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Risk stratification for the prognosis of patients with chemoresistant urothelial cancer treated with pembrolizumab

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Abstract

The use of immune checkpoint inhibitors to treat urothelial carcinoma (UC) is increasing rapidly without clear guidance for validated risk stratification. This multicenter retrospective study collected clinicopathological information on 463 patients, and 11 predefined variables were analyzed to develop a multivariate model predicting overall survival

(OS). The model was validated using an independent dataset of 292 patients. Patient characteristics and outcomes were well balanced between the discovery and validation cohorts, which had median OS times of 10.2 and 12.5 mo, respectively. The final validated multivariate model was defined by risk scores based on the hazard ratios (HRs) of independent prognostic factors including performance status, site of metastasis, hemoglobin levels, and the neutrophil-to-lymphocyte ratio. The median OS times (95% confidence intervals [CIs]) for the low-, intermediate-, and high-risk groups (discovery cohort) were not yet reached (NYR) (NYR–19.1), 6.8 mo (5.8-8.9), and 2.3 mo (1.2-2.6), respectively. The HRs (95% CI) for OS in the low- and intermediate-risk groups vs the high-risk group were 0.07 (0.04-0.11) and 0.23 (0.15-0.37), respectively. The objective response rates for in the low-, intermediate-, and high-risk groups were 48.3%, 28.8%, and 10.5%, respectively. These differential outcomes were well reproduced in the validation cohort and in patients who received pembrolizumab after perioperative or first-line chemotherapy (N = 584). In conclusion, the present study developed and validated a simple prognostic model predicting the oncological outcomes of pembrolizumab-treated patients with chemoresistant UC. The model provides useful information for external validation, patient counseling, and clinical trial design.

KEYWORDS

metastatic urothelial cancer, multivariate risk stratification model, overall survival, pembrolizumab, prognostic variables

1 | INTRODUCTION

No survival-prolonging therapy was available for treating chemoresistant surgically unresectable urothelial carcinoma (UC) prior to pembrolizumab, a humanized monoclonal antibody targeting PD-1 that has been demonstrated to improve overall survival (OS) in this setting.[1] Pembrolizumab has been approved in a number of countries and used to treat large numbers of patients. However, recently published results of >2 y of follow-up indicated that the 12-mo and 24-mo OS rates for this drug were only 44.2% and 26.9%, respectively, and the objective response rate was only 21.2%.[2] It was also reported that 62% of patients experienced treatment-related adverse events (AEs), and 12% of patients experienced serious AEs. However, the median OS among patients who achieved objective response exceeded 24 mo.

Considering the high heterogeneity in treatment responses, there is an urgent need for markers to predict the radiological and oncological outcomes of pembrolizumab treatment. However, the drug is widely used without large-scale, real-world data or clear guidance for risk stratification.

Various therapeutic agents are used or approved for use in the second-line setting after systemic chemotherapy for patients with UC in North America and Europe,[3-5] and this makes it difficult to evaluate the unmitigated outcomes of pembrolizumab treatment. In this regard, pembrolizumab has been used as a single treatment of choice in the second-line setting in Japan since it was approved in December 2017. Therefore, the real-world data in Japan would be useful for analyzing the outcomes of second-line pembrolizumab treatment. In this study, we collected and analyzed large-scale data for patients with UC outside clinical trial to develop a risk classification model for prognostic guidance.

2 | PATIENTS AND METHODS

2.1 | Study cohorts

In a multicenter retrospective study conducted under the Japan Urological Oncology Group framework, which was approved by Institutional Review Board (IRB) at Kyoto University Graduate School of Medicine (approval number R1783) and local IRB at each participating institute, the clinicopathological data of patients with surgically unresectable, chemoresistant UC who received pembrolizumab were collected (Appendix 1). The discovery cohort consisted of the first 463 patients (5 patients were excluded due to missing data) registered from 41 institutions across Japan. After the development of a multivariate prognostic model using data of the discovery cohort, another independent dataset of 292 patients was additionally collected from 21 institutes for external validation of the model (validation cohort). Consequently, 755 patients from 59 institutes (3 institutes provided data for both discovery and validation cohorts) were analyzed in the present study.

TABLE 1 Demographic and disease characteristics of the discovery and validation cohorts

	Discovery cohort (N = 463)	Validation cohort (N = 292)	*P*-value
Age, years, median (range)	71 (31-88)	71 (34-91)	.3171[b]
Sex, N (%)			
Male	357 (77.1)	211 (72.3)	
Female	106 (22.9)	81 (27.7)	.1416[c]
Smoking history, N (%)			
Yes	283 (61.1)	157 (53.8)	.0677[d]
No	169 (36.5)	122 (41.8)	
Unknown	11 (2.4)	13 (4.5)	
Primary site, N (%)			
Bladder/urethra	230 (49.7)	150 (51.4)	.4862[d]
Upper tract	179 (38.7)	115 (39.4)	
Both	27 (5.8)	25 (8.6)	
Unknown	1 (0.2)	2 (0.7)	
Surgical removal of primary site, N (%)			
Yes	272 (58.7)	152 (52.1)	.0711[c]
No	191 (41.3)	140 (47.9)	
History of NMIBC, N (%)			
Yes	101 (21.8)	48 (16.4)	.0707[c]
No	362 (78.2)	244 (83.6)	
Treatment lines			
1[a]	72(15.6)	41(14.0)	.644[d]
2	274(59.2)	184 (63.0)	
3	83 (17.9)	51 (17.5)	
4 or more	34 (7.3)	16 (5.5)	
Time since most recent chemotherapy, N (%)			
<90 d	221 (44.7)	134 (45.9)	.6215[c]
≥90 d	242 (55.3)	158 (54.1)	
ECOG PS, N (%)			
0	219 (47.3)	138 (47.3)	.7239[d]
1	154 (33.3)	91 (31.2)	
≥2	90 (19.4)	63 (21.6)	
Metastasis site, N (%)			
LN only	156 (33.7)	118 (40.4)	.0853[d]
Other organs	206 (44.5)	126 (41.2)	
Liver	101 (21.8)	48 (16.4)	
Hb, N (%)			
≥11 g/dL	202 (43.6)	121 (41.4)	.5536[c]
<11 g/dL	261 (56.4)	171 (58.6)	
NLR, N (%)			
≥3	271 (58.5)	170 (58.2)	.9325[c]
<3	192 (41.5)	122 (41.8)	

Abbreviations: NMIBC, non–muscle-invasive bladder cancer; ECOG PS, Eastern Cooperative Oncology Group performance status; Hb, hemoglobin; NLR, neutrophil-to-lymphocyte ratio.

[a]Secondary to perioperative systemic chemotherapy.

[b]Mann-Whitney U test.

[c]Fisher exact test.

[d]Chi-squared test.

[e]Patients receiving pembrolizumab for recurrent disease after surgery with perioperative systemic chemotherapy.

2.2 | Data collection

The cutoff date for data inclusion was January 24, 2020. The following data were collected using a standardized case report form: age; sex; smoking history; primary tumor site; history of primary tumor resection; history of non–muscle-invasive bladder cancer; treatment setting of pembrolizumab use (secondary to perioperative systemic chemotherapy, secondary to first-line therapeutic chemotherapy, third line, or fourth line or later); duration since last dose of chemotherapy; site of metastasis; Eastern Cooperative Oncology Group (ECOG) performance status (PS); complete blood count including leukocyte fraction; number of doses of pembrolizumab; presence of histological variants; and best objective response to pembrolizumab treatment based on Response Evaluation Criteria in Solid Tumors version 1.1. Blood test data within 2 wk before the initiation of pembrolizumab treatment were collected. The neutrophil-to-lymphocyte ratio (NLR) was calculated by dividing the number of neutrophils by that of lymphocytes. Information on any grade and ≥grade 3 AE, respectively, and subsequent therapy after pembrolizumab treatment were also collected.

2.3 | Statistical analysis and model development

The discovery cohort was used to develop the prognostic model, which was subsequently validated in the independent validation cohort. OS was defined as the time from the initiation of pembrolizumab treatment to death from any cause and estimated using Kaplan-Meier analysis with the log-rank test. Univariate and multivariate Cox proportional hazards models were used to examine the associations of OS with 11 predefined categorical and/or continuous variables.

The optimal cutoffs for hemoglobin (Hb) (g/dL) and NLR were screened via time-dependent receiver-operating characteristic (ROC) curve analysis.[6] Sensitivity analyses with regard to convenient cutoff values were performed, and an optimal cutoff was determined on the basis of the hazard ratios (HRs), frequency, and Harrell C-index.[7]

All 11 potential prognostic factors were incorporated in the multivariate Cox proportional hazard analysis. Least Absolute Shrinkage and Selection Operator (LASSO) methods were used for automatic variable selection to establish a prognostic model.[8] The prognostication model established based on discovery cohort was externally validated using an independent validation cohort by calibration plots that compared the predicted prognosis with the actual prognosis.[9,10] The prognostication accuracies of survival models were compared using Harrell C-index.[7] The association between risk stratification and the best objective response was evaluated using patients for whom data for objective responses to pembrolizumab were available. The results were depicted using mosaic (Marimekko) plots and statistically examined using the chi-squared test.

All statistical analyses were performed using JMP Pro 15.1.0 (SAS Institute, Cary, NC, USA), Prism 6 version 6.0 h (GraphPad Software, Inc, San Diego, CA, USA), and R glmnet and R survAUC packages, R version 3.2.2.[11]

3 | RESULTS

3.1 | Patients

Baseline demographics, treatment history, and disease characteristics were balanced between the discovery and validation cohorts (Table 1). At the data cutoff date, the median follow-up (interquartile range) periods were 17.7 (12.9-21.4) and 12.0 mo (6.1-17.9), respectively. The median OS times of the discovery and validation cohorts were estimated as 10.2 (95% confidence interval [CI] = 8.2-11.7) and 12.5 mo (95% CI = 8.4-17.3), respectively (Figure 1). Death occurred in 272 (58.7%) and 123 (42.1%) patients in the discovery and validation cohorts, respectively.

3.2 | Model development

The univariate Cox proportional hazards models found that 6 of the 11 variables were significantly associated with unfavorable OS: residual primary lesion, use of pembrolizumab within 90 d after the last treatment, poor ECOG PS (≥2 vs 1 vs 0), site of metastasis (liver vs other organs vs lymph nodes only), Hb < 11 g/dL, and NLR ≥ 3.

It was notable that Hb levels and NLR were significantly associated with OS both as continuous and binary variables at multiple cutoffs. The best cutoffs for Hb levels and NLR were screened via time-dependent ROC analysis. The optimal cutoff values (area under the ROC curve [AUC]) for Hb at 6, 12, 18 and 24 mo were ≤10.2

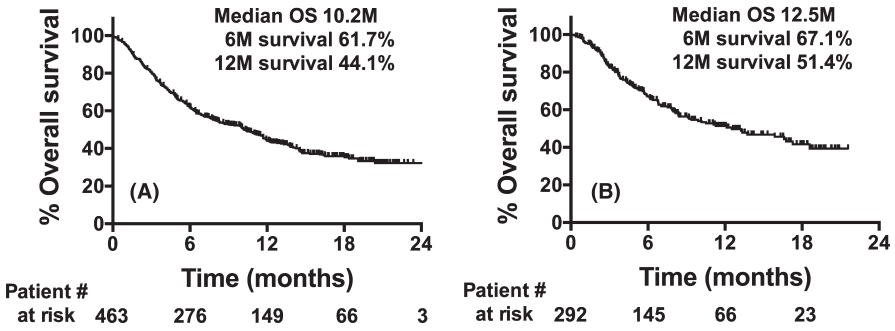

FIGURE 1 Kaplan-Meier plots displaying overall survival (OS) for 463 patients in the discovery cohort (A) and 292 patients in the validation cohort (B). The numbers of patients at risk are presented below the figure

TABLE 2 Univariate and multivariate Cox proportional hazard analyses of overall survival based on key parameters

		Univariate				*Multivariate*			
		HR	Lower 95%	Upper 95%	*P* value	HR	Lower 95%	Upper 95%	*P* value
Age	≥75 y	Ref.				Ref.			
	<75 y	0.8729	0.6797	1.1209	.2865	1.1948	0.9156	1.5591	.1901
Sex	Male	Ref.				Ref.			
	Female	1.1086	0.8354	1.4522	.4680	1.2737	0.9049	1.7929	.1655
Involvement of upper tract	Yes	1.0746	0.8457	1.3636	0.5549	1.0685	0.8246	1.3844	.6163
	No	Ref.				Ref.			
Surgical removal of primary site	Yes	Ref.				Ref.			
	No	1.3516	1.0635	1.7153	.0139	1.1842	0.9235	1.5186	.1827
History of NMIBC	Yes	Ref.				Ref.			
	No	1.2144	0.9089	1.6518	.1930	1.0430	0.7564	1.4383	.7972
Time since most recent chemotherapy	<90 d	1.3089	1.0317	1.6620	.0266	0.9872	0.7597	1.2595	.8643
	≥90 d	Ref.				Ref.			
Smoking history	Yes	1.1782	0.9233	1.5112	.1887	1.3979	1.0346	1.8889	.0291
	No	Ref.				Ref.			
NLR	<3	Ref.				Ref.			
	≥3	2.2397	1.7330	2.9210	<.0001	1.5736	1.1960	2.0703	.0012
Hb	≥11 g/dL	Ref.				Ref.			
	<11 g/dL	2.2490	1.7502	2.9100	<.0001	1.6559	1.2510	2.1918	.0004
Metastasis site	LN only	Ref.				Ref.			
	Other organs	1.7526	1.3069	2.3724	.0002	1.6304	1.2064	2.2033	.0015
	Liver	3.3093	2.3842	4.6079	<.0001	2.5368	1.7910	3.5930	<.0001
ECOG PS	0	Ref.				Ref.			
	1	1.7759	1.3403	2.3524	<.0001	1.5650	1.1687	2.0957	.0026
	≥2	4.3243	3.1815	5.8555	<.0001	2.9529	2.1357	4.0827	<.0001

Abbreviations: NMIBC, non–muscle-invasive bladder cancer; ECOG PS, Eastern Cooperative Oncology Group performance status; Hb, hemoglobin; NLR, neutrophil-to-lymphocyte ratio.

(0.656), ≤11.0 (0.672), ≤11.0 (0.639), and ≤11.0 (0.617) g/dL, respectively. The optimal cutoff values (AUC) for NLR at 6, 12, 18, and 24 mo were ≥3.46 (0.674), ≥3.37 (0.685), ≥3.04 (0.663), and ≥3.04 (0.650), respectively. Furthermore, sensitivity analyses of Hb and NLR indicated that Hb < 11 g/dL (Figure S1) and NLR ≥ 3 (Figure S2) were the optimal cutoff values for prognostication.

The multivariate Cox proportional hazards models revealed that 5 of the 11 variables were significantly and independently associated with unfavorable OS: smoking history (yes vs no), NLR (≥ 3 vs <3), Hb (<11 vs ≥11 g/dL), site of metastasis (liver vs other organs vs lymph nodes only), and poor ECOG PS (≥2 vs 1 vs 0) (Table 2). LASSO regression analysis automatically selected 6 independent prognosticators; surgical removal of primary site (no vs yes), smoking history (yes vs no), NLR (≥3 vs <3), Hb (<11 vs ≥11 g/dL), site of metastasis (liver vs other organs vs lymph nodes only), and poor ECOG PS (≥2 vs 1 vs 0) (Table S1).

We assigned the following scores to the 6 dichotomous or trichotomous variables and incorporate them into prognostic models: surgical removal of primary site no (1) or yes (0); smoking history yes (1) or no (0); NLR ≥ 3 (1) or <3 (0); Hb < 11 g/dL (1) or ≥11 g/dL (0); metastasis in the liver (2), other organs (1), or lymph nodes only (0) and ECOG PS ≥ 2 (2), 1 (1), or 0 (0). As shown in Table S2, a four-factor model (NLR, Hb, Metastasis site, ECOG PS) yielded an equivalent accuracy as assessed by C-index to five- or six-factor models using surgical removal of primary site and/or smoking history in addition to the 4 factors, whereas three-factor models showed lower C-indices. Due to simplicity and usability, the four-factor model was selected for further investigation. Each of the 4 variables discriminated the OS of the patients (Figure 2A-D) with HRs based on the assigned scores (Figure 2E).

In the four-factor model, each patient was assigned a risk score sum ranging from 0 to 6 with widely differing HRs for OS (Figures 3A

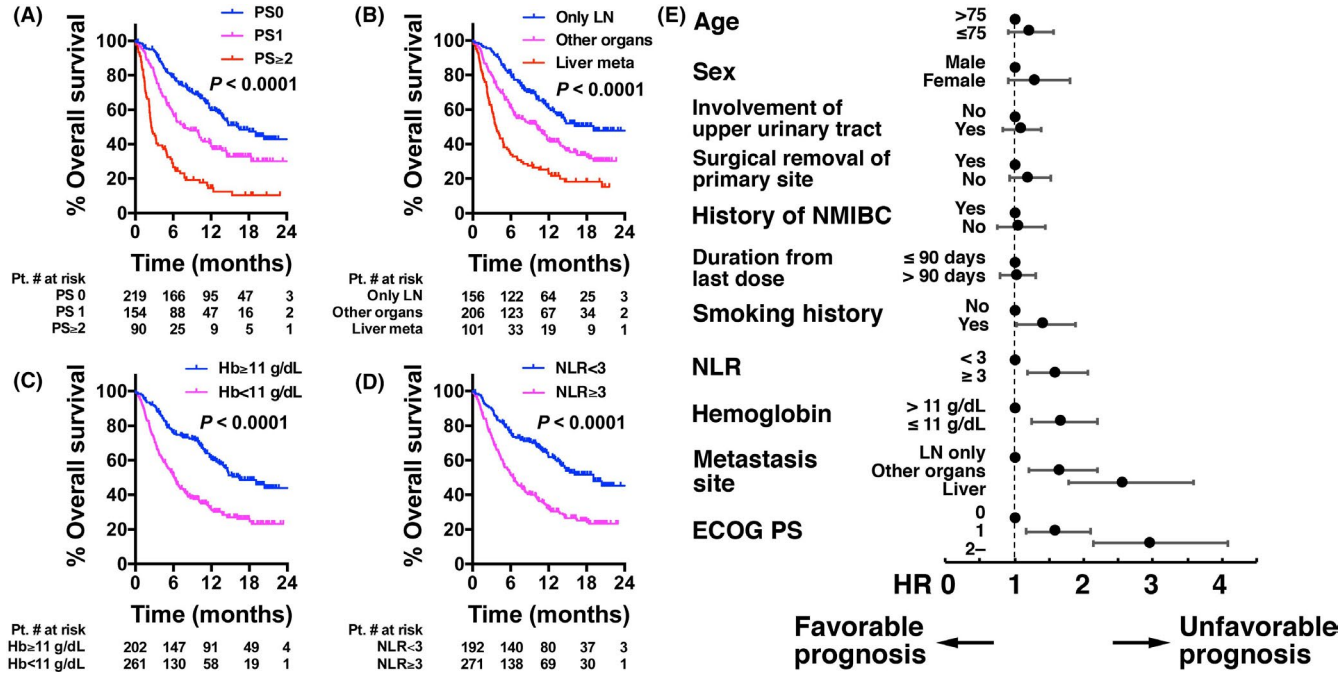

FIGURE 2 Kaplan-Meier plots displaying overall survival for 463 patients in the discovery cohort based on Eastern Cooperative Oncology Group performance status (PS; A), site of metastasis (B), hemoglobin (Hb) levels (C), and the neutrophil-to-lymphocyte ratio (NLR; D). E, Forest plot depicting hazard ratios (HRs) for overall survival with 95% confidence interval (CI) concerning the indicated clinical factors

and S3A) and a well balanced distribution (Figure 3B). Based on the HRs, we divided patients into 3 groups, namely low-risk (score = 0-1, n = 119, HR: 1.00-1.14), intermediate-risk (score = 2-5, n = 321, HR: 2.25-8.48), and high-risk (score = 6, n = 23, HR: 17.72) groups, yielding wide separations of the Kaplan-Meier curves of the groups ($P < .0001$, log-rank test; Figure 3C and Table 3).

Data for the best objective response were available for 422 (91.1%) patients in the discovery cohort. Importantly, the risk stratification revealed a highly significant correlation with objective response ($P < .0001$, chi-squared test, Figure 3D). An objective response based on tumor shrinkage (complete response [CR] or partial response [PR]) was obtained in 48.3%, 28.8%, and 10.5% of patients in the low-, intermediate-, and high-risk groups, respectively. It was confirmed that the best objective response was significantly associated with OS ($P < .0001$, log-rank test, Figure 3E).

3.3 | External validation of the model using an independent cohort

We applied the risk stratification criteria to the independent validation cohort (N = 292). The risk group distribution of patients in the validation was similar to that in the discovery cohort (Table 3 and Figure S3B). Based on the risk criteria, the prognoses of the patients in the validation cohort were successfully separated ($P < .0001$, log-rank test; Figure 3F and Table 3), as observed for the discovery cohort. The calibration plots demonstrated minimal deviation of the predicted prognoses from the actual patient survival (Figure S3C).

We compared our model with the previously reported models incorporating so-called Bellmunt factors (ECOG PS > 0, Hb <10 g/dL, the presence of liver metastases, and a time since the completion or discontinuation of previous therapy < 3M)[1,12,13] using the validation cohort. C-index for Bellmunt model was 0.700, whereas that for our model was 0.747. Bellmunt's 4 factors stratified the 292 patients into 5 groups (those harboring 0 to 4 factors) with good prognostications (Figure S4A), while similar survival curves were drawn for those with 1 or 2 risk factors and those with 3 or 4 risk factors (Figure S4B). Accordingly, a 3-group model comprising those with no risk factor, those with 1 or 2 risk factors and those with 3 or 4 risk factors showed a good prognosticating ability. Compared with the Bellmunt model, our risk stratification model was characterized by high HR for high-risk group (Figure S4C).

Data for the best objective response were available in 265 (90.8%) patients in the validation cohort. Similar to the results in the discovery cohort, the risk stratification exhibited a significant correlation with objective response ($P < .0001$, chi-squared test; Figure 3G) in the validation cohort. An objective response based on tumor shrinkage (CR or PR) was obtained in 29.4, 20.8, and 0% of patients in the low-, intermediate-, and high-risk groups, respectively. It was also confirmed that the best objective response was significantly associated with OS ($P < .0001$, log-rank test; Figure 3H).

When the model was applied to the prognoses of the patients in the overall 755 study patients, the prognostic model functioned well ($P < .0001$, log-rank test; Figure 3I), as observed for the 2 original cohorts. Similarly, the risk stratification was significantly correlated with objective response ($P < .0001$, chi-squared test; Figure 3J). It was also confirmed that the best objective response was significantly associated with OS ($P < .0001$, log-rank test; Figure 3K).

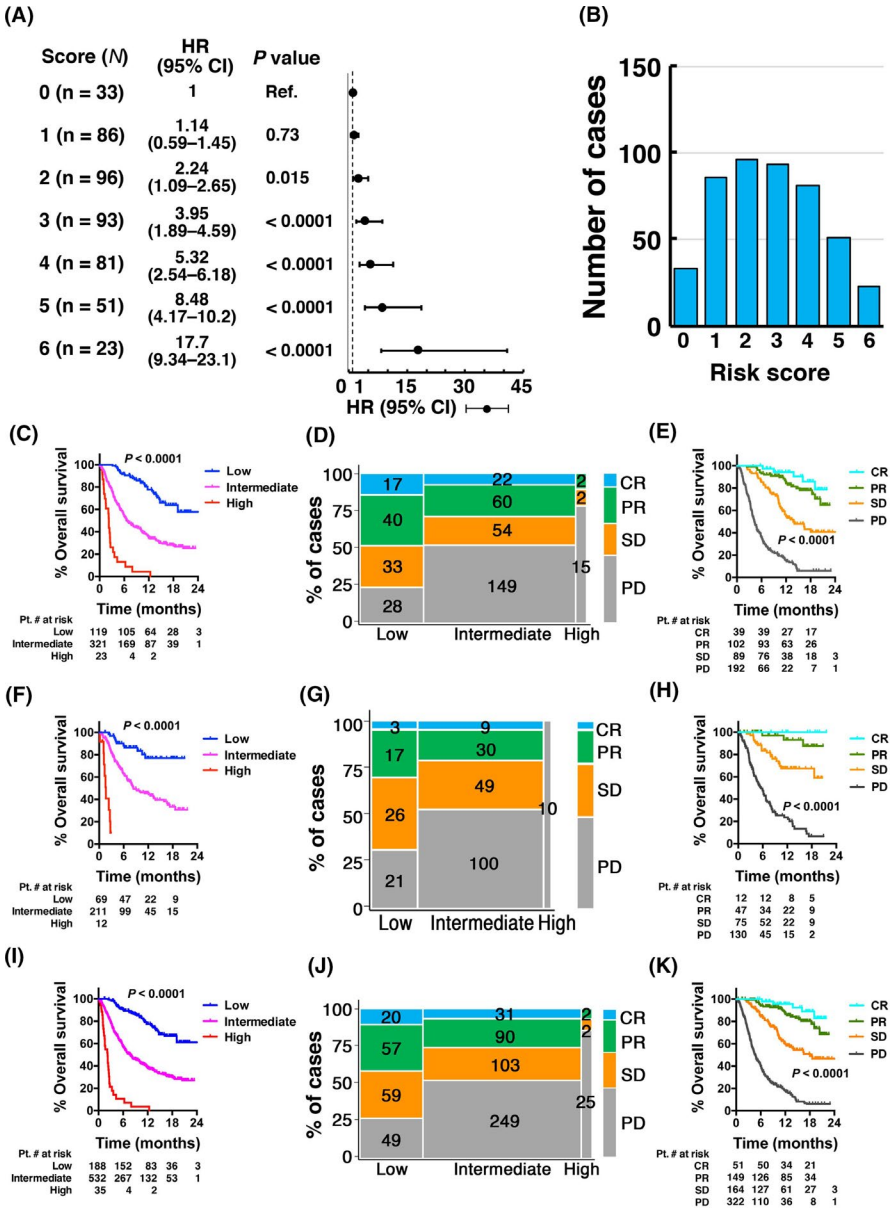

FIGURE 3 A, Forest plot depicting the hazard ratios (HRs) for overall survival with 95% confidence intervals [CIs] as error bars) concerning each risk score category. B, Histogram visualizing the distribution of patients assigned to each risk score category. C, Kaplan-Meier plot displaying overall survival for 463 patients in the discovery cohort by risk group. D, Mosaic (Marimekko) plot showing the cross-sectional distribution of the best objective response for each risk group. E, Kaplan-Meier plot displaying overall survival for 422 patients in the discovery cohort concerning the best objective response. F, Kaplan-Meier plot displaying overall survival for 292 patients in the validation cohort by risk group. G, Mosaic (Marimekko) plot showing the cross-sectional distribution of the best objective response by risk group. H, Kaplan-Meier plot displaying overall survival for 265 patients in the validation cohort concerning the best objective response. I, Kaplan-Meier plot displaying overall survival for all 755 study patients by risk group. J, Mosaic (Marimekko) plot showing the cross-sectional distribution of the best objective response by risk group. K, Kaplan-Meier plot displaying overall survival for all 755 study patients concerning the best objective response

3.4 | Use of the model in patients receiving pembrolizumab after perioperative or first-line chemotherapy and those receiving pembrolizumab in the third line or later

The discovery cohort included patients who received pembrolizumab in the second-line setting after perioperative chemotherapy (N = 72), first-line systemic chemotherapy (N = 274), second-line chemotherapy (N = 83), or third-line chemotherapy or later (N = 34), and the validation cohort similarly included patients who received pembrolizumab after perioperative chemotherapy (N = 41), first-line systemic chemotherapy (N = 184), second-line chemotherapy (N = 51), or third-line chemotherapy or later (N = 16) (P = .6442, chi-squared test). Because pembrolizumab is currently used in the second-line setting (after perioperative or first-line chemotherapy) in most cases, we tested the prognosticative ability of our model in patients who received pembrolizumab in the first or second lines. The analysis of 571 patients who received pembrolizumab after perioperative or first-line chemotherapy in both cohorts revealed that the risk stratification model had similar predictive ability for OS (P < .0001, log-rank test; Figure 4A) and best objective response (P < .0001, chi-squared test; Figure 4B). In this patient population, the best objective response was significantly associated with OS (n = 534, P < .0001, log-rank test; Figure 4C). Conversely, the analysis of 184 patients who received pembrolizumab in the third or later lines (after second-line chemotherapy or later) in both cohorts also demonstrated that the risk stratification model had similar predictive ability for OS (P < .0001, log-rank test; Figure 4D) and best objective response (P < .0001, chi-squared test; Figure 4E). Best objective response was significantly associated with OS in this population (n = 166, P < .0001, log-rank test; Figure 4F).

TABLE 3 Radiological and survival outcomes for the discovery and validation cohorts and for patients who received pembrolizumab in the first- or second-line setting

Cohort	N (%)	Median OS	Lower 95%CI	Higher 95%CI	HR[b]	Lower 95%CI	Higher 95%CI	*P*-value
Discovery	463 (100)							
Low	119 (25.7)	NYR	19.08	NYR	1.00	–	–	Ref.
Intermediate	321 (69.3)	6.82	5.83	8.69	3.57	2.49	5.13	<.0001
High	23 (5.0)	2.26	1.25	2.56	15.25	8.87	26.2	<.0001
Validation	292 (100)							
Low	69 (23.6)	NYR	NYR	NYR	1.00	–	–	Ref.
Intermediate	211 (72.3)	8.42	7.02	13.34	3.92	2.10	7.30	<.0001
High	12 (4.1)	1.64	1.08	2.62	40.96	15.99	104.9	<.0001
Earlier treatment[a]	571 (100)							
Low	142 (24.9)	NYR	19.08	NYR	1.00	–	–	Ref.
Intermediate	406 (71.1)	7.90	6.75	10.20	3.62	2.51	5.24	<.0001
High	23 (4.0)	1.54	1.05	2.39	24.78	14.05	43.71	<.0001

Abbreviations: OS, overall survival; CI; confidence interval, HR; hazard ratio, NYR; not yet reached.

[a]Patients who received pembrolizumab in the first or second line.

[b]Hazard ratio and *P*-value vs the high-risk group.

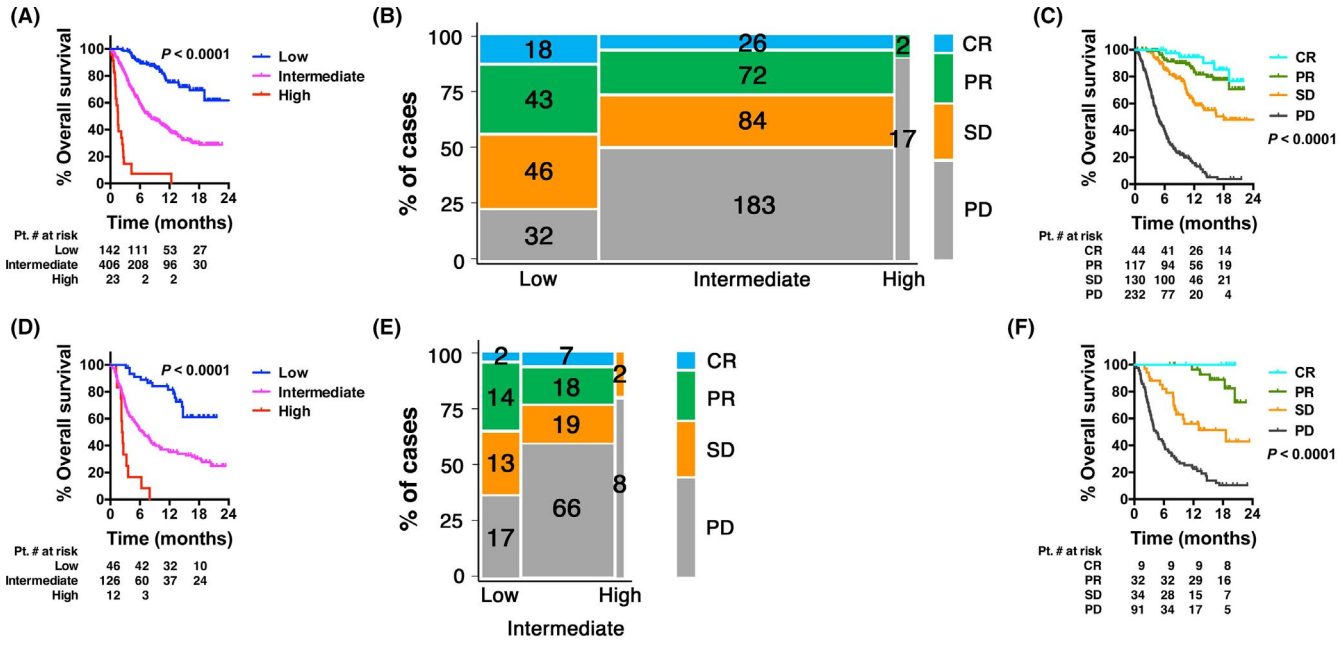

FIGURE 4 A, Kaplan-Meier plot displaying overall survival for 571 patients who received pembrolizumab in the first- or second-line setting by risk group. B, Mosaic (Marimekko) plot showing the cross-sectional distribution of the best objective response for 521 patients who received pembrolizumab in the first- or second-line setting by risk group. C, Kaplan-Meier plot displaying overall survival for 521 patients who received pembrolizumab in the first- or second-line setting by the best objective response. D, Kaplan-Meier plot displaying overall survival for 184 patients who received pembrolizumab in the third or later lines by risk group. E, Mosaic (Marimekko) plot showing the cross-sectional distribution of the best objective response for 166 patients who received pembrolizumab in the third or later lines by risk group. F, Kaplan-Meier plot displaying overall survival for 166 patients who received pembrolizumab in the third or later lines by the best objective response

3.5 | Other clinical factors and survival of the patients treated with pembrolizumab

Other clinical variables including the number of doses of pembrolizumab, front-line chemotherapeutic agents, serum creatinine, serum albumin and the number of metastasized organs were analyzed using data of overall 755 patients for the associations with OS after pembrolizumab treatment. Not surprisingly, the number of doses of pembrolizumab was positively correlated with OS (HR 0.773, 95% CI 0.747-0.798; $P < .0001$), which must reflect durable response to

the pembrolizumab treatment. Front-line chemotherapeutic agents (vs non-platinum, CDDP, HR 1.274, 95% CI 0.918-1.768, $P = .1473$; CBDCA, HR 1.190, 95% CI 0.829-1.707, $P = .3453$; CDGP, HR 1.090, 95% CI 0.574-2.070, $P = .7927$), serum creatinine (HR 0.969, 95% CI 0.853-1.072, $P = .5824$) and the presence of histological variants (HR 0.812, 95% CI 0.566-1.165, $P = .2584$) were not significantly associated with OS. Serum albumin was significantly correlated with ECOG PS (3.8 ± 0.03 g/dL for PS0, 3.6 ± 0.04 g/dL for PS1, 3.1 ± 0.05 g/dL for PS ≥ 2, $P < .0001$, Kruskal-Wallis test) and significantly associated with OS (HR 0.450, 95% CI 0.387-0.526, $P < .0001$). The number of metastasized organs was significantly correlated with metastasis site ($P < .0001$, chi-square test) and significantly associated with OS (single vs multiple organs, HR 0.390, 95% CI 0.320-0.476, $P < .0001$).

3.6 | Adverse events

AE reported for the overall 755 study patients are shown in Table 4. Rate of any grade AE was 36.6% (n = 276), while that of ≥grade 3 AE was 15.8% (n = 119).

3.7 | Subsequent treatment

At the data cutoff, pembrolizumab treatment was ongoing in 22.9% (n = 173) of patients, although it had been discontinued due to good disease control (4.2%, n = 32), AE (7.5%, n = 57), progressive disease (59.9%, n = 452) or other reasons (5.4%, n = 41) (Figure S5A). Of the 550 who ceased pembrolizumab not for good disease control, only 95 (17.3% of 550 or 12.6% of overall 755 patients) received subsequent anti-cancer treatment. Of the 95, 51 were given taxan-containing and 53 were given platinum-containing regimens, while 16 contained both and 6 contained neither (Figure S5B).

There was no significant difference between taxan-containing and platinum- containing regimens in best objective response (Figure S5C,D). Median OS of the 95 patients after initiation of subsequent treatment was 9.5 mo (Figure S5E) and there was no significant difference between taxan- and platinum-containing regimens ($P = .276$, log-rank test; Figure S5F). Neither were there statistically significant differences in OS between those discontinued pembrolizumab due to progressive disease or AE ($P = .290$, log-rank test; Figure S5G) or between those received 1 or 2 chemotherapy regimen prior to pembrolizumab treatment ($P = .752$, log-rank test; Figure S5H).

4 | DISCUSSION

Because few treatment options are available for patients with chemoresistant unresectable UC, pembrolizumab has been used without clear prognostic information. However, several novel therapeutic agents such as erdafitinib[4] and enfortumab vedotin[5] are being newly introduced for use in the second-line treatment of this malignancy.

TABLE 4 Adverse events in the 755 study patients

	Any grade, N (%)	grade ≥ 3, N (%)
Any event	277 (36.7)	120 (15.9)
Event leading to discontinuation of pembrolizumab	56 (7.4)	40 (5.3)
Event leading to death	6 (0.8)	6 (0.8)
Skin disorder	61 (8.1)	8 (1.1)
Endocrine disorder	64 (8.5)	15 (2.0)
Gastrointestinal disorder/diarrhea	52 (6.9)	16 (2.1)
Liver injury/hepatitis	41 (5.4)	15 (2.0)
Lung injury/interstitial pneumonitis	41 (5.4)	27 (3.6)
General fatigue	33 (4.4)	6 (0.8)
Infection	21 (2.8)	15 (2.0)
Kidney injury	18 (2.4)	13 (1.7)
Neurological disorder	18 (2.4)	2 (0.3)
Myelosuppression	15 (2.0)	3 (0.4)
Infusion reaction	5 (0.7)	0 (0.0)
Arthritis	3 (0.4)	1 (0.1)
Stomatitis	2 (0.3)	0 (0.0)
Diabetes mellitus	2 (0.3)	1 (0.1)
Pancreatitis	2 (0.3)	2 (0.3)
Uveitis	1 (0.1)	0 (0.0)
CK elevation	1 (0.1)	1 (0.1)
Pleural effusion	1 (0.1)	1 (0.1)
Edema	4 (0.5)	0 (0.0)
Polymyositis/Dermatitis	1 (0.1)	1 (0.1)
Heart failure	1 (0.1)	1 (0.1)
Meningoencephalitis	1 (0.1)	1 (0.1)
Thrombocytopenia	1 (0.1)	1 (0.1)
Sialadenitis	1 (0.1)	1 (0.1)
Hypercalcemia	1 (0.1)	1 (0.1)

Accordingly, there is an urgent need for better prognostication models for patients with UC in this setting.

The estimated OS at 12 mo in the discovery cohort was 44.1%, which is similar to the results of the initial report (43.9%),[1] the subsequent >2-y follow-up analysis (44.2%),[2] and a subgroup analysis of Japanese subjects (40.0%).[14] The results of the present study are important because they confirmed the prognosis observed in the pivotal clinical trial using large-scale, real-world data. OS was slightly better in the validation cohort in the present study, which may be attributable to the shorter follow-up time in this cohort. Another possible explanation is that the validation cohort included patients who received pembrolizumab in later period of time, which may be associated with the use of pembrolizumab in the earlier line of treatment or better clinical management including immune-related AEs.

In the present study, the newly proposed model using 4 clinical factors predicted OS after the initiation of pembrolizumab treatment. With the goal of developing a parsimonious, flexible, and readily applicable model, we decided to exclude 2 of the 6 factors selected by LASSO regression analysis; surgical removal of primary site and smoking history, the latter of which was associated with a modest HR and significance level in the Cox proportional hazard model of the discovery cohort. Indeed, a model incorporating the 5 or 6 prognosticators including surgical removal of primary site and/or smoking history did not improve prognostication accuracy assessed by C-index. The reduction of input items is desirable from the viewpoint of model simplification and usability. The 4 factors, namely PS, site of metastasis, Hb levels, and NLR, are evaluated in most patients with UC who receive systemic treatment and, therefore, this model can be easily applied and validated in independent cohorts. In the development of this model, we prioritized the discrimination of prognosis more than the balance of patient distribution. The risk stratification revealed a strong correlation with objective response to pembrolizumab treatment. It may indicate that the model predicts OS in patients based on the efficacy of pembrolizumab treatment, whereas it may simply reflected a time-dependent exposure. In any case, Our model may predict the best objective response by pembrolizumab treatment in addition to OS at the pretreatment setting.

The strength of our model is that it has been externally validated using an independent patient cohort showing minimal deviation of the predicted prognosis from the actually observed patient survival. Additionally, our model demonstrated an at least equivalent prognostic accuracy compared with a prognostic model using the Bellmunt factors. Our model adopted additional biomarkers NLR, and lymph node only metastasis as a relatively favorable factor in addition to liver metastasis as an unfavorable prognostic factor. It weighed PS ≥2 and liver metastasis by scoring 2 points. These modifications seemed to improve the prognostication accuracy of the model.

The high-risk criteria of the present model identified patients for whom OS is expected to range only a few months. Although only 4%-5% of patients in both cohorts in the present study were stratified into this group, the high-risk criteria aimed to identify the group of patients with the poorest prognosis, for whom pembrolizumab is not recommended despite being the only available treatment. All high-risk patients died within 1 y, and few exhibited objective responses to pembrolizumab treatment. Considering the risk of AEs and the cost of treatment, pembrolizumab may not be indicated for this population. Additionally, high-risk patients are not likely to be eligible for any clinical trial, but the use of newly introduced treatments is justified without direct comparisons with pembrolizumab.

Patients in the low-risk group had an OS of 60% at 18 mo with a high (30%-50%) objective response rate. Kaplan-Meier curves for this risk group appeared to feature a plateau in the right tail, suggesting long-term survival for the majority of patients in this risk group. High CR or PR rates also suggest durable responses, leading to long-term survival in this group.

Individuals in the intermediate-risk group, comprising the majority of the patients, displayed average OS outcomes. The patients in this group appeared to be highly heterogeneous with relatively wide variation in terms of risk factors and outcomes. Probably, patients meeting these risk criteria are the best candidates for future clinical trials comparing novel treatment strategies with pembrolizumab. The present study provides useful information for clinical trial design including patient recruitment, sample size estimation, and follow-up planning.

In our retrospective analysis, a shorter time since the most recent dose of chemotherapy (<90 d) was a significant factor for unfavorable prognosis, as reported elsewhere.[12] However, the HR was not large or significant in the multivariate analysis.

Instead, NLR was identified as an independent prognostic factor with a high HR. Therefore, we included NLR in addition to conventional predictive factors, namely PS, site of metastasis, and Hb levels. High NLR or lymphocytopenia has been reported to be associated with higher mortality rates in every setting of various malignant diseases.[15] Additionally, it has been reported that high NLR is associated with higher mortality rates in patients with a variety of non-malignant diseases,[16-20] as well as the general population.[21] These findings indicate that higher NLR may act as a strong prognosticator in any population.

Regarding UC, a number of reports identified high NLR as a prognosticator of unfavorable oncological outcomes. Moreover, NLR was reported to be predictive of outcome in a variety of settings, including non-muscle-invasive disease,[22-24] non-metastatic muscle-invasive disease (MIBC) treated via radical cystectomy with[25-27] or without[28-33] neoadjuvant chemotherapy, and MIBC after radical cystectomy.[34] In addition to surgical treatment, high NLR has been reported to be associated with unfavorable oncological outcomes in patients with surgically unresectable metastatic UC receiving first-line[35] or second-line[13,36,37] chemotherapy, in addition to being associated with lower pathological response rates to neoadjuvant chemotherapy.[38] Interestingly, most previous studies reported 2.5-3.0 as the optimal cutoff for NLR. The present study also identified 3.0 as an optimal cutoff based on time-dependent ROC analysis, HR, and patient frequency.

The accumulated evidence on the association between NLR and UC demonstrated the high potential of this variable as a prognosticator in patients with UC. Additionally, the importance of host immune status should be relatively high in UC compared with that in other malignancies considering its high immunogenicity as predicted by the immunotherapeutic effect of bacillus Calmette-Guérin vaccination[39] and the high somatic mutation burden resulting in increased neoantigen levels.[40-42] Indeed, UC is one of the first solid malignancies successfully treated with immune checkpoint inhibitors.[43] Furthermore, the efficacy of immune checkpoint inhibitors is strongly affected by the host immune status and vice versa. In this regard, several investigators have reported that NLR decrease in response to pembrolizumab treatment was associated with better survival,[44-46] although NLR change during pembrolizumab treatment was not available in the present study. Taken together, NLR is expected to be a useful

predictive factor for therapeutic efficacy, particularly in the use of immune checkpoint inhibitors in the treatment of UC.

PS also displayed strong prognostic ability as reported elsewhere.[1,13] Notably, 20% of patients in the present study had a PS of 2 or higher. The pivotal randomized clinical trial KEYNOTE-045 included few (about 1%) patients with a PS of 2. However, most patients with metastatic UC who experience disease progression after chemotherapy have poor PS. To date, few data on the efficacy of pembrolizumab in patients with chemoresistant UC and poor PS have been generated. In a previous report on the survival outcomes of atezolizumab in patients with chemoresistant UC, 10% of patients had a PS of 2, and these patients had significantly shorter survival.[47] Another study using real-world data also identified a negative impact of poor PS (≥2) only in patients who received immune checkpoint inhibitors in the first-line setting.[48] The present study illustrated that patients with poor PS has unfavorable OS, but pembrolizumab can be offered to such patients if they are categorized as intermediate risk based on the absence of other risk factors.

Concerning site of metastasis, the present study demonstrated that patients with only lymph node metastasis can expect better prognosis compared with those with metastatic lesions in other organs, such as the liver as reported previously.[1,13] It is not clear whether the differential survival outcomes arose from differences in the tumor progression rate or from biological differences in the response to pembrolizumab between liver and lymph node metastasis.

Regarding Hb, some previous studies identified 10 g/dL as an optimal cutoff for predicting the oncological outcomes of second-line chemotherapy in patients with UC.[13,49] However, in the present study, time-dependent ROC and sensitivity analyses for HR and frequency revealed that 11 g/dL was the optimal cutoff. Ethnicity, prior treatment burden, or the treatment modality given to the patients (chemotherapy vs. immunotherapy) might affect the association between Hb and prognosis.

Surgical removal of the primary site showed a positive effect on OS in univariate analysis but not in multivariate analysis, whereas smoking history showed a negative effect on OS in multivariate analysis, but not in univariate analysis. Surgical removal of the primary site has been reported to benefit metastatic UC patients, particularly those with lower metastasis burden,[50] although the more direct impact on immune checkpoint inhibitor treatment is unclear. Smoking history has been reported to be associated with higher tumor mutation burden in some tobacco-related cancers including non-small cell lung cancer[51] and head and neck squamous cell carcinoma,[52] suggesting a higher benefit from immune checkpoint inhibitor treatment although there is skeptical opinion for a usefulness as a predictive or prognostic biomarker.[53] Although the present study does not draw a definitive conclusion for the role of surgical removal of primary site or smoking history as a prognostic factor, these factors have been excluded from the final 4-factor model as the incorporation of the 2 factors did not improve prognostication ability of the model. Further studies should be warranted in the future to elucidate the role of these factors as a prognosticator.

The present study analyzed other clinically relevant factors with regard to the association with OS. Doses of pembrolizumab, serum albumin, and the number of metastasized organs were shown to be significantly associated with OS, whereas front-line chemotherapeutic agents, serum creatinine and the presence of histological variant were not. It is not surprising that doses of pembrolizumab was positively correlated with OS, as it must reflect a durable response to pembrolizumab. Unfortunately, it is not a pretreatment factor. Although serum albumin showed a highly significant association with OS, it was well correlated with ECOG PS and missed in quite a few patients, which made it difficult to incorporate to the multivariate prognostication model. The number of metastasized organs also showed strong association with OS, but it was strongly correlated with metastasis site (lymph node only, liver, or other organs). The authors were concerned that the number of lesions could not be taken into consideration such as discrimination between solitary and multiple lung/liver metastases, which were not collected distinguishably. Therefore, the authors decided to incorporate ECOG PS and metastasis site instead of serum albumin and number of metastasized organs, respectively.

The study patients had a heterogeneous treatment history. The majority (61%) received pembrolizumab after first-line systemic chemotherapy (as the second-line treatment), followed by those who received pembrolizumab after second-line chemotherapy (18%). In addition, 15% of patients received pembrolizumab after perioperative chemotherapy, and the remaining 6% of patients received the drug in the fourth line or later. A previous report has recorded the differential prognostic values of PS in relation to the treatment setting.[48] In this regard, our prognostic model worked very well in both earlier (after perioperative or first-line chemotherapy) and later treatment (third line or later), indicating its wide clinical applicability, which is a strength of the model.

There have been inconsistent results reported in terms of ethnicity and clinical benefit regarding the use immune checkpoint inhibitors in the treatment of chemoresistant unresectable UC. For pembrolizumab, no difference in outcomes was noted between global and Japanese populations in the pivotal randomized clinical trial.[1,14] Conversely, better progression-free survival and OS were reported in Japanese patients compared with in the global population in a phase 2 trial of nivolumab.[54] In the previous report, several risk factors including PS and liver metastasis favored the Japanese population, whereas the population of the present study included patients with PS ≥ 2. Because of differences in patient backgrounds, further studies are warranted to clarify the influence of race or ethnicity on the efficacy of immune checkpoint inhibitors in patients with UC.

The present study utilized 2 independent cohorts to develop and validate a prognostic model. This method is commonly used in this kind of studies on prognostic models.[8-10] Our analyses would have been statistically more powered if we had utilized all 755 patients from the initial setting. This might have resulted in several more variables being identified as statistically significant prognostic factors. However, the hazard ratio of additionally identified prognostic

factors would be usually modest and the model would have the concern of overfitting, which will not be likely to be externally validated in the subsequent studies. Additionally, a model with too many variables is not practically useful and we consider that 3 to 5 variables are the most user friendly in the clinical practice as reported previously.[7,55,56] Thus, with the goal of developing a simple, flexible, and readily applicable clinical model, we made a decision to follow this method using 463 patients for the development of the model and the subsequent 292 for external validation.

In terms of AE profile, any grade AE was reported in 36.7% of the patients in the present study, this percentage is lower compared with 60.9% at the initial report[1] and 62.0% at >2-y follow-up[2] of the participants in KEYNOTE-045 study. This is probably attributed to the difference in the way of AE screening and reporting between real-world practice and clinical trial. Our AE rate is comparable with real-world data reported for lung cancer patients treated with nivolumab or pembrolizumab.[57] Grade ≥ 3 AE was reported in 15.9% of the patients in the present study, which is consistent with 15.0% at the initial report[1] and 16.5% at >2-y follow-up[2] of the participants in KEYNOTE-045 study. Rates of specific severe AE, such as events leading to treatment discontinuation, events leading to death and pneumonitis were also comparable with those reported from KEYNOTE-045 study.[1,2]

For subsequent therapy, the majority of the patients in the present study received only best supportive care upon discontinuation of pembrolizumab treatment due to disease progression or AE, suggesting that the 95 patients who received subsequent anti-cancer therapy had been a highly selected population. Of those 95 patients, objective response was obtained approximately in 25%, this percentage is consistent with that of a previous report.[58] Median OS after initiation of subsequent therapy was 9.1 mo, which is also comparable with another previous report.[59] There seemed to be no significant differences between taxan-containing and platinum-containing regimens. Although we did not identify any predictive factors for the potential benefit of subsequent anti-cancer therapy after pembrolizumab treatment, the efficacy of front-line chemotherapy prior to pembrolizumab treatment or general status of the patients including PS may have some impact. At the data cutoff of the present study, 31% (n = 29) of the 95 patients were still on subsequent treatment and 27% (n = 205) of the overall study patients were alive with disease under control by pembrolizumab. Future studies based on further follow-up will clarify the issue of optimal target population and treatment regimens in the subsequent therapy following pembrolizumab. Nonetheless, our findings suggested that subsequent anti-cancer therapy is beneficial in highly selected patients with metastatic UC who discontinued pembrolizumab treatment due to progressive disease or AE.

Some limitations of this study should be acknowledged. Objective response or pathological diagnosis was determined in each study institute, and thus, the findings were not centralized. The characteristics of pembrolizumab treatment, particularly when to discontinue treatment, were not standardized throughout the study. Nonetheless, this was the largest study to analyze OS in patients receiving pembrolizumab for the treatment of chemoresistant UC. It is unclear how previous chemotherapy or current pembrolizumab treatment affected Hb and NLR in the study patients.

Unlike the pivotal randomized clinical trial,[1] PD-L1 expression or tumor mutation burden was not examined in the present study. Similarly, analysis of tumor-infiltrating lymphocytes in the context of the tumor microenvironment may have some role in predicting the efficacy of immune checkpoint inhibitors.[60] In the management of patients with advanced UC, we do not usually perform surgical resection of chemoresistant tumors, which may have accumulated additional mutation and altered PD-L1 expression compared with those at the pretreatment setting. Therefore, a prognostic model based on clinical variables is clinically relevant and useful until a novel biomarker is introduced to clinical practice in the future.

The clinical variables proposed in the present study may not be totally novel and some of previously reported clinical prognosticators such as C-reactive protein (CRP)[61] were not incorporated in the present study due to data availability. Nonetheless, the authors believe that the prognostic model is novel and worth reporting as one of most powerful tool for prognostication of chemoresistant UC patients treated with pembrolizumab.

Although those predictive biomarkers may be introduced in future studies, they are not widely available in real-world practice. Until then, our prognosis model using conventional clinical parameters should be used as a strong prognosticator. Furthermore, it is easy to validate and apply in real-world practice, and it will be useful and informative for the design of future clinical trials.

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CONFLICT OF INTEREST

All authors declare there is no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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APPENDIX 1

Study institutes: Osaka City University, Akita University, Hirosaki University, National Cancer Center Hospital, Hamamatsu University School of Medicine, Yamagata University Faculty of Medicine, Kyoto University, University of Tsukuba, Nara Medical University, Shizuoka General Hospital, University of the Ryukyus, Iwate Medical University, Oita University, Hiroshima University, Shimane University, Kansai Medical University, Osaka University, Kagawa University, University of Yamanashi, Japanese Red Cross Wakayama Medical Center, Kyoto Prefectural University of Medicine, Kobe City Nishi-Kobe Medical Center, Japanese Red Cross Osaka Hospital, Nagoya University, Harasanshin Hospital, Hokkaido University, Japanese Red Cross Otsu Hospital, Kagoshima University, Kyushu University, Shikoku Cancer Center, Tenri Hospital, Hakodate Goryoukaku Hospital, Kitasato University, Kyoto Katsura Hospital, National Hospital Organization Kyoto Medical Center, Kumamoto University, National Hospital Organization Himeji Medical Center, Tazuke Kofukai Medical Research Institute, Kitano Hospital, Toyooka Hospital, Hokkaido Cancer Center, University of Miyazaki, Hitachi General Hospital, The Jikei University Kashiwa Hospital, Shimada Municipal Hospital, Mie University, Yamaguchi University, Ibaraki Prefectural Central Hospital, Kyoto City Hospital, Kochi School of Medicine, Ijinkai Takeda General Hospital, University of Toyama, Otsu City Hospital, Sapporo Medical University, Kansai Electric Power Hospital, Kurume University, Hyogo College of Medicine, Hirakata Kohsai Hospital, Rakuwakai Otowa Memorial Hospital.