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International real-world study of DLL3 expression in patients with small cell lung cancer

Federico Rojo[a,*], Marcelo Corassa[b], Dimitrios Mavroudis[c], Aysim Büge Öz[d], Bonne Biesma[e], Luka Brcic[f], Patrick Pauwels[g], Verena Sailer[h], John Gosney[i], Darko Miljkovic[j], Carlos Hader[j], Meijing Wu[j], Todd Almarez[j], Frédérique Penault-Llorca[k]

[a] Hospital Universitario Fundación Jiménez Díaz CIBERONC, Madrid, Spain
[b] A.C. Camargo Cancer Center, São Paulo, Brazil
[c] University General Hospital of Heraklion, Heraklion, Greece
[d] Istanbul University Cerrahpaşa, Faculty of Medicine, Istanbul, Turkey
[e] Jeroen Bosch Ziekenhuis,' s-Hertogenbosch, Netherlands
[f] Medical University of Graz, Graz, Austria
[g] Centre for Oncological Research (CORE), University of Antwerp, and University Hospital Antwerp, Edegem, Belgium
[h] University Hospital Schleswig-Holstein, Lübeck, Germany
[i] Liverpool University Hospitals, Liverpool, United Kingdom
[j] AbbVie Inc., North Chicago, IL, United States
[k] University Clermont Auvergne, Jean Perrin Center, INSERM, IMoST, 63000 Clermont-Ferrand, France

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ABSTRACT

Objectives: Expression of the Notch-family ligand delta-like protein 3 (DLL3), a potential therapeutic target in small cell lung cancer (SCLC), has not been assessed in the real-world setting. To identify the real-world utility of DLL3 as an SCLC therapeutic target, we performed the largest retrospective international noninterventional study to date to evaluate DLL3 prevalence in SCLC patients.

Materials and Methods: DLL3 expression was assessed using immunohistochemistry in archived histological and cytological specimens (independent and paired) and correlated to patient demographics, clinical disease characteristics, and survival. The primary endpoint was the proportion of patients with DLL3 expression in ≥25 % of tumor cells. DLL3 expression concordance was assessed in paired specimens.

Results: Independent tumor specimens were collected from 1073 patients. The mean age at biopsy was 66 years (SD, 10); 682 (64 %) patients were male. Paired specimens were collected from 36 patients. The mean age at biopsy was 62 years (SD, 11); 16 (44 %) patients were male. Most patients had ECOG performance status of 0–1, were smokers/ex-smokers, and received ≥1 prior therapy. Positive DLL3 expression (defined as ≥25 % of tumor cells) was identified in 895/1050 (85 %) patients with 1 specimen and evaluable DLL3 expression; 719/1050 (68 %) patients had high DLL3 expression (defined as ≥75 % of tumor cells). DLL3 expression concordance was 88 % between paired specimens (n = 17; Cohen's kappa *P* value, .9412). There was no significant difference in median overall survival from SCLC diagnosis for evaluable patients with nonmissing data based on DLL3 expression (negative DLL3 expression [n = 139], 9.5 months; positive DLL3 expression [n = 747], 9.5 months; all evaluable patients [n = 893, 9.5 months).

Conclusion: These real-world epidemiologic findings indicate that DLL3 is robustly expressed across SCLC disease stages and remains stable despite treatment, consistent with available clinical trial data. There was no prognostic role for DLL3 observed in this study for overall survival.

Abbreviations: DLL3, delta-like ligand 3; ECOG, Eastern Cooperative Oncology Group; FFPE, formalin-fixed paraffin embedded; IHC, immunohistochemistry; IQR, interquartile range; SCLC, small cell lung cancer

* Corresponding author at: Hospital Universitario Fundación Jiménez Díaz CIBERONC, Avda. de los Reyes Católicos, 2 28040 Madrid, Spain.
E-mail address: frojo@fjd.es (F. Rojo).

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1. Introduction

Small cell lung cancer (SCLC), a poorly differentiated neuroendocrine carcinoma, is a distinct clinical and histological form of lung cancer characterized by cells with scant cytoplasms and poorly defined cell borders [1,2]. SCLC accounts for 13 % of newly diagnosed cases of lung cancer worldwide [1]; within the United States and Europe, lung cancer, including SCLC, remains the leading cause of cancer-related death in both men and women [2–4]. SCLC tumors are highly aggressive, rapidly growing, and often present with metastatic disease at time of diagnosis [2,5]. Despite impressive response to cytotoxic therapy, prognosis is still dismal.

SCLC is divided into limited and extensive stage disease [1,6]. Limited disease, which describes approximately 35 %–40 % of patients, is defined by tumor confinement to 1 hemithorax and associated regional lymph nodes (stages I-III, American Joint Committee on Cancer, AJCC, TNM staging system), with a total area that fits into a feasible radiotherapy field [6,7]. Conversely, extensive disease is defined by tumors that cannot be classified as limited (eg, tumors in patients with malignant pericardial and pleural effusion) [2,6] or patients who have large volumes of disease who are not amenable to definite chemoradiotherapy (stage IV). First-line treatment of metastatic SCLC with platinum-based combination chemotherapy leads to rapid responses; however, most patients relapse within 6 months, with less than 5 % surviving at 5 years [5,8,9]. Several cytotoxic agents, targeted therapies, and immunotherapies are under clinical investigation [10].

One emerging target, delta-like ligand 3 (DLL3), an atypical protein in the Notch family, is involved in cellular development under normal physiological conditions [11]. Transcription of DLL3 is regulated by the SCLC oncogenic driver achaete-scute homolog-1 [12,13], and as such, DLL3 has been implicated in neuroendocrine tumorigenesis [12,14,15]. Preclinical studies have demonstrated that DLL3 is widely expressed in human SCLC cell lines, whereby it functions to promote cell proliferation (both in vitro and in vivo) and mediate migration and invasion of SCLC cells through a Snail-dependent mechanism [16]. Furthermore, a DLL3-targeted antibody-drug conjugate reduced survival of tumor-initiating cells and induced durable responses in patient-derived xenograft models of SCLC [17]. Collectively, these findings suggest an oncogenic role for DLL3 in SCLC.

DLL3 expression has also been evaluated in formalin-fixed paraffin embedded (FFPE) patient material in previous clinical trials using immunohistochemistry (IHC), though assay methodologies, including antibodies and thresholds for defining positivity, have widely varied [18–22]. Present data show that DLL3 is expressed in approximately 85 % of SCLC tumors [18–22], with minimal expression in normal tissues, which makes it a potential therapeutic target [17]. Importantly, DLL3 expression remained stable in serial patient tumor biopsies and patient-derived xenografts from primary SCLC tumors collected pre- and post-chemotherapy [18]. These findings suggest that DLL3 expression in archival tissue accurately estimates expression following intervening therapies and highlights the practicality of testing on archived samples [18]. However, studies to date have not yet assessed DLL3 expression in the real-world setting or the utility of DLL3 as a tractable drug target for SCLC. Here, we present findings from the largest retrospective international noninterventional study performed to date to evaluate the prevalence of DLL3 expression in histological and cytological specimens from patients with SCLC and changes in DLL3 expression at different stages of disease and across lines of therapy.

2. Materials and methods

2.1. Study design, patients, and specimen selection

This multicenter international noninterventional study was conducted using archived histological and cytological specimens of diagnosed SCLC and previously consented for research use. Study specimens were obtained from 56 sites in 19 countries (Supplementary Table 1) in compliance with local regulations and ethics requirements at each site. Site selection was based on experience in lung cancer histopathologic evaluation and availability of biobank or stored tumor tissue for research purposes. Specimens collected between February 2008 and February 2017 were used and were retroactively selected starting from February 2017 until 15–20 patients were identified per site. Per inclusion criteria, selected specimens were from patients ≥18 years of age at time of obtaining samples with confirmed diagnosis of SCLC irrespective of duration of disease, tumor stage, location (ie, primary or metastasis), or treatment. Of note, tumor stage was determined using the Veterans Administration Lung Cancer Group (VALG) two-stage system (ie, limited versus extensive disease) given that most clinical trials in SCLC utilize this classification methodology and since the TNM approach has not be readily adopted in SCLC compared with non-SCLC [23,24].

All study material was derived from FFPE and obtained through resection, core biopsy, bronchial biopsy, or fine-needle aspiration in the form of cell blocks or alcohol-fixed cytological samples. Sites could identify independent or paired specimens, with the latter defined as 2 specimens from the same patient and same primary disease site, or as first specimen obtained at diagnosis and second obtained at relapse/recurrence using the same method of collection (eg, fine-needle aspiration). Information on treatment history, tumor stage, grade, demographics, and clinical outcome were collected by chart review in coded form, with no requirement for additional patient consent. Findings from this study did not affect therapeutic decisions of individual patients.

2.2. Determination of DLL3 expression

Expression of DLL3 in tumor cells was assayed by IHC using DLL3 antibody (clone SP347, Ventana, Tucson, AZ) following manufacturer's recommendations. Briefly, 4 µm tissue sections from FFPE samples were stained within 2 weeks of specimen collection. The OptiView DAB IHC Detection Kit system was used for specific antibody visualization. Ventana BenchMark GX, XT, or ULTRA automated staining instruments were recommended in lieu of manual scoring. Normal lung tissue was used as a negative tissue control, and rabbit immunoglobulin was used as a reagent control. Use of a positive tissue control was mandatory in accordance with the potential tissue options detailed in the Ventana package insert.

Qualified pathologists at individual laboratories at each study site were involved in interpreting DLL3 staining in conjunction with the proper controls. Multiple processes were enacted to ensure that staining quality was equal amongst participating centers. Foremost, each individual laboratory was responsible for validating the assay based on its respective internal standard operating procedure. Second, pathologists had mandatory training on DLL3 biology, the assay package insert (including assay overview, staining and performance, evaluation methodology with example cases, and pointers and artifacts), staining cutoffs, and a broad spectrum of guided case reviews that depicted a wide range of tumor cell staining. Lastly, technical support was made available by Ventana, and laboratories were instructed to submit inquiries as needed, particularly for challenging cases.

Specimens were evaluated for staining at 4x magnification. DLL3 positivity was determined based on the proportion of tumor cells with DLL3 staining present at the following thresholds for DLL3 scoring, which were used for patient classification in previous clinical studies [20,21]: negative (0 %–24 % of tumor cells), positive (≥25 %), non-high positive (25 %–74 %), and high positive (≥75 %). DLL3 staining was identified as present if tumor cells exhibited punctate and/or diffuse cytoplasmic and/or membranous staining that was either partial or circumferential. The percentage of cells expressing DLL3 was collected and defined the level of expression of DLL3.

2.3. Assessments

The primary endpoint was the proportion of patients with DLL3 expression in ≥25 % of tumor cells in the archived specimen. Secondary endpoints included the proportion of patients with DLL3 expression in ≥25 % of tumor cells at different stages, grades, or treatment therapies; and in ≥25 % of tumor cells from the primary and metastatic tumor sites. Other secondary endpoints were the proportion of specimens passing DLL3 assay quality control requirements; changes in DLL3 expression between paired specimens collected across lines of therapy (eg, surgery, chemotherapy, radiotherapy, hormonal therapy, targeted therapies); and DLL3 expression concordance of paired specimens based on the 25 % or 75 % cutoff. Survival from SCLC diagnosis stratified by DLL3 expression and from initiation of therapy was assessed as a secondary analysis objective.

2.4. Statistical analyses

The study design was descriptive and planned to collect at least 1000 specimens, which would allow for a sample size of approximately 600 patient specimens for the primary endpoint analysis. It was expected that 80 %–85 % of patients with SCLC have DLL3 expression [18–22]. With a sample size of 600 patients, the precision of the estimate of proportion of patients with DLL3 expression was 2.4 %–3.5 % as provided by half-width of 95 % CI of the various point estimates. All SCLC patient specimens that met study inclusion criteria and had adequate representative tumor content were included for analysis. Independent specimens were used for analysis of DLL3 prevalence and overall survival; paired specimens were used for concordance analyses.

For the primary analysis, adequately representative tumor content was determined by pathologists, with an estimated sample failure rate of 30 %. No hypothesis testing was performed for the primary analysis. The primary and secondary endpoints were described using descriptive summary statistics. DLL3 expression was analyzed as a continuous measure (median, interquartile range [IQR]) and categorically (negative, positive, non-high positive, and high positive).

DLL3 expression was analyzed in subgroups defined by age and sex, disease grade and stage, clinical baseline characteristics (eg, Eastern Cooperative Oncology Group [ECOG] performance status at diagnosis), lines of treatment, and regional distribution. Patient baseline characteristics were determined at time of biopsy. For paired specimens, DLL3 expression concordance on an ordinal scale (ie, negative, positive/non-high, and positive/high) was assessed using a weighted kappa; concordance between binary outcomes (ie, positive versus negative) was assessed using Cohen's kappa. Univariable logistic regression models were performed to evaluate candidate variables (eg, age, sex, ECOG performance status at diagnosis) and binary DLL3 outcomes for the final multivariable, mixed effects logistic regression models. Any candidate who met a significance level of 0.2 was included in the list of candidate variables for final multivariable model selection, except for extensive versus limited SCLC stage due to colinearity with metastatic versus primary SCLC stage. The final multivariable models were developed to identify factors associated with DLL3 expression (eg, tumor grade, treatment line, metastatic disease).

Survival curves were generated using Kaplan-Meier method by DLL3 expression status and different lines of therapy. Overall survival was defined as the time from SCLC diagnosis to date of death or last available contact. For survival by line of therapy, survival time was defined as the time from initiation of therapy to date of death or last contact. Treatment subgroups were not mutually exclusive (ie, the first-line therapy subgroup included those who went on to receive second-line therapy, and the second-line therapy subgroup included those who went on to receive third-line therapy). Survival estimates were generated using a delayed entry model to account for left truncation of survival time before initiation of therapy or biopsy, whichever was earlier.

Table 1
Patient demographics and clinical characteristics.

Charcteristic	Independent Specimens N = 1073	Paired Specimens N = 36
Age at biopsy, mean (SD), years[a]	66 (10)	62 (11)
< 65 years, n (%)	462 (43)	22 (61)
≥65 years, n (%)	595 (56)	14 (39)
Male, n (%)	682 (64)	16 (44)
ECOG at diagnosis, n (%)		
0	269 (25)	20 (56)
1	395 (37)	8 (22)
2	157 (15)	3 (8)
3	70 (7)	1 (3)
4	15 (1)	0
5	0	0
Missing	167 (16)	4 (11)
Metastases, yes, n (%)	682 (64)	13 (36)
SCLC stage, n (%)		
Limited	340 (32)	19 (53)
Extensive	672 (63)	12 (33)
Missing	61 (6)	5 (14)
Smoking history, n (%)		
Smoker	611 (57)	23 (64)
Ex-smoker	339 (32)	9 (25)
Never-smoker	79 (7)	3 (8)
Missing	44 (4)	1 (3)
Number of years smoked, median (IQR)[b]	40 (30–48)[c]	28 (20–37)[d]
Highest line of therapy received, n (%)		
No treatment received	234 (22)	9 (25)
First-line	446 (42)	12 (33)
Second-line	247 (23)	7 (19)
Third-line or greater	146 (14)	8 (22)

Abbreviations: ECOG, Eastern Cooperative Oncology Group; IQR, interquartile range; SCLC, small cell lung cancer.
[a] n = 1057.
[b] Among smokers and ex-smokers.
[c] n = 760.
[d] n = 18.

3. Results

3.1. Patient demographics and clinical characteristics

Independent tumor specimens were collected from 1073 patients (primary tumor, n = 342 [32 %]; metastatic tumor, n = 682 [64 %]; unknown, n = 49 [5%]). Paired specimens were collected from 36 patients (primary tumor, n = 20 [56 %]; metastatic tumor, n = 13 [36 %]; metastatic unknown, n = 3 [8%]). For patients with independent and paired specimens, respectively, the mean age at biopsy was 66 (SD, 10) and 62 (SD, 11) years, and 682 (64 %) and 16 (44 %) patients were male (Table 1). Most patients had an ECOG performance status of 0–1 (independent specimens, n = 664 [62 %]; paired specimens, n = 28 [78 %]). Most patients with independent specimens had metastases (n = 682 [64 %]) and extensive disease (n = 672 [63 %]) at diagnosis, whereas most patients with paired specimens lacked metastases (n = 20 [56 %]) and had limited disease (n = 19 [53 %]). The majority of patients were smokers (independent specimens, n = 950 [57 %]; paired specimens, n = 32 [64 %]), and many were ex-smokers (independent specimens, 32 %; paired specimens, 25 %). For those patients with independent and paired specimens, respectively, the median number of years smoked was 40 (IQR, 30–48) and 28 (IQR, 20–37) years. Of 1073 patients with independent and 36 with paired specimens, respectively, 234 (22 %) and 9 (25 %) received no prior treatment; 446 (42 %) and 12 (33 %) received first-line therapy; 247 (23 %) and 7 (19 %) received second-line therapy; and 146 (14 %) and 8 (22 %) received third-line or greater therapy.

Table 2
Tumor specimen characteristics.

Characteristic	Independent Specimens N = 1073	Paired Specimens N = 36
Timepoint specimen obtained, n (%)		
Diagnostic specimen	1032 (96)	33 (92)
Relapse/recurrence specimen	27 (3)	2 (6)
Missing	14 (1)	1 (3)
Method of collection, n (%)		
Resection	131 (12)	4 (11)
Core biopsy	692 (64)	25 (69)
Fine-needle aspiration	87 (8)	5 (14)
Forceps biopsy[a]	51 (5)	0
Missing	112 (10)	2 (6)
Type of fixation, n (%)		
Formalin	1033 (96)	32 (89)
Alcohol-based	23 (2)	3 (8)
Other	3 (0)	0
Missing	14 (1)	1 (3)

Patients with missing data are not captured in this table.
[a] An option only in the United Kingdom.

3.2. Tumor specimen and DLL3 assay characteristics

The majority of independent (N = 1073) and paired specimens (N = 36), respectively, were diagnostic (n = 1032 [96 %] and n = 33 [92 %]), collected via core biopsy (n = 692 [64 %] and n = 25 [69 %]), and formalin-fixed (n = 1033 [96 %] and n = 32 [89 %]; Table 2). DLL3 expression was determined in 1054 (98 %) independent specimens and 35 (97 %) paired specimens. The recommended staining protocol, as described in the study protocol, was followed for nearly all specimens (independent, n = 1035 [96 %]; paired, n = 34 [94 %]). Negative reagent control (ie, rabbit monoclonal immunoglobulin) was used for 844 (79 %) independent specimens and 21 (58 %) paired specimens. For the majority of independent and paired specimens, respectively, DLL3 expression was manually scored (n = 995 [93 %] and n = 34 [94 %]) using 4x magnification (n = 945 [88 %] and n = 26 [72 %]), per protocol specifications. Automated scoring and other magnification were used for 20 (2%) and 73 (7%) independent specimens, respectively. Although automated scoring was not used to assess DLL3 expression in paired specimens, a non-protocol-specified magnification was used for 9 (25 %) specimens. Overall, similar tumor specimen and DLL3 assay characteristics were observed irrespective of specimen site (ie, primary or metastatic tumor).

3.3. DLL3 expression level in independent specimens

A total of 1050 of 1073 patients with independent specimens had evaluable DLL3 expression. Of the specimens collected from these patients, the median percentage of DLL3-expressing tumor cells was 90 % (IQR, 60 %–100 %). Of specimens acquired from primary (n = 337/1050) and metastatic (n = 676/1050) sites, 85 % (IQR, 60 %–100 %) and 90 % (IQR, 60 %–100 %) of tumor cells, respectively, expressed DLL3. Among diagnostic specimens (n = 1023/1073), 90 % (IQR, 60 %–100 %) of tumor cells expressed DLL3; of these, 85 % (IQR, 50 %–100 %) and 90 % (IQR, 60 %–100 %) of tumor cells expressed DLL3 in diagnostic specimens acquired from primary tumor (n = 329/1023) and metastatic tumor (n = 657/1023), respectively. Comparatively, though sample sizes were smaller, a similar proportion of tumor cells from relapse/recurrent specimens (n = 27/1073) expressed DLL3 (90 %; IQR, 80 %–100 %). Of specimens acquired from primary tumor (n = 8/27) and metastatic tumor (n = 19/27), 98 % (IQR, 93 %–100 %) and 90 % (IQR, 75 %–100 %), respectively, of tumor cells expressed DLL3.

3.4. DLL3 expression level in paired specimens and expression concordance

A total of 57 specimens (36 patients provided a first specimen and 21 provided a second specimen) were collected for assessing concordance of DLL3 expression level between specimens collated at diagnosis and at relapse/recurrence. DLL3 expression level data were available in 53/57 specimens (35/36 and 18/21 patients who provided the first and second set of specimens, respectively). The median percentage of DLL3-expressing tumor cells in the 53 specimens with evaluable DLL3 expression data was 80 % (IQR, 60 %–100 %). Median percentage of DLL3-expressing tumor cells did not differ between the first set of specimens (80 %; IQR, 60 %–100 %) and the second set of specimens (80 %; IQR, 50 %–95 %) collected.

There was 88 % concordance in DLL3 positivity or negativity between paired specimens (n = 17; Cohen's kappa *P* value, .9412). Of DLL3-positive paired specimens, there was 77 % concordance between first and second specimens with high DLL3 expression compared with 65 % concordance between specimens with non-high DLL3 expression (weighted kappa *P* value, .0699).

3.5. Factors associated with classes of DLL3 expression

A total of 895/1050 (85 %) patients with 1 specimen and evaluable DLL3 expression had positive DLL3 expression (≥25 % of tumor cells; Table 3); 719/1050 (68 %) patients had high DLL3 expression (≥75 % of tumor cells). A similar proportion of patients had positive DLL3 expression irrespective of sex (male, 86 %; female, 84 %), age at biopsy (< 65 years, 87 %; ≥65 years, 84 %), metastatic disease (yes, 86 %; no, 83 %), SCLC stage (limited, 83 %; extensive, 86 %), ECOG performance status at diagnosis (0, 85 %; 1, 82 %; 2, 90 %; 3, 87 %; 4, 93 %), and highest line of therapy received (no treatment received, 89 %; received first-line, 83 %; received second-line, 84 %; received third-line or greater, 86 %).

In univariable logistic regression analyses, there was no association between DLL3 positivity and age, sex, SCLC stage, ECOG performance status at diagnosis, method of specimen collection, type of fixation used, or highest line of treatment prior to biopsy (Supplementary Tables 2–4). There was a significant association between high DLL3 expression and ECOG performance status at diagnosis (2 versus 0; *P* = .0222) and a trend toward statistical significance for SCLC stage at biopsy (extensive versus limited; *P* = .0626; Supplementary Table 3). In multivariable logistic regression analyses, there was no association between positive or high DLL3 expression and metastatic disease or ECOG performance status at diagnosis (Table 4). For patients with non-high DLL3 expression, there was no association between DLL3 expression and sex or metastatic disease in multivariable analyses (Table 4).

3.6. Survival

A decrease in survival was observed with each advancing line of therapy (Fig. 1). The median overall survival from SCLC diagnosis for all evaluable patients was 9.5 months, which was the same as that observed irrespective of DLL3 expression (negative, 9.5 months; positive, 9.5 months; Fig. 2). For patients with positive DLL3 expression, median survival did not differ based on the extent of positivity (non-high DLL3, 9.5 months; high DLL3, 9.5 months). Median survival time from diagnosis decreased with increasing ECOG performance status at diagnosis and was greater for patients with metastases (14.6 months versus 7.6 months for patients without metastases) and limited disease (14.6 months versus 7.5 months for patients with extensive disease; Supplementary Table 5).

4. Discussion

The inhibitory Notch pathway ligand DLL3 is robustly and aberrantly expressed on the cell surface in SCLC tumors and other high-

Table 3
Patient demographics and clinical characteristics by DLL3 expression for patients with 1 specimen.

Characteristic	Specimens With Evaluable DLL3 Expression[a] n = 1050		
	No. in Each Category	Negative 0%–24 %	Positive[b] 25 %–100 %
All patients, n (%)	1050	155 (15)	895 (85)
Sex, n (%)			
Male	677	94 (14)	583 (86)
Female	373	61 (16)	312 (84)
Missing	0	0	0
Age at biopsy, n (%)			
< 65 years	460	62 (13)	398 (87)
≥65 years	587	93 (16)	494 (84)
Missing	3	0	3 (100)
Metastatic			
Yes	676	93 (14)	583 (86)
No	337	57 (17)	280 (83)
Missing	37	5 (14)	32 (86)
SCLC stage, n (%)			
Limited	335	57 (17)	278 (83)
Extensive	667	92 (14)	575 (86)
Missing	48	6 (13)	42 (88)
ECOG performance status, n (%)			
0	265	41 (15)	224 (85)
1	393	71 (18)	322 (82)
2	156	15 (10)	141 (90)
3	69	9 (13)	60 (87)
4	15	1 (7)	14 (93)
5	0	0	0
Missing	152	18 (12)	134 (88)
Highest line of therapy received, n (%)			
No treatment received	218	23 (11)	195 (89)
First-line	442	74 (17)	368 (83)
Second-line	244	38 (16)	206 (84)
Third-line or greater	146	20 (14)	126 (86)

Percentages presented within the table are row percentages.
Abbreviations: DLL3, delta-like ligand 3; ECOG, Eastern Cooperative Oncology Group; SCLC, small cell lung cancer.
[a] DLL3 expression was evaluable in 1050 of 1073 patients who provided independent tumor specimens; patients without DLL3 expression data (n = 23) are not captured in this table.
[b] Positive is the sum of non-high positive and high positive.

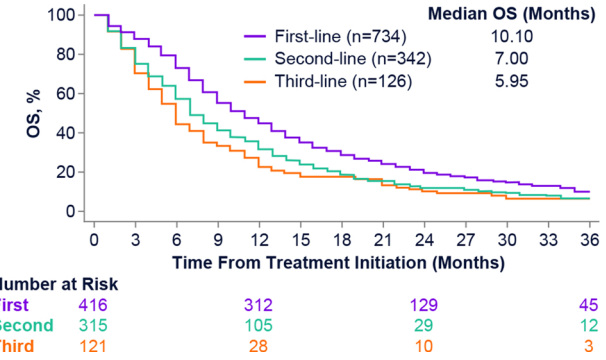

Fig. 1. Survival from initiation of SCLC therapy. The 3 treatment subgroups shown here are not mutually exclusive (ie, the first-line therapy subgroup includes those who went on to receive second-line therapy, and the second-line therapy subgroup includes those who went on to receive third-line therapy). Abbreviations: OS, overall survival; SCLC, small cell lung cancer.

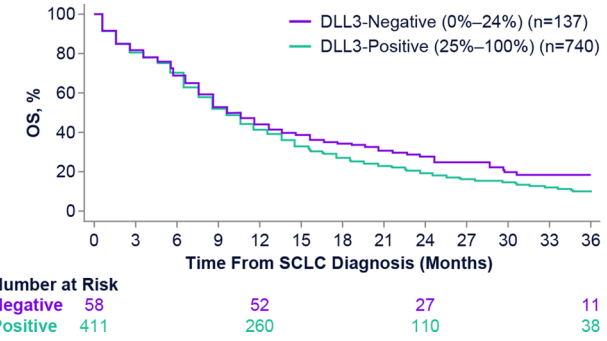

Fig. 2. Survival from SCLC diagnosis stratified by DLL3 expression. Negative and positive DLL3 expression were defined as expression in 0%–24 % and ≥25 % of tumor cells, respectively. Abbreviations: DLL3, delta-like ligand 3; OS, overall survival; SCLC, small cell lung cancer.

grade neuroendocrine tumors, including carcinoids [18,20,25,26]. Conversely, expression of DLL3 in normal cell types (eg, neurons, pituitary cells, testis cells) is limited and exclusively cytoplasmic [20,25,27]. For this reason, DLL3 has emerged as a therapeutic target in SCLC in the last decade. However, therapeutic success of DLL3 targeted therapy was not achieved, and gaps remain in understanding the epidemiology of DLL3 expression and SCLC geographically and temporally throughout the disease course.

This retrospective international noninterventional study was the largest epidemiologic study performed to date to evaluate the prevalence of DLL3 expression in patients with SCLC at various stages of disease and across lines of therapy. Tumor specimens were collected from 56 sites in 19 countries, with 1073 patients contributing independent specimens and 36 contributing paired specimens. It is well established that while SCLC has a propensity to respond to first-line cytotoxic chemotherapy, most patients quickly develop resistant

Table 4
Adjusted associations for DLL3 expression, per multivariable logistic regression.

Outcome	Parameter	N	OR (95 % CI)	*P* Value
Positive (25 %–100 %) vs negative (0 %–24 %) DLL3 expression	SCLC stage (metastatic vs primary)	897	0.767 (0.511, 1.151)	.1995
	ECOG at diagnosis			
	1 vs 0		1.252 (0.792, 1.978)	.3359
	2 vs 0		0.646 (0.333, 1.255)	.1967
	3 vs 0		0.959 (0.422, 2.184)	.9214
	4 vs 0		0.445 (0.054, 3.644)	.4499
High positive (75 %–100 %) vs (negative [0 %–24 %] + non-high positive [25 %–74 %]) DLL3 expression	SCLC stage (metastatic vs primary)	897	0.752 (0.545, 1.039)	.0835
	ECOG at diagnosis			
	1 vs 0		1.105 (0.769, 1.589)	.5879
	2 vs 0		0.694 (0.428, 1.125)	.1384
	3 vs 0		0.817 (0.430, 1.554)	.5372
	4 vs 0		0.346 (0.072, 1.653)	.1833
High positive (75 %–100 %) vs non-high positive (25 %–74%) DLL3 expression	SCLC stage (metastatic vs primary)	863	1.357 (0.937, 1.965)	.1061
	Sex (female vs male)		1.450 (0.987, 2.129)	.0580

Abbreviations: DLL3, delta-like ligand 3; ECOG, Eastern Cooperative Oncology Group; OR, odds ratio; SCLC, small cell lung cancer.

disease that is increasing refractory to additional lines of therapy [23,28]. As was expected, a decrease in survival was observed with each advancing line of therapy in this study, highlighting that the study population was not biased but rather representative of patients with SCLC collectively.

Of patients with 1 specimen in this study, 85 % (n = 895/1050) had positive DLL3 expression (≥25 % of tumor cells), and 68 % (n = 719/1050) had high DLL3 expression (≥75 % of tumor cells). These real-world findings are consistent with available clinical trial data showing that DLL3 expression is common among SCLC tumors [18–22]. Although DLL3 expression thresholds and IHC assay methodologies varied across the aforementioned studies, limiting direct comparisons, the literature, in conjunction with this study, supports robust DLL3 expression in SCLC across different stages of disease and lines of therapy. Furthermore, concordance analyses of DLL3 positivity or negativity between paired specimens in this study demonstrated that DLL3 expression remained stable over time, consistent with other reports [18].

Positive DLL3 expression was observed in this study irrespective of sex, age at biopsy, metastatic disease, SCLC stage, ECOG performance status at diagnosis, and number of lines of therapy received. In both univariable and multivariable logistic regression analyses, no associations were demonstrated between DLL3 positivity and a number of factors, including SCLC stage or ECOG performance status at diagnosis, consistent with other studies [22]. Notably, 1 study reported that median progression-free survival was 4.5 months (95 % CI, 3.0–5.4 months) in patients with high DLL3 expression (≥50 % of tumor cells) versus 2.3 months (95 % CI, 1.3–3.3 months) for those with low DLL3 expression (< 50 % of tumor cells), suggesting potential utility of DLL3 as a predictive biomarker in SCLC [21]. Yet, other studies have demonstrated comparable efficacy outcomes, including progression-free survival and overall survival, among patients with high versus low DLL3 expression [19,22]. Correspondingly, in this study, there was no significant difference in the median overall survival from SCLC diagnosis for evaluable patients based on DLL3 expression status, indicating no prognostic role for DLL3 for overall survival.

5. Conclusion

These real-world international epidemiologic findings indicate that DLL3 is robustly expressed in SCLC across different stages of disease, consistent with available clinical trial data [18,20–22]. DLL3 expression was commonly detected across lines of therapy, and its expression remained stable over time despite intervening treatment, indicating that DLL3 expression in archival tissue is consistent pre- and post-therapy in patients with SCLC. However, no prognostic role for DLL3 was observed in this study. These data establish that archived SCLC specimens are suitable for DLL3 biomarker evaluation and provide an in-depth global understanding of DLL3 prevalence. Taken together, these data may inform ongoing and future clinical studies of DLL3-specific therapies in development for the treatment of SCLC [25].

Data sharing statement

This clinical trial data can be requested by any qualified researchers who engage in rigorous, independent scientific research, and will be provided following review and approval of a research proposal and Statistical Analysis Plan (SAP) and execution of a Data Sharing Agreement (DSA). Data requests can be submitted at any time, and the data will be accessible for 12 months, with possible extensions considered. For more information on the process, or to submit a request, visit the following link: https://www.abbvie.com/our-science/clinical-trials/clinical-trials-data-and-information-sharing/data-and-information-sharing-with-qualified-researchers.html.

CRediT authorship contribution statement

Federico Rojo: Investigation, Resources, Data curation, Writing - review & editing. **Marcelo Corassa:** Investigation, Writing - review & editing. **Dimitrios Mavroudis:** Investigation, Resources, Writing - review & editing. **Aysim Büge Öz:** Investigation, Resources, Writing - review & editing. **Bonne Biesma:** Investigation, Resources. **Luka Brcic:** Investigation, Resources, Data curation, Writing - review & editing. **Patrick Pauwels:** Conceptualization, Investigation, Resources, Writing - review & editing. **Verena Sailer:** Investigation, Resources, Writing - review & editing. **John Gosney:** Validation, Investigation, Resources, Writing - review & editing. **Darko Miljkovic:** Conceptualization, Methodology, Resources, Data curation, Visualization, Supervision, Project administration, Writing - review & editing. **Carlos Hader:** Conceptualization, Methodology, Visualization, Supervision, Project administration, Funding acquisition, Writing - review & editing. **Meijing Wu:** Validation, Formal analysis, Writing - review & editing. **Todd Almarez:** Conceptualization, Methodology, Resources, Supervision, Project administration, Funding acquisition, Writing - review & editing. **Frédérique Penault-Llorca:** Investigation, Resources, Supervision, Writing - review & editing.

Declaration of Competing Interest

F. Rojo: Honoraria from Bristol-Myers Squibb, Merck Sharp and Dohme, Merck, AstraZeneca, Roche, Genomic Health, Pfizer, and Novartis; research support from Roche, Merck, Pfizer, and Novartis; and grants ISCIII-CIBERONC, ISCIII-PT17−0015-0006, and ISCIII-PI18/00382. **M. Corassa, D. Mavroudis, A. Büge Öz, B. Biesma, and V. Sailer:** No relevant financial relationships to disclose. **L. Brcic:** Honoraria from AbbVie, AstraZeneca, Roche, Boehringer-Ingelheim, Merck Sharp and Dohme, Merck, and Takeda; nonfinancial support from AstraZeneca, Roche, Boehringer-Ingelheim, Merck Sharp and Dohme, and AbbVie; and research funding from AstraZeneca. **P. Pauwels:** Honoraria from AbbVie, AstraZeneca, Bayer, Boehringer-Ingelheim, Pfizer, and Roche; research funding from AstraZeneca, Boehringer-Ingelheim, Pfizer, and Roche. **J. Gosney:** Consultancy/advisory role for AbbVie, Amgen, AstraZeneca, Bayer, Boehringer-Ingelheim, Bristol-Myers Squibb, Diaceutics, Guidepoint, Eli Lilly & Co, Merck Sharp and Dohme, Novartis, Pfizer, Roche, and Takeda Oncology; research funding from AstraZeneca and Eli Lilly & Co. **D. Miljkovic, C. Hader, M. Wu, and T. Almarez:** Employment with AbbVie; may own stock or options. **F. Penault-Llorca:** Consultancy/advisory role for AbbVie and Ventana and research support from Ventana.

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