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ORIGINAL ARTICLE

Predictive factors for hyperprogressive disease during nivolumab as anti-PD1 treatment in patients with advanced gastric cancer

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Abstract

Background Hyperprogressive disease (HPD) during treatment with anti-programmed death-1/programmed death-ligand 1 monoclonal antibodies has anecdotally been reported in some types of cancers, but is not well-characterized in patients with advanced gastric cancer (AGC).

Methods Total 62 AGC patients treated with nivolumab in a single institution from September 2017 to April 2018 were enrolled in this study. Tumor responses were assessed according to Response Evaluation Criteria in Solid Tumors version 1.1, and HPD was defined as ≥ two fold increase in tumor growth rate. Clinicopathological and molecular characteristics associated with HPD were also investigated.

Results Thirteen of 62 patients (21%) developed HPD after nivolumab treatment. Overall survival (OS) and progression-free survival (PFS) were significantly shorter in patients with HPD than in patients without HPD (median OS: 2.3 months vs. not reached, $P < 0.001$; median PFS: 0.7 months vs. 2.4 months, $P < 0.001$). Liver metastases (77% vs. 41%), Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 1 or 2 (77% vs. 29%), and a large sum of target lesion diameters at baseline (median 104.2 mm vs. 44.9 mm) were significantly associated with HPD. Absolute neutrophil count (ANC) and C-reactive protein (CRP) level significantly increased in the first 4 weeks in only patients with HPD.

Conclusions HPD was observed in AGC patients treated with nivolumab and correlated with some clinicopathological characteristics. Elevations in ANC and CRP levels upon treatment might indicate HPD.

Keywords Nivolumab · PD-1 inhibitor · Gastric cancer · Hyperprogressive disease

Abbreviations

PD-1	Anti-programmed death-1
PD-L1	Programmed death-ligand 1
mAbs	Monoclonal antibodies
HPD	Hyperprogressive disease
AGC	Advanced gastric cancer
OS	Overall survival
PFS	Progression-free survival
ECOG PS	Eastern Cooperative Oncology Group performance status
ANC	Absolute neutrophil count
CRP	C-reactive protein
HER2	Human epidermal growth factor receptor 2
MMR	Mismatch repair
EBV	Epstein–Barr virus
IHC	Immunohistochemistry
FISH	Fluorescence in situ hybridization
TC	Tumor cell
IC	Immune cell
CPS	Combined positive score
MLH1	Anti-mutL homolog 1
MSH2	Anti-mutS homolog 2
PMS2	Anti-postmeiotic segregation increased 2
MSH6	Anti-mutS homolog 6
EBER	EBV-encoded RNA
TMB	Tumor mutation burden

Akinori Sasaki and Yoshiaki Nakamura contributed equally to this work.

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mt/MB	Mutations/megabase
NLR	Neutrophil-to-lymphocyte ratio
LDH	Lactate dehydrogenase
TGK	Tumor growth kinetics
TGK_R	TGK ratio
HR	Hazard ratio
CI	Confidence interval
PR	Partial response
SD	Stable disease
PD	Progressive disease
MAPK	Mitogen-activated protein kinase
MDSC	Myeloid-derived suppressor cell

Introduction

Gastric cancer (GC) is the fifth most common cancer and the third leading cause of cancer mortality worldwide [1]. Although some chemotherapy regimens, including a platinum and fluoropyrimidine combination, trastuzumab (for HER2-positive cases), taxanes, irinotecan, and ramucirumab have been shown to improve the survival outcomes of patients with advanced GC (AGC) [2–6], the prognosis remains poor, with the median survival being approximately 1 year. Therefore, further therapeutic development is needed for AGC.

Nivolumab, a fully human IgG4 monoclonal antibody (mAb) against programmed death-1 (PD-1), is an immune checkpoint inhibitor that enhances antitumor T-cell activity through the inhibition of immune checkpoints. It has been shown to have efficacy against various types of malignancies [7–11]. A recent randomized phase III trial, ATTRACTION-2 (ONO-4538-12), demonstrated that nivolumab treatment after two or more previous chemotherapy regimens in AGC patients had a survival benefit compared to that associated with placebo [12]. Pembrolizumab, another PD-1 mAb, also demonstrated encouraging antitumor activity with acceptable safety for programmed death-ligand 1 (PD-L1)-positive AGC in phase II and III trials [13, 14].

Recently, anti-PD-1/PD-L1 mAbs have anecdotally been reported to cause rapid progression of some types of cancers, which is called hyperprogressive disease (HPD) [15–18]. To avoid the potential harm, it is necessary to identify clinical or molecular factors for predicting HPD. However, the characteristics of HPD in AGC patients treated with anti-PD-1/PD-L-1 mAbs remain unclear. Therefore, in this study, we assessed HPD by estimating tumor growth kinetics during nivolumab treatment in AGC and evaluated the clinicopathological and molecular factors associated with HPD.

Methods

Patients

A retrospective study was performed to evaluate HPD in patients with AGC who received nivolumab from September 2017 to April 2018 at the National Cancer Center Hospital East. Patients who met the following criteria were included: (1) presence of histologically proven gastric adenocarcinoma; (2) receipt of treatment with two or more previous chemotherapy regimens; (3) underwent nivolumab treatment; (4) an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 to 2; and (5) availability of a computed tomography (CT) scan during previous chemotherapy (pre-baseline CT), before initiation of nivolumab treatment (baseline CT), and within 3 months after the initiation of nivolumab treatment (post-CT). Tumor responses were assessed according to Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST v1.1) [19]. Patients who did not have any measurable lesions, as judged based on RECIST v1.1, were excluded. All CT scans were independently reviewed by two or more physicians (A. S., Y. N., and K. S.). All patients provided written, informed consent prior to participating in this observational study. The study protocol was approved by the Institutional Review Board at the National Cancer Center.

Molecular characteristics

Molecular characteristics, such as the status of the human epidermal growth factor receptor 2 (HER2), PD-L1, mismatch repair (MMR), Epstein–Barr virus (EBV), and genomic alterations, were analyzed with formalin-fixed paraffin-embedded tissue specimens from archival tissue samples if available. Immunohistochemistry (IHC) using a monoclonal anti-HER2 antibody (PATHWAY HER2 [4B5], Ventana, Tucson, AZ) and fluorescence in situ hybridization (FISH) using the PathVysion HER-2 probe kit (Abbott Laboratories, Abbott Park, IL, USA) were performed to assess HER2 status, and HER2 positivity was defined as IHC 3 + or IHC 2 + and FISH positive. IHC for PD-L1 was performed using an anti-PD-L1 rabbit monoclonal antibody [VENTANA PD-L1 (SP142) Assay, Ventana], and PD-L1 positivity on tumor cells (TCs) or immune cell (ICs) was defined as the presence of ≥ 1% of TCs or ICs with membrane staining. The combined positive score (CPS), which was the number of cells showing PD-L1 staining (TCs, lymphocytes, and macrophages) divided by the total number of viable TCs multiplied by 100, was also determined. MMR status was assessed by

IHC using monoclonal anti-mutL homolog 1 (MLH1, ES05), anti-mutS homolog 2 (MSH2, FE11), anti-postmeiotic segregation increased 2 (PMS2, EP51), and anti-mutS homolog 6 antibodies (MSH6, EP49) (Agilent Technologies, Santa Clara, CA), and tumors lacking MLH1, MSH2, PMS2, or MSH6 expression were considered as MMR deficient (D-MMR). Chromogenic in situ hybridization for EBV-encoded RNA (EBER) using fluorescein-labeled oligonucleotide probes (INFORM EBER Probe, Ventana) was performed to assess EBV status [20]. Genomic alterations were assessed using Oncomine™ Comprehensive Assay version 3 or Oncomine™ Cancer Research Panel (Thermo Fisher Scientific, Waltham, MA), which allows detection of gene mutations, copy number variants, and fusions across multiple genes (Additional File 1: Table S1). Tumor mutation burden (TMB) was defined as the number of non-synonymous mutations, including indel, per megabase (mt/Mb) of genome in tumor tissue. Known germline variants in dbSNP and East Asian population of 1000 Genomes or ExAC database were not counted.

Laboratory data

To determine whether serial monitoring of laboratory data can predict HPD, the following laboratory data at the beginning of nivolumab treatment and at the first 2 and 4 weeks of therapy were collected: carcinoembryonic antigen (CEA), carbohydrate antigen (CA)19-9, C-reactive protein (CRP), absolute neutrophil count (ANC), lymphocyte count, neutrophil-to-lymphocyte ratio (NLR), lactate dehydrogenase (LDH) level, and albumin level.

Definition of HPD

The time of pre-baseline, baseline, and post-CT scanning was defined as T_{PRE}, T_0, and T_{POST}, respectively. The sum of the largest diameters of the target lesions according to RECIST v1.1 at pre-baseline, baseline, and post-CT was defined as S_{PRE}, S_0, and S_{POST}, respectively [16].

Tumor growth kinetics (TGK) were assessed as described previously [16]. Briefly, TGK_{PRE} was calculated as the difference of the sum of the largest diameters of the target lesions per unit of time between pre-baseline and baseline imaging: $(S_0 - S_{PRE})/(T_0 - T_{PRE})$. Similarly, TGK_{POST} was calculated as $(S_{POST} - S_0)/(T_{POST} - T_0)$. We defined TGK_{POST}/TGK_{PRE} as TGK ratio (TGK_R). According to the previous studies [15–17], HPD was defined as $TGK_R \geq 2$ and > 50% increase in tumor burden compared to that at pre-treatment imaging.

Statistical analyses

Statistical comparisons of baseline characteristics between HPD and non-HPD patients were performed using χ^2 or Fisher's exact test for categorical data and Student's test or Mann–Whitney's test for continuous variables. Progression-free survival (PFS) was estimated from the date of initiation of nivolumab treatment to the date of disease progression or death from any cause. Overall survival (OS) was defined as the interval between the date of initiation of nivolumab treatment and the date of mortality due to any cause. PFS and OS were determined and presented graphically using the Kaplan–Meier method. Survival rates at various times and 95% confidence intervals were also determined. The survival curves were compared using the long-rank test and hazard ratio (HR) and 95% confidence interval (CI) was determined using the Cox's proportional-hazards model for the comparison of the HPD and non-HPD groups. Multivariate analysis was conducted to adjust HR using clinical factors which showed difference between HPD and non-HPD groups. All statistical analyses were performed with 5% alpha risk or 95% confidence interval using SPSS version 25 (IBM, Chicago, IL, USA).

Results

Patients' characteristics

Among 73 AGC patients treated with nivolumab between September 2017 and April 2018, 11 were excluded because of the absence of post-CT imaging (four patients) or the absence of measurable disease on CT imaging (seven patients). Thus, in total, 62 patients were eligible for inclusion in this study (Fig. 1).

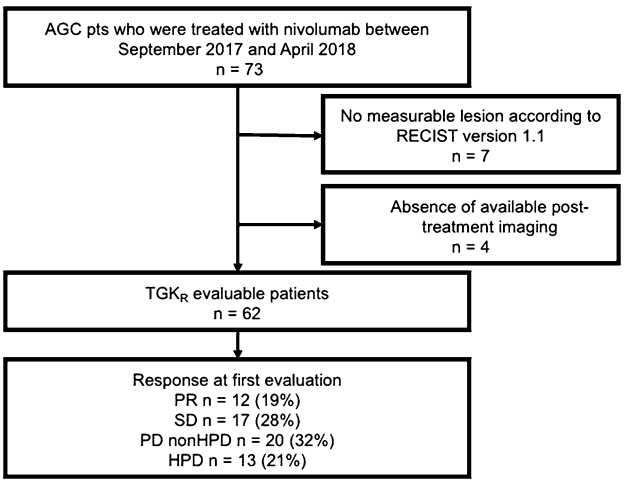

Fig. 1 Consort flow diagram

Table 1 Patients' characteristics

Features	Non-HPD ($n=49$)	HPD ($n=13$)	HPD ratio (%)	OR	P value
Age, ≥ 65, *n* (%)	31 (63.3)	9 (69.2)	22.5	1.3	0.76
Male, *n* (%)	33 (71.4)	12 (92.3)	26.7	4.8	0.16
ECOG PS, *n* (%)					
0	35 (71.4)	3 (23.1)	7.9	Ref.	0.003
1 or 2	14 (28.6)	10 (76.9)	41.7	8.3	
Histology, *n* (%)					
Intestinal	24 (49.0)	6 (46.2)	20	Ref.	0.81
Diffuse	25 (51.0)	7 (53.8)	21.9	1.1	
Previous treatment regimens, *n* (%)					
2	24 (49.0)	4 (30.8)	14.3	Ref.	0.35
≥ 3	25 (51.0)	9 (69.2)	26.5	2.2	
Previous gastrectomy, *n* (%)					
No	28 (57.1)	10 (76.9)	26.3	Ref.	0.22
Yes	21 (42.9)	3 (23.1)	12.5	0.4	
Previous radiation therapy, *n* (%)					
No	46 (93.9)	12 (92.3)	20.7	Ref.	1.00
Yes	3 (6.1)	1 (7.7)	25	1.3	
Organs with metastases, *n* (%)					
≤ 2	35 (71.4)	6 (46.2)	14.6	Ref.	0.19
≥ 3	14 (28.6)	7 (53.8)	33.3	2.9	
Site of metastases, *n* (%)					
Liver	20 (40.8)	10 (76.9)	33.3	4.8	0.029
Lung	9 (18.4)	2 (15.4)	18.2	0.81	1.00
Peritoneum	25 (51.0)	6 (46.2)	19.4	0.82	1.00
Lymph node	42 (85.7)	11 (84.6)	20.8	0.92	1.00
Other	14 (28.6)	4 (30.8)	22.2	1.1	1.00
S_0 (mm), ≥ median, *n* (%)	20 (40.8)	11 (84.6)	35.5	8.0	0.003

HPD hyperprogressive disease, *OR* odds ratio, *ECOG PS* Eastern Cooperative Oncology Group performance status, *ref* reference, S_0 the sum of the largest diameters of target lesions estimated according to RECIST v1.1 at baseline

Table 1 shows patient characteristics. The median patient age was 67 (range 25–86) years, and 47 patients (76%) were male. Thirty-eight patients (61%) had an ECOG PS of 0, whereas the remaining 24 patients (39%) had a PS of 1 or 2 at the initiation of nivolumab treatment. Thirty-four patients (56%) had been treated with three or more lines of the previous chemotherapies before nivolumab treatment. The most common metastatic site was the lymph node (85%), followed by the liver and peritoneum. The median S_0 (the sum of the diameters of the target lesion at baseline) was 59.4 (range 16.0–260.9) mm.

Data on HER2, PD-L1, MMR, EBV, and genomic alteration statuses were available in 61, 53, 55, 57, and 47 patients. Fourteen patients showed HER2-positive tumors. PD-L1 expression on TCs or ICs was identified in 47 patients. Fourteen patients (26.4%) showed tumors with a PD-L1 CPS of 10 or higher. Eight patients (14.5%) were found to have a D-MMR status, and four patients showed EBV-positive tumors (7.1%).

Response to treatment

The median follow-up time was 8.1 (range 5.9–10.0) months. The best overall responses were partial response (PR) in 12 patients (19%), stable disease (SD) in 17 patients (28%), and progressive disease (PD) in 33 patients (55%). Thirteen patients (21%) met the HPD criteria. Among HPD patients, TGK_R ranged from 2.0 to 25.9. No progressive disease followed by tumor shrinkage (i.e., pseudoprogression) was observed. The changes in the sum of the largest diameters of target lesions according to response classification are shown in Fig. 2.

The median PFS according to RECIST v1.1 was 2.0 (95% CI, 1.5–2.5) months, and the median OS was not reached. Patients who showed HPD had a significantly shorter PFS (median: 0.7 months vs. 2.4 months, $P<0.001$, HR 4.8) (Fig. 3a), and OS (median: 2.3 months vs. not reached, $P<0.001$, HR 9.2) than those of the patients who did not show HPD (Fig. 3b).

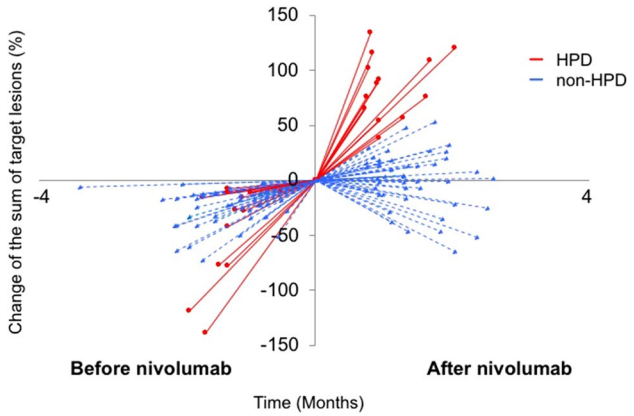

Fig. 2 Spider plot depicting percentage change in the sum of the largest diameters of target lesions over time according to hyperprogressive disease (HPD) status

Clinicopathological and molecular features associated with HPD

Among baseline clinical characteristics, the frequencies of an ECOG PS of 1 or 2 (77% vs. 29%, $P = 0.003$) and liver metastases (77% vs. 41%, $P = 0.029$) were significantly higher in the HPD group than in the non-HPD group (Table 1). Furthermore, the sum of diameters of target lesions at baseline was significantly larger in the HPD group (median 104.2 mm vs. 44.9 mm, $P = 0.003$) than in the non-HPD group (Table 1). While nine of the 13 (69%) patients who showed HPD presented with all of these clinical factors—an ECOG PS of 1 or 2, liver metastases, and sum of diameters larger than or equal to the median—only one patient who did not present these factors showed HPD (Additional File 1: Table S2). There were no other clinical factors significantly associated with HPD. OS was worse with HPD than non-HPD even after adjusting other clinical factors including ECOG PS, liver metastasis, and sum of diameters ($P < 0.001$, HR 6.1) (Additional File 1: Table S3).

There were no significant differences in HER2, PD-L1, MMR, and EBV statuses between the two groups (Table 2). Each one of the patients with the D-MMR and positive EBV experienced HPD. One patient with the D-MMR had an ECOG PS of 2, liver and skin metastases, S_0 larger than the median, and somatic mutations in multiple genes, including *FBXW7* and *PTEN*. Another patient positive for EBV had *FBXW7* mutation and *MYC* amplification.

In the HPD group, three patients showed *KRAS* amplification, while three others showed *FBXW7* mutation; these findings were not observed in the non-HPD group ($P = 0.009$). Mutations in *CDKN2A* and *PTEN* and *FGF19* amplification were also observed in only the HPD group; however, there were no statistically significant differences between the HPD and the non-HPD groups (Table 2).

With regard to laboratory data, baseline ANC (median: 4,490/μl vs. 2,720/μl, $P = 0.002$), CRP level (median: 4.0 mg/dl vs. 0.50 mg/dl, $P = 0.006$), and LDH level (median: 396.0 U/l vs. 179.5 U/l, $P = 0.006$) were significantly higher in the HPD group than in the non-HPD group (Table 3). Furthermore, analyses of serial laboratory data showed that ANC and CRP levels were significantly elevated at 4 weeks after the initiation of nivolumab treatment (ANC, 4490/μl vs. 7740/μl; CRP, 4.0 mg/dl vs. 8.3 mg/dl) in the HPD group (Fig. 4). On the other hand, in the non-HPD group, there were no significant differences in laboratory data during treatment courses.

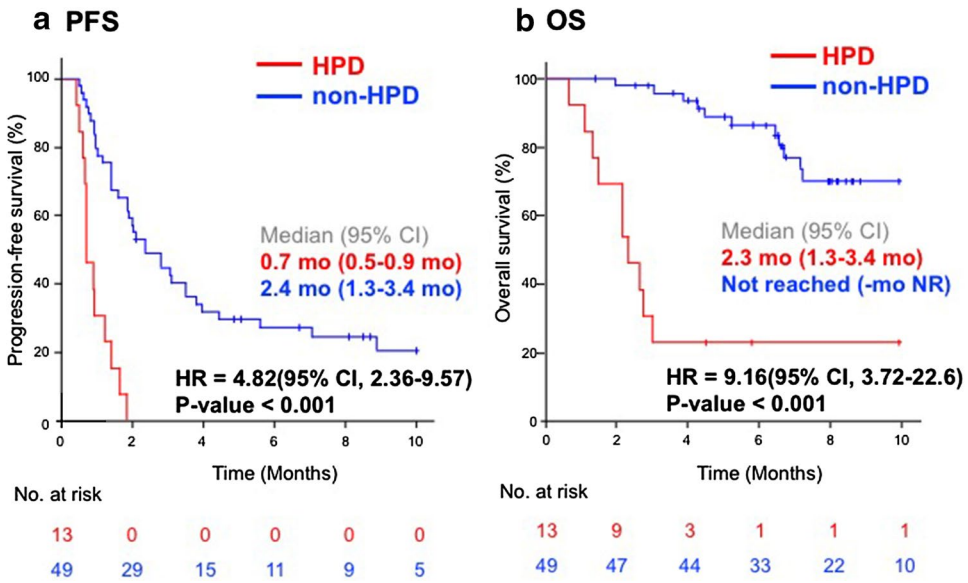

Fig. 3 Progression-free survival (PFS) **a** and overall survival (OS) **b** according to hyperprogressive disease (HPD) status

Table 2 Patients' characteristics (molecular profiles)

Features	Available	Non-HPD ($n=49$)	HPD ($n=13$)	HPD ratio (%)	OR	*P* value
HER2, *n* (%)	61					
Negative		36 (75.0)	11 (84.6)	23.4	Ref.	0.71
Positive		12 (25.0)	2 (15.4)	14.3	0.56	
MMR, *n* (%)	55					
Proficient		36 (83.7)	11 (91.7)	23.4	Ref.	0.66
Deficient		7 (16.3)	1 (8.3)	12.5	0.48	
EBV, *n* (%)	57					
Negative		41 (93.2)	12 (92.3)	22.6	Ref.	1.00
Positive		3 (6.8)	1 (7.7)	25	1.1	
PD-L1, *n* (%)	53					
Positive in TC		11 (26.8)	1 (8.3)	8.3	0.25	0.26
Positive in IC		36 (87.8)	11 (91.7)	23.4	1.5	1.00
Any expression		36 (87.8)	11 (91.7)	23.4	Ref.	1.00
No expression		5 (12.2)	1 (8.3)	16.7	0.67	
PD-L1 CPS, *n* (%)	53					
< 10		30 (73.2)	9 (75.0)	23.1	Ref.	1.00
≥ 10		11 (26.8)	3 (25.0)	21.4	0.91	
TMB, *n* (%)	47					
< 10		16 (43.2)	1 (10.0)	5.9	Ref.	0.067
≥ 10		21 (56.8)	9 (90.0)	30	7.3	
Genomic alterations	47					
CCND1 amplification		1	0	0	0.83	1.00
CCNE1 amplification		3	0	0	0.33	0.55
CDKN2A mutation		0	1	100	9.3	0.28
ERBB2 mutation		1	1	50	2.8	0.48
ERBB2 amplification		6	2	25	0.85	1.00
FGF19 amplification		0	1	100	9.3	0.28
FGFR2 amplification		1	0	0	0.83	1.00
FBXW7 mutation		0	3	100	26.2	0.018
KRAS mutation		2	1	33.3	1.3	1.00
KRAS amplification		0	3	100	26.2	0.018
MDM2 amplification		1	1	50	2.8	0.48
MYC amplification		1	1	50	2.8	0.48
NF1 mutation		1	0	0	0.83	1.00
PIK3CA mutation		4	3	42.9	2.3	0.38
PTEN mutation		0	1	100	9.3	0.28
RHOA mutation		1	1	50	2.8	0.48
TP53 mutation		17	7	29.2	1.2	1.00

HER2 human epidermal growth factor receptor related 2, *MMR* mismatch repair *EBV* Epstein–Barr virus, *PD-L1* programmed death-ligand 1, *TC* tumor cell, *CPS* combined positive score, *TMB* tumor mutation burden

Discussion

We assessed HPD by tumor growth kinetics in patients with AGC who received nivolumab treatment, and evaluated the clinicopathological and molecular factors associated with HPD. In this study, 22% of patients with AGC showed HPD during nivolumab treatment and several factors were associated with HPD. To the best of our knowledge, this is the first study to report the characteristics of HPD in AGC patients. Previous studies reported that HPD was observed in 9–29% of various types of cancers treated with anti-PD-1/PD-L1 mAbs [15, 16]; this finding is in agreement with the results of our study. In the ATTRACTION-2 trial, no significant difference of the frequency of patients with early tumor progression was reported between nivolumab and placebo group [21]. However, since they did not assess the tumor growth rate in the previous treatment, which is needed to determine HPD status,

Table 3 Patients' characteristics (baseline laboratory data)

Features	Non-HPD ($n=49$)	HPD ($n=13$)	OR (≥median vs. <median)	P value
CEA (ng/ml), range	17.7 (1.0–1789.0)	5.7 (4.0–415.2)	0.65	0.74
CA19-9 (U/ml), range	26.6 (5.3–4158.0)	294.0 (0.6–5545.0)	1.5	0.63
ANC (x10^2/μl), range	27.2 (8.3–100.6)	44.9 (31.1–103.8)	8.0	0.002
Lymphocyte (x10^2/μl), range	12.9 (4.1–19.0)	10.4 (4.5–18.5)	0.82	0.60
NLR, range	2.2 (0.7–24.5)	4.9 (2.4–11.3)	3.0	0.008
CRP (mg/dl), range	0.5 (0.05–10.3)	4.0 (0.4–8.7)	4.1	0.006
LDH (U/l), range	179.5 (121.0–524.0)	396.0 (182.0–2392.0)	5.3	0.006
Albumin (g/dl), range	3.4 (2.5–4.2)	3.1 (2.1–4.2)	0.56	0.24

HPD hyperprogressive disease, *OR* odds ratio, *ANC* absolute neutrophil count, *NLR* neutrophil-to-lymphocyte ratio, *CRP* C-reactive protein, *LDH* lactate dehydrogenase

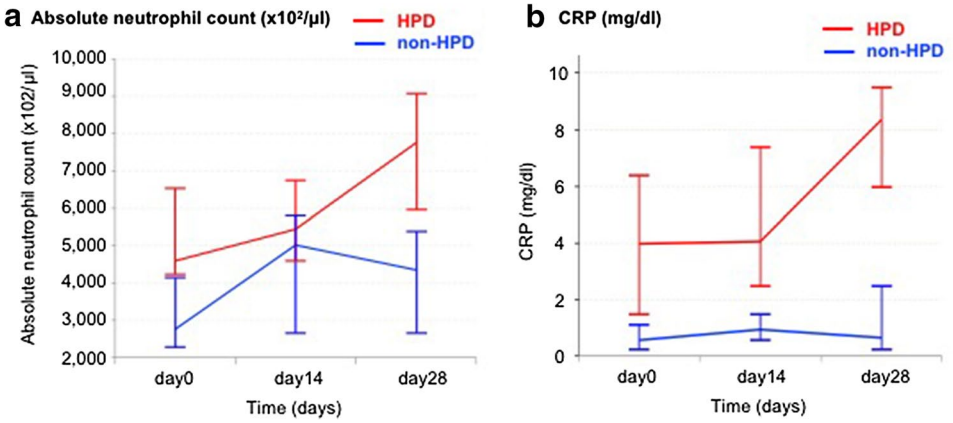

Fig. 4 Transition of absolute neutrophil count (ANC) (**a**) and CRP (**b**) after nivolumab

it was not evaluated whether tumor increase was accelerated or not in each group.

In this study, survival outcome of patients with HPD was shorter than those with non-HPD as previously reported in other cancer type, though the impact of HPD on the survival was not clear because of the lack of control. Actually, OS of patients with PD treated with nivolumab was not worse than those with placebo in the ATTRACTION-2 [22]. However, exact OS after HPD remains unclear; thus, further analysis might be necessary.

Although the mechanism of HPD is unclear, upregulation of alternative immune checkpoints resulting in further immune suppression [23], acceleration of cell growth by blocking of cancer intrinsic PD-1 [24], low number of senescent CD4 T cells, and FcR triggering of clustered macrophages with a specific immunophenotyped [25] have been suggested to cause rapid disease progression by PD1 blockade [17]. Most recently, we reported an increase in regulatory T cells with proliferative capacity among tumor-infiltrating lymphocytes in AGC patients who showed HPD after treatment with an anti-PD-1 mAb [26]. Furthermore, an in vitro study showed that PD-1 blockade activated not only effector T cells, but also Treg cells, which promoted tumor progression in a fraction of patients [26].

In the previous studies, only higher age and regional recurrence in head and neck cancer were suggested as clinical factors associated with HPD [15, 16, 18]. However, we found that AGC patients with poor PS, liver metastasis, or a large tumor at baseline more commonly experienced hyperprogression after nivolumab treatment compared to those without these factors. Several studies suggested an association between resistance to immune checkpoint inhibitors and low baseline PS in various types of cancers, including gastric cancer [7, 9, 13, 14, 27]. Severe exacerbations of primary diseases in NSCLC patients with poor PS were also reported in a case series [28]. Although the exact cause of worse outcome in patients with poor PS remains unclear, patients with poor PS may not stay on treatment long enough to achieve a response. Another study suggested higher clearance of anti-PD1 therapy in patients with cancer cachexia and catabolic clearance to be a cause of HPD; however, it may not be sufficient to induce HPD [29].

Liver metastasis has also been suggested to decrease the probability of a response to anti-PD-1/PD-L1 mAbs by liver-induced immune tolerance [30–33]. Tumor burden was

shown to negatively affect tumor response and survival after anti-PD-1 mAb treatment, especially when T-cell reinvigoration in peripheral blood was not sufficient [34, 35]. Our findings indicated that these factors could be associated with not only resistance to anti-PD-1 therapy, but also rapid tumor progression caused by inhibition of PD-1 in AGC.

Although all patients with *KRAS* amplification or *FBXW7* mutation showed HPD in our study, the association between these genetic alterations and HPD was not clear. These alterations could be only prognostic factors. Therefore, a validation in a larger cohort with nivolumab and other treatments are needed. A previous study regarding genomic analyses of patients with HPD during treatment with immune checkpoint inhibitors suggested the association between *MDM2* amplification and HPD, but one of the two patients with *MDM2* amplification did not show HPD in our study.

Although a D-MMR status and EBV positivity were suggested to be associated with better responses to anti-PD-1/PD-L1 mAbs in previous case series [36, 37], each all patients with the D-MMR status or EBV positivity showed HPD in our cohort. The patient with the D-MMR had some HPD-associated features, such as an ECOG PS of 2, liver metastases, and a large tumor size. In addition, both patients received nivolumab as fourth line treatment, which suggested that late initiation of nivolumab treatment could affect the poor outcome in these patients. These findings suggest that even patients with favorable molecular characteristics for anti-PD-1/PD-L1 therapy can have HPD, especially when having HPD-associated factors.

We also found that ANC and CRP levels significantly increased during nivolumab treatment in patients with HPD. Increased ANC generally reflects a premature release of myeloid cells from the bone marrow. Recent evidence suggests that accumulation of myeloid-derived suppressor cells (MDSCs) is related to resistance to immune checkpoint inhibitors [38]. MDSC counts were also reported to be positively correlated with CRP levels [39]. Although we did not assess the MDSC fraction in neutrophils, the increased ANC and CRP level could be due to the increase in the MDSC fraction, which potentially reflected hyperprogressive status in our study. Changes in these laboratory data can be good on-treatment markers for early HPD prediction, but further investigations of these immune phenotypes are needed.

It is important to note the limitations of the present study. First, this was a retrospective and single-institution study with a limited sample size. Therefore, the differences of clinicopathological characteristics between HPD and non-HPD group contribute to poor PFS and OS by HPD. Second, since we did not evaluate TGKs of patients treated with nivolumab in comparison with those of patients treated with chemotherapy or supportive care alone, we could not accurately assess whether HPD in our patient population was specifically caused by nivolumab or was a natural course. Prospective observational study is necessary to compare treatment course after each treatment. Third, we used RECIST v1.1 for assessing tumor burden, because of which a limited number of tumors were considered in each organ. Thus, the total tumor burden was potentially underestimated. Moreover, we could not evaluate non-target lesions, including ascites, pleural effusion, and bone metastases. Finally, we applied the definition of HPD used in the previous studies [15–17], which has not been standardized. Although the ordinary definition of HPD has been based on a hypothesis that tumor growth rate is stable over time without treatment, it has been suggested to be affected by several factors, including histologic subtypes and tumor size [40]. Because of all these limitations, the current study is only hypothesis-generating, and mechanisms and biomarkers of HPD in AGC treated with nivolumab need to be further evaluated.

Conclusions

Although the small sample size is the major limitation of our study, our study suggested that some clinicopathological and molecular characteristics might be associated with HPD in AGC patients treated with nivolumab. The identification of these characteristics will potentially allow careful management of AGC during nivolumab treatment. However, further investigations in larger cohorts are needed to confirm HPD-associated biomarkers.

Author contributions AS, YN, SM, AK, and KS designed the study, collected data, performed data analysis, and wrote manuscript. YK, HB, TD, TY, TK, and TA were involved in data interpretation and critically reviewing the manuscript. TK was involved in testing tumor tissue as well as critically reviewing the manuscript. All authors read and approved the final manuscript.

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Data Availability All data analyzed during this study have been included within the article.

Compliance with ethical standards

Ethics approval and consent to participate All procedures followed in this study were in accordance with the Declaration of Helsinki of 1964 and later versions and the Japanese Ethical Guidelines for Medical and Health Research Involving Human Subjects. Informed consent for it was obtained from all patients for their being included in the study.

Consent for publication This is not applicable for this study.

Conflict of interest AS has nothing to disclose. YN reports personal fees from Chugai. SM has nothing to disclose. AK reports research funding from Ono, Sumitomo Dainippon, and Taiho. YK reports consulting or advisory role for Takeda; personal fees from Bayer, Lilly,

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