



## ORIGINAL ARTICLE

# Analysis of topoisomerase I expression and identification of predictive markers for efficacy of topotecan chemotherapy in small cell lung cancer

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## Keywords

Second-line chemotherapy; small cell lung cancer; topoisomerase-1; topotecan.

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## Abstract

**Background:** We evaluated topoisomerase I (TOPO1) expression in patients with small cell lung cancer (SCLC) and identified predictive factors for the efficacy of second-line topotecan chemotherapy.

**Methods:** We retrospectively evaluated the records of SCLC patients treated in our department from January 2007 to December 2016 who received second-line topotecan chemotherapy. Patients with archived tumor samples were enrolled. TOPO1 expression levels were evaluated by immunohistochemistry, and the relationships between TOPO1 expression, clinical factors, chemotherapy efficacy, and survival were analyzed.

**Results:** Of the 78 patients enrolled, 67 showed TOPO1 expression (85.9%). Patients were divided into strong ( $n = 43$ ) or weak ( $n = 35$ ) expression groups based on staining intensity. Disease control rates for topotecan were 39.5% and 14.3% in the strong and weak groups, respectively ( $P = 0.014$ ). Second-line median progression-free survival was 2.2 and 2.0 months ( $P = 0.057$ ), and median overall survival was 8.1 and 6.0 months ( $P = 0.199$ ) in the strong and weak positive groups, respectively. Patients were also divided into sensitive ( $n = 47$ ) and refractory ( $n = 31$ ) disease groups according to the duration from the onset of first-line therapy to relapse. Median second-line progression-free survival was 2.2 and 1.8 months in the sensitive and refractory relapse groups, respectively ( $P = 0.005$ ).

**Conclusions:** TOPO1 expression was prevalent in SCLC patients. Strong expression was associated with an elevated disease control rate after second-line topotecan chemotherapy. Patients with sensitive disease that relapsed after first-line chemotherapy had better survival than refractory patients who received second-line topotecan chemotherapy.

## Introduction

Lung cancer is a severe worldwide health problem, particularly in China.<sup>1,2</sup> Small cell lung cancer (SCLC) is the most

aggressive subtype and accounts for approximately 14% of all lung cancers.<sup>3</sup> In the United States (US), approximately 31 000 patients are diagnosed with SCLC annually. Despite numerous clinical trials since the 1970s, including at least

40 phase III trials, systemic treatment for patients with SCLC has not changed significantly over the past few decades. Consequently, the five-year survival rate for this type of lung cancer remains low at < 7%, with the majority of patients surviving less than a year after diagnosis.<sup>4</sup>

Currently, the main treatments for SCLC include chemotherapy and radiotherapy, with a response rate of approximately 70% to first-line chemotherapy.<sup>5–7</sup> However, the efficacy of second-line therapy is low because of drug resistance, resulting in poor prognosis.<sup>8,9</sup> At present, topotecan is the only agent approved by the US Food and Drug Administration (FDA) for recurrent or progressive SCLC.<sup>10,11</sup> Topotecan works by inhibiting topoisomerase I (TOPO1), a nuclear enzyme that relieves torsional strain during DNA replication by creating single strand breaks.<sup>12,13</sup> Topotecan binding to the DNA cleavage complex prevents TOPO1 from re-ligating the nicked DNA strand after relieving the strain. This intercalation therefore traps TOPO1, leading to DNA damage.<sup>14</sup> The unbroken DNA strand then breaks, resulting in a double-stranded break that mammalian cells are unable to efficiently repair.<sup>15</sup> This action prevents DNA replication and ultimately leads to cancer cell death.

However, response rates to topotecan in cases of relapsed SCLC are reported at approximately 20%.<sup>9,16,17</sup> Effective predictive markers are needed to improve the selection of sensitive patients.<sup>18</sup> *in vitro* studies have shown that high TOPO1 expression levels are associated with improved sensitivity to TOPO1 inhibitors in cancer cells.<sup>19</sup> However, it is not clear whether TOPO1 could serve as a predictive marker for topotecan efficacy in patients with SCLC.<sup>20,21</sup> In this study, therefore, we examine TOPO1 expression levels and explore the potential predictive markers for the response to topotecan chemotherapy in SCLC patients.

## Methods

### Patients

The study was approved by the Ethics Committee of the Peking University Cancer Hospital in Beijing, China. Consent was obtained from all patients diagnosed with SCLC who received topotecan chemotherapy in our department between January 2007 and December 2016, identified through the hospital database. Inclusion criteria were: pathological diagnosis of SCLC; topotecan administered as second-line chemotherapy; Eastern Cooperative Oncology Group performance status (ECOG-PS) 0–2; and the availability of archived tissue samples for immunohistochemical analysis. Disease recurrence was assessed by computed tomography (CT) scanning, magnetic resonance imaging (MRI), bone scanning, and various tumor markers. All staging procedures were carried out using the 7th Union

for International Cancer Control tumor node metastasis (TNM) classification. We collected details on the dates from initiation of chemotherapy to disease progression, gender, age, smoking status, disease stage, chemotherapy response, survival and pathologic subtype, and ECOG PS.<sup>22</sup> The therapy response was assessed according to Revised Evaluation Criteria in Solid Tumors (RECIST) version 1.1.<sup>23</sup> Second-line progression-free survival (PFS) was measured from the date of topotecan administration to the date of documented disease progression. Second-line overall survival (OS) was calculated as the date of topotecan administration to the date of death. First-line OS was measured from the date first-line chemotherapy was commenced to the date of death.

### Immunohistochemical analysis

Samples were obtained at the time of diagnosis in 69 (88.5%) patients and 9 (11.5%) provided re-biopsy samples at the time of disease progression before commencing topotecan chemotherapy. TOPO1 expression was examined by immunohistochemistry (IHC) using an anti-TOPO1 antibody (ab85038, Abcam, Cambridge, UK).<sup>24,25</sup> Two central pathologists who were not involved in patient management decisions and were blinded to study treatment verified the IHC results. Five fields of view were analyzed in each slide, and a semi-quantitative H-score ranging from 0 to 300 was calculated by multiplying the percentage of positively stained cells by an intensity score (0, absent; 1, weak; 2, moderate; 3, strong). Patients with an H-score of 200–300 were classified into the “strong expression” group, and the remainder was classified into the “weak expression” group, including those with negative expression.

### Statistical analysis

Frequencies and proportions were reported for categorical variables, and the mean and median for continuous variables. Univariate analysis was performed using chi-square or Fisher's exact tests for categorical variables, and independent sample *t* or Wilcoxon rank sum tests for normal and non-normal continuous variables, respectively.

We used the Kaplan–Meier method to describe OS and PFS. The log-rank test was utilized to test for differences in OS and PFS between groups. A log-log survival plot of the categorical variables was used to determine whether the proportional hazards assumption was appropriate, and all variables were fit to the proportional hazards assumption. The impact of TOPO1 expression on overall and cancer-specific survival was assessed by Cox proportional hazard models with and without risk-adjustment for age, gender, smoking status, tumor stage, response to first-line

chemotherapy, response to second-line chemotherapy, and type of relapse.

Statistical analysis was performed with R software version 3.3.3 (R Foundation for Statistical computing, Vienna, Austria). The reported significance levels were all two-sided, with statistical significance set at 0.05.

## Results

### Patient characteristics

A total of 78 patients were included in the present study. The median age was 61 years (range: 39–80), and the proportion of male and female patients was 82.0% and 18.0%, respectively. The demographic and clinical characteristics of the primary cohort are shown in Table 1. There were 24 limited and 54 extensive stage cases. First-line chemotherapy regimens comprised etoposide plus cisplatin (EP) or etoposide plus carboplatin (EC), and had a response rate (RR) of 87.2% and a median PFS of 5.6 months. Second-line chemotherapy consisted of single-agent topotecan, and had a disease control rate (DCR) of 28.2% and a PFS of 2.1 months. The OS for second-line chemotherapy was 8.5 months.

Adverse effects included gastrointestinal (71.5%), hematological (62.3%), and liver and kidney (23.5%) toxicities. Most adverse effects were tolerable, and no patients withdrew from treatment or died as a result of chemotherapy toxicity.

### Topoisomerase 1 (TOPO1) expression analysis by immunohistochemistry

TOPO1 staining was positive in 67 patients (85.9%) and negative in 11 (14.1%) (Fig 1). All patients were divided into two groups according to the extent of TOPO1 expression: 43 (55.1%) were categorized into the strong, and 35 (44.9%) into the weak expression group. There were no significant differences in age, gender, stage, and first-line RRs between the two groups (Table 1). No significant difference was detected in the TOPO1 strong expression ratio between those with limited and extensive stages of cancer (50% vs. 57.4%, respectively;  $P = 0.544$ ) (Table 1).

### Topotecan efficacy in patients with different TOPO1 expression levels

Topotecan efficacy was compared between the strong and weak expression groups. In the strong expression group there were 3 cases of partial remission (PR), 14 stable disease (SD), and 26 progressive disease (PD), while in the weak group there were no cases of PR, 5 SD, and 30 PD. There was a significant difference in the DCR between the

**Table 1** Demographic and clinical characteristics of the TOPO1 expression groups

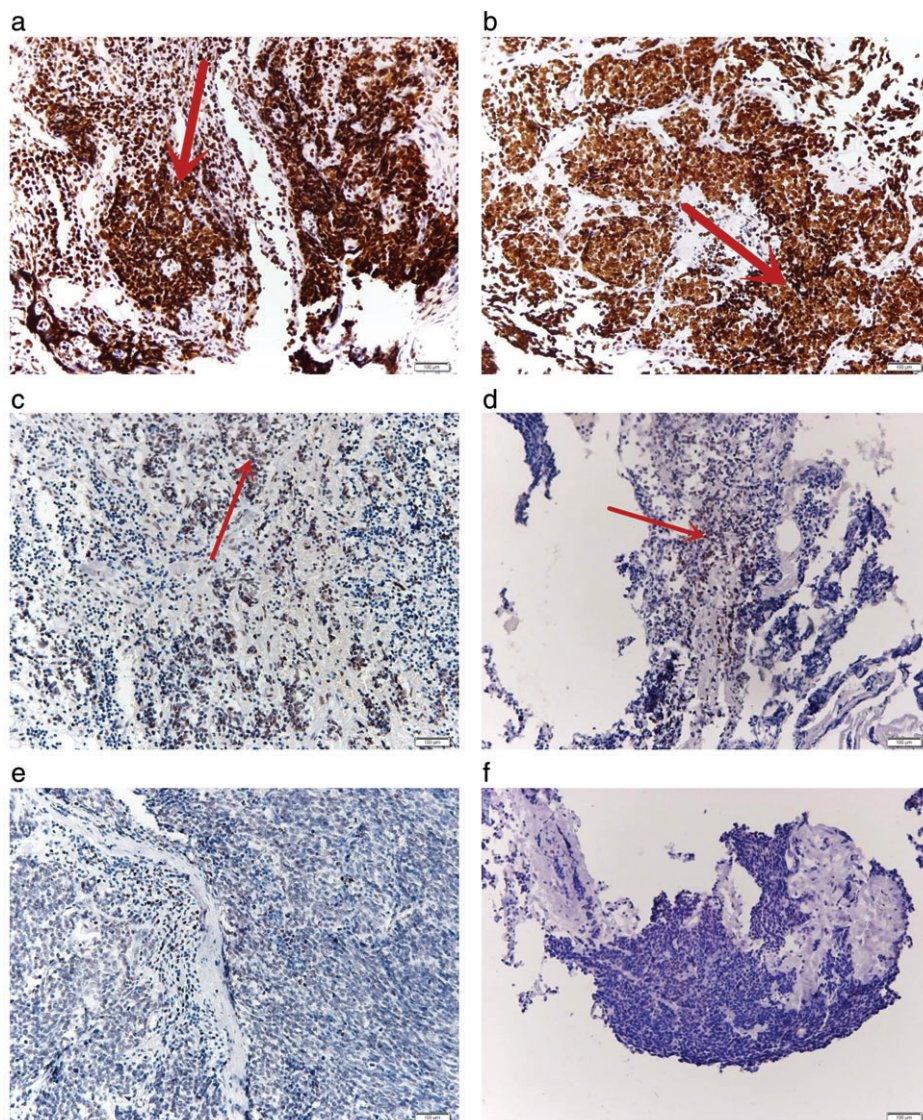
Characteristic	Weak TOPO1 expression ( <i>n</i> = 35)	Strong TOPO1 expression ( <i>n</i> = 43)	<i>P</i>
Age			
Median (interquartile range)	59 (54–67)	61 (57–69)	0.577
< 65 years	24 (68.6%)	28 (65.1%)	0.747
≥ 65 years	11 (31.4%)	15 (34.9%)	
Gender			
Female	5 (14.3%)	9 (20.9%)	0.447
Male	30 (85.7%)	34 (79.1%)	
Smoking status			
Non-smoker	8 (22.9%)	13 (30.2%)	0.465
Smoker	27 (77.1%)	30 (69.8%)	
Clinical stage			
Limited	12 (34.3%)	12 (27.9%)	0.544
extensive	23 (65.7%)	31 (72.1%)	
Response to first-line chemotherapy			
Partial response	30 (85.7%)	38 (88.4%)	0.727
Stable disease	5 (14.3%)	5 (11.6%)	
Type of relapse			
Resistant	11 (31.4%)	20 (46.5%)	0.176
Sensitive	24 (68.6%)	23 (53.5%)	
Response to second-line chemotherapy			
Disease control	5 (14.3%)	17 (39.5%)	0.014
Disease progression	30 (85.7%)	26 (60.5%)	

TOPO1, topoisomerase 1.

strong and weak expression groups (39.5% vs. 14.3%, respectively;  $P = 0.014$ ) (Table 1), but no significant difference was detected in survival between the groups. Median second-line PFS was 2.2 (range 2.0–not applicable [NA]) and 2.0 (range 1.9–2.3) months in the strong and weak expression groups, respectively ( $P = 0.057$ ) (Fig 2a). Median second-line OS was 8.1 (range 6.3–11.2) and 6.0 (range 5.7–8.8) months in the strong and weak expression groups, respectively ( $P = 0.199$ ) (Fig 2b). Median first-line OS was 15.3 (range: 12.7–17.4) and 12.5 (range 10.1–14.5) months in the strong and weak expression groups, respectively ( $P = 0.134$ ) (Fig 2c).

The results of univariate analysis are shown in Table 2. Multivariate analysis demonstrated that TOPO1 expression, clinical stage, and type of relapse were independent risk factors for poor second-line PFS in response to topotecan therapy (Table 3). However, TOPO1 expression failed to show any predictive value for OS or survival after topotecan chemotherapy. In addition, smoking status, clinical stage, and response to topotecan therapy were independent risk factors for poor survival after topotecan therapy. Additional risk factors for poor OS were also type of relapse and response to first-line therapy.

**Figure 1** Immunohistochemical staining of topoisomerase 1 in small cell lung cancer tumors. Examples of (a,b) strong positive, (c,d) weak positive, and (e,f) negative staining (magnification 100 $\times$ , scale bar 100  $\mu$ m).

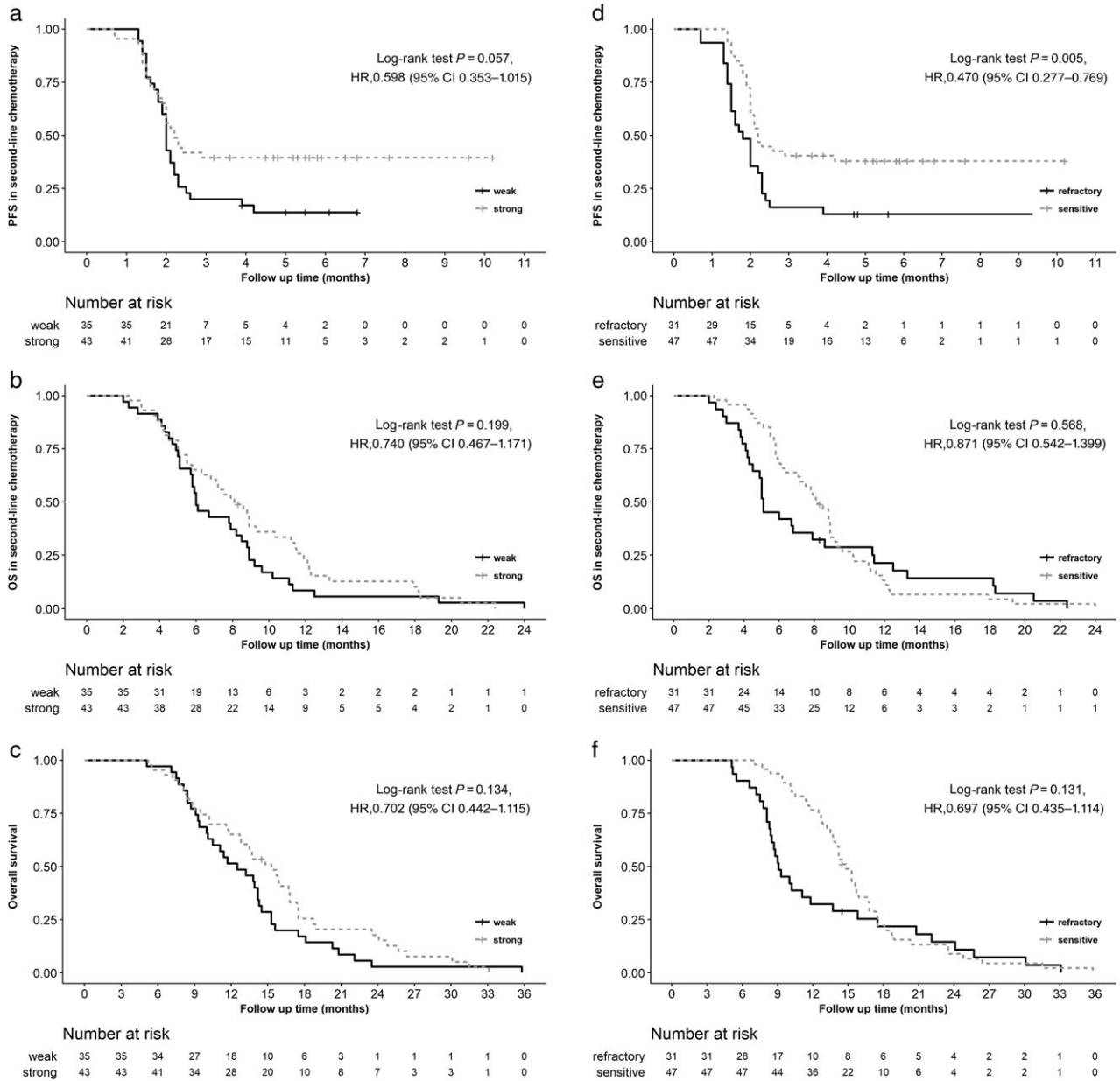


Patients were then divided into two groups based on TOPO1 expression: 67 patients had positive and 11 had negative expression. There were no significant differences in age, gender, clinical stage, or response to first-line chemotherapy between these groups (Table S1). The DCR in response to topotecan therapy was much higher in the TOPO1 positive compared to the negative group (32.8% vs. 0%, respectively;  $P = 0.028$ ) (Table S1). The median PFS after second-line chemotherapy was 2.2 (interquartile range [IQR] 2.0–2.6) and 1.9 (IQR 1.7–NA) months in the positive and negative groups, respectively ( $P = 0.022$ ; Fig S1), while the median OS after second-line chemotherapy was 7.8 (6.1–8.9) and 5.9 (5.1–NA) months, respectively ( $P = 0.453$ ) (Fig S2).

### Topotecan efficacy is associated with relapse time after initial therapy

It has been shown that the response to second-line therapy is highly dependent on the time interval from the onset of first-line therapy to relapse. If the interval is < 3 months (refractory disease), the response to most agents or regimens is < 10%, and if > 3 months have elapsed (sensitive disease), the expected response rates are approximately 25%.<sup>26</sup>

Based on these findings, patients in this study were divided into two groups: those with refractory ( $n = 31$ ) and sensitive ( $n = 47$ ) disease. In the sensitive disease group, responses to second-line chemotherapy included



**Figure 2** Kaplan–Meier survival curves for 78 patients with small cell lung cancer receiving topotecan therapy, grouped by (a–c) weak and strong tumor topoisomerase 1 expression or (d–f) refractory and sensitive type of relapse. CI, confidence interval; HR, hazard ratio; OS, overall survival; PFS, progression-free survival.

3 PR, 15 SD, and 29 PD, while in the refractory disease group there were no cases of PR, 4 SD, and 27 PD. Hence, there was a significant difference in the DCR between the sensitive and refractory groups (38.3% vs. 12.9%, respectively;  $P = 0.038$ ).

The median PFS after second-line chemotherapy was 2.2 (IQR 2.0–NA) and 1.8 (IQR 1.5–2.3) months ( $P = 0.005$ ) in the sensitive and refractory groups, respectively (Fig 2d). The median OS after second-line chemotherapy was 8.2 (IQR 7.1–9.2) and 5.1 (IQR 4.5–11.3) months in the

sensitive and refractory groups, respectively ( $P = 0.568$ ) (Fig 2e). The median OS after first-line chemotherapy was 14.8 (IQR 13.8–16.8) and 9.1 (IQR 8.4–15.8) months in the sensitive and refractory groups, respectively ( $P = 0.131$ ) (Fig 2f).

## Discussion

To the best of our knowledge, this is the first study to show that TOPO1 expression levels are associated with DCR

**Table 2** Univariate analysis of the primary cohort (*n* = 78)

Characteristic	PFS after topotecan therapy		Survival after topotecan therapy		OS	
	HR (95% CI)	<i>P</i>	HR (95% CI)	<i>P</i>	HR (95% CI)	<i>P</i>
Smoking status: Smoker	1.119 (0.610–2.050)	0.717	1.652 (0.968–2.821)	0.066	1.698 (0.994–2.901)	0.053
Clinical stage: Disseminated	1.884 (1.025–3.464)	0.041	2.347 (1.366–4.033)	0.002	2.047 (1.201–3.488)	0.008
TOPO1 expression: Strong positive	0.598 (0.352–1.015)	0.057	0.739 (0.467–1.171)	0.199	0.702 (0.442–1.115)	0.134
Response to first-line chemotherapy: Stable disease	0.710 (0.304–1.658)	0.429	1.221 (0.622–2.398)	0.561	1.371 (0.696–2.703)	0.362
Type of relapse: Sensitive disease	0.470 (0.277–0.796)	0.005	0.871 (0.542–1.399)	0.568	0.697 (0.435–1.114)	0.131
Response to second-line chemotherapy: Disease progression	—	—	2.830 (1.662–4.820)	<0.001	2.934 (1.699–5.068)	< 0.001

CI, confidence interval; HR, hazard ratio; OS, overall survival; PFS, progression-free survival; TOPO1, topoisomerase 1.

**Table 3** Multivariate analysis of the primary cohort (*n* = 78)

Characteristic	PFS after topotecan therapy		Survival after topotecan therapy		OS	
	HR (95% CI)	<i>P</i>	HR (95% CI)	<i>P</i>	HR (95% CI)	<i>P</i>
Smoking status: Smoker	—	—	1.915 (1.076–3.406)	0.027	2.472 (1.339–4.562)	0.004
Clinical stage: Disseminated	2.856 (1.487–5.487)	0.001	2.421 (1.352–4.337)	0.003	2.213 (1.252–3.912)	0.006
TOPO1 expression: Strong positive	0.431 (0.247–0.753)	0.003	0.937 (0.572–1.537)	0.797	0.766 (0.444–1.319)	0.336
Response to first-line chemotherapy: Stable disease	—	—	1.887 (0.934–3.814)	0.076	2.212 (1.077–4.542)	0.031
Type of relapse: Sensitive disease	0.313 (0.176–0.557)	< 0.001	—	—	0.498 (0.273–0.909)	0.023
Response to second-line chemotherapy: Disease progression	—	—	2.732 (1.522–4.905)	< 0.001	2.323 (1.238–4.357)	0.008

CI, confidence interval; HR, hazard ratio; OS, overall survival; PFS, progression-free survival; TOPO1, topoisomerase 1.

after topotecan therapy in Chinese patients with SCLC. The DCR was much higher in the strong than in the weak positive expression group. We also confirmed that in relapse cases, patients with sensitive disease had better survival after topotecan therapy than those with refractory disease. The proportion of patients with positive TOPO1 expression (85.9%), the first-line chemotherapy RR (87.2%), and DCR after topotecan therapy (28.2%) in our study were similar to data reported in previous studies.<sup>27–29</sup>

Previous *in vitro* studies have revealed that higher TOPO1 inhibitor efficacy is associated with increased TOPO1 expression levels.<sup>19,20,30</sup> However, results from clinical trials on solid tumors are controversial. For instance, in 2014, Nygård *et al.* reported that there was no significant correlation between TOPO1 expression levels and irinotecan (a TOPO1 inhibitor) efficacy in colorectal cancer.<sup>31</sup> Conversely, Guo *et al.* studied 50 cases of SCLC and reported that TOPO1 expression levels were higher in late stage than in early stage disease, and that high TOPO1

expression is a rationale to indicate TOPO1 inhibitor treatment of malignancies.<sup>27</sup> Brunner *et al.* reported that low TOPO1 levels identified a group of patients with stage III colon cancer that would not benefit from irinotecan adjuvant treatment.<sup>32</sup> Unlike prior studies, we used the semi-quantitative H-score method to evaluate TOPO1 protein levels, and found that the DCR was significantly higher in the strong than in the weak expression group. Similarly, the DCR in patients with positive TOPO1 expression of any intensity was better than that in those with no expression. Our results indicated that the TOPO1 expression level evaluated using the H-score method could serve as a potential predictor for topotecan efficacy in SCLC.

We explored the prognostic value of TOPO1 expression. No significant difference in survival was observed between the groups with strong and weak TOPO1 expression in Kaplan–Meier survival analysis, nor was a significant relationship observed between TOPO1 expression level and survival in univariate regression analysis. However, in

multivariate regression analysis, TOPO1 expression was significantly associated with second-line PFS ( $P = 0.003$ ). This discrepancy may be caused by the sample size and confounding factors such as age, staging, and topotecan response. We conclude that OS was affected by subsequent chemotherapy and local radiotherapy. In the strong TOPO1 expression group, 14 (32.6%) patients received third-line chemotherapy, while in the weak expression group, 13 (37.1%) patients received subsequent chemotherapy. Thirty-one (72.1%) and 28 (80%) patients received local radiotherapy (thorax, brain, or bone) in the strong and weak TOPO1 expression groups, respectively. Our results suggest the possibility that TOPO1 expression level might be a predictor for PFS after topotecan therapy.

Our data demonstrate that among relapsed patients, those with sensitive disease could benefit more than those with refractory disease after second-line chemotherapy; this result was consistent with those of a previous study.<sup>33</sup> Topotecan is effective for patients with sensitive disease; however, second-line chemotherapy efficacy was low for those with refractory disease. Therefore, patients with refractory disease require therapy with alternative, more efficient agents. In recent years, new agents such as amrubicin and immunotherapy with antibodies against PD-1 and CTLA-4 represent promising alternatives for patients with SCLC.<sup>11,12,34</sup>

Guo *et al.* reported that clinical staging of SCLC was associated with TOPO1 expression levels; patients with advanced stage cancer exhibit higher TOPO1 expression than early stage patients.<sup>27</sup> However, we did not find any significant differences in TOPO1 levels between limited and advanced stage patients (50% and 57.4% of patients with strong positive TOPO1 expression, respectively;  $P = 0.544$ ). Possible reasons for this could be clinicopathological differences in enrolled patients, examination techniques, or the antibodies used. Further investigation is therefore warranted.

There were some limitations to this retrospective study. The limited sample size made it difficult to detect a significant difference in PFS between the groups with strong and weak TOPO1 expression. The imbalance in patient numbers between the TOPO1 positive and negative groups (67 vs. 11 cases) could also influence the reliability of these conclusions. In future, large scale prospective studies combined with multigene analysis could help to identify more valuable prognostic and predictive markers in SCLC.

In conclusion, the results of our study show that TOPO1 expression is highly prevalent in SCLC. In the second-line topotecan chemotherapy group, patients with strong TOPO1 expression had better DCR than those with weak expression, and sensitive relapse patients had longer PFS than those with refractory disease. Our findings indicate that the TOPO1 expression level could serve

as a potential predictive marker for topotecan efficacy in SCLC.

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## Disclosure

No authors report any conflict of interest.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

**Table S1** Clinical characteristics of the negative and positive topoisomerase 1 (TOPO1) expression groups

**Figure S1** CI, confidence interval; HR, hazard ratio; PFS, progression-free survival.

**Figure S2** Overall survival after second-line topotecan chemotherapy in the topoisomerase-1 positive group compared to the negative group.