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Research paper

High expression of topoisomerase-II predicts favorable clinical outcomes in patients with relapsed small cell lung cancers receiving amrubicin

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ABSTRACT

Objectives: Amrubicin monotherapy is a treatment option for patients with relapsed small cell lung cancers (SCLCs). Topoisomerase-II (Topo-II) – a target of amrubicin – has been reported as a predictive or prognostic marker for chemosensitivity or outcomes in patients with various malignancies. Here, we investigated the prognostic role of Topo-II expression in patients with relapsed SCLCs who underwent amrubicin monotherapy.
Materials and methods: Eighty-three patients with relapsed SCLCs who received amrubicin monotherapy between 2004 and 2015, after progression beyond first-line chemotherapy, were enrolled in the study. We retrospectively collected clinical data from their medical records, and evaluated the expression levels of Topo-II, by immunohistochemical staining of archival tumor specimens obtained through surgical resections or biopsies.
Results: Most of the enrolled patients were elderly men (89%), with a median age of 70 years (range, 49–83); 16% of these patients showed Topo-II overexpression. Compared to patients with sensitive relapses, those with refractory relapses showed significantly higher Topo-II expression levels ($P = 0.03$). The overall response rates in patients with high and low Topo-II expression were 38.5% and 25.7%, respectively ($P = 0.34$). Multivariate analysis confirmed that patients with a higher Topo-II expression level had significantly longer progression-free survival (hazard ratio (HR), 0.39; $P < 0.01$) and overall survival (HR, 0.48; $P = 0.04$), compared to patients with a lower Topo-II expression level.
Conclusion: Our study identified Topo-II expression as a significant biomarker for the prediction of favorable outcomes in patients with relapsed SCLCs who underwent treatment with amrubicin, a Topo-II inhibitor. Thus, Topo-II expression may be a promising predictor of the efficacy of amrubicin.

1. Introduction

Small cell lung cancers (SCLCs) are distinct neuroendocrine tumors with aggressive features, and account for 13% of all newly diagnosed cases of lung cancer [1]. Despite showing high response rates to initial combination chemotherapy [2,3], most patients with SCLCs experience either recurrences or disease progression. Thus, most patients with relapsed SCLCs need effective salvage chemotherapy. However, standard chemotherapy for this purpose has not yet been established despite extensive efforts to develop new strategies for relapsed SCLCs.

In the United States, topotecan, a specific DNA topoisomerase-I inhibitor, is the only agent approved for second-line therapy in patients with relapsed SCLCs, from among the agents recommended in the National Comprehensive Cancer Network (NCCN) guidelines [4] (based on the results of previous randomized phase III trials [5,6]). However, the results of a Japanese prospective study of patients treated with topotecan were disappointing, especially those pertaining to refractory relapses, demonstrating overall response rates (ORRs) of 0%, a median progression-free survival (PFS) of 1.5 months, and a median overall survival (OS) of 5.4 months [7].

Amrubicin, a fully synthetic 9-aminoanthracycline derivative, is converted to the active metabolite amrubicinol in the body via the reduction of a ketone motif at its 13th position; this serves as a DNA topoisomerase-II (Topo-II) inhibitor, and not mainly as a DNA intercalator [8]. In Japan, amrubicin was approved for use in 2002, and is one of the treatment options available for patients with relapsed SCLCs.

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Several phase II studies have shown promising efficacy of amrubicin in patients, with ORRs of 21–67%, median PFS of 3.2–5.4 months, and median OS of 6.0–14.4 months [7,9–12]. Although the largest randomized phase III trial conducted could not confirm the superiority of amrubicin therapy over that of topotecan therapy in terms of patient survivals, subset analysis indicated that SCLC patients with refractory relapses could benefit greatly from undergoing amrubicin therapy [13]. This has encouraged further investigations into the clinical characteristics or biological features that may result in higher anti-tumor effects of amrubicin therapy. To date, to the best of our knowledge, no studies have elucidated any potential biomarkers that may predict tumor responses or clinical outcomes in patients with SCLC treated with amrubicin, except in case of a polymorphism of NAD(P)H quinone oxidoreductase 1 [14]; however, the predictive value of this biomarker has not been validated in prospective trials.

Topo-II, a target of amrubicin, has been evaluated as a potential biomarker in various malignancies, including breast [15,16], ovarian [17], and lung cancers [18,19]. A large-scale prospective pooled analysis reported that Topo-II expression was a significant biomarker in predicting the benefits of adjuvant anthracycline chemotherapy in breast cancer [16]. However, its role as a biomarker remains unclear in patients with relapsed SCLCs who receive amrubicin therapy. Therefore, our present study examines whether the expression levels of Topo-II, determined by immunohistochemistry, can be correlated with chemosensitivity or clinical outcomes in patients with relapsed SCLCs who received amrubicin monotherapy.

2. Material and methods

2.1. Patients

We screened 116 patients with either SCLC or large cell neuroendocrine carcinoma (LCNEC) treated at the Shibukawa Medical Center or the Gunma University Hospital between July 2004 and July 2015. All patients experienced relapse after first-line chemotherapy, and subsequently received amrubicin monotherapy. Thirty-three patients were excluded due to non-availability of tumor specimens (n = 21), inaccurate diagnoses (n = 2), or receipt of amrubicin as first-line therapy (n = 10). A total of 83 patients were eventually enrolled in the present study.

2.2. Data collection

We retrospectively collected data on patient characteristics such as response to chemotherapy, pathological findings, and survival, from patient medical records. The clinical stage at diagnosis was classified into limited disease (LD) or extensive disease (ED) [20]. The histopathological types were assessed according to the 2004 World Health Organization histological classification [21]. Types of relapse were classified as sensitive or refractory relapses, according to the length of the treatment-failure interval (TFI). We defined TFI as the period from the date of completion of first-line therapy to the date of recurrence. As defined in most clinical trials, relapses with TFI ≥ 90 days were defined as sensitive relapses; relapses with TFI < 90 days were defined as refractory relapses. The tumor responses were assessed according to the Response Evaluation Criteria in Solid Tumors version 1.1 [22]. PFS was defined as the time interval between the date of amrubicin treatment initiation and the date of disease progression or of death due to any cause. Similarly, OS was calculated as the time interval between the date of amrubicin treatment initiation and the date of death or the last follow-up consultation. This study was conducted in compliance with the Declaration of Helsinki. The study protocol was approved by the Institutional Review Boards of the Gunma University Hospital (ref. 1287) and the National Hospital Organization Shibukawa Medical Center (ref. 15-03-05).

2.3. Immunohistochemical analysis

Tumor samples were obtained via surgical resections or biopsies obtained prior to first-line chemotherapy; 10 samples (12%) were obtained via surgical resections, and 69 (83%) via biopsies. Information about the collection procedure was not available for 4 samples (5%). The procedure used for immunohistochemical staining has been described previously [23]. An anti-Topo-II rabbit polyclonal antibody (ab180393, Abcam, Tokyo, Japan, 1:100 dilution) was used in this study. Cells were deemed positive for Topo-II if positive staining was present in the nuclei. The proportion of Topo-II-positive cells was assessed by using a semi-quantitative scoring method, wherein samples were assigned a score based on the percentage of positive cells: Score 1, < 10% positive cells; 2, 10% to < 25%; 3, 25 to < 50% positive cells; 4, 50 to < 75%, and 5, ≥75% positive cells [24,25]. We compared tumor responses and survival data between the groups that showed high (Topo-II-high group) and low (Topo-II-low group) Topo-II expression, with various cut-off scores for Topo-II expression. In the present study, expression scores between 1 and 4 signified low Topo-II expression, and a score of 5 signified high Topo-II expression. Given that most samples were biopsy specimens, only the presence, but not the intensity, of the staining was used for analysis. Sections were examined under light microscopy by at least two investigators in a blinded fashion. In case of discrepancies, both investigators simultaneously evaluated the slides until a consensus was reached.

2.4. Statistical analysis

Statistical significance was set at $P < 0.05$. The association between immunohistochemical staining and the clinicopathological factors was examined using the Fisher's exact test. The difference in mean Topo-II scores between the two groups was analyzed by the nonparametric Mann-Whitney test. The Kaplan-Meier method was used to estimate survivals, and the survival difference between groups was analyzed by the log-rank test. Multivariate analyses were performed using the Cox proportional hazards model to identify independent prognostic factors. Statistical analysis was performed using GraphPad Prism 6 software (Graph Pad Software, San Diego, CA, USA) and EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan) for Windows.

3. Results

3.1. Patient characteristics according to Topo-II expression

The characteristics of the patients included in this study are summarized in Table 1A. Most eligible patients were elderly men (89%), with a median age of 70 years (range, 49–83). Most tumor specimens showed histology characteristic of SCLC (92%). Sixty-four patients (77%) had favorable performance status (PS) scores of 0 or 1, while 19 patients (23%) had unfavorable PS scores of 2, 3, or 4. Sensitive relapses occurred in 24 patients (29%), and refractory relapses in 59 (71%). Amrubicin was administered as second-line therapy in 61 patients (74%), and administered as third-line or more in 22 patients (26%). After progression beyond amrubicin monotherapy, 49 patients (59%) received subsequent chemotherapy, whereas 34 (41%) received no further chemotherapy.

The clinicopathological features of patients according to Topo-II expression are summarized in Table 1B. The Topo-II-high group had a significantly lower proportion of patients treated with irinotecan-based chemotherapy, compared to the Topo-II-low group ($P = 0.03$). More refractory relapses and lower incidence of subsequent chemotherapy after amrubicin monotherapy were seen in the Topo-II-high group, although these differences were not statistically significant. The patients in the Topo-II-high group tended to receive amrubicin as second-line therapy rather than as third-line or more.

Table 1
Baseline characteristics of total patients (A) and those according to Topo-II expression (B).

A

Characteristics (N = 83)	Number of pts. (%)
Age	median, 70 (range, 49–83)
Sex	
Male	74 (89)
Female	9 (11)
PS	
0 or 1	64 (77)
2, 3, or 4	19 (23)
Histology	
Small cell carcinoma	76 (92)
Large cell neuroendocrine carcinoma	5 (6)
Combined small cell carcinoma	2 (2)
Stage at diagnosis	
Limited disease	30 (36)
Extensive disease	53 (64)
Relapse type	
Sensitive relapse	24 (29)
Refractory relapse	59 (71)
1st-line regimen[a]	
VP16-based	64 (77)
CPT11-based	19 (23)
Response to 1st-line treatment	
CR	7 (8)
PR	51 (61)
SD	14 (17)
PD	5 (6)
NE	6[b] (7)
Timing of AMR	
2nd-line	61 (73)
beyond 2nd-line	22 (27)
Doses of AMR[c]	
≥35 mg/m^2	74 (89)
< 35 mg/m^2	9 (11)
Course of AMR administration	median, 2 (range, 1–24)
1–2 courses	46 (55)
≥3 courses	37 (45)
Post-progression chemotherapy	
Yes	49 (59)
No	34 (41)
Discontinuation due to AE	
Yes	12 (14)
No	71 (86)

B

Characteristics	Topo-II		
	Low (N = 70)	High (N = 13)	*P* value
Age			
< 70 yrs	33	7	0.77
≥ 70 yrs	37	6	
Sex			
Male	61	13	0.34
Female	9	0	
PS			
0 or 1	53	11	0.72
2, 3, or 4	17	2	
Stage at diagnosis			
Limited disease	27	3	0.36
Extensive disease	43	10	
Relapse type			
Sensitive relapse	23	1	0.10
Refractory relapse	47	12	
1st-line regimen[a]			
VP16-based	52	13	0.03*
CPT11-based	19	0	
Response to 1st-line treatment			
CR/PR	47	11	0.33
SD/PD/NE	23	2	
Timing of AMR therapy			
2nd-line	49	12	0.17
beyond 2nd-line	21	1	
Doses of AMR[c]			
≥35 mg/m^2	61	12	1.00
< 35 mg/m^2	7	1	
Post-progression chemotherapy			
Yes	31	3	0.22
No	39	10	
Discontinuation due to AE			
Yes	10	2	1.00
No	60	11	

Abbreviations: Topo-II, topoisomerase-II; PS, performance status; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluated; AMR, amrubicin; AE, adverse event.

[a] One patient who received etoposide-based concurrent chemo-radiation therapy followed by chemotherapy with cisplatin plus irinotecan was included in both etoposide-based- and irinotecan-based 1st-line regimen.

[b] The effect of 1st-line treatment was classified as NE due to the lack of measurable lesions in 5 of 6 patients treated with adjuvant chemotherapy after surgical resection.

[c] Information of doses of AMR was not collected in 2 patients.

* *P* values are statistically significant ($P < 0.05$).

3.2. Immunohistochemical findings

Immunohistochemical analysis of Topo-II expression was conducted using tumor samples from 69 primary sites and 11 metastatic sites; the information in 3 samples was not recorded. Topo-II expression was successfully evaluated in all 83 specimens. Representative images of Topo-II staining are shown in Fig. 1, and the distribution of Topo-II expression scores is shown in Fig. 2A. Thirteen (16%) tumors showed high Topo-II expression. Topo-II overexpression was observed in 1 in 7 tumors (14%) with non-SCLC (LCNEC and combined SCLC). The mean Topo-II expression score was 3.52 ± 1.10 in all tumors, and was significantly higher in patients with refractory relapses than in those with sensitive relapses (3.68 ± 1.07 vs. 3.13 ± 1.08; $P = 0.03$; Fig. 2B); this suggested the presence of a significant correlation between Topo-II expression level and relapse type in these patients.

3.3. Efficacy of amrubicin

The ORR and the disease control rate (DCR) in all patients were 27.4% (95% confidence interval (CI), 17.8–36.9) and 67.9% (95% CI, 57.9–77.8), respectively. The ORRs in the Topo-II-high and low groups were 38.5% and 25.7%, respectively (odds ratio (OR), 1.81; $P = 0.34$). The DCRs in the Topo-II-high and low groups were 61.5% and 68.6%, respectively (OR, 0.73; $P = 0.75$). There was no significant correlation between clinicopathological characteristics and the ORR or the DCR.

3.4. Survival analysis according to Topo-II expression

As of the data cutoff date (March 31, 2016), all 83 patients had experienced disease progression after undergoing amrubicin monotherapy. Within the median follow-up period of 6.7 months (range, 0.4–58.2), 82 patients died. An OS event in 1 patient was censored because he was lost to follow-up. The median PFS and OS in all patients were 2.2 and 6.7 months, respectively. The median PFS in patients in the Topo-II-high group ($N = 13$) was significantly prolonged, compared to that in the Topo-II-low group ($N = 70$) (3.3 vs. 1.7 months; hazard ratio (HR), 0.47; $P < 0.01$; Fig. 3A). The median OS was longer in the Topo-II-high group than in the Topo-II-low group; this result was not statistically significant (7.3 vs. 6.0 months; HR, 0.68; $P = 0.14$; Fig. 3B). Among patients with refractory relapses ($N = 59$), those in the

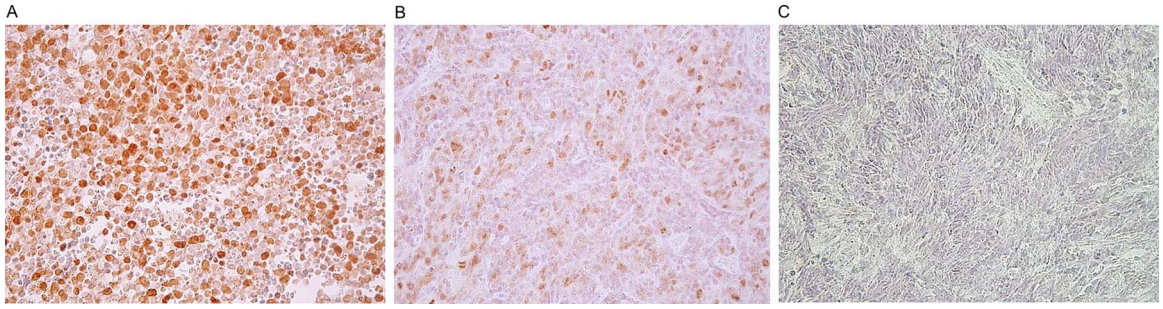

Fig. 1. Immunohistochemical staining for Topo-II expression in tumor samples with SCLCs (400x magnification). (A) High Topo-II expression is observed in the nuclei (score 5). (B, C) Low Topo-II expression can be seen (scores 3 and 1, respectively).

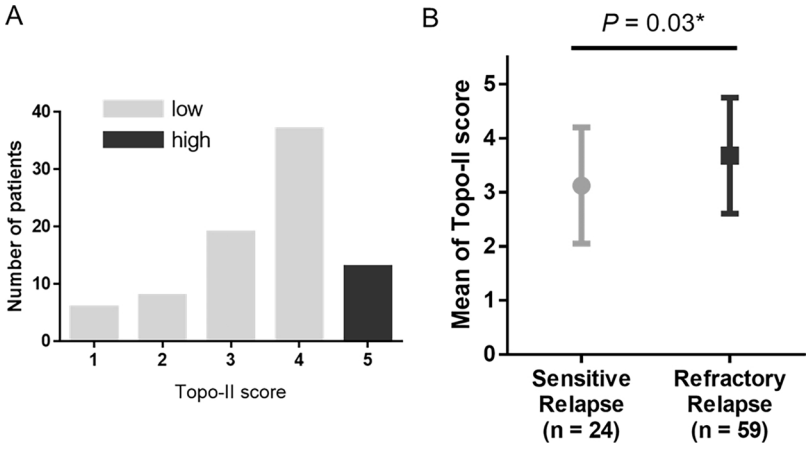

Fig. 2. (A) The distribution of Topo-II expression scores as per immunohistochemical staining is shown. Grey bars indicate low expression, and the black bar indicates high expression. (B) The mean Topo-II expression scores according to the type of relapse experienced by patients are shown. Patients with refractory relapses had a significantly higher mean Topo-II expression score, compared to those with sensitive relapses (3.68 ± 1.07 vs. 3.13 ± 1.08, $P = 0.03$).

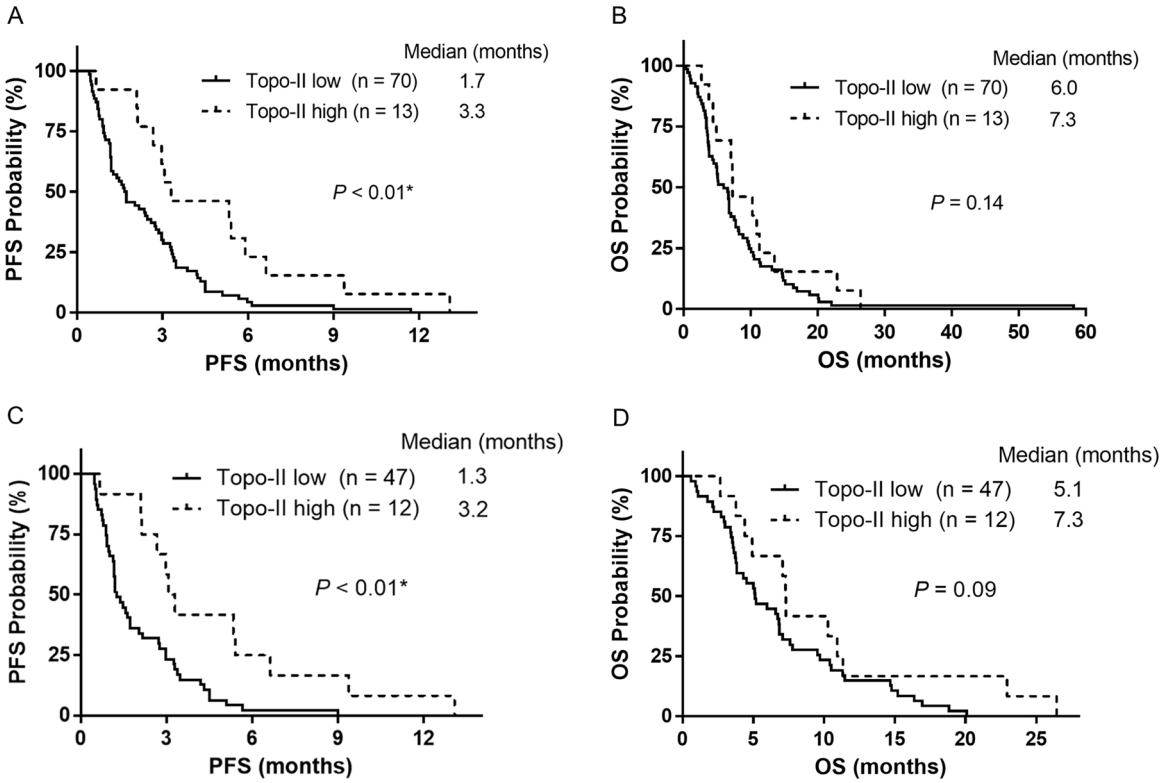

Fig. 3. Survival analysis according to Topo-II expression using the Kaplan-Meier method in all patients (A, B) and in patients with refractory relapses (C, D). (A) The median PFS in patients with high Topo-II expression was significantly longer than in patients with low Topo-II expression (median, 3.3 vs. 1.7 months; HR, 0.47; $P < 0.01$). (B) There was a trend toward longer OS in patients with high Topo-II expression than in those with low Topo-II expression (median, 7.3 vs. 6.0 months; HR, 0.68; $P = 0.14$). (C) Among patients with refractory relapses, those with high Topo-II expression showed significantly longer PFS that those with low Topo-II expression (median, 3.2 vs. 1.3 months; HR, 0.43; $P < 0.01$). (D) Among patients with refractory relapses, there was a trend toward longer OS in those with high Topo-II expression than in those with low Topo-II expression (median, 7.3 vs. 5.1 months; HR, 0.60; $P = 0.09$).

Table 2
Univariate analysis of progression-free survival (A) and overall survival (B) from the initiation of AMR chemotherapy.

A

Factors	PFS		
	HR	95% CI	*P* value
Age (< 70 yrs. vs ≥70 yrs.)	1.03	0.67–1.59	0.88
Sex (Male vs. Female)	1.13	0.59–2.19	0.72
PS (0–1 vs. 2–4)	0.59	0.29–0.96	0.04*
Stage at diagnosis (LD vs. ED)	1.03	0.66–1.61	0.9
Relapse type (Sensitive vs. Refractory)	0.79	0.50–1.24	0.31
1st-line regimen (VP16-based vs CPT11-based)	0.91	0.53–1.54	0.71
Response to 1st-line treatment (CR/PR vs. SD/PD/NE)	0.64	0.36–0.99	0.05*
Dose of AMR therapy (≥35 mg/m^2 vs. < 35 mg/m^2)	0.61	0.23–1.23	0.15
Discontinuation due to AE (No vs. Yes)	0.54	0.19–0.92	0.04*
Timing of AMR therapy (2nd-line vs. 3rd-line ~)	0.99	0.60–1.61	0.95
Topo-II (high vs. low)	0.47	0.31–0.80	< 0.01*

B

Factors	OS		
	HR	95% CI	*P* value
Age (< 70 yrs. vs. ≥70 yrs.)	0.91	0.59–1.40	0.67
Sex (Male vs. Female)	1.26	0.67–2.39	0.49
PS (0–1 vs. 2–4)	0.38	0.11–0.47	0.0001*
Stage at diagnosis (LD vs. ED)	0.97	0.62–1.51	0.88
Relapse type (Sensitive vs. Refractory)	0.78	0.49–1.23	0.29
1st-line regimen (VP16-based vs. CPT11-based)	0.91	0.53–1.55	0.72
Response to 1st-line therapy (CR/PR vs. SD/PD/NE)	0.96	0.61–1.53	0.87
Dose of AMR therapy (≥35 mg/m^2 vs. < 35 mg/m^2)	0.52	0.16–1.01	0.06
Discontinuation due to AE (No vs. Yes)	0.41	0.10–0.58	< 0.01*
Timing of AMR therapy (2nd-line vs. 3rd-line ~)	0.83	0.48–1.35	0.47
Post-PD treatment (Yes vs. No)	0.51	0.31–0.75	< 0.01*
Response to AMR therapy (CR/PR vs. SD/PD/NE)	0.67	0.43–1.07	0.10
Response to AMR therapy (CR/PR/SD vs. PD/NE)	0.55	0.28–0.81	< 0.01*
Topo-II (high vs. low)	0.68	0.41–1.18	0.14

Abbreviations: AMRamrubicin; PFSprogression-free survival; OSoverall survival; HRhazard ratio; CIconfidence interval; PSperformance status; LDlimited disease; EDextensive disease; VP16etoposide; CPT11irinotecan; CRcomplete response; PRpartial response; SDstable disease; PDprogressive disease; NEnot evaluated; Topo-IItopoisomerase-II.

* *P* values are statistically significant ($P < 0.05$).

Table 3
Multivariate analysis of progression-free survival, and overall survival of AMR chemotherapy.

Variables	PFS			OS		
	HR	95% CI	*P* value	HR	95% CI	*P* value
PS (0–1 vs. 2–4)	0.65	0.38–1.11	0.11	0.43	0.23–0.78	< 0.01*
Discontinuation due to AE (No vs. Yes)	0.53	0.28–1.00	0.048*	0.40	0.20–0.81	0.01*
Relapse type (Sensitive vs. Refractory)	0.65	0.39–1.08	0.10	0.63	0.37–1.07	0.09
Post-PD treatment (Yes vs. No)	–	–	–	0.51	0.30–0.88	0.02*
Response to AMR therapy (CR/PR/SD vs. PD/NE)	–	–	–	0.60	0.35–1.00	0.052
Topo-II (high vs. low)	0.39	0.20–0.74	< 0.01*	0.48	0.24–0.96	0.04*

Abbreviations: AMR, amrubicin; PFS, progression-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval; PS, performance status; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; Topo-II, topoisomerase-II.

* *P* values are statistically significant ($P < 0.05$).

Topo-II-high group ($N = 12$) had significantly longer median PFS (3.2 vs. 1.3 months; HR, 0.43; $P < 0.01$; Fig. 3C) and a trend towards longer median OS, compared to those in the Topo-II-high group (7.3 vs. 5.1 months; HR, 0.60; $P = 0.09$; Fig. 3D). Because only 1 patient from the Topo-II-high group presented with a sensitive relapse, we did not perform subset survival analysis of patients with sensitive relapses.

3.5. Univariate and multivariate analysis

Univariate analysis showed that a favorable PS, response to first-line chemotherapy, and high Topo-II expression were significantly associated with a prolonged PFS (Table 2). In addition, a favorable PS, no discontinuation of amrubicin treatment due to adverse events (AEs) subsequent chemotherapy after amrubicin therapy, and responsiveness to amrubicin therapy (complete response (CR)/partial response (PR)/stable disease (SD) vs. progressive disease (PD)/not evaluated (NE)) were significantly linked with favorable OS; Topo-II expression was not significantly linked with a favorable OS (Table 2).

Multivariate analysis revealed that no discontinuation of amrubicin therapy due to AE (HR, 0.53; $P = 0.048$) and high Topo-II expression (HR, 0.39; $P < 0.01$) were independent prognostic factors for longer PFS (Table 3). Furthermore, a favorable PS (HR, 0.43; $P < 0.01$), no discontinuation of amrubicin therapy due to AE (HR, 0.40; $P = 0.01$), subsequent chemotherapy after undergoing amrubicin therapy (HR, 0.51; $P = 0.02$), and a high Topo-II expression (HR, 0.48; $P = 0.04$) were independent prognostic factors for longer OS (Table 3).

4. Discussion

In our study, Topo-II expression was identified as an independent prognostic factor for predicting favorable PFS and OS in patients with relapsed SCLCs who received amrubicin monotherapy. Additionally, this study revealed that patients with refractory relapses demonstrated significantly higher levels of Topo-II expression than those with sensitive relapses. Specifically, we found that evaluation of Topo-II expression was useful as a significant predictor of favorable outcomes in patients with SCLCs who received amrubicin monotherapy.

The clinical utility of Topo-II expression has been tested extensively in previously untreated patients with SCLC who received combination chemotherapy with agents including etoposide (a representative Topo-II inhibitor) and platinum agents [18,26,27]. Generally, these studies have displayed a correlation between upregulation of Topo-II expression and unfavorable outcomes in patients, in agreement with our finding that patients with refractory relapses exhibited significantly higher Topo-II expression than those with sensitive relapses. As observed in cases of prostate cancer [28,29] and hepatocellular carcinoma [30], the overexpression or gene amplification of Topo-II may be involved in promoting cell proliferation and aggressive behavior of tumors, leading to earlier relapse of disease.

Most therapeutics that target Topo-II, such as anthracyclines and etoposide, elicit cytotoxicity by the generation of Topo-II-DNA covalent complexes that are dependent, in part, on Topo-II levels [31]. In preclinical studies, Topo-II activity or expression has been reported to be downregulated in cells that are resistant to treatment with etoposide or anthracyclines [32,33]. In lymphoma cells, a pooled shRNA screening approach has revealed that the suppression of Topo-II expression induced resistance to doxorubicin treatment in both *in vitro* and *in vivo* conditions [34]. Moreover, an *in-vitro* study has demonstrated that enhanced expression of the Topo-II protein via exogenous activation was associated with an increased sensitivity to treatment with doxorubicin and etoposide [35]. In accordance with these preclinical observations, several clinical studies have reported that Topo-II overexpression was closely associated with hypersensitivity to anthracycline-based chemotherapy [36,37]. Furthermore, a pivotal phase III study reported a higher activity of amrubicin in a subset of patients with refractory relapses [13]; based on our present findings, we

postulate that this population may have included a higher proportion of patients with Topo-II overexpression (Table 1B, Fig. 2B). Collectively, these findings robustly support a significant correlation between elevated Topo-II expression and a favorable prognosis for amrubicin therapy, as seen in our study.

The advances in translational research have indicated that the identification of strong positivity of a direct chemotherapeutic target as a predictive biomarker provides valuable implications for clinical practice. The recent success in this regard is in the case of anti-programmed death-1 (anti-PD-1) therapy for non-small cell lung cancers (NSCLCs). A randomized phase III trial has demonstrated that untreated patients with advanced NSCLCs and expression of programmed death ligand-1 (PD-L1), a direct target for the anti-PD-1 inhibitor, on at least 50% of tumor cells gained survival benefits from anti-PD-1 blockade, compared with patients who underwent chemotherapy [38]. Consistent with this result, our present data have suggested that elevated expression of Topo-II, a direct target of amrubicin, predicted favorable prognosis for patients who received amrubicin. Considering the previous evidence that Topo-II expression was associated with hypersensitivity to treatment with Topo-II inhibitors [32–37] and with favorable prognoses [39,40] in various cancers, it is plausible that elevated Topo-II expression favorably affects clinical outcomes in pretreated SCLC patients who received amrubicin treatment. Therefore, Topo-II expression may be a potential predictive biomarker for amrubicin treatment in patients with relapsed SCLCs, although further validation with prospective studies is indicated.

In this study, however, a correlation between Topo-II expression and response to amrubicin therapy was not observed. Consistent with our study, studies of other malignancies have similarly been unable to establish a relationship between chemosensitivity and Topo-II expression [39,40]. In experimental studies, it has been shown that treatment with Topo-II inhibitors induced a downregulation of Topo-II, and treatment with Topo-I inhibitors led to an upregulation of Topo-II [41,42]. Additionally, other preclinical data have suggested that the expression levels of Topo-II transcripts or proteins may not always accurately reflect Topo-II activity in various tumor types [43–45]. These findings have implied that Topo-II activity could be modulated by various factors. Therefore, further investigations are required to evaluate the role of Topo-II in promoting amrubicin responsiveness and favorable clinical outcomes.

There are several limitations of this study. First, our study was retrospective and had a small sample size. However, this is the largest study of previously treated patients with SCLC who received amrubicin treatment after relapse. Second, analyzed specimens were obtained mainly via biopsies at initial diagnosis; hence, intratumoral heterogeneity or chemotherapy-induced alteration of Topo-II expression may have influenced the results. Owing to the rapid progression of disease, it was difficult to obtain samples via surgical resections or repeat biopsies prior to amrubicin administration. However, this study has shown that biopsy samples at initial diagnoses may facilitate the prediction of disease prognosis in patients subsequently treated with amrubicin therapy.

5. Conclusion

We identified Topo-II overexpression as a significant biomarker for the prediction of favorable outcomes in patients with relapsed SCLCs who received amrubicin, a Topo-II inhibitor. Topo-II expression may be a promising indicator of the efficacy of amrubicin. Further prospective studies to verify the prognostic significance of Topo-II overexpression are warranted.

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Conflict of interest statement

The authors declare that they have no conflicts of interest.

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