Thoracic Cancer

WILEY

ORIGINAL ARTICLE OPEN ACCESS

Impact of DLL3 Expression as Prognostic Factor in Extensive Stage of Small Cell Lung Cancer Treated With First-Line Chemotherapy

Hohyung Nam[1] | Soon-Hee Jung[2] | Jii Bum Lee[3] | Jee Hyun Kong[4,5] | Seungtaek Lim[4]

[1]Department of Hematology, Seoul St. Mary's Hospital, College of Medicine, Catholic University, Seoul, Republic of Korea | [2]Department of Pathology, Yonsei University Wonju College of Medicine, Wonju, Republic of Korea | [3]Division of Medical Oncology, Department of Internal Medicine, Yonsei Cancer Center, Yonsei University College of Medicine, Seoul, Republic of Korea | [4]Department of Hematology-Oncology, Division of Internal Medicine, Wonju Severance Christian Hospital, Yonsei University Wonju College of Medicine, Wonju, Korea | [5]Center of Evidence-Based Medicine, Institute of Convergence Science, Yonsei University, Seoul, Republic of Korea

Correspondence: Seungtaek Lim (darksgtlim@naver.com)

Received: 5 July 2024 | **Revised:** 4 December 2024 | **Accepted:** 13 December 2024

Funding: The authors received no specific funding for this work.

Keywords: DLL3 protein | human | prognosis | small cell lung carcinoma

ABSTRACT

Introduction: Small cell lung cancer (SCLC) is known for its high proliferative rate and poor prognosis. Although Delta-like ligand 3 (DLL3) is specifically expressed on the surface of SCLC, the association of DLL3 with prognosis in SCLC remains uncertain. Hence, we aimed to evaluate prognostic role of DLL3 in extensive stage of SCLC treated with first-line chemotherapy.

Materials and Methods: A total of 54 patients with extensive stage of SCLC (ES-SCLC) who were treated with first-line chemotherapy were included for our analysis. In addition, tissue specimen should be available for immuno-histochemical staining for DLL3, and their clinico-pathologic data, including progression-free survival (PFS) and overall survival (OS), were obtained. DLL3 expression and the percentage of tumor cells with DLL3 positive among total cancer cells were analyzed microscopically and DLL3 high and DLL3 low were defined as the percentage of DLL3 positive tumor cells versus total cancer cells $\geqq 75\%$ and $< 75\%$, respectively.

Results: DLL3 expression was not associated with any of the clinico-pathological characteristics such as age at diagnosis, sex, response to first-line chemotherapy, second-line chemotherapy (Yes or No), and number of metastatic sites. However, response to first-line chemotherapy and number of metastatic sites were correlated to PFS, while DLL3 expression and number of metastatic sites were correlated to OS.

Conclusion: DLL3 was highly expressed in SCLC, and not associated with any clinico-pathological characteristics. In survival outcome, DLL3 was correlated with worse OS, which suggests the prognostic role of DLL3 in ES-SCLC.

1 | Introduction

Small cell lung cancer (SCLC), accounting for 15% of all lung cancers, is known for its high proliferative rate and poor prognosis [1]. For the first line treatment of extensive-stage small cell lung cancer (ES-SCLC), immune checkpoint inhibitors (ICIs) such as atezolizumab or durvalumab in combination with platinum-based chemotherapy became the standard treatment option due to survival benefit shown in large phase III randomized clinical trials [2, 3]. ES-SCLC is well known for its initial sensitivity to chemotherapy, but it rapidly acquires resistance to the chemotherapy, eventually culminating in patient death [4].

Although topotecan has been approved for relapsed SCLC based on a phase III clinical trial in patients who progressed after platinum doublet chemotherapy, the survival benefit was only modest, resulting in a dismal prognosis of ES-SCLC patients [5]. Hence, there is clearly an unmet need for new therapeutic options for highly lethal malignancy.

On the surface of SCLC tumor cells, delta-like ligand 3 (DLL3) is specifically expressed [6]. DLL3 expression promotes SCLC migration and invasion through controlling the epithelial mesenchymal transition protein, SNAI1 [7]. Various therapeutic options targeting DLL3 have been investigated for the treatment of relapsed ES-SCLC. Rovalpituzumab tesirine (Rova-T), a first-in-class antibody-drug conjugate directed against DLL3, is a promising targeted therapeutic for individuals with SCLC [6, 8]. In a phase I trial [9], Rova-T demonstrated a higher response rate in patients with tumors with a higher level of DLL3 than those with a lower level of DLL3 expression, indicating DLL3 expression can be considered a potential biomarker for response or Rova-T. Although Rova-T failed to demonstrate survival benefit compared with topotecan in a phase III clinical trial [10], clinical trials using other DLL-3 inhibitors are ongoing based on promising results in early phase clinical trials [11, 12]. Especially, tarlatamab, a first-in-class DLL3-targeted bispecific T-cell engager obtained FDA approval for pre-treated ES-SCLC based on the efficacy shown previous clinical trial [11]. Therefore, targeting DLL-3 is still considered a promising therapeutic approach in the treatment of ES-SCLC. However, incidence of high DLL3 expression and its prognostic value in SCLC patients remains to be discussed.

Hence, we herein investigate the value of DLL3 as a prognostic factor in ES-SCLC patients.

2 | Materials and Methods

2.1 | Patients and Data Collection

For our analysis, we enrolled patients who were diagnosed with ES-SCLC and treated with palliative chemotherapy at the Department of Hemato-oncology, Wonju Severance Christian Hospital, Yonsei University, from 2015 to 2018. We included patients whose tumor samples were available for immunohistochemical staining of DLL3. Patients' information including sex, age at diagnosis, biopsy site, metastatic site, first-line and second-line chemotherapy regimen, response to first-line chemotherapy, and survival data (status at the end of 2018, date of death or date of last follow-up), were obtained through medical chart review. This study was approved by the institutional review board of Wonju Severance Christian Hospital, Yonsei University (IRB number: CR322117) and conducted in accordance with the Declaration of Helsinki and Ethical Guidelines for Medical Research Involving Human Subjects.

2.2 | Immuno-Histochemical Analysis

All tissue specimen were prepared for immunohistochemical staining in 4-μm-thick formalin-fixed, paraffin-embedded tissue sections mounted on glass slides. DLL3 staining was performed via an anti-DLL3 mouse monoclonal antibody (AbbVie-Stemcentrx, North Chicago, Illinois, USA). In this study, DLL3 positive was defined as any cytoplasmic or membranous staining at any intensity in tumor cells and was determined by a pathologist specialized in thoracic oncology. DLL3 expression and the percentage of tumor cells with DLL3 positive among total cancer cells were analyzed microscopically. DLL3 high and DLL3 low were defined as the percentage of DLL3 positive tumor cells versus total cancer cells $\geqq 75\%$ and $< 75\%$, respectively, as described previously [13].

2.3 | Statistical Analysis

Differences in clinico-pathological factors, including age at biopsy, sex, response to first-line chemotherapy, history of second-line chemotherapy, and number of metastatic sites between DLL3 expression were assessed based on Student's *t*-test and Chi-square test. Overall survival (OS) was defined as the time from the first day of treatment to death, and progression-free survival (PFS) was calculated from the first day of treatment to disease progression or death. Univariate analysis of association between patients' characteristics and survival outcomes was conducted on the Kaplan–Meier test with log rank test. Multivariate analysis of association between survival outcomes and various clinico-pathological factors was conducted by the Cox proportional hazard model. A p value of < 0.05 was considered statistically significant. All statistical analysis was performed via SPSS Statistics 29.0 (IBM, Armonk, New York, USA).

3 | Results

3.1 | Patient Characteristics

The baseline characteristics of the participants in this study were summarized in Table 1. A total of 54 patients diagnosed with ES-SCLC at the Department of Hemato-oncology, Wonju Severance Christian Hospital, Yonsei University from 2015 to 2018 were analyzed in the study. The median age was 66.5 with a range of 44–86, and the majority ($n = 48$, 87.3%) were male. Most commonly used regimen was etoposide-cisplatin ($n = 50$, 92.6%), followed by etoposide-carboplatin ($n = 3$, 5.5%) and belotecan ($n = 1$, 1.8%). The frequent metastatic site was lymph nodes ($n = 39$, 72.2%), lung and bone ($n = 20$, 37.0%, each) and pleural effusion ($n = 14$, 25.9%). A total of 47 patients were evaluable for response to first-line chemotherapy, and the number of patients, and overall response rate (ORR) to 1st line chemotherapy was 61.7% (29 out of 47).

3.2 | Expression Status of DLL3 and Relationship Between Clinical Parameters and Survival

All 54 tumor specimens were available for immuno-histochemical staining for DLL3, whose representative images are shown in Figure 1. The dot plot for the percentage of tumor cells positively stained for DLL3 was presented in Figure 2. Among all 54

TABLE 1 | General characteristics of study subjects.

Characteristics	*n*	%
Age (years)		
Median	66.5	
Range	44–86	
Age at diagnosis		
≧65 years	38	70.4
<65 years	16	29.6
Sex		
Male	48	88.9
Female	6	11.1
First-line chemotherapy		
Etoposide + cisplatin	50	92.6
Etoposide + carboplatin	3	5.6
Belotecan	1	1.8
Second-line chemotherapy		
Yes	19	35.2
No	35	64.8
Response to first-line chemotherapy		
CR + PR	29	61.7
SD + PD	18	38.3
Metastatic sites		
Liver	15	27.8
Lung	20	37.0
Bone	20	37.0
Brain	9	16.7
Adrenal gland	2	3.7
Pericardial effusion	1	1.9
Pleural effusion	14	25.9
Lymph nodes	39	72.2
Number of metastatic sites		
≧3	15	28.3
<3	38	71.7

Abbreviations: CR: complete response; PD: progressive disease; PR: partial response; SD: stable disease.

patients, DLL3 high was observed in 70.4% of patients ($n = 38$). The mean of DLL3 expression and the standard deviation of DLL3 were 76.3% and 22.9, respectively. Baseline characteristics of patients were summarized in Table 1. The expression level of DLL3 was not correlated to any characteristics: sex, age at biopsy, response to first-line chemotherapy, history of second-line chemotherapy, and number of metastatic sites (Table 2).

3.3 | Survival Outcomes According to DLL3 Expression

The median PFS and OS in all patients was 175 (95% CI: 131–219) days and 250 (95% CI: 210–316) days, respectively. We performed survival analysis of all 54 patients according to clinical characteristics and DLL3 expression (Table 3). In univariate analysis, response to first-line chemotherapy and the number of metastatic sites were correlated with both PFS and OS. Patients with higher DLL3 expression tended to have worse median PFS (159 vs. 221 days, $p = 0.373$, 95% CI: 0.405–1.404) and worse median OS (226 vs. 312 days, $p = 0.098$, 95% CI: 0.306–1.105), although both had no statistical significance (Table 3, Figure 3).

We also conducted multivariate analysis for PFS and OS. For PFS, response to first-line chemotherapy and number of metastatic sites were independent adverse prognostic factors of PFS. Furthermore, DLL3 high expression was shown for an independent adverse prognostic factor of OS ($p = 0.031$) as well as number of metastatic sites.

4 | Discussion

The primary aim of this study was to evaluate DLL3 as a prognostic factor in ES-SCLC patients who received first-line chemotherapy. Univariate analysis showed that expression of DLL3 was not associated with PFS or OS, although it showed a tendency of worse PFS and OS. Response to first-line chemotherapy and number of metastatic sites were identified as prognostic factors for both worse PFS and OS. In addition, we performed multivariate analysis which revealed that expression of DLL3 was an independent prognostic factor for worse OS as well as number of metastatic sites. This result suggests the prognostic role of DLL3 in ES-SCLC patients treated with first-line chemotherapy.

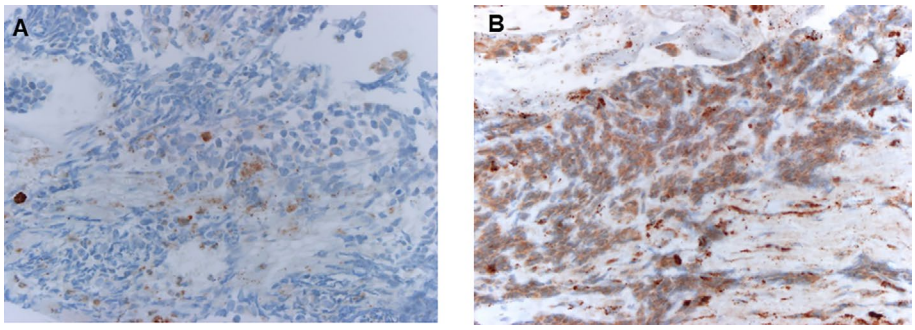
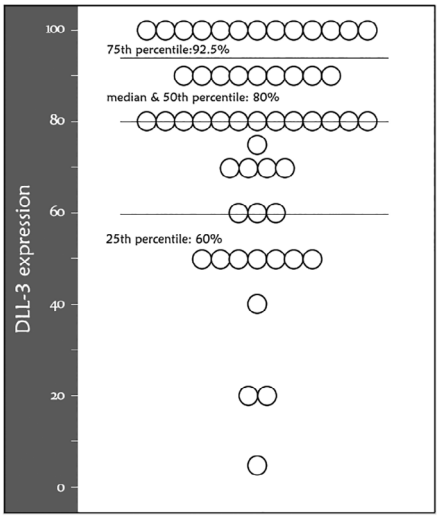


FIGURE 1 | Representative immunohistochemical staining of DLL3 in SCLC tissue specimens. (A) 5% cancer cells (+) for DLL-3 (low). (B) 100% cancer cells (+) for DLL-3 (high).



DLL-3 positive cells/total cancer cells	Number of patients (%)
≧75%(DLL-3 high)	38(70.4)
<75%(DLL-3 low)	16(29.6)

FIGURE 2 | Dot plot for the percentage of tumor cells staining positive for DLL3 in all patients. The median DLL3 expression was 80%, and 25th percentile was 60%, 50th percentile 80%, and 75th percentile was 92.5%.

TABLE 2 | Association between various patients' characteristics and DLL3 expression.

Factor	Number	*p*
Sex		
Male vs. female	48 vs. 6	0.833
Age at diagnosis (years)		
≧65 vs. <65	31 vs. 23	0.256
Response to first-line chemotherapy		
CR + PR vs. SD + PD	29 vs. 18	0.869
Second-line chemotherapy		
Received vs. None	19 vs. 35	0.309
Number of metastatic sites		
≧3 vs. <3	15 vs. 39	0.767

Abbreviations: CR: complete response; PD: progressive disease; PR: partial response; SD: stable disease.

Previous studies have showed conflicting results regarding the prognostic role of DLL3 (Table 4). Some studies showed that patients with high DLL3 expression had worse OS [14], whereas other studies indicated that DLL3 had no correlation with survival [15] or better prognostic factor [16]. The reason for this discrepancy has not been well understood. Ethnic difference of the prognostic value of DLL3 in different population has been suggested for one reason. Meta-analysis conducted by Li et al. [17] proposed that DLL3 expression was correlated with poor survival in Asian population, not in European or American population. Consistent with the conclusion, our study showed that DLL3 expression was prognostic factor for worse overall survival. Moreover, the discrepancy between studies regarding prognostic role of DLL3 could be explained by heterogeneity in the various detection methods and cut-off values for DLL3 expression used in each study. In this study, DLL3-high was initially defined as a value of ≥ 50%. After we found that there was no survival difference according to DLL3 expression, we decided to use different cutoff value of 75% as used in previous literatures [13], and found that patients with DLL3 high expression (defined as ≧75%) was associated with the longer OS in multivariate analysis. Further research for optimal cutoff value and detection methods for DLL3 expression is warranted.

The inconsistent results on the survival of DLL3 expression among studies shown in Table 4 might suggest that it is unlikely to affect survival. As mentioned in our paper and previous articles, there has been no consensus regarding the prognostic role of DLL3 expression in SCLC. It might not be a strong prognostic and/or predictive factor like EGFR mutation in NSCLC. But, as seen in the clinical trial of tarlatamab, targeting DLL3 is important therapeutic strategy to treat this lethal malignancy, and we believed that understanding the clinical relevance of DLL3 expression regarding the prognostic impact of SCLC is still worthy of evaluation.

This study had some strengths and limitation over other studies. In previous studies, the patient population was mainly heterogeneous, especially in line of therapy and SCLC stage. To overcome this limitation, we exclusively included patients who were diagnosed with ES-SCLC and treated with first-line chemotherapy (mainly platinum-based doublet chemotherapy). Moreover, we investigated the association of DLL3 with PFS and ORR as well as OS. Finally, we conducted multivariate analysis of PFS and OS, which revealed prognostic impact of DLL3 on OS. This study had also several limitations. The main limitation of our study is its retrospective nature and small number of patients. Moreover, the 1st line chemotherapy regimen used in the study population should be pointed out. Since Immune Checkpoint Inhibitor (ICIs) plus platinum-based chemotherapy, the current standard treatment based on large phase III trial result [2], was not reimbursed before 2018 in Korea, all 54 patients in this study did not receive any immune-oncologic therapy as first-line therapy at that time. Another limitation of this study is only one pathologist was involved in DLL3 assessment. Although two or three pathologists could make more independent and reliable interpretation regarding DLL3 expression, only one pathologist who was specialized and dedicated to lung pathology was available for our study.

5 | Conclusion

In this single center retrospective study, we found that DLL3 was highly expressed in ES-SCLC, and higher expression of

TABLE 3 | Univariate and multivariate analysis of patients' characteristics on (A) PFS, and (B) OS.

(A) PFS

	Univariate analysis		Multivariate analysis			
Characteristics	**MST (days)**	***p***	**Factor**	**HR**	**95% CI**	***p***
Sex		0.454		Not included		
Male	175					
Female	156					
Age		0.203	≧65 vs. <65	1.910	0.944–3.864	0.072
≧65	152					
<65	233					
Response to first-line chemotherapy		*<0.001*	CR + PR vs. SD + PD	0.265	0.127–0.553	*<0.001*
CR + PR	203					
SD + PD	103					
Second-line chemotherapy		0.483		Not included		
None	159					
Received	233					
DLL3 expression		0.370	High vs. low	1.333	0.678–2.622	0.405
High	159					
Low	221					
Number of metastatic sites		*<0.001*	≧3 vs. <3	4.428	2.012–9.746	*<0.001*
≧3	90					
<3	222					

B. OS

	Univariate analysis		Multivariate analysis			
Characteristics	**MST (days)**	***p***	**Factor**	**HR**	**95% CI**	***p***
Sex		0.454		Not included		
Male	226					
Female	542					
Age		0.203	≧65 vs. <65	1.719	0.857–3.447	0.127
≧65	165					
<65	312					
Response to first-line chemotherapy		*<0.001*	CR + PR vs. SD + PD	0.594	0.281–1.255	0.172
CR + PR	372					
SD + PD	159					
Second-line chemotherapy		0.483		Not included		
None	165					
Received	288					
DLL3 expression		0.370	High vs. low	2.343	1.078–5.090	*0.031*
High	226					
Low	312					
Number of metastatic sites		*<0.001*	≧3 vs. <3	2.641	1.200–5.811	*0.016*
≧3	123					
<3	288					

Note: Italic indicate statistically significant values.
Abbreviations: CR: complete response; DLL3: Delta-like ligand 3; HR: hazard ratio; MST: median survival time; PD: progressive disease; PFS: progression-free survival; PR: partial response; SD: stable disease.

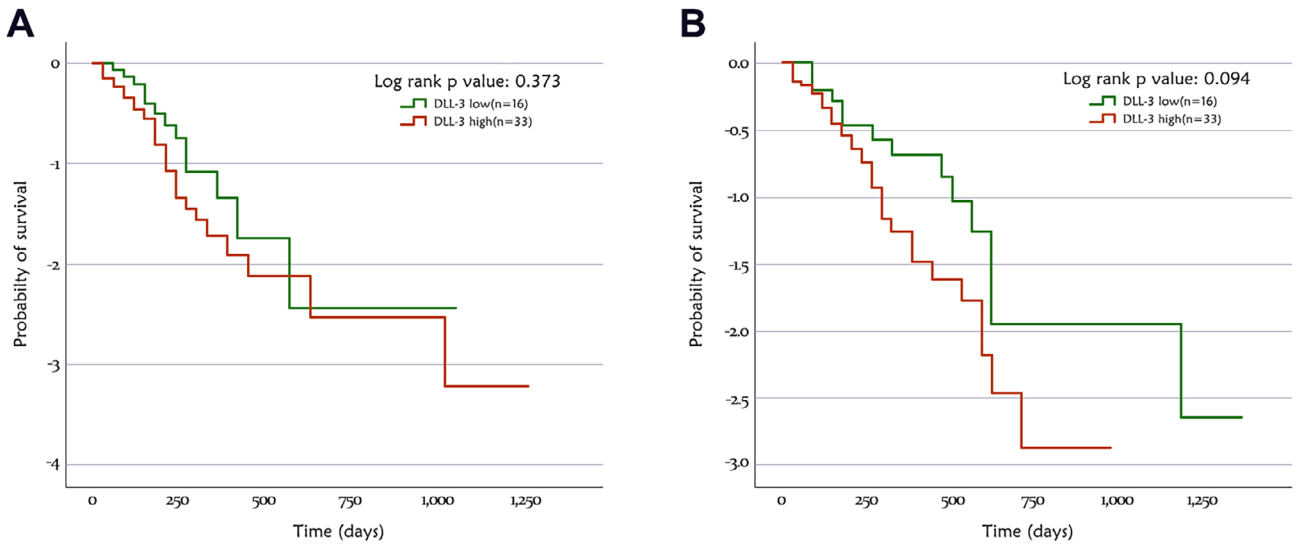


FIGURE 3 | Survival data study plotted via Kaplan–Meier curves: (A) PFS according to DLL3 expression, (B) OS of according to DLL3 expression. DLL3: Delta like ligand 3; OS: overall survival; PFS: progression-free survival.

TABLE 4 | Previous studies on the prognostic role of DLL3.

n	DLL3 high (%)	Cutoff	Results	References
335	62.4	H-score≧150	Worse OS	Yan 2019 [14]
44	79.5	≧50%	Better OS	Xie 2019 [16]
63	23	≧50%	No difference	Tanaka 2018 [15]
38	52.6	H-score≧135	Worse OS	Regzedmaa 2019 [18]
72	31.9	H-score≧6	Worse OS/ PFS/RR	Huang 2019 [19]
93	44	≧75%	No difference	Furuta 2019 [20]
1073	68	≧75%	No difference	Rojo 2020 [13]
54	70.4	≧75%	Worse OS	This study

DLL3 was correlated with poor OS, which suggests the prognostic role of DLL3 in ES-SCLC.

Author Contributions

Conceptualization and design: Seungtaek Lim and Jee Hyun Kong. Literature screening and selection: Seungtaek Lim. Pathological data acquisition: Soon-Hee Jung. Data generation and statistical analyses: Hohyung Nam. Manuscript writing and preparation: Hohyung Nam and Jii Bum Lee. Final approval of manuscript: all authors.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. C. M. Rudin, E. Brambilla, C. Faivre-Finn, and J. Sage, "Small-Cell Lung Cancer," *Nature Reviews. Disease Primers* 7 (2021): 3.

2. L. Horn, A. S. Mansfield, A. Szczesna, et al., "First-Line Atezolizumab Plus Chemotherapy in Extensive-Stage Small-Cell Lung Cancer," *New England Journal of Medicine* 379 (2018): 2220–2229.

3. L. Paz-Ares, M. Dvorkin, Y. Chen, et al., "Durvalumab Plus Platinum-Etoposide Versus Platinum-Etoposide in First-Line Treatment of Extensive-Stage Small-Cell Lung Cancer (CASPIAN): A Randomised, Controlled, Open-Label, Phase 3 Trial," *Lancet* 394 (2019): 1929–1939.

4. R. Stupp, C. Monnerat, A. T. Turrisi, M. C. Perry, and S. Leyvraz, "Small Cell Lung Cancer: State of the Art and Future Perspectives," *Lung Cancer* 45 (2004): 105–117.

5. J. H. Schiller, S. Adak, D. Cella, R. F. DeVore, and D. H. Johnson, "Topotecan Versus Observation After Cisplatin Plus Etoposide in Extensive-Stage Small-Cell Lung Cancer: E7593—A Phase III Trial of the Eastern Cooperative Oncology Group," *Journal of Clinical Oncology* 19 (2001): 2114–2122.

6. L. R. Saunders, A. J. Bankovich, W. C. Anderson, et al., "A DLL3-Targeted Antibody-Drug Conjugate Eradicates High-Grade Pulmonary Neuroendocrine Tumor-Initiating Cells In Vivo," *Science Translational Medicine* 7 (2015): 302ra136.

7. M. Furuta, H. Kikuchi, T. Shoji, et al., "DLL3 Regulates the Migration and Invasion of Small Cell Lung Cancer by Modulating Snail," *Cancer Science* 110 (2019): 1599–1608.

8. D. H. Owen, M. J. Giffin, J. M. Bailis, M. D. Smit, D. P. Carbone, and K. He, "DLL3: An Emerging Target in Small Cell Lung Cancer," *Journal of Hematology & Oncology* 12 (2019): 61.

9. C. M. Rudin, M. C. Pietanza, T. M. Bauer, et al., "Rovalpituzumab Tesirine, a DLL3-Targeted Antibody-Drug Conjugate, in Recurrent Small-Cell Lung Cancer: A First-In-Human, First-In-Class, Open-Label, Phase 1 Study," *Lancet Oncology* 18 (2017): 42–51.

10. F. Blackhall, K. Jao, L. Greillier, et al., "Efficacy and Safety of Rovalpituzumab Tesirine Compared With Topotecan as Second-Line Therapy in DLL3-High SCLC: Results From the Phase 3 TAHOE Study," *Journal of Thoracic Oncology* 16 (2021): 1547–1558.

11. L. Paz-Ares, S. Champiat, W. V. Lai, et al., "Tarlatamab, a First-In-Class DLL3-Targeted Bispecific T-Cell Engager, in Recurrent Small-Cell

Lung Cancer: An Open-Label Phase I Study," *Journal of Clinical Oncology* 41 (2023): 2893–2903.

12. M. Wermke, E. Felip, V. Gambardella, et al., "Phase I Trial of the DLL3/CD3 Bispecific T-Cell Engager BI 764532 in DLL3-Positive Small-Cell Lung Cancer and Neuroendocrine Carcinomas," *Future Oncology* 18 (2022): 2639–2649.

13. F. Rojo, M. Corassa, D. Mavroudis, et al., "International Real-World Study of DLL3 Expression in Patients With Small Cell Lung Cancer," *Lung Cancer* 147 (2020): 237–243.

14. L. X. Yan, Y. H. Liu, Z. Li, et al., "Prognostic Value of Delta-Like Protein 3 Combined With Thyroid Transcription Factor-1 in Small-Cell Lung Cancer," *Oncology Letters* 18 (2019): 2254–2261.

15. K. Tanaka, K. Isse, T. Fujihira, et al., "Prevalence of Delta-Like Protein 3 Expression in Patients With Small Cell Lung Cancer," *Lung Cancer* 115 (2018): 116–120.

16. H. Xie, J. M. Boland, J. J. Maleszewski, et al., "Expression of Delta-Like Protein 3 Is Reproducibly Present in a Subset of Small Cell Lung Carcinomas and Pulmonary Carcinoid Tumors," *Lung Cancer* 135 (2019): 73–79.

17. B. Chen, H. Li, C. Liu, et al., "Potential Prognostic Value of delta-Like Protein 3 in Small Cell Lung Cancer: A meta-Analysis," *World Journal of Surgical Oncology* 18 (2020): 226.

18. O. Regzedmaa, Y. Li, Y. Li, et al., "Prevalence of DLL3, CTLA-4 and MSTN Expression in Patients With Small Cell Lung Cancer," *Oncotargets and Therapy* 12 (2019): 10043–10055.

19. R. S. P. Huang, B. F. Holmes, C. Powell, et al., "Delta-Like Protein 3 Prevalence in Small Cell Lung Cancer and DLL3 (SP347) Assay Characteristics," *Archives of Pathology & Laboratory Medicine* 143 (2019): 1373–1377.

20. M. Furuta, J. Sakakibara-Konishi, H. Kikuchi, et al., "Analysis of DLL3 and ASCL1 in Surgically Resected Small Cell Lung Cancer (HOT1702)," *Oncologist* 24 (2019): e1172–e1179.