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[Title]

A maintained absolute lymphocyte count predicts the overall survival benefit from eribulin therapy, including eribulin re-administration, in HER2-negative advanced breast cancer patients: A single institutional experience

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All authors contributed to the study conception and design. Material preparation, data collection and analyses were performed by Junichiro Watanabe and Shogo Nakamoto. The first draft of the manuscript was written by Junichiro Watanabe, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

[Running Title]

Maintained ALC predict OS benefit from eribulin therapy in HER2-ABC

[Abstract (234 words)]

Purpose: Eribulin methylate (eribulin) improved the overall survival (OS) of HER2-negative advanced breast cancer (HER2-ABC) patients; however, the mechanism underlying the OS improvement has not been clarified. Several reports suggest that eribulin promotes antitumor immunity via tumor microenvironment conditioning. Recently, a maintained baseline lymphocyte count was proposed as predictive marker for eribulin therapy in HER2-ABC patients; however, no associations with the OS have been noted. We retrospectively investigated the neutrophil-to-lymphocyte ratio (NLR) and absolute lymphocyte count (ALC) in HER2-ABC patients receiving eribulin and assessed the utility of eribulin re-administration for further OS improvement.

Methods: HER2-ABC patients who received eribulin therapy at Shizuoka Cancer Center between November 2011 and December 2018 were retrospectively analyzed.

Results: A total of 144 HER2-ABC (108 estrogen receptor-positive [ER+], 36 ER-) patients were identified, and 32 patients (28 ER+, 4 ER-) were re-administered eribulin. In the ER+ subgroup, a multivariate analysis showed that an ALC ≥1000/μL and re-administration were significantly associated with the OS (hazard ratio [HR] 0.503; $P = 0.034$ and HR 0.366; $P < 0.0001$, respectively), and an ALC ≥1000/μL was also identified as the only predictive factor for re-administration (HR 0.329; $P = 0.033$). In contrast, a multivariate analysis in the ER- subgroup identified no predictive markers.

Conclusion: In HER2-ER+ABC patients, ALC was identified as a predictive marker for eribulin therapy, and the re-administration of eribulin is considered a valid therapeutic option for further improvement of the OS.

[Key words]

HER2 negative, metastatic breast cancer, overall survival, eribulin, absolute lymphocyte count, re-administration

[Introduction]

Eribulin mesylate (eribulin) significantly prolonged the overall survival (OS) of HER2-negative (HER2-) advanced breast cancer (ABC) patients in the EMBRACE study (study 305) [**1**] and showed a strong trend for OS prolongation in comparison to capecitabine in earlier-line therapy in HER2- ABC patients (study 301) [**2**]. Based on this evidence, eribulin is recognized as a viable option for treating HER2- ABC that is resistant to anthracycline-based regimens and/or taxane therapy [**3**].

Eribulin exerts its antitumor activity by suppressing the elongation of microtubules as an anti-tubulin agent [**4**], resulting in various effects being reported, including the suppression of epithelial-mesenchymal transition (EMT) [**5, 6**], improvement of the tumor microenvironment [**7**] and modification of tumor immunity [**8**]. Some of these additional effects, such as the suppression of new metastasis [**9, 10**] and promotion of tumor immunity, might be related to the improvement of the OS by eribulin [**8**].

According to basic studies, the promotion of tumor immunity in particular is responsible for the antitumor activity of not only immune-checkpoint inhibitors [**11**] but also conventional anticancer agents [**12**]; however, inflammation associated with neoplasms is well known to suppress tumor immunity [**13, 14**].

Several recent clinical studies showed that the baseline neutrophil-to-lymphocyte ratio (NLR) is a prognostic or predictive marker in various carcinomas, including breast cancer

[**15-19**]. However, while the absolute lymphocyte count (ALC) may also be a useful immune-related marker, its clinical significance in breast cancer patients has not been well studied [**20**].

Once a cancer has shown resistance to an anticancer agent, the re-administration of the agent might not be considered in the same patient, due to documented drug resistance; however, taxane or anthracycline may be offered to patients with recurrent breast cancer, even if they received these drugs during perioperative therapy [**3**]. Assuming that eribulin has a unique mode of action, we considered that it could be potentially effective in the same patient with ABC who had previously received eribulin and who also met some certain conditions; such as an achievement of better progression-free survival (PFS) or a new lesion-free in first-round of eribulin therapy.

In the present study, we retrospectively evaluated the significance of the NLR and ALC of HER2- ABC patients who underwent eribulin monotherapy and evaluated the utility of the re-administration of eribulin for further improving the OS in the real-world setting.

[Methods]

Patients background

HER2- ABC patients who received at least two cycles of eribulin monotherapy at Shizuoka Cancer Center hospital as a practice from November 2011 to December 2018 were selected from the medical records, and the patient backgrounds and outcomes were retrospectively reviewed. In Japan, eribulin can be selected as any line of therapy for HER2- ABC, and it is now approved for health insurance reimbursement, including for re-administration in the same patient.

Eribulin therapy

HER2- ABC patients who showed resistance to an anthracycline-based regimen and/or taxane were considered as candidates for the first round of eribulin therapy. Patients received eribulin at the maximum dose of 1.4mg/m^2 of eribulin on days 1 and 8 of a 3-week cycle. Dose reduction or interruption of eribulin was performed according to toxicities, and the therapy was discontinued when disease-progression, intolerable toxicity or withdrawal of consent was documented. To avoid frequent visits, the prophylactic use of granulocyte colony-stimulating factor (G-CSF) was not planned. Patients who had been considered responders to their first round of eribulin therapy (*e.g.*, those with a longer time-to-treatment failure [TTF] or who were new lesion-free) without significant toxicity and who maintained a good to fair PS were offered the re-administration of eribulin as a late-line therapy after

several other regimens had been applied and after they provided their sufficient informed consent.

Blood sampling and analysis

Whole blood samples were drawn from patients just before the administration of eribulin or within seven days before its administration, and a complete blood count was performed by a Sysmex XN-3100 or XE-5000 automated hematology system (Sysmex Co., Kobe, Japan).

Statistical analysis

The Kaplan-Meier method was used for the survival analysis. The chi-squared test or Fisher's exact test was used to compare two groups, and a Cox regression analysis was used for univariate and multivariate analyses. For all analyses, a P-value of <0.05 was considered statistically significant. The JMP software program, Japanese version 13.2.0 (SAS Institute Inc., Cary, NC, USA), was used for these statistical analyses.

[Results]

Overall efficacy and safety analyses

We identified 144 HER2- ABC patients (108 estrogen receptor-positive [ER+] and 36 ER-) who met the criteria described above, and the patients' details are shown in **Table 1**.

The median TTF of the first eribulin therapy was 140.0 days (95% confidence interval [CI] 125.0-168.0) in the ER+ subgroup and 104.0 days (95% CI 57.0-153.0) in the ER- group, which was similar to the findings in previous reports.

Dose reduction and/or dose interruption of the first round of eribulin therapy were documented in 105 of 108 patients (97.2%) in the ER+ subgroup and 37/38 (97.4%) in the ER- subgroup, mainly because of neutropenia. No prophylactic use of G-CSF was documented. The median relative dose intensity (mRDI) of first-round eribulin therapy in the ER+ subgroup was 68.5% (range 22.4-101.6), and there was no significant relationship between RDI and TTF (Spearman's $\rho = -0.0662$, $P > 0.5$). Similarly, in the ER- subgroup, the mRDI was 64.3% (range 32.8-103.0), and there was no significant relationship between RDI and TTF (Spearman's $\rho = -0.2740$, $P = 0.1058$).

Most (140/144, 97.2%) of the patients discontinued eribulin at the time of data cut-off for the following reasons: progression of known lesion (KL), 103 (73.6%); development of new lesion (NL), 22 (15.7%); intolerable toxicity, 6 (4.3%) and other reasons, 9 (6.4%). Development of NL was more frequently seen in the ER- subgroup than in the ER+ subgroup,

although not to a significant degree (Fisher's exact test).

At the time of data cut-off, 28 of 108 (25.9%) patients in the ER+ subgroup and 4 of 36 (11.1%) patients in the ER- subgroup received a second attempt of eribulin therapy. The median OS from the initiation of their first round of eribulin therapy, with or without second round of eribulin therapy, was 589.0 days (95% CI 503.0-692.0) in the ER+ subgroup and 472.5 days (95% CI 229.0-573.0) in the ER- subgroup, without a significant difference between the subgroups (P = 0.0953, log-rank).

In the ER+ subgroup, patients who showed progression of disease (PD) by the progression of KL (N = 82) strongly tended to have a superior OS than those who showed PD by the development of NL (N = 14) at their first eribulin therapy (median OS 632.0 vs. 462.0 days, respectively, P = 0.0919, log-rank) regardless of eribulin re-administration.

No significant or new safety signals related to the eribulin therapies, including the re-administration of eribulin, were noted within the observation period.

The clinical utility of the re-administration of eribulin in the ER+ subgroup

Twenty-eight (25.9%) of the 108 ER+ patients who were considered responders to the first attempt at eribulin therapy were offered a second attempt of eribulin, mainly as a substitute for gemcitabine or vinorelbine.

Of these 28 patients, the median TTF of their first eribulin therapy was significantly

better than that of the patients who were not offered second eribulin therapy (median 224.0 days vs. 125.0 days; P = 0.0036, log-rank). Patients received a median of 2 (range 1-5) regimens between the first and second round of eribulin therapies, and the median number of regimens patients received prior to the second round of eribulin therapy, including the first round of eribulin therapy, was 4 (range 2-10). A majority (25/28, 89.3%) of patients received chemotherapy with a median of 1 regimen (range 1-4) prior to the second round of eribulin therapy. The median TTF of the therapies (22 chemotherapy, 5 endocrine therapy ± targeted therapy, excluding 1 drug-holiday) just before the second round of eribulin therapy was 150.5 days (95% CI 112.0-195.0), and the main cause of discontinuation of the therapy was progression of KL (22/27, 81.5%; excluding 1 patient who underwent a drug-holiday). Other details are summarized in **Table 1**.

The median TTF of the second round of eribulin therapy was 97.0 days (95% CI 62.0-132.0) with an mRDI of 64.3% (range 32.8-103.0), and the causes of discontinuation (excluding 3 patients still on treatment) were as follows: progression of KL, 15 (60.0%); development of NL, 4 (16.0%) and other reasons, 6 (24.0%). Among those patients who discontinued the second attempt at eribulin therapy, 5 (20.0%) achieved stable disease for ≥24 weeks. Subsequent systemic therapy was introduced to 17 out of 25 (68.0%) patients, and 13 patients received chemotherapy. The median OS from the induction of the second round of eribulin therapy was 266.0 days (95% CI 135.0-612.0), and the median OS from the

induction of the first round of eribulin therapy was 890.5 days (**Figure 1**). There was a significant relationship between the TTF of the second round of eribulin therapy and the OS from the induction of the second round of eribulin therapy (P <0.001, Spearman's coefficient analysis), and the patients who received a second round of eribulin therapy showed a significantly longer median OS not only from the initiation of the second round of eribulin therapy but also from the initiation of their first chemotherapy regimen (1449.5 vs. 1016.0 days, P = 0.0137, log-rank, hazard ratio [HR] 0.58, 95% CI 0.35-0.93, **Supplemental Fig. 1**).

NLR and ALC analyses for the ER+ subgroup

Significant relationships between patients' outcomes and hematologic parameters at the initiation of the first round of eribulin therapy were revealed in the present study.

As shown in **Table 2**, ER+ patients who had a low (<3 or <2.5) NLR and maintained their ALC (≥1500 or ≥1000/μL) at the initiation of the first round of eribulin therapy showed an improved TTF and OS on log-rank tests, excluding the TTF in patients who had an ALC ≥1500/μL. Furthermore, there was a significant relationship between the ALC at the initiation of the first round of eribulin therapy and the pattern of PD (P = 0.0498, **Figure 2**) at the end of the first round of eribulin therapy.

While the majority of the patients who underwent the second round of eribulin therapy had an NLR <3 or <2.5 or an ALC ≥1000/μL at the initiation of the first round of

eribulin therapy, we performed further analyses using multivariate Cox regression analyses to clarify the significance of the NLR, ALC and the second round of eribulin therapy on the OS from the first round of eribulin therapy for all ER+ ABC patients. As shown in **Table 3**, an ALC of ≥1000/μL at the initiation of the first round of eribulin therapy and the re-administration of eribulin were independently associated with OS, with HRs of 0.503 (95% CI 0.264-0.949, P = 0.034) and 0.366 (95% CI 0.200-0.649, P <0.0001), respectively, regardless of other factors.

Further analyses were conducted to extract predictive factors for the second round of eribulin therapy (**Table 4**). The univariate analysis showed that an ALC ≥1000/μL at the initiation of the second round of eribulin therapy was the only significant predictive factor, although the presence of visceral metastasis showed a strong trend for a poor outcome. Thus, in the multivariate analysis, we selected factors that would commonly be observed when considering the re-administration of eribulin in the real world, and ALC ≥1000/μL at the initiation of the second round of eribulin therapy was the only significant predictive factor identified by the multivariate analysis (HR 0.329, 95% CI 0.116-0.910, P = 0.033).

NLR and ALC analyses for the ER- subgroup

In contrast to the ER+ subgroup, no immune-related predictive factors for eribulin therapy were identified in the ER- subgroup. According to the Kaplan-Meier method with log-rank

test, patients who had an NLR <3 or ALC ≥1000/μL at the initiation of the first round of eribulin therapy showed a significantly superior median OS to those with NLR ≥3 or ALC <1000/μL (NLR <3: 573.0 vs. 140.0 days, *P* = 0.0309, **Supplemental Fig. 2-A**; ALC ≥1000/μL: 545.0 vs. 133.0 days, *P* = 0.0106, **Supplemental Fig. 2-B**) by log-rank tests; however, no independent predictive markers was identified by a multivariate analysis.

Only four patients in the ER- subgroup underwent a second round of eribulin therapy, presumably due to the aggressive nature of their cancer; therefore, the benefit from a second round of eribulin therapy was not evaluable.

[Discussion]

Our present findings suggest that, when considering eribulin therapy for patients with HER2- ABC, especially those with ER+HER2- ABC, a maintained ALC of ≥1000/μL at the initiation of the first round of eribulin therapy is a useful biomarker for predicting OS. Furthermore, when subsequent therapies fail in eribulin-pretreated ER+HER2- ABC patients and an ALC of ≥1000/μL is maintained, the re-administration of eribulin should be considered in order to improve the subsequent OS.

It has been well reported in various carcinomas, including ABC, that a low baseline NLR is associated with a better outcome, with the opposite holding true as well [**16, 17**]. Furthermore, the NLR at the initiation of anticancer therapy is also known to be a predictive marker for patients with operable breast cancer [**18, 19**]. However, the usefulness of the baseline NLR as a biomarker in patients with ABC has not been well studied. Miyagawa et al. [**21**] reported that an NLR of <3 at the start of treatment was associated with superior PFS (HR 0.37, 95% CI 0.18-0.71, P = 0.0032), and a strong trend toward OS prolongation (HR 0.44, 95% CI 0.17-1.01, P = 0.058) in the eribulin group; however, this was not true for the *nab*-paclitaxel group. However, few reports have described the association of baseline lymphopenia (<700 or <1000/μL) and a poor outcome (i.e. lymphopenia as an adverse prognostic factor) in patients with ABC or other advanced malignancies [**22-24**]. Indeed, to our knowledge, there is only one report from Araki et al. [**25**] that described a significant

association between a maintained ALC (≥1500/μL) and the PFS of anti-HER2 therapy in HER2+ ABC patients receiving eribulin or *nab*-paclitaxel combined with trastuzumab and pertuzumab. According to our present study, an ALC of ≥1000/μL was an independent hematologic parameter influencing the OS in the ER+ subgroup, but not in the ER- subgroup. Furthermore, we found that an ALC ≥1000/μL at the initiation of eribulin therapy, both in earlier and later lines of therapy, was a significant predictor of the subsequent OS in ER+ ABC patients.

As previously mentioned, discussions are ongoing regarding whether the NLR or ALC is the more useful biomarker for eribulin monotherapy, and the cut-off values for those immune-related markers are still controversial. Our study confirmed that an ALC ≥1000/μL at the initiation of eribulin therapy was improved their survival, regardless of other factors (e.g. tumor load, established resistance to anticancer agents and even a history of eribulin therapy in the ER+ subgroup). These findings suggest that the ALC is a more useful immune-related marker than the NLR in patients with ER+HER2- ABC who undergo eribulin therapy. However, we failed to detect any independent immune-related markers by a multivariate analysis in ER- patients, although the aggressive nature of ER-HER2- ABC made any conclusions regarding the clinical utility of ALC at the initiation of eribulin therapy difficult to determine.

The tumor microenvironment consists of tumor cells and host cells; such as

endothelial cells, fibroblasts and immune-associated cells [**13, 14**], and it is well known that the local antitumor activity is enhanced by tumor-infiltrating lymphocytes (TILs) in early breast cancer (EBC) patients, especially those with the triple-negative (TN) subtype, who undergo neoadjuvant chemotherapy (NAC). Afghani et al. [**26**] reported in their retrospective large-scale cohort study that operable TN patients who underwent NAC and showed no lymphopenia (<1000/μL) during NAC had a reduced risk of mortality from breast cancer. While their sample size was relatively small (N = 70), they showed a significant positive relationship between the ALC and TILs in their exploratory analysis. Consequently, their report suggested a positive relationship between systemic immune-related markers (i.e. a maintained ALC) and local antitumor activity.

Anti-HER2 monoclonal antibodies, such as trastuzumab, induce antibody-dependent cell cytotoxicity [**27**] and upregulate local and systemic tumor immunity [**28**]; however, conventional cytotoxic drugs do not directly enhance the tumor immunity itself. In contrast, eribulin, which is classified as a tubulin inhibitor, has been reported to have additional modes of action absent in conventional drugs, such as EMT reversal [**5, 6**], re-oxygenation via vascular re-modeling both *in vitro* and *in vivo* [**7**] and the upregulation of tumor immunity [**8**]. Recent reports, including translational research, have indicated that eribulin is involved in tumor immunity [**8, 10, 29, 30**]; eribulin is thus considered to improve the tumor microenvironment which is disseminated widely to other organs and thereby activate

systemic antitumor immunity. Given these findings, it is reasonable to suspect that systemic immune-related markers, such as the ALC or NLR, may predict the systemic antitumor activity aroused by eribulin in patients with ABC, regardless of subtype.

The usefulness of the re-administration of anticancer agents has not been well studied; however, several reports [**31, 32**] regarding lung cancer have been published. With ABC, known treatment options, such as the crossover use of taxanes in patients who have been exposed to taxanes in the perioperative treatment [**3**]; however, to our knowledge, there are no reports on the re-administration of the same anticancer drug in patients with HER2- ABC. One hypothesis asserts that the effect of post-treatment will be enhanced by eribulin, as an explanation for the improvement in the OS in the EMBRACE study (study 305) [**1**] and the integrated analysis of the 305 and 301 trials [**33**]. In the present study, upon obtaining adequate informed consent, we re-administered eribulin to patients who had responded well to the initial eribulin therapy with tolerable toxicity. While the median PFS or TTF of the 4th-line treatment for ER+HER2- ABC was roughly 3 months, regardless of the regimen [**34**], the median TTF was almost the same in this study (97.0 days). Furthermore, the median OS from the first administration of eribulin was found to be about twice as long as that in the eribulin re-challenge group and others. Because there were no significant differences in the patient background characteristics at the first administration of eribulin, such as the age, tumor burden or treatment line, and because the TTF of the subsequent therapies tended to be

longer in the eribulin re-administration population than in patients who did not undergo the second round of eribulin therapy (median 160.5 vs. 117.0 days, *P* = 0.1852, log-rank, **Supplemental Fig. 3**), a favorable influence (e.g. re-booting tumor immunity) is presumed to have been applied post-treatment. Taking the above into account, it is consistent that ALC ≥ 1000/μL is a biomarker for second-round eribulin therapy in ER+HER2- ABC patients.

This study has several limitations, such as its retrospective nature, relatively small numbers of patients and lack of a control arm inside the study. However, our previous real-world report [**35**], which discussed the improvement of OS by eribulin therapy, revealed that eribulin monotherapy significantly improved the OS of ER+HER2- ABC patients in the identical dataset currently used in this study, with an HR of 0.55 (95% CI 0.36-0.85, *P* = 0.01). Furthermore, in that study [**35**], no patient received second-round eribulin therapy at the time of data cut-off, so the report supports the usefulness of second-round eribulin therapy as a historical control. Thus, this study is strengthened by its single-institutional setting, as patients were followed diligently (e.g., in this study, only 4 patients [2.8%] were untraceable, all deceased patients died in our hospital, and treatment strategies were consistent).

[Conclusion]

In this article, we discussed the clinical significance of peripheral immune-related markers in in HER2- ABC patients receiving eribulin therapy, and we originally reported the utility of the re-administration of eribulin to ER+HER2- ABC patients.

In conclusion, our study showed that, in ER+HER2- ABC, a maintained ALC of ≥1000/μL at the initiation of eribulin therapy is a consistent and universal predictive marker that is also noninvasive and inexpensive and which better predicts the outcome not only in eribulin-naïve patients but also in eribulin-pretreated patients. However, these findings need to be further evaluated through a prospective, randomized clinical trial.

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[Compliance with Ethical Standards]

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This study was conducted in full compliance of the laws and after obtaining approval from the institutional review board of institutions where the study was conducted.

Informed consent was obtained from all patients included in the study.

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Table 1: Patient characteristics and outcomes

		ER-positive			
	ER-negative	Overall	Second round of eribulin therapy		*P* for (a) vs. (b)
			Received (a)	Not received (b)	
Patient characteristics					
N	36 (100.0)	108 (100.0)	28 (25.9)	80 (74.1)	⋯
Median age, years (range)	56.5 (33-71)	58 (30-77)	54.5 (31-71)	59.5 (30-77)	⋯

Female, N (%)	36 (100.0)	108 (100.0)	···	···	···
Japanese, N (%)	36 (100.0)	108 (100.0)	···	···	···
Diagnosis					
Advanced, N (%)	10 (27.8)	30 (27.8)	7 (25.0)	23 (28.8)	NS*
Recurrent, N (%)	26 (72.2)	78 (72.2)	21 (75.0)	57 (71.2)	
Involved organ at the initiation of the first round of eribulin therapy					
Lung, N (%)	10 (27.8)	41 (38.0)	6 (21.4)	35 (43.8)	0.043*
Liver, N (%)	16 (44.4)	65 (60.2)	19 (67.9)	46 (57.5)	NS*
Bone, N (%)	15 (41.7)	73 (67.6)	18 (64.3)	55 (66.8)	NS*
Soft tissue, N (%)	22 (61.1)	69 (63.9)	17 (60.7)	52 (65.0)	NS*
CNS, N (%)	5 (13.9)	12 (11.1)	0 (0.0)	12 (15.0)	NS*

Tumor load					
Visceral metastasis, N (%)	25 (69.4)	87 (80.6)	22 (78.1)	65 (81.3)	NS*
Median numbers of organs involved, N (range)	2 (0-5)	2 (1-5)	2 (1-4)	3 (1-5)	⋯
≥3 organs involved, N (%)	7 (19.4)	56 (51.9)	12 (42.9)	44 (54.0)	NS*
Treatment history prior to the first round of eribulin therapy					
Anthracycline for EBC, N (%)	19 (52.8)	41 (38.0)	13 (46.4)	28 (35.0)	NS*
Anthracycline for ABC, N (%)	16 (44.4)	29 (26.9)	7 (25.0)	22 (27.5)	NS*
Taxane for EBC, N (%)	13 (36.1)	31 (28.7)	6 (21.4)	25 (31.3)	NS*
Taxane for ABC, N (%)	19 (52.8)	53 (49.1)	16 (57.1)	37 (46.3)	NS*
Bevacizumab for ABC, N (%)	14 (38.9)	35 (32.4)	10 (35.7)	25 (31.3)	NS*
Median number of previous regimens for ABC (range)	2 (0-8)	1 (0-6)	1 (0-6)	1 (0-6)	⋯
Concomitant therapy					

BMA, N (%)	12 (33.3)	69 (63.9)	17 (60.7)	52 (65.0)	NS*
Outcome of the first round of eribulin therapy					
Discontinued, N, %	36 (100.0)	104/108 (96.3)	28/28 (100.0)	76/80 (95.0)	···
Cause of discontinuation					
Progression of known lesion, N (%)	21 (58.3)	82/104 (78.8)	24/28 (85.7)	58/76 (76.3)	NS*
Development of new lesion, N (%)	8 (22.2)	14/104 (13.6)	1/28 (3.6)	13/76 (17.1)	
Toxicity, N (%)	2 (5.6)	4/104 (3.8)	3/28 (10.7)	1/76 (1.3)	NS*
Other, N (%)	5 (13.9)	4/104 (3.8)	0/28 (0.0)	4/76 (5.3)	NS*
Ongoing, N	0 (0.0)	4/108	0/28	4/80	NS*
Median TTF of the first round of eribulin therapy, days (95% CI)	104.0 (57.0-153.0	140.0 (125.0-168.0)	224.0 (146.0-274.0	125.0 (90.0-146.0)	0.0036 **

))

* Fisher's exact test, ** log-rank test

ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; CNS, central nervous system; EBC, early breast cancer; ABC, advanced breast cancer; BMA, bone-modifying agent; TTF, time-to-treatment failure; CI, confidence interval.

Table 2: Patients' background characteristics at the initiation of the first round of eribulin therapy and outcomes of ER+ subgroup

			Status at the initiation of 1st eribulin therapy †					TTF of 1st eribulin therapy				2nd round of eribulin therapy	OS from 1st eribulin therapy			
Factor	Cut-off value	N	Median numbers of prior regimens (range)	≥3 prior regimens (%)	Visceral involvement (%)	≥3 involved organs (%)	Liver metastasis (%)	median (days)	95% CI		*P* *	(%)	median (days)	95% CI		*P* *
	Overall	108	1 (0-6)	20 (18.5)	87 (80.6)	56 (51.9)	65 (60.2)	140.0	125.0	- 168.0	･･･	28 (25.9)	589.0	503.0	- 692.0	･･･
NLR	≥3	36	1 (0-6)	9 (25.0)	29 (80.6)	23 (63.9)	20 (55.6)	119.0	83.0	- 146.0	0.0325	5	503.0	369.0	- 692.0	0.0082

												(13.9)					
	<3	72	1 (0-6)	11 (15.3)	58 (80.6)	33 (45.8)	45 (62.5)	147.0	132.0	-	168.0		23 (31.9)	622.0	547.0	-	755.0	
	≥2.5	46	1 (0-6)	9 (19.6)	36 (78.3)	26 (56.5)	25 (54.3)	124.5	87.0	-	146.0		6 (13.0)	458.0	321.0	-	689.0	
												0.0077						0.0008
	<2.5	62	1 (0-6)	11 (17.7)	51 (82.3)	30 (48.4)	40 (64.5)	168.0	132.0	-	209.0		22 (32.3)	638.0	554.0	-	772.0	
ALC (/μL)	≥1500	42	1 (0-6)	7 (16.7)	32 (76.2)	23 (54.8)	24 (57.1)	147.0	130.0	-	220.0		18 (42.9)	735.0	602.0	-	898.0	
												0.2836						0.0072
	<1500	66	1 (0-6)	13 (19.7)	55 (83.3)	33 (50.0)	41 (62.1)	132.0	110.0	-	168.0		10 (15.2)	462.0	369.0	-	574.0	
	≥1000	78	1 (0-6)	13 (16.7)	63 (80.8)	41 (52.6)	48 (61.5)	147.0	133.0	-	168.0	0.0271	24	692.0	589.0	-	772.0	0.0049

												(30.8)				
												4				
<1000		1 (0-6)	7 (23.3)	24 (80.0)	15 (50.0)	17 (56.7)	106.5	69.0	-	146.0			364.0	296.0	-	526.0
	30											(13.3)				

TTF, time-to-treatment failure; OS, overall survival; CI, confidence interval; NLR, neutrophil-to-lymphocyte rate; ALC, absolute lymphocyte count

*: log-rank test, †: no significant differences between two groups in each subset by Fisher's exact test

Table 3: Univariate and multivariate analyses for factors affecting the overall survival from the initiation of the first round of eribulin therapy in the ER+ subgroup

		Univariate analysis			Multivariate analysis		
Factor	N (%)	HR	*P*	95% CI	HR	*P*	95% CI
Age ≥65 years old	31 (28.7)	0.871	0.565	0.532 - 1.380	⋯	⋯	⋯ - ⋯
Lung metastasis	41 (38.0)	1.614	0.040	1.023 - 2.518	1.348	0.309	0.757 - 2.388
Liver metastasis	65 (61.2)	1.070	0.762	0.694 - 1.677	⋯	⋯	⋯ - ⋯
Bone metastasis	73 (67.6)	1.735	0.021	1.084 - 2.876	1.298	0.427	0.684 - 2.501
CNS metastasis	12 (11.1)	2.656	0.006	1.350 - 4.808	0.822	0.631	0.355 - 1.801
Soft tissue metastasis	69 (63.9)	1.030	0.895	0.664 - 1.631	⋯	⋯	⋯ - ⋯
Visceral metastasis	87 (80.6)	1.539	0.117	0.903 - 2.807	⋯	⋯	⋯ - ⋯

≥3 involved organs	56 (51.9)	1.549	0.046	1.009	-	2.391	1.324	0.385	0.703	-	2.507
≥3 lines of chemotherapy	20 (18.5)	1.075	0.781	0.627	-	1.757	···	···	···	-	···
NLR <3	72 (66.7)	0.542	0.011	0.343	-	0.866	2.701	0.053	0.989	-	6.760
NLR <2.5	61 (56.5)	0.475	0.001	0.303	-	0.744	0.424	0.069	0.183	-	1.075
ALC ≥1500/μL	42 (38.9)	0.545	0.007	0.344	-	0.847	0.866	0.633	0.481	-	1.568
ALC ≥1000/μL	78 (72.2)	0.457	0.001	0.292	-	0.729	0.503	0.034	0.264	-	0.949
TTF of 1st ERI >120 days	62 (57.4)	0.443	<0.001	0.286	-	0.690	0.829	0.553	0.439	-	1.531
TTF of 1st ERI >180 days	38 (35.2)	0.507	0.003	0.319	-	0.789	0.768	0.400	0.420	-	1.431
PD by known lesion	82 (75.9)	0.752	0.280	0.462	-	1.276	···	···	···	-	···
Re-administration	28 (25.9)	0.273	<0.0001	0.158	-	0.454	0.366	0.0005	0.200	-	0.649

HR, hazard ratio; CI, confidence interval; CNS, central nervous system; NLR, neutrophil to lymphocyte ratio; ALC, absolute lymphocyte count; TTF, time-to-treatment failure; ERI, eribulin therapy; PD, progressive disease

Table 4: Univariate and multivariate analyses for factors affecting the overall survival from the initiation of the second round of eribulin therapy in the ER+ subgroup

		Univariate analysis			Multivariate analysis		
Factor	N (%)	HR	*P*	95% CI	HR	*P*	95% CI
Age ≥65 years old	11 (39.3)	0.755	0.537	0.287 - 1.805	⋯	⋯	⋯ - ⋯
Lung metastasis	7 (25.0)	1.731	0.255	0.653 - 4.181	⋯	⋯	⋯ - ⋯
Liver metastasis	19 (67.9)	1.608	0.310	0.656 - 4.507	⋯	⋯	⋯ - ⋯
Bone metastasis	16 (57.1)	2.011	0.118	0.842 - 5.300	⋯	⋯	⋯ - ⋯
CNS metastasis	3 (10.7)	2.649	0.181	0.589 - 8.776	⋯	⋯	⋯ - ⋯
Soft tissue metastasis	18 (64.3)	0.842	0.712	0.352 - 2.219	⋯	⋯	⋯ - ⋯
Visceral metastasis	21 (75.0)	2.538	0.069	0.935 - 8.855	2.003	0.215	0.685 - 7.332

≥3 involved organs	12 (42.9)	1.754	0.192	0.752	-	4.179	⋯	⋯	⋯	-	⋯
≥5 lines of chemotherapy	22 (78.6)	1.013	0.980	0.330	-	2.583	0.942	0.912	0.294	-	2.536
TTF of 1st ERI >120 days	25 (89.3)	0.700	0.538	0.258	-	2.442	0.951	0.931	0.328	-	3.447
TTF of 1st ERI >180 days	16 (57.1)	1.135	0.764	0.498	-	2.664	⋯	⋯	⋯	-	⋯
NLR <3	10 (35.7)	0.899	0.814	0.381	-	2.271	⋯	⋯	⋯	-	⋯
NLR <2.5	13 (46.4)	0.635	0.319	0.239	-	1.532	⋯	⋯	⋯	-	⋯
ALC ≥1500/μL	9 (32.1)	0.461	0.084	0.174	-	1.105	⋯	⋯	⋯	-	⋯
ALC ≥1000/μL	18 (64.3)	0.279	0.013	0.100	-	0.757	0.329	0.033	0.116	-	0.910

HR, hazard ratio; CI, confidence interval; CNS, central nervous system; TTF, time-to-treatment failure, ERI, eribulin therapy; NLR, neutrophil to lymphocyte ratio; ALC, absolute lymphocyte count

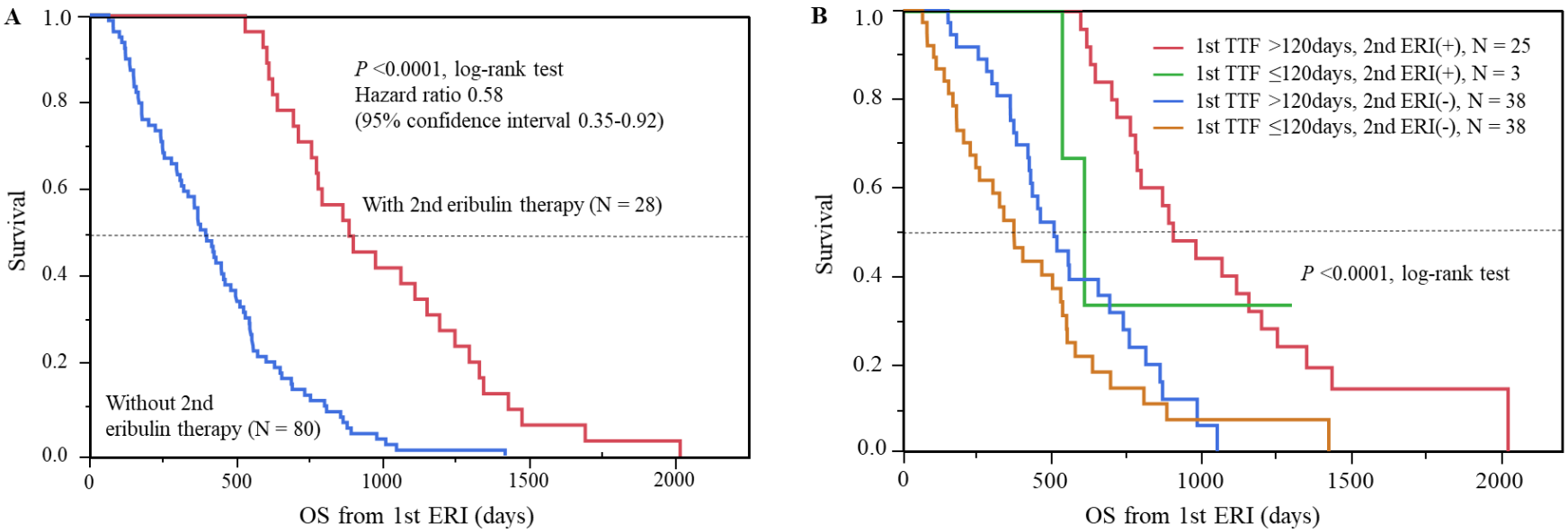


Fig. 1 Overall survival after the initiation of the first eribulin therapy in ER+ patients according to **A** with/without re-administration of eribulin (P <0.0001), and **B** TTF of the first eribulin therapy and with/without re-administration of eribulin; excluding 4 patients who were still being treated by the first eribulin (P <0.0001). *1st ERI* first eribulin therapy, *1st TTF* time-to-treatment failure of the first eribulin therapy.

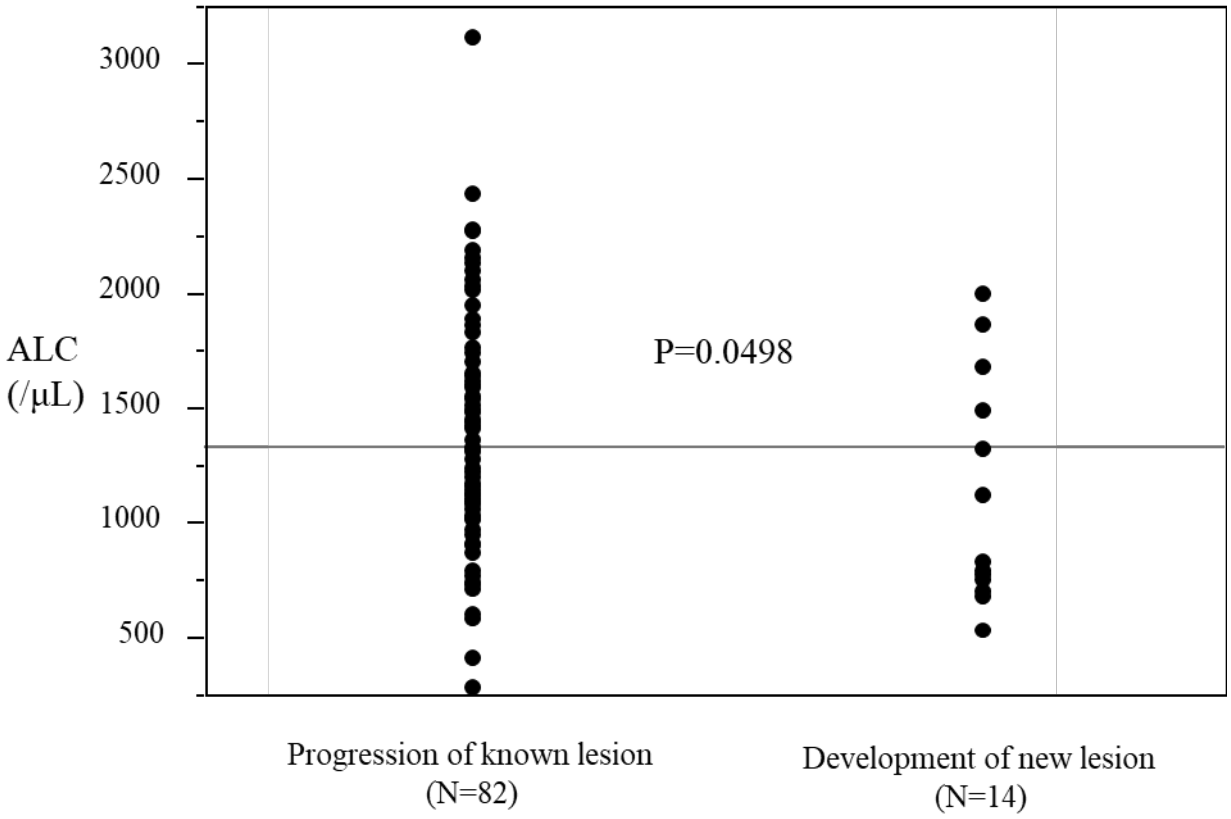
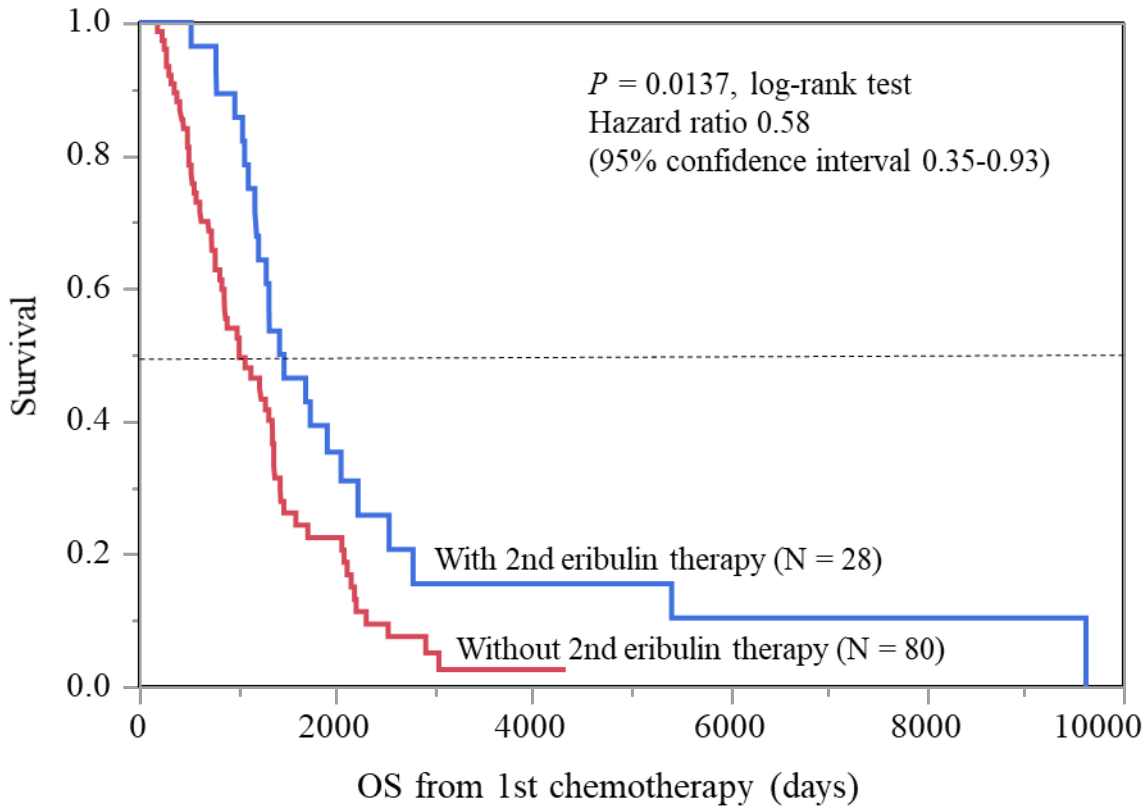
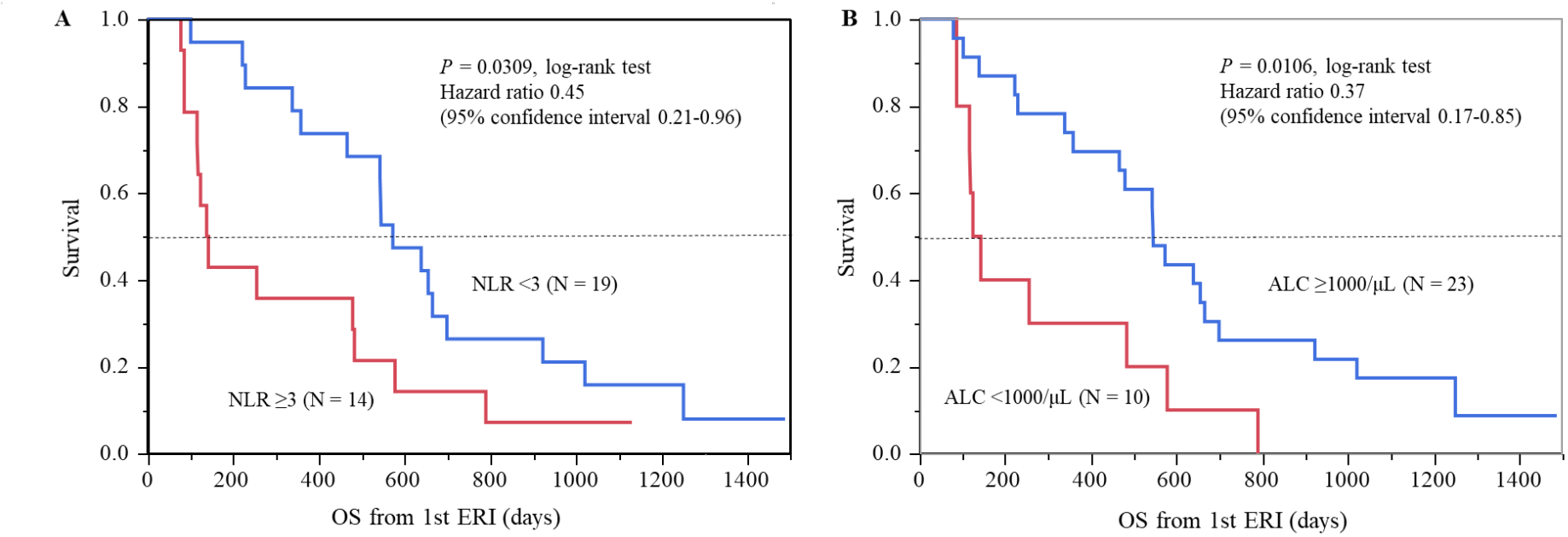


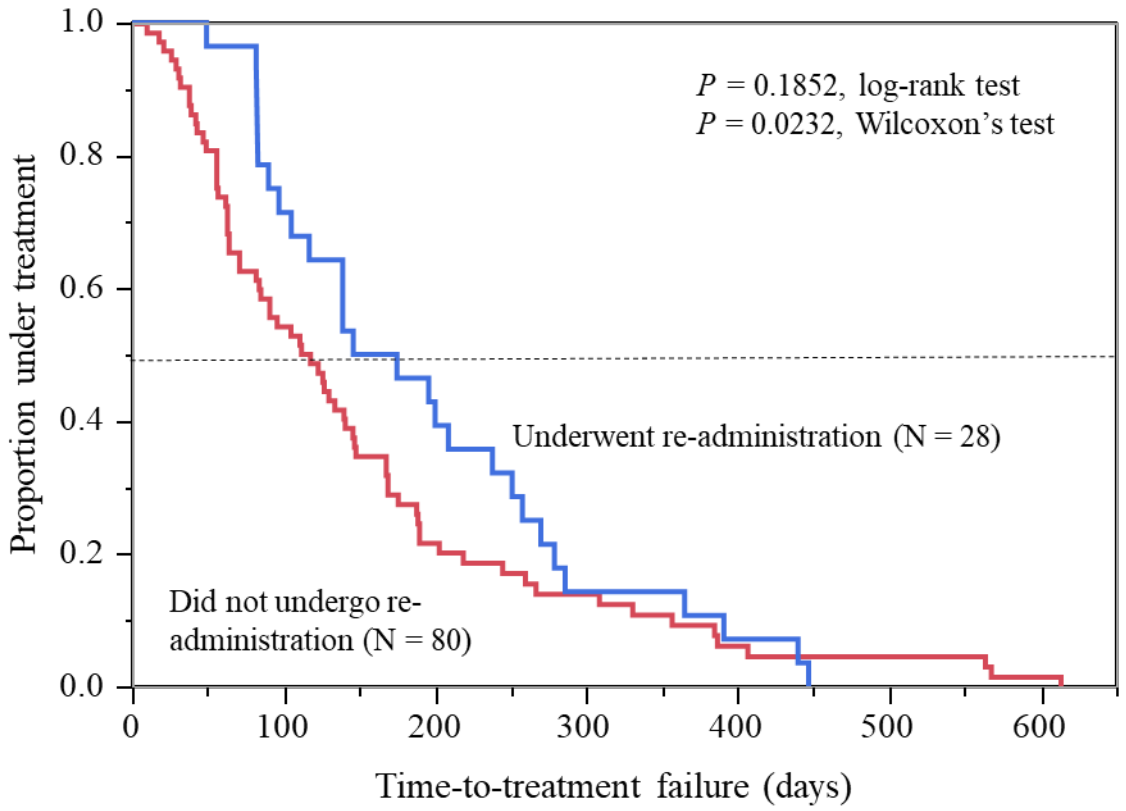
Fig. 2 Wilcoxon/Kruskal-Wallis test for relationship between absolute lymphocyte count at the initiation of first eribulin therapy and pattern of disease progression. *ALC* absolute lymphocyte count



Supplemental Fig. 1 Overall survival after the initiation of the first-line chemotherapy in ER+ patients according to with/without re-administration of eribulin.



Supplemental Fig. 2 Overall survival after the initiation of the first eribulin therapy in ER-negative patients excluding 3 patients missing absolute lymphocyte count at the initiation of 1st eribulin therapy according to **A** NLR <3/≥3 (P = 0.0309), and **B** ALC (/μL) ≥1000/<1000 (P = 0.0106). *OS* overall survival, *NLR* neutrophil-to-lymphocyte rate, *ALC* absolute lymphocyte count, *1st ERI* first eribulin therapy.



Supplemental Fig. 3 Time-to-treatment failure of the subsequent therapy after the first round of eribulin therapy in ER+ patients according to re-administration.