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Dynamic Changes in Absolute Lymphocyte Counts During Eribulin Therapy Are Associated With Survival Benefit

SHOGO NAKAMOTO, MASAHIKO IKEDA, SHINICHIRO KUBO, MARI YAMAMOTO and TETSUMASA YAMASHITA

Division of Breast and Thyroid Gland Surgery, Fukuyama City Hospital, Hiroshima, Japan

Abstract. *Background/Aim: We investigated the usefulness of dynamic changes in absolute lymphocyte count (ALC) and neutrophil-to-lymphocyte ratio (NLR) during eribulin therapy as predictive markers for survival benefit including post-progression survival (PPS). Patients and Methods: We retrospectively investigated 94 advanced breast cancer (ABC) patients who underwent eribulin therapy between July 2011 and June 2020. Results: The multivariate analysis showed that high baseline ALC and low NLR were independent predictive markers for overall survival (OS) (p=0.007 and p=0.011, respectively) and PPS (p=0.005 and p=0.007, respectively). Dynamic changes in ALC were also associated with OS and PPS (p=0.015, and p=0.026, respectively) and were an independent predictive marker for PPS (p=0.021). Conclusion: Baseline ALC and NLR and dynamic changes in ALC during eribulin therapy were significantly associated with survival benefit including PPS for patients with ABC.*

As eribulin methylate (eribulin) has reportedly improved overall survival (OS) in several clinical trials (1, 2), it is a preferred option for patients with human epidermal growth factor receptor-2 (HER2)-negative advanced breast cancer (ABC). In the EMBRACE study, eribulin significantly prolonged OS compared with the treatment of the physician's choice [hazard ratio (HR)=0.81; 95%confidence interval (CI)=0.66-0.99; *p*=0.041] (3) without prolonging progression-free survival (PFS). In the 301 study, eribulin showed a strong tendency for improved OS compared with capecitabine (HR=0.88; 95%CI=0.77-1.00; *p*=0.056) (4) without prolonging PFS. Pooled analyses demonstrated the significant OS benefit of eribulin compared with controls (5, 6). Data from several real-world studies have shown an OS improvement with eribulin compared with conventional chemotherapy for patients with ABC (7, 8). However, the biomarkers predicting survival from eribulin therapy remain unknown.

Reports have demonstrated that eribulin has various effects (in addition to its antitumor activity), which include vascular remodeling (9, 10), inhibition of epithelial–mesenchymal transition (EMT) (11), improvement of the tumor micro-environment (10, 12), and biological effects on the immune system (13), that might be associated with OS improvement. We therefore hypothesized that eribulin could improve OS by prolonging post-progression survival (PPS) without improving PFS, thereby influencing post-eribulin therapy.

Studies have indicated that systemic immunity markers, including the absolute lymphocyte count (ALC) and neutrophil-to-lymphocyte ratio (NLR), are useful prognostic factors in various cancers including breast cancer (14-17). In addition to being prognostic factors, baseline ALC and NLR have been identified as predictive markers for patients with ABC undergoing eribulin and bevacizumab therapy (18-21). However, the immune system and the tumor microenvironment are not static and can undergo dynamic changes during treatment. Li *et al*. reported that the activation of the systemic immune system evaluated by kinetic changes in serum inflammatory factors was associated with a good response to bevacizumab for patients with advanced non–small-cell lung cancer (22). Thus, dynamic changes in systemic immunity markers during a certain treatment could be associated with a survival benefit.

We therefore evaluated the usefulness of the dynamic changes in systemic immunity markers during eribulin therapy as predictive markers for survival benefit including PPS.

Patients and Methods

Patients. We retrospectively investigated patients with HER2-negative ABC who underwent eribulin therapy at Fukuyama City Hospital between July 2011 and June 2020, with a data cutoff date of August 31, 2020. The tumor response was assessed according to the Response Evaluation Criteria in Solid Tumors version 1.1 (23).

Correspondence to: Shogo Nakamoto (ORCID ID SN: 0000-0001-8043-8608), Division of Breast and Thyroid Gland Surgery, Fukuyama City Hospital, 5-23-1 Zao, Fukuyama, Hiroshima, 7218511, Japan. Tel: +81 849415151, Fax: +81 849415159, e-mail: p92c9f20@s.okayama-u.ac.jp

Key Words: Advanced breast cancer, eribulin, overall survival, post-progression survival, dynamic changes, absolute lymphocyte count.

The subtypes included estrogen receptor (ER), progesterone receptor (PgR), and HER2 expression. We defined HER2 over-expression according to the American Society of Clinical Oncology/College of American Pathologists guidelines (24).

This retrospective study was approved by the review board of Fukuyama City Hospital (approval number: 538). All procedures that involved human participants were performed in accordance with the ethical standards of the institutional and/or national research committees and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants in the form of an opt-out on the website included in the study.

Treatments. The study patients underwent eribulin therapy, which was administered intravenously at a dose of 1.4 mg/m^2 on days 1 and 8 for each 21-day cycle (3). Eribulin therapy was continued until disease progression, unacceptable toxic effects, changes in the physician's judgment, or changes in the patient's preferences. Dose modification, interruption, and discontinuation of the therapy were decided based on daily clinical practice. In Japan, physicians can use eribulin in any chemotherapy line for patients with ABC if anthracyclines and taxanes have been previously administered. We administered eribulin again, with sufficient informed consent, as a late-line therapy after several other regimens had been administered (20).

Measurements of systemic immunity markers. Neutrophil and lymphocyte counts were measured automatically by Sysmex XE-2100 or XE-5000 automated hematology system (Sysmex Co., Kobe, Japan). ALC and NLR were calculated from blood cell counts at baseline (just prior to administering eribulin), at the start of the second cycle, and at the end of the therapy. Baseline ALC and NLR cutoff values were set on the basis of previous studies: 1500/μl for ALC and 3 for NLR (17, 18, 25). As for the dynamic changes in immunity markers, if ALC or NLR at the start of the second cycle changed compared to baseline, we defined the changes as "increased ALC(SO2nd)," "decreased ALC(SO2nd)," "increased NLR(SO2nd)," or "decreased NLR(SO2nd)." If ALC or NLR at the end of the therapy changed compared to baseline, we defined the changes as "increased ALC(EOT)," "decreased ALC(EOT)," "increased NLR(EOT)," or "decreased NLR(EOT)."

Statistical analysis. Mann–Whitney *U*-test was used to compare the continuous variables and Fisher's exact test to compare the proportions of the categorical variables between the groups. Survival analyses were performed using the Kaplan–Meier method, and differences were analyzed using the log-rank test. Univariate and multivariate Cox regression analyses were used to determine the association between baseline patient characteristics and survival benefit. Given that the baseline ALC and NLR and the dynamic changes in ALC and NLR apparently intertwined, we did not include these six markers simultaneously in the multivariate analysis. Thus, they were independently included as one confounder in each multivariate analysis on survival benefit. Lastly, we performed this analysis 16 times. *p*-Values <0.05 were considered statistically significant, and all statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria) (26).

We defined time-to-treatment failure (TTF) as the time from administration of eribulin to discontinuation of treatment due to any reason, including disease progression, treatment toxicity, patient/physician decision, and death from any cause. OS was defined as the time from administration of eribulin to the date of death due to any cause. PPS was defined as the time from the end of eribulin therapy to death due to any cause.

Results

Patient characteristics and overall efficacy. We assessed 94 patients with HER2-negative ABC. Table I shows the patients' baseline characteristics at the start of eribulin therapy. Of the 94 patients, 64 (68.1%) were ER-positive and/or PgR-positive, 75 (79.8%) had ≥3 metastatic sites, and 77 (81.9%) had visceral metastases. The median number of prior chemotherapies before eribulin therapy was 2 (range=0-9).

Of the 94 patients, we assessed 80 patients who underwent ≥2 cycles of eribulin therapy when we analyzed the relationship between the dynamic changes in immunity markers and the survival benefit. We assessed 70 patients who underwent subsequent treatment after eribulin therapy when we analyzed the relationship between the immunity markers at baseline and PPS. We also assessed 64 patients who underwent ≥2 cycles of eribulin and subsequent treatment when we analyzed the relationship between the immunity markers, including dynamic changes and PPS. The overall efficacy of eribulin therapy was as follows: median TTF, 92 days (95%CI=70-126); median OS, 313 days (95%CI=238-426); and median PPS, 259 days (95%CI=197-349).

Relationship between systemic immunity markers at baseline and survival benefit. We employed the cutoff values for ALC (high, >1,500/μl; low, ≤1,500/μl) and NLR (high, >3; low, ≤3) and compared the median TTF, OS, and PPS according to the baseline ALC and NLR (Figure 1).

The median TTF of patients with high ALC and low NLR was significantly longer than that of patients with low ALC (201 *vs*. 83 days, HR=0.54, 95%CI=0.31-0.94, log-rank p=0.028; Figure 1A) and high NLR (128 *vs*. 60 days, HR=0.46, 95%CI=0.30-0.73, log-rank p<0.001; Figure 1B), respectively.

The median OS of patients with high ALC and low NLR was significantly longer than that of patients with low ALC (not reached *vs*. 289 days, HR=0.22, 95%CI=0.10-0.52, log-rank p<0.001; Figure 1C) and high NLR (469 *vs*. 234 days, HR=0.36, 95%CI=0.22-0.59, log-rank p<0.001; Figure 1D), respectively.

The median PPS of patients with high ALC and low NLR was significantly longer than that of patients with low ALC (819 *vs*. 226 days, HR=0.23, 95% CI=0.09-0.59, log-rank p<0.001; Figure 1E) and high NLR (384 *vs*. 197 days, HR=0.41, 95%CI=0.23-0.72, log-rank p=0.001; Figure 1F), respectively.

We performed univariate and multivariate analyses to identify the independent predictors of survival with eribulin therapy (Tables II and III). Each of the six multivariate

Table I. *Patient characteristics at the time eribulin therapy was administered.*

Variables	Overall	Dynamic change in ALC[a]			Dynamic change in NLR[a]		
	(n=94)	Decreased (n=30)	Increased (n=50)	*p*-Value	Decreased (n=55)	Increased (n=25)	*p*-Value
Age, years	62 (37-83)	63 (45-83)	62 (37-75)	0.191	62 (37-77)	66 (40-83)	0.264
ER and/or PgR status, n (%)							
Positive	64 (68.1)	20 (66.7)	35 (70.0)	0.806	33 (60.0)	22 (88.0)	**0.018**
Negative	30 (31.9)	10 (33.3)	15 (30.0)		22 (40.0)	3 (12.0)	
Diagnosis, n (%)							
Advanced	26 (27.7)	6 (20.0)	11 (22.0)	1.000	12 (21.8)	5 (20.0)	1.000
Recurrence	68 (72.3)	24 (80.0)	39 (78.0)		43 (78.2)	20 (80.0)	
Metastatic sites, n (%)							
Central nervous system	16 (17.0)	3 (10.0)	9 (18.0)	0.520	8 (14.5)	4 (16.0)	1.000
Bone	54 (57.4)	16 (53.3)	30 (60.0)	0.643	28 (50.9)	18 (72.0)	0.092
Lungs	48 (51.1)	14 (46.7)	25 (50.0)	0.820	27 (49.1)	12 (48.0)	1.000
Pleura and/or lymphangiopathy	39 (41.5)	14 (46.7)	16 (32.0)	0.236	22 (40.0)	8 (32.0)	0.620
Lymph nodes	67 (71.3)	23 (76.7)	32 (64.0)	0.321	38 (69.1)	17 (68.0)	1.000
Liver	50 (53.2)	11 (36.7)	32 (64.0)	**0.022**	30 (54.5)	13 (52.0)	1.000
Soft tissue	49 (52.1)	11 (36.7)	28 (56.0)	0.110	29 (52.7)	10 (40.0)	0.340
Type of metastases, n (%)							
Visceral	77 (81.9)	21 (70.0)	45 (90.0)	**0.033**	45 (81.8)	21 (84.0)	1.000
Non-visceral	17 (18.1)	9 (30.0)	5 (10.0)		10 (18.2)	4 (16.0)	
Number of metastatic sites	4 (1-8)	3.5 (1-6)	4 (1-8)	0.429	4 (1-8)	4 (1-6)	0.660
Number of metastatic sites, n (%)							
≥3	75 (79.8)	23 (76.7)	39 (78.0)	1.000	43 (78.2)	19 (76.0)	1.000
<3	19 (20.2)	7 (23.3)	11 (22.0)		12 (21.8)	6 (24.0)	
Perioperative chemotherapy[b], n (%)							
Yes	43 (45.7)	15 (50.0)	25 (50.0)	1.000	32 (58.2)	8 (32.0)	0.053
No	51 (54.3)	15 (50.0)	25 (50.0)		23 (41.8)	17 (68.0)	
Disease-free interval, n (%)							
<24 months	47 (50.0)	11 (36.7)	25 (50.0)	0.353	29 (52.7)	7 (28.0)	0.053
≥24 months	47 (50.0)	19 (63.3)	25 (50.0)		26 (47.3)	18 (72.0)	
Number of previous therapies							
Chemotherapy	2 (0-9)	1.5 (0-5)	2 (0-9)	0.465	2 (0-7)	2 (0-9)	0.261
Hormone therapy	1.5 (0-9)	1.5 (0-9)	2 (0-8)	0.473	1 (0-9)	3 (0-7)	**0.021**

ALC: Absolute lymphocyte count; ER: estrogen receptor; NLR: neutrophil-to-lymphocyte ratio; PgR: progesterone receptor. Values are in median (range) unless otherwise noted. [a]Dynamic changes at the start of the second cycle; [b]Chemotherapy included anthracycline and/or taxane-based regimen. Statistically significant *p*-Values are shown in bold

analyses identified baseline high ALC and low NLR as independent predictive markers for OS (HR=0.29, 95%CI=0.12-0.71, p=0.007 for high ALC; HR=0.51, 95%CI=0.30-0.86, p=0.011 for low NLR) and PPS (HR=0.23, 95%CI=0.08-0.64, p=0.005 for high ALC; HR=0.44, 95%CI=0.24-0.80, p=0.007 for low NLR), respectively. Although baseline high ALC and low NLR showed a favorable tendency in TTF, there was no significant difference (HR=0.66, 95%CI=0.36-1.22, p=0.18 for high ALC; HR=0.62, 95%CI=0.37-1.04, p=0.067 for low NLR).

Relationship between dynamic changes in systemic immunity markers at the start of the second cycle and survival benefit. We compared the median TTF, OS, and PPS according to the dynamic changes in ALC and NLR at the start of the second cycle. TTF, OS, and PPS were all significantly worse for patients with increased ALC(SO2nd) than for those with decreased ALC(SO2nd) (TTF; 110 *vs*. 166 days, HR=1.65, 95%CI=1.01-2.70, log-rank p=0.044; Figure 2A, OS; 360 *vs*. 582 days, HR=2.03, 95%CI=1.13-3.62, log-rank p=0.015; Figure 2C, PPS; 255 *vs*. 504 days, HR=2.04, 95%CI=1.09-3.81, log-rank p=0.026; Figure 2E). However, there were no differences between increased and decreased NLR(SO2nd) (TTF; 117 *vs*. 126 days, HR=0.74, 95%CI=0.44-1.25, log-rank p=0.254; Figure 2B, OS; 405 *vs*. 406 days, HR=1.15, 95%CI=0.64-2.06, log-rank p=0.635; Figure 2D, PPS; 266 *vs*. 316 days, HR=1.40, 95%CI=0.74-2.62, log-rank p=0.295; Figure 2F).

The results of univariate and multivariate analyses are shown in Tables II and III. Each of the six multivariate analyses identified increased ALC(SO2nd) as an independent

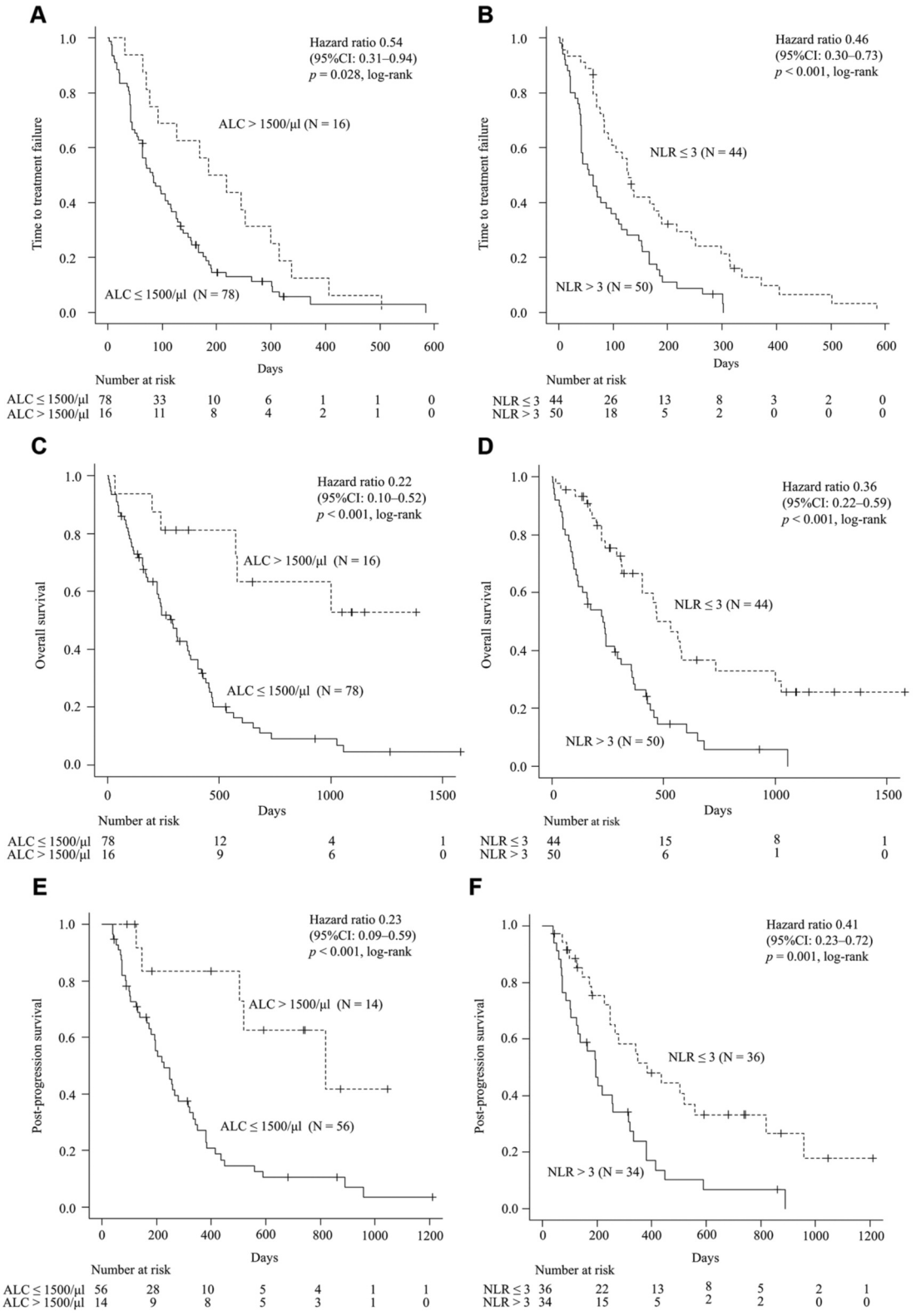

Figure 1. *Time-to-treatment failure according to baseline levels of (A) ALC and (B) NLR and overall survival according to baseline levels of (C) ALC and (D) NLR and post-progression survival according to baseline levels of (E) ALC and (F) NLR in patients treated with eribulin. ALC: Absolute lymphocyte count; NLR: neutrophil-to-lymphocyte ratio.*

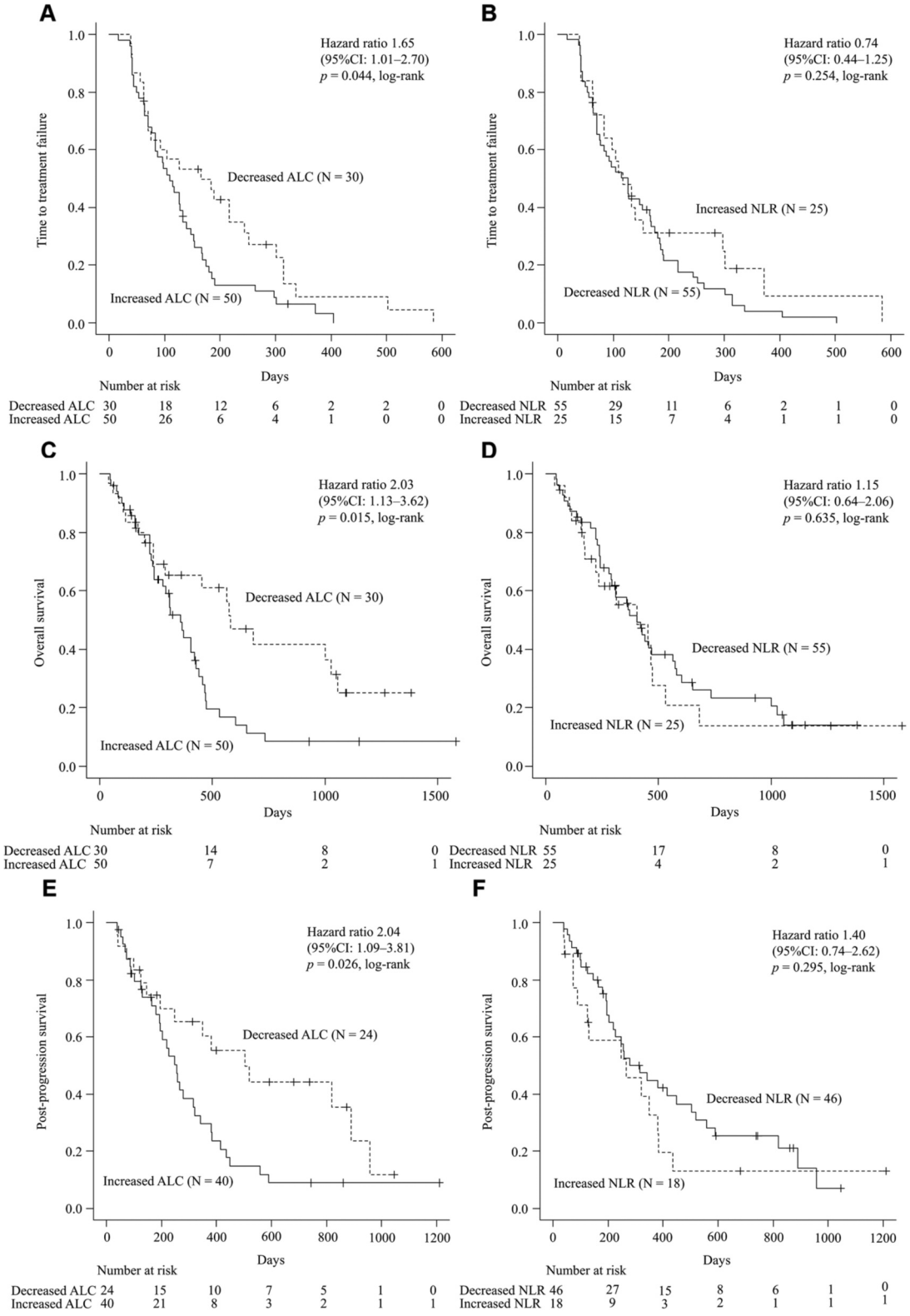

Figure 2. *Time-to-treatment failure according to the dynamic changes in (A) ALC and (B) NLR at the start of the second cycle and overall survival according to the dynamic changes in (C) ALC and (D) NLR at the start of the second cycle and post-progression survival according to the dynamic changes in (E) ALC and (F) NLR at the start of the second cycle in patients treated with eribulin. ALC: Absolute lymphocyte count; NLR: neutrophil-to-lymphocyte ratio.*

Table II. *Univariate analysis of time-to-treatment failure, overall survival, and post-progression survival (Cox hazard model).*

Variables	TTF			OS			PPS		
	HR	95%CI	*p*-Value	HR	95%CI	*p*-Value	HR	95%CI	*p*-Value
Age	0.97	0.95-0.99	**0.012**	0.98	0.96-1.01	0.202	0.98	0.96-1.01	0.256
ER and/or PgR (negative *vs*. positive)	1.55	0.99-2.43	0.054	1.35	0.82-2.20	0.235	0.93	0.51-1.68	0.799
Diagnosis (recurrence *vs*. advanced)	0.49	0.30-0.80	**0.004**	0.58	0.35-0.97	**0.038**	0.72	0.39-1.33	0.295
Metastatic sites (yes *vs*. no)									
Central nervous system	1.67	0.97-2.89	0.066	1.53	0.81-2.88	0.189	1.47	0.71-3.07	0.299
Bone	0.73	0.48-1.13	0.154	0.75	0.47-1.20	0.232	0.93	0.54-1.62	0.804
Lungs	1.12	0.74-1.71	0.593	1.22	0.76-1.97	0.405	1.29	0.74-2.25	0.369
Pleura and/or lymphangiopathy	1.05	0.69-1.62	0.808	1.12	0.70-1.80	0.644	0.94	0.54-1.64	0.822
Lymph nodes	1.56	0.97-2.49	0.064	1.54	0.91-2.61	0.111	1.17	0.65-2.10	0.596
Liver	1.40	0.91-2.18	0.130	1.73	1.05-2.84	**0.030**	2.04	1.15-3.61	**0.014**
Soft tissue	1.91	1.21-3.01	**0.005**	1.78	1.10-2.88	**0.019**	1.65	0.96-2.84	0.071
Visceral metastasis (yes *vs*. no)	0.96	0.56-1.67	0.897	1.49	0.81-2.75	0.204	2.09	1.01-4.31	**0.046**
Number of metastatic sites (≥3 *vs*. <3)	1.43	0.83-2.45	0.193	1.87	1.02-3.43	**0.045**	2.14	1.06-4.28	**0.033**
Perioperative chemotherapya (yes *vs*. no)	0.70	0.45-1.10	0.119	0.51	0.31-0.83	**0.007**	0.48	0.28-0.83	**0.008**
Disease-free interval (<24 months *vs*. ≥24)	2.15	1.39-3.32	**<0.001**	2.06	1.27-3.33	**0.003**	1.78	1.03-3.07	**0.040**
Number of eribulin regimen lines	1.19	1.08-1.33	**<0.001**	1.21	1.09-1.34	**<0.001**	1.19	1.05-1.35	**0.006**
Eribulin readministration	-	-	-	0.11	0.01-0.79	**0.029**	0.12	0.02-0.88	**0.037**
Marker of systemic immunity at baseline									
ALC >1,500 *vs*. ALC ≤1,500	0.54	0.31-0.94	**0.031**	0.22	0.10-0.52	**<0.001**	0.23	0.09-0.59	**0.002**
NLR ≤3 *vs*. NLR >3	0.46	0.30-0.73	**<0.001**	0.36	0.22-0.59	**<0.001**	0.41	0.23-0.72	**0.002**
Dynamic changes at the start of the second cycle									
Increased ALC *vs*. decreased ALC	1.65	1.01-2.70	**0.047**	2.03	1.13-3.61	**0.017**	2.04	1.09-3.81	**0.026**
Increased NLR *vs*. decreased NLR	0.74	0.44-1.25	0.259	1.15	0.64-2.06	0.636	1.40	0.74-2.62	0.297
Dynamic changes at the end of therapy									
Increased ALC *vs*. decreased ALC	-	-	-	1.25	0.73-2.14	0.422	1.37	0.76-2.46	0.289
Increased NLR *vs*. decreased NLR	-	-	-	1.12	0.65-1.92	0.689	1.43	0.80-2.55	0.229

ALC: Absolute lymphocyte count; CI: confidence interval; ER: estrogen receptor; HR: hazard ratio; NLR: neutrophil-to-lymphocyte ratio; OS: overall survival; PgR: progesterone receptor; PPS: post-progression survival; TTF: time-to-treatment failure. [a]Chemotherapy included anthracycline and/or taxane. Statistically significant *p*-Values are shown in bold.

Table III. *Multivariate analysis of time-to-treatment failure, overall survival, and post-progression survival (Cox hazard model).*

Variable	TTF			OS			PPS		
	HR	95%CI	*p*-Value	HR	95%CI	*p*-Value	HR	95%CI	*p*-Value
Marker of systemic immunity at baseline									
ALC >1,500 *vs*. ALC ≤1,500	0.66	0.36-1.22	0.184	0.29	0.12-0.71	**0.007**	0.23	0.08-0.64	**0.005**
NLR ≤3 *vs*. NLR >3	0.62	0.37-1.04	0.067	0.51	0.30-0.86	**0.011**	0.44	0.24-0.80	**0.007**
Dynamic changes at the start of the second cycle									
Increased ALC *vs*. decreased ALC	0.95	0.55-1.65	0.856	1.97	0.97-4.02	0.061	2.56	1.15-5.69	**0.021**
Increased NLR *vs*. decreased NLR	0.92	0.52-1.62	0.777	1.18	0.59-2.35	0.641	1.20	0.59-2.43	0.609
Dynamic changes at the end of therapy									
Increased ALC *vs*. decreased ALC	-	-	-	0.94	0.49-1.80	0.849	1.17	0.58-2.34	0.663
Increased NLR *vs*. decreased NLR	-	-	-	1.09	0.60-1.96	0.789	1.44	0.76-2.73	0.263

ALC: Absolute lymphocyte count; CI: confidence interval; HR: hazard ratio; NLR: neutrophil-to-lymphocyte ratio; OS: overall survival; PPS: post-progression survival; TTF: time-to-treatment failure. Statistically significant *p*-Values are shown in bold.

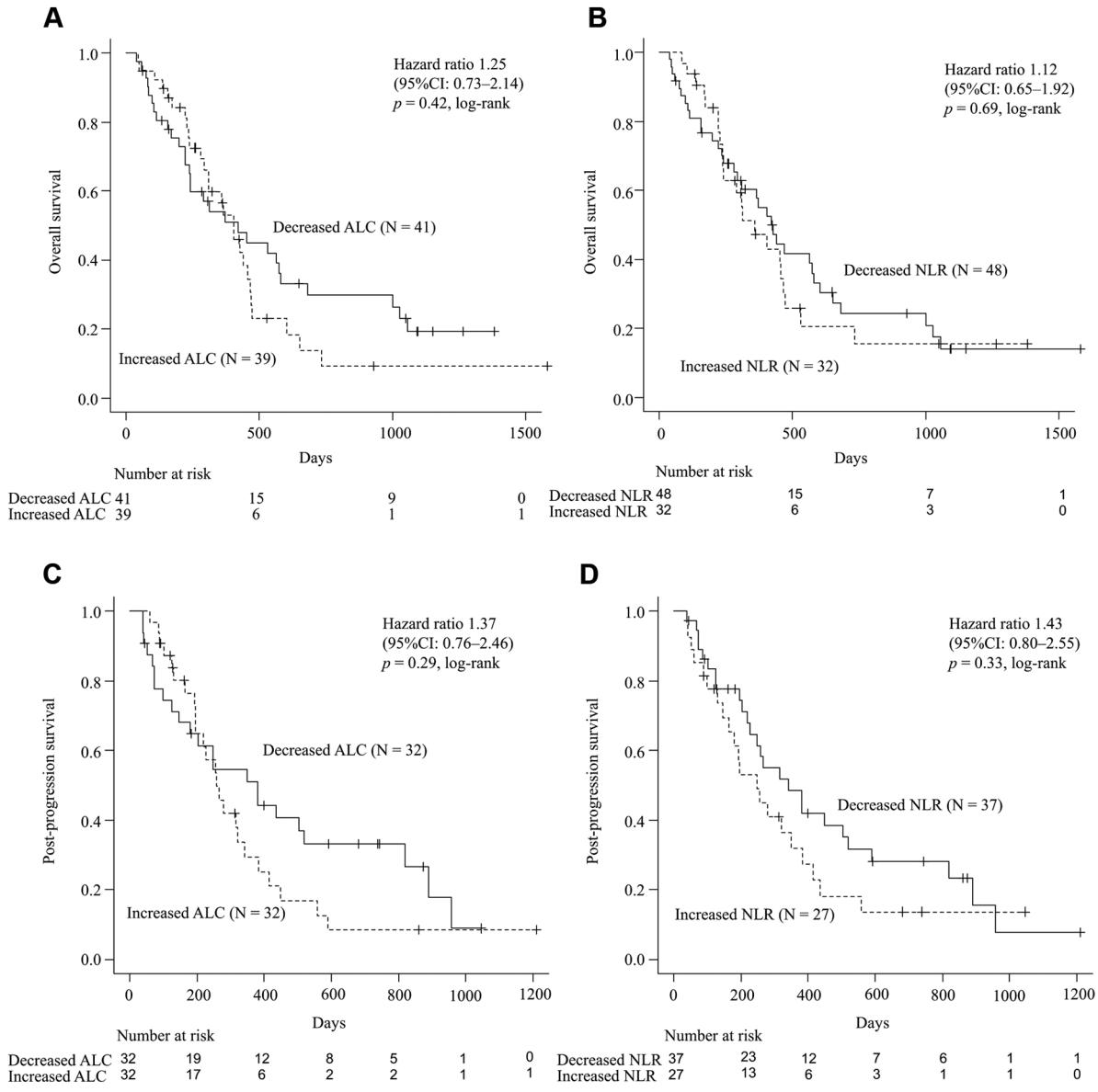

Figure 3. *Overall survival according to the dynamic changes in (A) ALC and (B) NLR at the end of the therapy and post-progression survival according to the dynamic changes in (C) ALC and (D) NLR at the end of the therapy in the patients treated with eribulin. ALC: Absolute lymphocyte count; NLR: neutrophil-to-lymphocyte ratio.*

predictive marker for PPS (HR=2.56, 95%CI=1.15-5.69, p=0.021). Although increased ALC(SO2nd) showed a favorable tendency, there was no significant difference in OS (HR=1.97, 95%CI=0.97-4.02, p=0.061).

Relationship between dynamic changes in systemic immunity markers at the end of therapy and survival benefit. The dynamic changes in ALC(EOT) and NLR(EOT) were not significantly associated with OS and PPS (Figure 3), and each of the four multivariate analyses showed that the dynamic changes in ALC (EOT) and NLR (EOT) at the end of the therapy had no significant association with OS or PPS (Tables II and III).

Discussion

This retrospective observational study showed that baseline high ALC and low NLR were significantly associated with improved TTF and OS in patients with HER2-negative ABC who underwent eribulin therapy and were independent predictive markers for improved PPS, TTF, and OS in the

multivariate analysis. Furthermore, as for the dynamic changes in the immunity markers, decreased ALC(SO2nd) was significantly associated with improved TTF, OS, and PPS compared with increased ALC(SO2nd), and decreased ALC(SO2nd) was an independent predictive marker for improved PPS in the multivariate analysis. To the best of our knowledge, our study is the first to demonstrate that the dynamic changes in ALC are useful in predicting TTF, OS, and PPS in patients with HER2-negative ABC.

Studies have reported that baseline ALC and NLR, as markers of immune status, are prognostic markers in various cancers including breast cancer (14-17). These markers have also been identified as independent predictive markers for improved OS in patients with ABC who undergo eribulin and bevacizumab therapy (18-21). The post hoc analysis of the EMBRACE study showed that baseline high ALC was significantly associated with improved OS in the eribulin group (18). A multicenter retrospective study showed that the OS of patients with baseline high ALC and low NLR was significantly longer (HR=0.30, 95%CI=0.13-0.62, log-rank p=0.001 and HR=0.39, 95%C=0.20-0.73, log-rank p=0.003, respectively) (19). A single-center retrospective study showed that baseline high ALC was significantly associated with improved OS in patients with ER-positive HER2-negative ABC (HR=0.50, 95%CI=0.26-0.95, p=0.034) (20). In the current study, baseline high ALC and low NLR were significantly associated with improved survival in patients with HER2-negative ABC undergoing eribulin therapy (Figure 1, Table III). Our findings support the results of these previous studies and are meaningful for selecting patients who can benefit from eribulin therapy in clinical practice.

Furthermore, our results showed that baseline high ALC and low NLR were significantly associated with improved PPS. In some diseases and treatment settings, improved PFS does not always result in improved OS. Given that OS is defined as the sum of PFS and PPS, the median PPS affects OS. Thus, PPS has a critical role (as does PFS) in defining OS (27). Although the probability of observing a statistically significant difference in OS depended on the median PPS and the magnitude of the observed PFS difference (27), eribulin improved OS without improving PFS in a number of clinical trials (3, 4). We therefore hypothesized that eribulin had a positive effect on PPS by increasing the antitumor activity of subsequent treatments with an anticancer drug through additional effects (10) and that ALC and NLR might predict PPS improvement with eribulin therapy. We focused on the effect of eribulin on PPS and investigated the usefulness of baseline ALC and NLR as predictive markers for PPS in patients treated with eribulin. We demonstrated that both baseline ALC and NLR were independent predictive markers for improved PPS in the multivariate analysis. Based on our hypotheses, eribulin could prolong PPS, and baseline ALC and NLR could predict an improved PPS.

As demonstrated in the previous studies and in our study, baseline systemic immunity markers were significantly associated with survival. However, the immune system and tumor microenvironment are not static and can undergo dynamic changes during treatment (22), especially during eribulin therapy. The various effects of eribulin in addition to its antitumor activity have been demonstrated by assessing changes in biological markers before and after eribulin therapy. A study compared the changes in the tissue concentrations of oxyhemoglobin, deoxyhemoglobin (HHb), oxygen saturation (SO2), and plasma transforming growth factor-β1 (TGF-β1) of breast tumors before and at day 7 after the first eribulin administration in patients with ABC and showed that eribulin therapy increased the tumor SO2 and decreased the tumor concentration of HHb and plasma TGF-β1 concentration when compared with the values prior to the eribulin therapy (9). Another study, compared the changes in the expression of markers for EMT and cellular hypoxia in patients with ABC before and after eribulin therapy and demonstrated that eribulin therapy increased E-cadherin and decreased N-cadherin, vimentin, and carbonic anhydrase 9 expression after eribulin therapy (28). Furthermore, a study examining the changes in the tumor microenvironment in patients with ABC before and after eribulin therapy reported decreased expression levels of programmed death-1, programmed death ligand 1 (PD-L1), and forkhead box P3 (FOXP3) in all responders to eribulin, and the response to eribulin was significantly associated with programmed death ligand 1 and forkhead box P3 negative conversion (p=0.024 and p=0.004, respectively) (13). The results suggest that the immune system and tumor microenvironment undergo dynamic changes during eribulin therapy.

Furthermore, it has been reported that dynamic changes in systemic immunity markers during treatment could play an important role. A multicenter retrospective study assessing the usefulness of NLR for determining the treatment efficacy of trastuzumab emtansine in HER2-positive ABC showed that NLR was significantly decreased and ALC was significantly increased after one treatment cycle (p=0.0010 and p=0.0005, respectively), and the OS of patients whose NLR changed from high to low after one cycle was improved (29). Li *et al*. demonstrated that increased NLR was an independent risk factor for death in multivariate analyses (HR=2.36, p=0.008), and dynamic changes in systemic immunity markers predicted the outcomes of patients with advanced non-small-cell lung cancer treated with bevacizumab (22). Thus, we investigated the usefulness of dynamic changes in systemic immunity markers during eribulin therapy as predictive markers, and demonstrated that increased ALC(SO2nd) was significantly associated with shorter TTF, OS, and PPS compared with decreased ALC. Therefore, in addition to the baseline ALC and NLR,

calculating the dynamic changes in systemic immunity markers at the start of the second cycle could help identify patients who will benefit from eribulin therapy.

Our results showed a significant association between survival benefit and the dynamic changes in ALC compared with NLR. A study reported that lymphocyte counts could reflect an immune reaction or potential immunity against cancer cells (29). In a post hoc analysis of the EMBRACE study, NLR was associated with prolonged PFS and OS, not only in the eribulin group but also in the group administered the treatment of the physician's choice. NLR was therefore assumed to be a general prognostic marker rather than a specific predictor of OS for eribulin, and ALC was assumed to be superior to NLR in predicting improved OS with eribulin (18). A single-center retrospective study also suggested that ALC was a more useful immunity marker than NLR for patients undergoing eribulin therapy (20), which is supported by our results.

Our study had several limitations. First, the study was a retrospective single-center study comprising a relatively small number of patients and a heterogeneous patient population. Although we made adjustments by performing a multivariate analysis, a subjective bias that could have affected the results cannot be completely ruled out. However, our study's strength lies in the fact that our results provide realistic findings observed in actual clinical practice, given that patients in the real world are usually more heterogeneous. Second, it remains unclear whether the dynamic changes in ALC are a specific predictor for eribulin therapy. Third, other causes, such as infections and steroids, may have affected ALC and NLR in this study. Therefore, further research, especially prospective studies with large numbers of patients, is warranted to confirm these issues.

In conclusion, our results showed that baseline ALC and NLR were significantly associated with longer PPS, TTF, and OS in patients with HER2-negative ABC undergoing eribulin therapy. Furthermore, we demonstrated that increased ALC(SO2nd) was significantly associated with shorter PPS compared with decreased ALC. These results could help identify patients likely to experience survival benefits from eribulin therapy in actual clinical practice and could help physicians make decisions for managing HER2-negative ABC.

Conflicts of Interest

Shogo Nakamoto has received lecture fees from Chugai Pharmaceuticals, Eisai, and Taiho Pharmaceuticals. Masahiko Ikeda has received lecture fees from AstraZeneca, Chugai Pharmaceuticals, Daiichi-Sankyo, Eisai, Eli-Lilly, Kyowa Kirin, Pfizer, Nippon Kayaku, Novartis, Mundipharma, Celltrion Healthcare, and Sawai Pharmaceuticals outside the submitted work. Shinichiro Kubo has received lecture fees from Eli-Lilly outside the submitted work. Mari Yamamoto has received lecture fees from Bayer outside the submitted work. Tetsumasa Yamashita has no conflicts of interest to declare.

Authors' Contributions

All Authors contributed to the study conception and design. Material preparation and data collection were performed and the first draft of the manuscript was written by Shogo Nakamoto. All Authors commented on the previous versions of the manuscript and read and approved the final manuscript.

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