ORIGINAL RESEARCH

A prognostic model, including the EBV status of tumor cells, for primary gastric diffuse large B-cell lymphoma in the rituximab era

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Abstract
EBV-positive diffuse large B-cell lymphoma (DLBCL), not otherwise specified (NOS), often affects the gastrointestinal tract. However, the prognostic significance of EBV associated with primary gastric DLBCL (gDLBCL) has not been established. This retrospective study included 240 patients with primary gDLBCL, diagnosed between 1995 and 2015. Tumor specimens were analyzed with EBER in situ hybridization. In 25 (10%) cases, tumor cells harbored EBV. The EBV+ group more frequently exhibited programmed death-ligand 1 (PD-L1) expression in microenvironment immune cells, but not tumor cells, compared to the EBV− group (86% vs 43%, \( P = .006 \)). Among 156 patients that received rituximab-containing chemotherapy, the EBV+ group had a significantly worse overall survival (OS) than the EBV− group (86% vs 43%, \( P = .006 \)). Multivariate analyses identified 3 independent adverse prognostic factors of OS: multiple gastric lesions (\( P = .002 \)), EBER positivity (\( P = .003 \)), and B symptoms (\( P = .018 \)). These factors were combined to develop a gDLBCL prognostic (gDLP) model that significantly stratified the patients into 3 distinct risk groups (Scores: good = 0, intermediate = 1, and poor = 2/3, \( P < .0001 \)) with 5-year OS rates of 100%, 81%, and 39%, respectively. Patients with EBV+ gDLBCL commonly exhibited microenvironmental PD-L1 expression and showed a significantly worse prognosis than subjects with EBV− gDLBCL. Our gDLP model, which included EBV+ tumor cells, provided good predictions of clinical outcome and may be useful for selecting patients in trials in the immune-oncology era.

KEYWORDS
diffuse large B-cell lymphoma, Epstein-Barr virus, gastric lymphoma, PD-L1, rituximab
1 | INTRODUCTION

Within the gastrointestinal (GI) tract, the stomach is the most common site of non-Hodgkin lymphoma (NHL). About half the gastric lymphoma cases are diagnosed as diffuse large B-cell lymphoma (DLBCL), which is a clinically, pathologically, and molecularly heterogeneous entity. Epstein-Barr virus (EBV), a member of the herpes virus family, is implicated in numerous reactive and neoplastic processes of the immune system. EBV-positive (EBV+) DLBCL, not otherwise specified (NOS), often shows an aggressive clinical course with frequent extranodal disease that affects the GI tract. Previous reports have detected EBV in 5%-15% of patients with DLBCL. In the current rituximab era, the prognostic significance of this finding is controversial. Therefore, the clinicopathological and prognostic significance of EBV associated with primary gastric DLBCL (gDLBCL) has not been well established.

Targeted therapies that use antibodies against programmed cell death 1 (PD-1) and its ligand (PD-L1) have recently shown great promise in treating various malignancies, including relapsed or refractory DLBCL. Overexpression of PD-L1 on either DLBCL cells or tumor-infiltrating immune cells has been associated with tumor cells that harbor EBV. Additionally, clinical trial studies have reported that the immune microenvironment of tumors generally correlates with the response rate to anti-PD-1/PD-L1 therapies.

In patients with gDLBCL, outcome is typically predicted with the Lugano staging system for GI tract lymphoma and the International Prognostic Index (IPI) for aggressive NHL. However, these classification systems were devised in the pre-rituximab era; that is, before rituximab was routinely added to chemotherapy, due to its positive impact in patients with DLBCL. With the advent of novel immune-based therapies, there is a need to find alternative methods for identifying candidate targets for therapeutic immune checkpoint inhibitors.

In this study, we investigated 240 cases of primary gDLBCL to shed light on EBV-harboring tumor cells. We aimed to develop a gDLBCL-specific model designed for the rituximab era, which could be useful in selecting patients for clinical trials for immune-oncology therapeutics.

2 | MATERIALS AND METHODS

2.1 | Patient selection

This retrospective study included data on 240 patients with primary gDLBCL diagnosed between 1995 and 2015 at Nagoya University Hospital and 30 affiliated institutions. All clinical and laboratory data were obtained from the medical records at each institution. The diagnosis was established according to histopathologic and immunohistochemical criteria, based on the 2017 WHO classification system. All cases satisfied the criteria for primary gastrointestinal lymphoma as defined by Lewin et al. The best method for discriminating primary gastric DLBCL from systemic DLBCL involving the stomach is not clear. Lymphoma at the stomach was considered primary if the main bulk of disease is located in the stomach. The clinical stage was evaluated according to the Lugano classification for gastrointestinal NHL. The endoscopic appearance of the tumors was determined based on the classification of Watanabe et al, with some modifications; that is, we added the following tumor classification features: superficial-spreading type, mass-forming type, diffuse-infiltrating type, and mixed type. The mass-forming tumor type was further classified into ulcerated and polyoid types. Multiple gastric lesions were defined as the presence of 2 or more lesions in the stomach, with adjacent, non-neoplastic mucosa. The endoscopic findings were evaluated by 3 experienced endoscopists (E.I., K.F., and S.N.). All cases were reviewed by E.I., S.K., and S.N., and the consensus for diagnosis was reached with a multi-headed microscope. The study was approved by the Institutional Review Board of Nagoya University.

2.2 | Immunohistochemistry and in situ hybridization studies

Tissue samples were fixed in 10% formalin and embedded in paraffin. The samples were cut into 5-μm-thick sections and then stained with hematoxylin and eosin. The monoclonal antibodies used for immunohistochemistry are listed in the Table S1.

Cases were designated as either a germinal center B cell (GCB) or a non-GCB immunophenotype, based on the Hans criteria. Lymphoid cell staining was considered positive for PD-L1, when ≥5% of the neoplastic lymphoid cells showed moderate or strong membrane staining with a PD-L1-specific antibody (clone SP142). A case was considered positive for PD-L1 in the microenvironment when, among the total tissue cellularity, ≥20% comprised nonmalignant cells with moderate or strong membrane or cytoplasmic PD-L1-specific staining. The threshold used here is comparable to that from a prior publication involving the same clone antibody. To verify/falsify PD-L1 expression on tumor and nonmalignant large lymphoid cells in controversial cases, PD-L1/PAX5 and PD-L1/CD68 double staining were applied and performed analogously as described elsewhere. All cases were tested for EBV-encoded small RNA (EBER) with in situ hybridization (ISH), as described previously. Cases were considered EBER-positive when nuclear expression of EBER was observed in ≥80% of tumor cells. Immunohistochemical double staining for EBER-ISH and CD3 or CD79a was performed in selected cases.
2.3 | Statistical analysis

Correlations between 2 groups were determined with the Fisher exact test and Mann-Whitney U test. Survival distributions were estimated with the Kaplan-Meier method, and groups were compared with the log-rank test. Univariate Cox regression analyses were performed to assess the effects of prognostic factors. Only variables that were statistically significant in the univariate analysis were subsequently evaluated in the multivariate analysis. The multivariate analysis was performed with a forward/backward stepwise method, and \( P < .05 \) was the threshold for inclusion in the model. All statistical analyses were performed with the STATA software package, version 15 (Stata Corporation, College Station, TX, USA).

3 | RESULTS

3.1 | Clinicopathological characteristics of primary gastric DLBCL

The study cohort consisted of 240 patients with gDLBCL, including 136 males and 104 females (male: female ratio = 1.3:1), with a median age of 67 years (range, 32-89 years). Clinical and treatment information was available for 239 patients. Their clinicopathologic and endoscopic data are summarized in Table 1. Of these, 129 patients (54%) were in Lugano stage I/II1 and 110 patients (46%) were in Lugano stage II2/IIIE/IV; 69 patients (29%) had an IPI of high-intermediate/high (HI/H); 57 patients (24%) exhibited B symptoms; and 1 patient (0.4%) had perforation during rituximab-containing chemotherapy. Among the patients evaluated endoscopically (n = 128), 3 tumors (2%) were the superficial-spreading type, 111 (87%) were the mass-forming type, 2 (2%) were the diffuse-infiltrating type, and 12 (9%) were the mixed type. In addition, 42 patients (33%) had multiple gastric lesions (Figure S1).

Among the 240 patients enrolled in this study, all the punch biopsies (n = 237, 99%) and surgically resected specimens (n = 3, 1%) exhibited a predominant proliferation of medium-to-large lymphoid cells without evidence of concomitant low-grade lesions. Paraffin-section immunohistochemistry specimens were available for all patients. Of these 240 specimens, 231 (96%) showed CD20 positivity on tumor cells. The remaining CD20-negative cases (n = 9, 4%) were positive for CD79a. Twenty-five (10%) cases showed EBV harboring on ≥80% of their tumor cells by EBER-ISH, but EBV was not detected in the background cells. Of 54 cases examined for PD-L1, 29 (54%) showed positive PD-L1 signals; these signals were detected in microenvironmental immune cells, but not in tumor cells.

3.2 | Treatment of primary gastric DLBCL

In the proportion of patients principally treated with chemotherapy (n = 148), with no surgical resection or irradiation, overall survival (OS) was significantly different between the subgroups treated with or without rituximab (n = 91 and 57, respectively). Patients that received rituximab showed a more favorable course (\( P < .001 \)). Based on this finding, we limited subsequent survival analyses to the group of patients that received rituximab-containing chemotherapy.

Of 239 patients with primary gDLBCL, 156 (65%) received multi-agent chemotherapy combined with rituximab. Of these, 91 (58%) received only rituximab-containing chemotherapy, and 55 (35%) received additional irradiation. Of the 156 patients that received chemotherapy, 150 (96%) received anthracycline-based chemotherapy, including 146 (94%) that also received CHOP (cyclophosphamide, doxorubicin, vincristine, prednisolone).

3.3 | Treatment response and prognosis of primary gastric DLBCL in the rituximab era

Among 156 patients with gDLBCL that received rituximab-containing chemotherapy, 135 (87%) achieved a complete response (CR) and 7 (4%) developed progressive disease (PD). In this group, the 5-year OS and progression-free survival (PFS) rates were 83% and 75%, respectively, with a median follow-up of 60 months (range, 4-141 months). The 5-year OS rates for patients with Lugano stages I (n = 54), II1 (n = 37), II2 (n = 12), IIE (n = 8), and IV (n = 45) were 94%, 100%, 61%, 63%, and 66%, respectively. On the other hand, the 5-year OS rates for patients with IPI values of low (n = 87), low-intermediate (n = 24), high-intermediate (n = 26), and high (n = 19) were 94%, 77%, 78%, and 45%, respectively. No statistically significant difference was noted in OS between cases with positive and negative detection of PD-L1 in the microenvironment.

3.4 | Clinicopathological characteristics of EBV+ primary gastric DLBCL

Twenty-five (10%) of 240 cases with gDLBCL showed EBV harboring on ≥80% of their tumors by EBER-ISH. In 22 of 25 EBV+ gDLBCL cases with available FFPE sections, LMP1 and EBNA2 were found in 15 (68%) and 12 (55%) of them, respectively. They were diagnosed as EBV latency II and III for 4 (18%) and 12 cases (55%), respectively, in our series. We found no significant difference in clinicopathological findings between the EBV+ and EBV- gDLBCL subgroups, except for the endoscopic features and microenvironmental PD-L1 expression (Table 1). Endoscopic findings showed that the EBV+ subgroup had a higher prevalence of the diffuse-infiltrating tumor...
| Characteristics                        | Total (n=240) | EBV-positive (n=25) | EBV-negative (n=215) | P     |
|---------------------------------------|---------------|---------------------|----------------------|-------|
|                                       | No.  | %    | No.  | %    | No.  | %    |        |
| Sex (male/female)                    | 136/104 | 1.3  | 12/13 | 0.9  | 124/91 | 1.4  | .39    |
| Age (y), median (range)              | 67 (32-89) | 69 (37-85) | 67 (32-89) | .90  |
| Age >60 y                             | 170/240 | 71   | 18/25 | 72   | 152/215 | 71   | 1.00  |
| Abdominal pain                        | 39/81  | 48   | 2/7  | 29   | 37/74  | 50   | .43    |
| Perforation                           | 1/230  | 0.4  | 0/22 | 0    | 1/208  | 0.5  | 1.00  |
| PS 2-4                                | 28/239 | 12   | 2/25 | 8    | 26/214 | 12   | .75    |
| Lugano stage II/IIIE/IV               | 110/239 | 46   | 13/25 | 52   | 97/214 | 45   | .76    |
| Serum LDH > normal                   | 87/237  | 37   | 11/25 | 44   | 76/212  | 36   | .53    |
| sIL-2R ≥1000 U/mL                    | 101/211 | 48   | 9/19 | 47   | 92/192  | 48   | 1.00  |
| Extracranial involvement >1 site     | 61/239  | 26   | 4/25 | 16   | 57/214  | 27   | .34    |
| IPI High-int, High                   | 69/237  | 29   | 7/25 | 28   | 62/212  | 29   | 1.00  |
| B symptoms present                   | 57/239  | 24   | 9/25 | 36   | 48/214  | 22   | .14    |
| Helicobacter pylori infection         | 49/72   | 68   | 5/7  | 71   | 44/65   | 68   | 1.00  |
| Endoscopic appearance                |        |      |      |      |        |      |        |
| Superficial-spreading type           | 3/128  | 2    | 0/21 | 0    | 3/107  | 3    | 1.00  |
| Mass-forming type                    | 111/128 | 87   | 18/21 | 86   | 93/107  | 87   | 1.00  |
| Ulcerated type                       | 93/128 | 73   | 13/21 | 62   | 80/107  | 75   | .28    |
| Polypoid type                        | 18/128 | 14   | 5/21 | 24   | 13/107  | 12   | .18    |
| Diffuse-infiltrating type            | 2/128  | 2    | 2/21 | 10   | 0/107   | 0    | .026   |
| Mixed type                           | 12/128 | 9    | 1/21 | 5    | 11/107  | 10   | .69    |
| Multiple gastric lesions             | 42/128 | 33   | 9/21 | 43   | 33/107  | 31   | .32    |
| Bulky mass present                   | 34/240 | 14   | 3/25 | 12   | 31/215  | 14   | 1.00  |
| Immunophenotype                      |        |      |      |      |        |      |        |
| CD5                                  | 7/233  | 3    | 1/23 | 4    | 6/210   | 3    | .52    |
| CD10                                 | 58/234 | 25   | 7/23 | 30   | 51/211  | 24   | .61    |
| CD20                                 | 231/240 | 96   | 22/25 | 88   | 209/215  | 97   | .055   |
| CD30                                 | 4/52   | 8    | 1/13 | 8    | 3/39    | 8    | 1.00   |
| BCL-2                                | 95/212 | 45   | 12/22 | 55   | 83/190  | 44   | .37    |
| BCL-6                                | 139/228 | 61   | 12/23 | 52   | 127/205  | 62   | .38    |
| MUM1                                 | 163/228 | 72   | 17/23 | 74   | 146/205  | 71   | 1.00   |
| nPD-L1 (≥5%)                         | 0/54   | 0    | 0/14 | 0    | 0/40    | 0    | —      |
| miPD-L1 (≥20%)                       | 29/54  | 54   | 12/14 | 86   | 17/40   | 43   | .006   |
| Non-GCB immunophenotype              | 145/231 | 63   | 16/24 | 67   | 129/207  | 62   | .82    |
| Treatment                            |        |      |      |      |        |      |        |
| R-containing CTx                     | 156/239 | 65   | 12/25 | 48   | 144/214  | 67   | .075   |
| R-CTx                                | 91/156 | 58   | 8/12 | 67   | 83/144  | 58   | .76    |
| R-CTx + RT                           | 55/156 | 35   | 3/12 | 25   | 52/144  | 36   | .54    |
| R-CTx + Surgery                      | 9/156  | 6    | 0/12 | 0    | 9/144   | 6    | 1.00   |
| R-CTx + Surgery + RT                 | 1/156  | 0.6  | 1/12 | 8    | 0/144   | 0    | .077   |
| No. of cycles, median (range)        | 5 (1-8) | 3.5 (2-8) | 5 (1-8) | .22 |
| No treatment                         | 5/239  | 2    | 3/25 | 12   | 2/214   | 1    | .009   |

(Continues)
type (10%) than the EBV− subgroup (0%, \( P = .026 \)). Histological results showed that PD-L1 was detected in microenvironmental immune cells at a significantly higher rate in the EBV+ gDLBCL group (n = 12/14, 86%) than in the EBV− group (n = 17/40, 43%; \( P = .006 \); Figure S2).

### 3.5 Comparison of clinical courses in patients with EBV+ and EBV− primary gastric DLBCL in the rituximab era

We observed no significant difference in therapeutic response between the EBV+ and EBV− subgroups. However, among the patients with gDLBCL that received rituximab-containing chemotherapy, the OS and PFS rates were significantly worse in the EBV+ subgroup than in EBV− subgroup (5-year OS: 58% vs 84%; \( P = .0029 \); 5-year PFS: 47% vs 77%; \( P = .0043 \); Figure 1).

### 3.6 Clinical course of EBV+ primary gastric DLBCL

Among patients with EBV+ gDLBCL principally treated with chemotherapy (n = 16), with no surgical resection or irradiation, there was no benefit from the addition of rituximab (\( P = .6068 \), Figure S3). Therefore, we performed a univariate analysis for further stratification of our patients with EBV+ gDLBCL that received chemotherapy (with or without rituximab) (n = 21). This analysis identified 3 factors associated with a poor prognosis: multiple gastric lesions (\( P = .0031 \)), Lugano stage II2/IIE/IV (\( P = .0173 \)), and high levels of sIL-2R (\( P = .0434 \); Table 2). Based on this result, we stratified patients with EBV+ gDLBCL treated with chemotherapy into 3 groups by combination of Lugano stage classification and the number of gastric lesions: those with a single gastric lesion in Lugano stage I (n = 4), those with a single gastric lesion in Lugano stage

### TABLE 1 (Continued)

| Characteristics | Total (n=240) | EBV-positive (n=25) | EBV-negative (n=215) | \( P^a \) |
|-----------------|--------------|---------------------|---------------------|---------|
| Therapeutic response (R-containing CTx) | | | | |
| CR | 135/156 87 | 9/12 75 | 126/144 88 | .21 |
| PR | 13/156 8 | 1/12 8 | 12/144 8 | 1.00 |
| SD | 1/156 1 | 1/12 8 | 0/144 0 | .077 |
| PD | 7/156 4 | 1/12 8 | 6/144 4 | .44 |

CR, complete remission; CTx, chemotherapy; GCB, germinal center B-cell; High-int, high-intermediate; IPI, International Prognostic Index; LDH, lactate dehydrogenase; miPD-L1, microenvironmental programmed cell death ligand 1; nPD-L1, neoplastic programmed cell death ligand 1; PD, progressive disease; PR, partial remission; PS, performance status; sIL-2R, soluble interleukin-2 receptors; R, rituximab; RT, radiotherapy; SD, stable disease.

\( P^a \) value are for the comparison of EBV-positive and EBV-negative primary gastric DLBCL patients.

### FIGURE 1

Survival analysis of patients with gDLBCL treated with rituximab-containing chemotherapy. A, Overall survival; B, Progression-free survival according to EBV status on tumor cells
III/II2/IIE/IV (n = 8), and those with multiple gastric lesions (n = 6). The 5-year OS rates for these 3 subgroups were 100%, 63%, and 0%, respectively. This classification significantly stratified patients with EBV+ gDLBCL by survival risk ($P = .0036$, Figure 2).

### 3.7 Prognostic factors of primary gastric DLBCL in the rituximab era

A univariate analysis identified 9 prognostic factors for survival in patients with gDLBCL that received rituximab-containing chemotherapy (Table 3). Based on these 9 factors, survival was unfavorable for patients with Lugano stage III/II2/IIE/IV ($P < .0001$), more than 1 site of extranodal involvement ($P = .0001$), high levels of serum LDH ($P = .0002$), IPI HI/H ($P = .0002$), multiple gastric lesions ($P = .0004$), high levels of soluble interleukin-2 receptors (sIL-2R $\geq 1000$ U/mL, $P = .0021$), EBER positivity ($P = .0029$), B symptoms ($P = .0066$), and BCL-2 positivity ($P = .003$), and B symptoms ($P = .018$) were the only independent adverse prognostic factors.

### 3.8 Prognostic model of primary gastric DLBCL in the rituximab era

We constructed a gDLBCL prognostic (gDLP) model by combining the 3 independent prognostic variables; that is, multiple gastric lesions, EBER positivity, and B symptoms. Patients were scored according to the following gDLP scores: 0 = no adverse factors (n = 52); 1 = positive
for 1 factor (n = 36); 2 = positive for 2 factors (n = 14); and 3 = positive for 3 factors (n = 1). The 5-year OS rates for these 4 groups were 100%, 81%, 42%, and 0%, respectively. Our prognostic model stratified the patients into gDLP groups with good (gDLP score 0 [n = 52]), intermediate (gDLP score 1 [n = 36]), and poor (gDLP score 2/3 [n = 15]) prognoses. In good-gDLP group, 33 (63%) of 52 were treated with rituximab-containing chemotherapy alone, 15 (29%) received additional irradiation, and 4 (8%) underwent surgical resection initially. On the other hand, in poor-gDLP group, 13 (87%) of 15 received rituximab-containing chemotherapy alone and two (13%) received additional irradiation. With this model, the patients with gDLBCL were accurately stratified by survival risk (P < .0001, Figure 3).

**4 | DISCUSSION**

This study aimed to clarify the prognostic significance of EBV associated with gDLBCL and define the characteristics of patients with a poor prognosis to facilitate patient selection for clinical trials in the current era of immunoncology. To that end, we analyzed 240 primary gDLBCL patients, of whom 156 had been treated with rituximab-containing chemotherapy. Our results revealed that EBV+ gDLBCL occurred uncommonly, but it was often aggressive and life-threatening. In addition, this disease featured high PD-L1 expression in microenvironmental immune cells and relatively diverse in biologic behavior as shown in Figure 2. We developed a prognostic model that comprised 3 independent factors associated with adverse outcome: multiple gastric lesions, B symptoms, and the presence of EBV-harboring tumor cells. This model could accurately stratify patients with primary gDLBCL into 3 distinct prognostic groups. Further studies are warranted to establish optimal strategies for treating EBV+ tumor cells in primary gDLBCL.

Previous studies have described clinicopathological features that served as prognostic indicators of poor clinical outcome in gDLBCL, including *Helicobacter pylori* negativity,27 an absence of gene translocation involving the immunoglobulin heavy-chain,28 Lugano stage II/IIIE/IV, elevated serum LDH levels,29 and so on. Given the marked...
relatively low percentage of EBV+ tumor cells limits firm conclusions regarding EBV+ DLBCL and it is dubious whether in such cases the virus represents a true pathogenic event. Indeed, the above-mentioned 4 studies used thresholds as 30%, 20%, 10%, and 10%, respectively, to define EBV+ DLBCL. In this study, we adapted the threshold of 80% for the diagnosis of EBV+ gDLBCL, which is recommended in the 2017 WHO classification, to avoid the inclusion of cases in which EBV may be a bystander in non-neoplastic cells.

Very few studies have analyzed the significance of the endoscopic appearance and the abundance of gastric lesions in gDLBCL. In our series, approximately 90% of cases involved the mass-forming type of tumor, similar to the rate reported by Nakamura et al28 in patients with gDLBCL without mucosa-associated lymphoid tissue lymphoma. On the other hand, multifocal lesions were reported to be associated with secondary gastric NHL.31 To our knowledge, the significance of multiple gastric lesions on the clinical outcome of patients with gDLBCL had not been fully elucidated previously. In this study, we showed that, in addition to tumor cells that harbored EBV, multiple gastric lesions were independently associated with a poor OS in gDLBCL.

The IPI, which incorporates 5 clinical variables, remains a valuable prognostic tool for DLBCL in the rituximab era.32 The Lugano classification, which emphasizes the distribution of nodal and extranodal disease sites and the extension to adjacent organs, was proposed for GI tract lymphoma.20 However, due to tumor heterogeneity, risk-stratification specific to gDLBCL has not been elucidated. In the present study, the Lugano classification distinguished 5 separate groups with 5-year OS rates, ranging from 61% to 100%. In contrast, the IPI segregated patients into 4 outcome groups with 5-year OS ranging from 45% to 94%. Based on our findings, we proposed a new gDLP model, which integrated 3 clinical parameters: multiple gastric lesions (P = .002), EBER positivity (P = .003), and B symptoms (P = .018). This model succeeded in identifying a low-risk gDLP group with no adverse factors and an extremely favorable outcome in the rituximab era. In this group, 100% of patients remained alive after a median follow-up of 56 months (range 5-126 months). In contrast, patients in the high-risk gDLP group, which consisted of 5 patients with EBV+ and 10 with EBV+ gDLBCL, was shown to have the most aggressive disease, with a 5-year OS rate of only 39%.

Notably, among the 18 cases with EBV+ gDLBCL treated with chemotherapy, 6 had multiple gastric lesions. Aggressive disease in these 6 patients resulted in death within 3 years of diagnosis. Recent studies have shown that, blocking the interaction of PD-1 with its ligands, PD-L1 and PD-L2, led to impressive antitumor responses and clinical benefit in a subset of patients,33 including those with relapsed and refractory DLBCL.16,17 However, predicting tumor responses to PD-1 blockade remains a major challenge. Some studies reported that responses to PD-1/PD-L1 blockade immunotherapies were observed in patients with PD-L1 expression in tumor-infiltrating immune cells.19 In addition, Kiyasu et al18 showed a close association between PD-L1 expression in microenvironmental immune cells and EBV+ tumor cells.
consistent with our results. Given those findings, we believe that patients with EBV+ gDLBCL represent good candidates for future clinical trial, when they show significant PD-L1 expression in microenvironmental immune cells, and particularly, when multiple gastric lesions are present, due to their dismal prognosis.

In contrast to those patients, we identified 4 EBV+ cases with a single gastric lesion in Lugano stage I that displayed a plateau in the survival curve after diagnosis. The combination of these clinical features evoked the possibility of an EBV+ mucocutaneous ulcer (EBVMCU), characterized by indolent behavior and a self-limited clinical course. However, in our series, 2 of these cases had polypoid tumors, and the remaining 2 cases had large ulcerated tumors, but unlike the superficial lesion commonly observed for EBVMCU. In the original report by Dojcinov et al, EBVMCU was shown to affect the esophagus, colon, and rectum, but not the stomach. Nevertheless, the favorable clinical course we observed for patients with a single gastric lesion in Lugano stage I suggested that this condition might warrant special recognition as a separate class from the other EBV+ gDLBCL cases.

In the present study, of 54 gDLBCL cases examined, all were negative for PD-L1 in tumor cells, including 14 patients with EBV+ gDLBCL. This finding was considerably different from findings in previous studies, where PD-L1 expression in tumor cells was observed in 26% of DLBCL, NOS cases examined with immunohistochemistry. The frequent up-regulation of PD-L1 was also shown in EBV+ post-transplant lymphoproliferative disorders and EBV+ DLBCL. EBV was reported to provide an intrinsic signal to augment PD-L1 expression through EBV-LMP1 increasing PD-L1 promoter and enhancer activity. In this study, none of EBV+ gDLBCL cases expressed PD-L1 on tumor cells, although LMP1 was positive in 7 of 14 EBV+ cases evaluated. One potential explanation for these discrepancies could be the antibodies used. For example, Kataoka et al reported that a PD-L1 3'-UTR disruption caused a lack of PD-L1 detection in virus-related tumors that were probed with the SP142 antibody, which was directed against the C-terminal domain. Therefore, we conducted a preliminary study to compare our results with results obtained with the E1J2J antibody, which is directed against the N-terminal domain (n = 9 cases of EBV+ gDLBCL). However, again, we did not detect PD-L1 expression on tumor cells of those patients. Another potential explanation for these discrepancies could be the antibody, which was directed against the C-terminal domain. Therefore, we conducted a preliminary study to compare our results with results obtained with the E1J2J antibody, which is directed against the N-terminal domain (n = 9 cases of EBV+ gDLBCL). However, again, we did not detect PD-L1 expression on tumor cells of those patients. Another potential explanation for these discrepancies could be the difference among the primary sites of involvement (gastric site vs nodal). This issue should be examined further in the future studies.

In summary, our prognostic model, which included EBV+ tumor cells, multiple gastric lesions, and B symptoms, provided accurate definitions of 3 prognostic groups, including 1 group with the worst prognosis. In addition, EBV+ gDLBCL featured PD-L1 expression in microenvironmental immune cells and those with multiple gastric lesions were most likely to have highly aggressive disease. We recommend that EBV should be routinely examined in patients with gDLBCL to provide better assessments of prognosis and better predictions of the therapeutic effects of immune-oncology. This approach might facilitate patient selection in the future clinical trials. Our results warrant further validation in future studies.

ACKNOWLEDGMENTS

The authors thank Y. Katayama, Y. Inagaki, K. Matsubara and K. Kito for technical assistance and the following collaborators for providing patient clinical data and specimens: Aichi Cancer Center Hospital, Anjo Kosei Hospital, Chubu Rosai Hospital, Ekisaikai Hospital, Hyogo Cancer Center Hospital, Ichinomiya Municipal Hospital, Ishikawa Prefectural Central Hospital, Japanese Red Cross Nagoya Daiichi Hospital, Kani Tono Hospital, Koseiren Takaoka Hospital, Kyoto Kuramaguchi Medical Center, Matsue City Hospital, Ogaki Municipal Hospital, Oita Prefectural Hospital, Okazaki Municipal Hospital, Omihachiman Community Medical Center, Suzuka Chuo General Hospital, Takatsuki General Hospital, Toyama Prefectural Central Hospital, Toyohashi Municipal Hospital, Toyota Kosei Hospital, University Hospital Kyoto Prefectural University of Medicine and University of Fukui Hospital.

CONFLICT OF INTERESTS

The authors have no significant relationships with or financial interests in any commercial companies pertaining to this article.

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REFERENCES

1. Koch P, del Valle F, Berdel WE, et al. Primary gastrointestinal non-Hodgkin’s lymphoma: I. Anatomic and histologic distribution, clinical features, and survival data of 371 patients registered in the German Multicenter Study GIT NHL 01/92. J Clin Oncol. 2001;19:3861-3873.
2. Nakamura S, Matsumoto T, Iida M, Yao T, Tsumeyoshi M. Primary gastrointestinal lymphoma in Japan: A clinicopathologic analysis of 455 patients with special reference to its time trends. Cancer. 2003;97:2462-2473.
3. Chan WC, Armitage JO, Gascoyne R, et al. A clinical evaluation of the International Lymphoma Study Group classification of non-Hodgkin’s lymphoma. *Blood*. 1997;89:3909-3918.

4. Hans CP, Weisenburger DD, Greiner TC, et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. *Blood*. 2004;103:275-282.

5. Dubois S, Vially PJ, Mareschal S, et al. Next-generation sequencing in diffuse large B-cell lymphoma highlights molecular divergence and therapeutic opportunities: a LYSA study. *Clin Cancer Res*. 2016;22:2919-2928.

6. Cohen JI. Epstein-Barr virus infection. *N Engl J Med*. 2000;343:481-492.

7. Young LS, Rickinson AB. Epstein-Barr virus: 40 years on.

8. Oyama T, Yamamoto K, Asano N, et al. Age-related EBV-positive diffuse large B-cell lymphoma of the elderly in the rituximab era. *Cancer Sci*. 2014;105:1170-1175.

9. Sato A, Nakamura N, Kojima M, et al. Clinical outcome of Epstein-Barr virus-positive diffuse large B-cell lymphoma of the elderly. *Mod Pathol*. 2012;25:968-982.

10. Park S, Lee J, Ko YH, et al. The impact of Epstein-Barr virus status on clinical outcome in diffuse large B-cell lymphoma. *Blood*. 2007;110:972-978.

11. Morales D, Beltran B, De Mendoza FH, et al. Epstein-Barr virus as a prognostic factor in de novo nodal diffuse large B-cell lymphoma. *Leuk Lymphoma*. 2010;51:66-72.

12. Ahn JS, Yang DH, Choi YD, et al. Clinical outcome of elderly patients with Epstein-Barr virus positive diffuse large B-cell lymphoma treated with a combination of rituximab and CHOP chemotherapy. *Am J Hematol*. 2013;88:774-779.

13. Ok CY, Ye Q, Li L, et al. Age cutoff for Epstein-Barr virus-positive diffuse large B-cell lymphoma - is it necessary? *Oncotarget*. 2015;6:13935-13946.

14. Hong JY, Yoon DH, Suh C, et al. EBV-positive diffuse large B-cell lymphoma in young adults: is this a distinct disease entity? *Ann Oncol*. 2015;26:548-555.

15. Lesokhin AM, Ansell SM, Armand P, et al. Nivolumab in patients with relapsed or refractory hematologic malignancy: preliminary results of a Phase Ib study. *J Clin Oncol*. 2016;34:2698-2704.

16. Kiyasu J, Miyoshi H, Hirata A, et al. Expression of programmed cell death ligand 1 is associated with poor overall survival in patients with diffuse large B-cell lymphoma. *Blood*. 2015;126:2193-2201.

17. Herbst RS, Soria JC, Kowanetz M, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature*. 2014;515:563-567.

18. Rohatiner A, Damore F, Coiffier B, et al. Report on a workshop convened to discuss the pathological and staging classifications of gastrointestinal-tract lymphoma. *Ann Oncol*. 1994;5:397-400.

19. Shipp MA, Harrington DP, Anderson JR, et al. A predictive model for aggressive non-Hodgkins-Lymphoma. *N Engl J Med*. 1993;329:987-994.

20. Habermann TM, Weller EA, Morrison VA, et al. Rituximab-CHOP versus CHOP alone or with maintenance rituximab in older patients with diffuse large B-cell lymphoma. *J Clin Oncol*. 2006;24:3121-3127.

21. Watanabe H. Malignant lymphoma of the stomach 87 collected cases. *Stomach Intestine*. 1980;15:909-910.

22. Menter T, Bodmer-Haecki A, Dirnhofer S, Trankov A. Evaluation of the diagnostic and prognostic value of PDL1 expression in Hodgkin and B-cell lymphomas. *Hum Pathol*. 2016;54:17-24.

23. park S, Lee J, Ko YH, et al. Helicobacter pylori-related diffuse large B-cell lymphoma of the stomach: a distinct entity with lower aggressiveness and higher chemosensitivity. *Blood Cancer J*. 2014;4:7.

24. Nakamura S, Ye H, Bacon CM, et al. Translocations involving the immunoglobulin heavy chain gene locus predict better survival in gastric diffuse large B-cell lymphoma. *Clin Cancer Res*. 2008;14:3002-3010.

25. Tanaka T, Shimada K, Yamamoto K, et al. Retrospective analysis of primary gastric diffuse large B cell lymphoma in the rituximab era: a multicenter study of 95 patients in Japan. *Ann Hematol*. 2012;91:383-390.

26. Nicolae A, Pittaluga S, Abdullah S, et al. EBV-positive large B-cell lymphomas in young patients: a nodal lymphoma with evidence for a tolerogenic immune environment. *Blood*. 2015;126:863-872.

27. Kolve M, Fischbach W, Greiner A, Wils K. Differences in endoscopic and clinicopathological features of primary and secondary gastric non-Hodgkin’s lymphoma. German Gastrointestinal Lymphoma Study Group. *Gastrointest Endosc*. 1999;49:307-315.

28. Sehn LH, Berry B, Chhanabhai M, et al. The revised International Prognostic Index (R-IPI) is a better predictor of outcome than the standard IPI for patients with diffuse large B-cell lymphoma treated with R-CHOP. *Blood*. 2007;109:1857-1861.

29. Balar AV, Weber JS. PD-1 and PD-L1 antibodies in cancer: current status and future directions. *Cancer Immunol Immunother*. 2017;66:551-564.

30. Dojcincov SD, Venkataraman G, Raffeld M, Pittaluga S, Jaffe ES. EBV positive mucocutaneous Ulcer-A study of 26 cases associated with various sources of immunosuppression. *Am J Surg Pathol*. 2010;34:405-417.

31. Green MR, Rodig S, Juszczynski P, et al. Constitutive AP-1 activity and EBV infection induce PD-L1 in Hodgkin Lymphomas and posttransplant lymphoproliferative disorders: implications for targeted therapy. *Clin Cancer Res*. 2012;18:1611-1618.

32. Chen BJ, Chapuy B, Jing OY, et al. PD-L1 expression is characteristic of a subset of aggressive B-cell lymphomas and virus-associated malignancies. *Clin Cancer Res*. 2013;19:3462-3473.
37. Kataoka K, Shiraishi Y, Takeda Y, et al. Aberrant PD-L1 expression through 3′-UTR disruption in multiple cancers. Nature. 2016;534:402-406.

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**How to cite this article:** Ishikawa E, Tanaka T, Shimada K, et al. A prognostic model, including the EBV status of tumor cells, for primary gastric diffuse large B-cell lymphoma in the rituximab era. Cancer Med. 2018;7:3510–3520. [https://doi.org/10.1002/cam4.1595](https://doi.org/10.1002/cam4.1595)