rate.

Table 5. The combinatorial analysis of BLN and TMB in response.

			ORR			DCR	
	No. of patients	No. of responses	%	*p*-value	No. of responses	%	*p*-value
Subgroups				0.042			0.037
BLN^{L} and TMB^{H}	8	3	37.5		5	62.5	
$BLN^{H}TMB^{H}$/$BLN^{L}TMB^{L}$	31	4	12.9		14	45.2	
BLN^{H} and TMB^{L}	15	0	0.00		2	13.3	

The cut-off value of BLN and TMB were 5 and 12 mutations/Mb, respectively.
BLN, the baseline number of metastasis lesion; BLN^{H}, patients with high BLN; BLN^{L}, patients with low BLN; DCR, disease control rate; ORR, objective response rate; TMB, tumor mutation burden; TMB^{H}, patients with high TMB; TMB^{L}, patients with low TMB.

$BLN^{low}TMB^{high}$ group and the survival curves are presented in Figure 2(a) ($p < 0.05$). Best and worst mOS were shown in patients of the $BLN^{low}TMB^{high}$ and $BLN^{high}TMB^{low}$ group with good separation of survival curves [$p < 0.05$, Figure 2(b)]. The $BLN^{high}TMB^{low}$ group identified a group of patients barely benefiting from PD-1 inhibitors, with no PR, six of patients (42.9%) achieving PD, and the only two patients achieving stable disease (SD) (13.3%) had very short PFS.

Discussion

Baseline tumor size has been used as surrogate markers of tumor burden and investigated as prognostic factors for cancer patients receiving immunotherapy.[23–25] BLN is an indicator for tumor burden, which has been barely investigated in immunotherapy. Our study firstly indicated that BLN was an efficacy predictive and independent prognostic factor for PD-1 inhibitors in AGC. More importantly, we were the first to combine BLN and TMB to further risk-stratify AGC patients for immunotherapy. We found that lower BLN was related to improved clinical outcomes. Among three indicators (BLN, SLS and MTS) about tumor burden, BLN was the most important factor for immunotherapy of AGC, rather than tumor size. In our multivariate analysis, BLN was an independent predictive factor for both mPFS and mOS. Better tumor response was achieved in patients with low BLN. The combinatorial analysis of the BLN and TMB could further stratify patients with different responses and outcomes. This method identified a subgroup of patients ($BLN^{high}TMB^{low}$) with extremely inferior efficacy (no PR and very low DCR) and survival after PD-1 inhibitor treatment. The comprehensive consideration of BLN and TMB perhaps brings broader and more accurate clinic application.

The measurement of tumor burden contains various methods in previous studies, which could influence the results and hinder the application into clinical practice.[20–25] The overall tumor burden was frequently defined as SLS. However, it was often calculated with RECIST version 1.1, which requires lesions to be measurable and ignores non-target lesions.[26] Thus, it could not completely reflect the biological behavior of

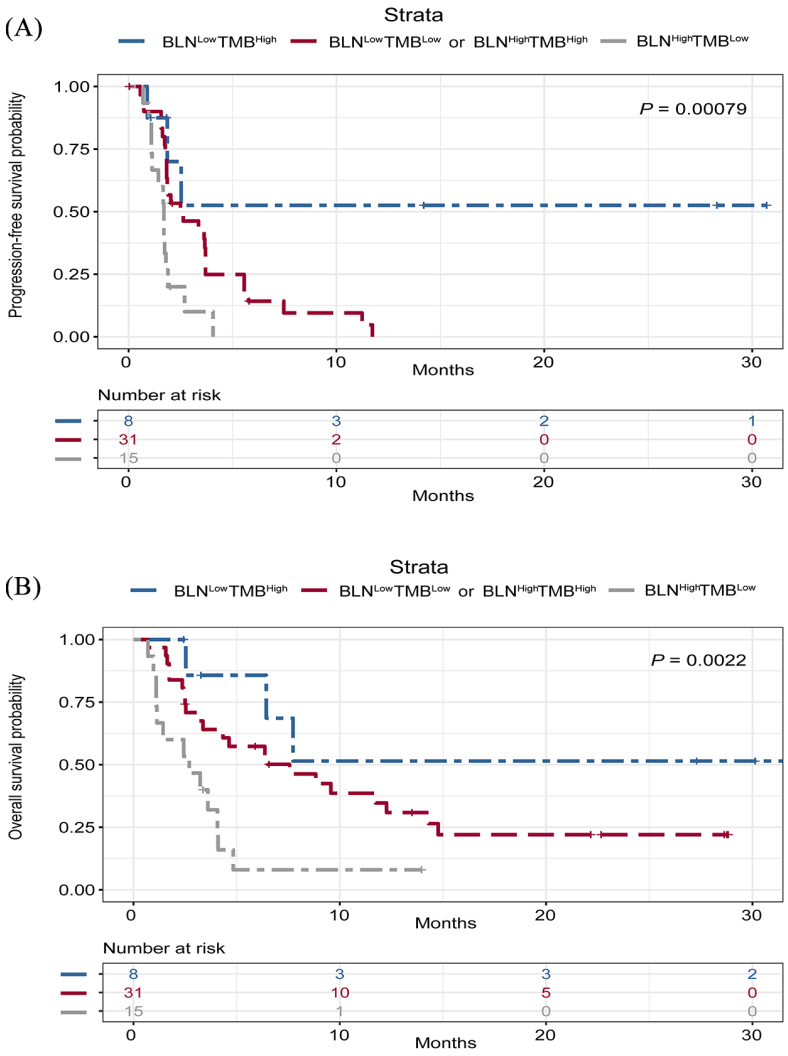

Figure 2. Kaplan–Meier plots of progression-free survival and overall survival stratified by BLN (BLN ⩽ 5/BLN > 5) and TMB (TMB ⩽ 12Muts/Mb/TMB > 12Muts/Mb) in treatment-refractory advanced gastric cancer patients receiving PD-1 inhibitor. (a) Progression-free survival; (b) Overall survival.
Patients were stratified into three subgroups: $BLN^{low}TMB^{high}$ ($n = 8$), $BLN^{high}TMB^{high}$ or $BLN^{low}TMB^{low}$ ($n = 31$) and $BLN^{high}TMB^{low}$ ($n = 15$). The progression-free survival and overall survival was the best for $BLN^{low}TMB^{high}$ group and the worst for $BLN^{high}TMB^{low}$ group (both $p < 0.05$).
BLN, baseline lesion number; PD-1, programmed cell death-1; TMB, tumor mutation burden.

tumors. Another indicator is MTS, which only shows the largest potential of tumor proliferation for single lesion. Most of studies regard the size of tumor as tumor burden and ignore the number of lesions. However, in our multivariate analysis, BLN was an independent prognostic factor for mPFS and mOS instead of SLS or MTS. Significantly better DCR and survival was shown in low BLN group. Superior ORR was shown in low BLN group (15.38% *versus* 5.26%), while the difference was insignificant ($p > 0.05$). The small sample size could be the cause. Another study also demonstrated the importance of lesion number, rather than tumor size, in immunotherapy. Roberto *et al.* indicated that high number of metastasis was related to hyperprogressive disease in patients treated with PD-1 inhibitor, while no association was shown in baseline tumor size.[27]

Our study suggested that BLN could be a better factor to reflect the tumor biological features impacting sensitivity to immunotherapy, which is worth more attention than tumor size. One mechanism supporting this is infiltration growth pattern (INF).[28–32] INFa is inclined to expanding growth with large tumor size while INFc is inclined to invasive growth with more metastasis lesions.[28–32] Poor survival was shown in patients with INFc in

various cancer including AGC.[28–32] Thus, high BLN could be an indicator suggesting more aggressive tumors. Another supportive theoretical basis is the key role of anti-tumor immunity to limit tumor metastasis.[33,34] Patients showing better response of immunotherapy are generally those with abundant invigoration of immune cells in tumor microenvironment, such as MSI-H/dMMR tumors.[33–37] Interestingly, this specific subtype of tumors was more inclined to form bulky tumors but not metastatic lesions.[35–37] The tumor burden feature of this subtype of tumors also supports that lesion number may be a more important clinical indicator than tumor size in their predictive value for immunotherapy. The pre-existing anti-tumor immunity in these tumors may contribute to limit metastasis. We speculate that tumors with higher BLN may have a higher potential to be cold tumors, which lose the anti-tumor immunity to restrict metastasis. But this hypothetical mechanism needs to be investigated in further studies.

Because the association between BLN and the efficacy of PD-1 inhibitor in AGC has not been studied, it is difficult to compare our results with previous researches to further confirm their predictive value. The value of BLN in AGC needs to be verified by larger studies, and further studies about its value in other tumors are also meaningful. BLN is a very convenient clinical indicator, which could be applied to clinical practice easily.

In order to further risk-stratify patients, we evaluated the joint utility of the BLN and TMB in predicting response, mPFS and mOS. The ORR was 37.5% in the $BLN^{low}TMB^{high}$ group, while 0% in $BLN^{high}TMB^{low}$ group ($p < 0.05$). The best and worst DCRs were shown in the $BLN^{low}TMB^{high}$ group and $BLN^{high}TMB^{low}$ group, respectively (62.5% *versus* 13.3%, $p < 0.05$). Patients with $BLN^{low}TMB^{high}$ and $BLN^{high}TMB^{low}$ showed best and worst mPFS and mOS, respectively with good separation of survival curves ($p < 0.05$). $BLN^{high}TMB^{low}$ group was shown to have a very poor response and prognosis, with about half of patients (42.9%) showing primary resistance to PD-1 inhibitor. Although two patients in $BLN^{high}TMB^{low}$ group were PD-L1 overexpressed, they both had PD. Two in $BLN^{high}TMB^{low}$ group achieved SD. However, their mPFS was quite short and one of them passed away 4 months later, which suggested that the SD could be attributed to low proliferation rate of tumor instead of the efficacy of immunotherapy. Our results indicated that patients with $BLN^{low}TMB^{high}$ could benefit from immunotherapy mostly and physicians should be more prudent to use PD-1 inhibitor for patients with $BLN^{high}TMB^{low}$.

The underlying mechanism of the combinatory predictive value of BLN and TMB may be related to the balance between tumor burden and invigoration of immune cells. Zhang *et al.* recently demonstrated that improved prognosis could be achieved in AGC with high levels of T-cell invigoration, suggesting T-cell invigoration reflects the anti-tumor immune response.[38] The ratio of T-cell invigoration to tumor burden was associated with the clinical efficacy of PD-1 inhibitors in melanoma.[39] Poor clinical outcomes could appear when tumor burden is high, while T-cells have been exhausted. In our study, BLN was an appropriate indicator to reflect tumor burden. TMB is related to the load of tumor antigens, which influences the invigoration of immune cells.[16] Thus, it could explain our results that the worst tumor response and prognosis was shown in patients with $BLN^{high}TMB^{low}$.

There are several potential clinical implications of our study. Firstly, our results recommended consideration of BLN to risk-stratify AGC patients for PD-1 inhibitors. And it suggested to use PD-1 inhibitors in early lines of treatment with lower tumor burden and sufficient T-cell invigoration. Further, high BLN was related with poor efficacy of PD-1 inhibitors, which indicated application of more aggressive approaches like the combination with other therapy. Last but not least, BLN was an independent prognostic factor and easy to acquire in clinical practice, and TMB had also been suggested to be a predictive indicator. Hence, BLN and TMB could be incorporated into trial design as stratification factors in AGC, if further studies validate our results.

We noted several limitations in this study. Firstly, there may be a risk of patient selection bias because it was based on a clinical trial, that means our results does not adapt to all patients. Secondly, the sample size is relatively small especially in subgroup analysis. Thirdly, the information for T-cell invigoration is unavailable, thus we could not further confirm the mechanism to support our study. Last but not least, a prospective evaluation is required to validate our study.

In conclusion, BLN is considered to be an independent prognostic factor for immunotherapy and it could combine TMB to further risk-stratify patients receiving PD-1 inhibitor in AGC. It

could be regarded as a valuable representative indicator for tumor burden and deserves more attention in immunotherapy than tumor size.

Authors' contributions

XRH, WF, WFH and WXL designed the study; XJY, WDS, CDL, RC, and LJN collected and interpreted data; WXL and XJY performed data analysis and wrote the manuscript. XRH, WF and WFH reviewed and revised manuscript. All authors approved final manuscript.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: National Key R&D Program of China (2018YFC1313300); Science and Technology Program of Guangdong (2019B020227002); CAMS Innovation Fund for Medical Sciences (2019-I2M-5-036); Guangdong Basic and Applied Basic Research Foundation (2019A1515110171).

Ethics approval and consent to participate

Our study was based on the data from a clinical trial approved by the institutional ethics committee of Sun Yat-sen University Cancer Center (approval number: A2016-046) and conducted in accordance with the Declaration of Helsinki.

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Availability of data and materials

Main data are shown in this article and additional data about this study can be obtained from the corresponding author on reasonable request.

Supplemental material

Supplemental material for this article is available online.

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