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# Expression of delta-like protein 3 is reproducibly present in a subset of small cell lung carcinomas and pulmonary carcinoid tumors*

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ABSTRACT

*Objectives:* Delta-like protein 3 (DLL3), an inhibitory Notch ligand, is the target for rovalpituzumab tesirine in development for the treatment of small cell lung cancer (SCLC). We studied the expression of DLL3, its reproducibility and prognostic role in pulmonary neuroendocrine tumors.

*Materials and Methods:* Institutional pathology files were searched for resected pulmonary neuroendocrine tumors (1995–2017). Expression of DLL3 (clone SP347) was categorized as high (≥50% of tumor cells) or low (< 50%). Interobserver agreement among 5 thoracic pathologists was measured by Krippendorff's α coefficient. Staging (N = 148) was performed according to the 8th AJCC.

*Results:* Our study included 157 patients with a median age of 62.2 years (range 23.2–88.1) including 59 men (37.6%). Tumors included 44 (28.0%) SCLC, 46 (29.3%) atypical and 67 (42.7%) typical carcinoid tumors at stages I (N = 83, 56.1%), II (N = 28, 18.9%), and III/IV (N = 37, 25.0%). Interobserver agreement for high vs low DLL3 expression (N = 70) was 82.9% (α = 0.79, substantial). High DLL3 expression was observed in 35 (79.5%) SCLC, 17 (37.0%) atypical and 22 (32.8%) typical carcinoid tumors. High DLL3 was associated with SCLC morphology ($p < 0.0001$). During a median follow-up of 4.2 years (range, 2 days–20.3 years), 70 patients died; 19 died from disease. High DLL3 expression was associated with better overall survival in SCLC ($p = 0.049$) but not after adjusting for age, tumor size and stage.

*Conclusions:* DLL3 expression is reliably quantifiable by pathologists and is highly expressed in the majority of SCLC and a subset of carcinoid tumors, making it an attractive target for anti-DLL3 treatment.

## 1. Introduction

Pulmonary neuroendocrine tumors represent a spectrum of neoplasms ranging from low and intermediate grade lesions such as typical and atypical carcinoid tumors, respectively to high grade malignancies including small cell lung carcinomas (SCLC) and large cell neuroendocrine carcinomas (LCNEC). The outcome of these tumors differs with reported 5-years survival of 79 to 99% for typical carcinoid tumors [1–3] to only 1 to 2% for extensive stage SCLC [4,5].

Whereas there has been successful development of many targeted therapies for adenocarcinomas of the lung and immunotherapies for adenocarcinomas, squamous cell carcinomas and small cell carcinomas, there has been very little development beyond targeting somatostatin receptors for pulmonary neuroendocrine tumors. These somatostatin receptors are often lacking in high grade neuroendocrine tumors. Therefore, more effective targeted therapies are sought to specifically treat patients with high grade pulmonary neuroendocrine carcinomas, but also patients with carcinoid tumors who are not curable with surgery alone. In addition, while morphology and stage are important prognostic parameters, other predictive factors are sought for individualized treatment and management of these patients.

The Notch pathway has been implicated in the oncogenesis of SCLC [6]. Specifically, Notch signaling has been found to suppress tumor growth in neuroendocrine tumors [6,7]. Delta-like protein 3 (DLL3), a member of the Notch family, has been identified as an inhibitory ligand of the Notch signaling pathway [8]. Initial studies suggested that DLL3

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is highly upregulated and aberrantly expressed on the cell surface of SCLC. If confirmed, DLL3 could represent a potential therapeutic target in that disease, as an antibody-drug conjugate with high specificity for DLL3, rovalpituzumab tesirine (Rova-T), has been recently identified [9]. In vivo studies have shown that Rova-T induces durable tumor regression in multiple patient-derived xenograft tumor models of SCLC with a strong correlation between level of DLL3 expression and therapeutic activity [9]. A subsequent phase I clinical trial including patients with SCLC and LCNEC confirmed encouraging anti-tumor activity in 11 of 60 (18%) patients who received active doses of Rova-T having an objective response [10]. Ten of 26 (35%) patients with available tissue that showed high DLL3 expression (50% or more of tumor cells expressing DLL3) had a confirmed objective response in contrast to 8 of 8 (100%) patients with low DLL3 expression who did not show an objective response. Similarly, Rova-T treatment prevented tumor growth for over 100 days in a patient derived small cell bladder cancer xenograft animal model [11]. Furthermore, DLL3-expressing *IDH* mutant tumorsphere lines were shown to be susceptible to Rova-T in contrast to *IDH* wildtype DLL3-negative cell lines [12].

Other DLL3-targeting agents are in development, including a bispecific anti-DLL3/CD3 antibody (AMG 757, NCT03319940) and chimeric antigen receptor T cells targeting DLL3 (AMG 119, NCT03392064). Collectively, these reports support a potential role for anti-DLL3 treatment of highly aggressive tumors, specifically small cell carcinomas, when the drug is combined with a DNA-damaging toxin. These findings support the importance of testing of DLL3 expression in these tumors and implicate DLL3 as a predictive biomarker for the therapy for high grade pulmonary neuroendocrine carcinomas. However, the rate of expression and potential prognostic role of DLL3 in pulmonary neuroendocrine tumors needs to be studied more thoroughly. In addition, interobserver reproducibility of DLL3 expression has not been investigated.

We studied the expression of DLL3 in pulmonary neuroendocrine tumors and investigated the reproducibility of DLL3 expression among multiple thoracic pathologists. We also explored clinical features that might be associated with DLL3 expression and the prognostic role of this marker in pulmonary neuroendocrine tumors.

## 2. Material and methods

### 2.1. Cohort

Surgical pathology files of Mayo Clinic Rochester were searched for pulmonary carcinoid tumors, SCLC, and LCNEC (1995–2017). All cases of atypical carcinoid tumor, SCLC and LCNEC that were resected during the time period were included in the study. Typical carcinoid tumors were randomly chosen. To avoid bias due to potential heterogeneity of DLL3 expression only resection specimens were included. Given the low number of atypical carcinoid tumors in contrast to typical carcinoid tumors, the study was enriched for atypical carcinoid tumors to a number that was similar to SCLC. Only a single case of resected LCNEC was identified which was not included in the study as statistical analysis could not be performed. Cases were reviewed by a thoracic pathologist (ACR) to confirm the diagnosis. Tumors were classified according to the current WHO classification into typical and atypical carcinoid tumors, and SCLC [13]. Representative tissue blocks were selected for immunohistochemistry. Cases were staged according to the 8th edition of the AJCC/UICC staging (TNM classification) [14]. Clinical information was abstracted from medical records. The study was approved by the Mayo Clinic Rochester Institutional Review Board (#16-000581).

### 2.2. Immunohistochemistry

Formalin-fixed, paraffin-embedded (FFPE) tissue blocks were cut at 4 microns. Consecutive slides were stained with hematoxylin-eosin (H&E), and antibody against DLL3 (clone SP346, Ventana-Roche Diagnostics, Indianapolis, IN, USA). DLL3 expression, as defined by percent of tumor cells with membranous staining, was determined by a thoracic pathologist (ACR, reviewer 1). A subset of tumors was also scored, independently, by four additional thoracic pathologists (JMB, JJM, JEY, MCA) to assess interoberserver variability. Expression of DLL3 was categorized as high (≥50% of tumor cells express DLL3) or low (< 50%), as previously suggested [10]. Intensity of expression was scored as 0 (negative), 1+ (weak), 2 + (moderate), or 3+ (strong) staining (Supplemental Figure). While staining intensity and percent tumor cell staining in 5% increments were recorded, the results were not formally analyzed following the methods utilized by Rudin et al. [10].

### 2.3. Statistical analysis

Continuous variables were summarized as medians and interquartile ranges (IQR), and categorical or ordinal variables were summarized with frequency counts and percentages. Continuous and ordinal characteristics were compared between groups with Wilcoxon rank-sum tests, and categorical variables were compared between groups with Fisher's exact tests. Overall survival (OS) was calculated from the date of tumor resection to the date of death (or censored at last follow-up). Disease-free survival (DFS) was calculated from the date of tumor resection to the date of either disease recurrence including local recurrence and metastases (or censored at time of last follow-up or death). Five year OS and DFS estimates were calculated using the Kaplan-Meier method. Cox proportional hazards models were used to compare OS and DFS between high versus low DLL3, stratified by diagnosis (SCLC, atypical, and typical carcinoid tumors). Hazard ratios, 95% confidence intervals, and likelihood ratio test p-values are reported. Interobserver variability of DLL3 expression between the five reviewers was quantified using agreement and Krippendorff's α. A Krippendorff's α value of 0.21-0.40 was regarded as fair agreement, 0.41-0.60 as moderate, 0.61-0.80 as substantial, and 0.81–1.00 as almost perfect agreement [15]. Data from reviewer 1 were used for final statistical analysis. All statistical tests were two-sided. A p-value < 0.05 was considered statistically significant. Analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC), or R [16].

## 3. Results

### 3.1. Patient demographics, clinical characteristics and morphologic findings

The study included 157 patients with typical carcinoid tumors (N = 67, 42.7%), atypical carcinoid tumors (N = 46, 29.3%), and SCLC (N = 44, 28.0%). Patient demographics and clinical and histologic findings are summarized in Table 1. Patients with SCLC were older than patients with atypical or typical carcinoid tumors (median age 69.8 years versus 63.1 and 58.5, respectively, p < 0.001). SCLC and atypical carcinoids were larger than typical carcinoid tumors (median tumor size 2.6 cm and 2.8 cm versus 2.0 cm, respectively, p = 0.02). Patients with carcinoid tumors underwent more extensive procedures such as lobectomy (67.4% and 67.2% for atypical and typical carcinoid tumors, respectively) in contrast to patients with SCLC who usually underwent wedge resection (61.4%, p < 0.001). Stage was available in 148 tumors, including 83 stage I (56.1%), 28 stage II (18.9%), and 37 stage III/IV (25.0%) tumors. Typical carcinoid tumors were more commonly resected at stage I (N = 49; 73.1%) in contrast to atypical carcinoid tumors (N = 19; 41.3%) and SCLC (N = 15; 42.9%). On the other hand, SCLC and atypical carcinoid tumors presented more often at stage III/IV (N = 16, 45.7% and N = 15, 32.6%, respectively) than typical carcinoid tumors (N = 6, 9.0%; p < 0.001 for diagnosis versus stage, overall).

**Table 1**
Demographics and clinical and histologic features of study population.

| Feature | All patients (N = 157) | Small cell lung carcinomas (N = 44) | Atypical carcinoid tumors (N = 46) | Typical carcinoid tumors (N = 67) | P-value |
|---|---|---|---|---|---|
| Age, median (range) | 63.4 (23.2-88.1) | 69.8 (41.9-88.1) | 63.1 (24.3-83.2) | 58.5 (23.2-87.0) | < 0.001 |
| Male sex, N (%) | 59 (37.6) | 19 (43.2) | 15 (32.6) | 25 (37.3) | 0.58 |
| Tumor size, median in cm (range) | 2.3 (0.8-8.3) | 2.6 (0.9-6.8) | 2.8 (0.9-8.3) | 2.0 (0.8-5.5) | 0.02 |
| Surgical procedure, N (%) | | | | | < 0.001 |
| Wedge resection | 41 (26.1) | 27 (61.4) | 6 (13.0) | 8 (11.9) | |
| Lobectomy | 90 (57.3) | 14 (31.8) | 31 (67.4) | 45 (67.2) | |
| Pneumonectomy | 6 (3.8) | 1 (2.3) | 4 (8.7) | 1 (1.5) | |
| Segmentectomy | 10 (6.4) | 0 (0.0) | 2 (4.3) | 8 (11.9) | |
| Sleeve resection | 5 (3.2) | 0 (0.0) | 1 (2.2) | 4 (6.0) | |
| Bilobectomy | 5 (3.2) | 2 (4.5) | 2 (4.3) | 1 (1.5) | |
| Completeness of resection, N (%) | | | | | 0.79 |
| Complete resection | 150 (98.0) | 40 (100) | 45 (97.8) | 65 (97.0) | |
| Incomplete resection | 3 (2.0) | 0 (0) | 1 (2.2) | 2 (3.0) | |
| Cannot be assessed[a] | 4 | 4 | 0 | 0 | |
| Pathologic AJCC Stage, N (%) | | | | | < 0.001 |
| IA1 | 10 (6.8) | 1 (2.9) | 1 (2.2) | 8 (11.9) | |
| IA2 | 41 (27.7) | 4 (11.4) | 12 (26.1) | 25 (37.3) | |
| IA3 | 19 (12.8) | 5 (14.3) | 4 (8.7) | 10 (14.9) | |
| IB | 13 (8.8) | 5 (14.3) | 2 (4.3) | 6 (9.0) | |
| IIA | 7 (4.7) | 2 (5.7) | 4 (8.7) | 1 (1.5) | |
| IIB | 21 (14.2) | 2 (5.7) | 8 (17.4) | 11 (16.4) | |
| IIIA | 25 (16.9) | 11 (31.4) | 8 (17.4) | 6 (9.0) | |
| IIIB | 4 (2.7) | 2 (5.7) | 2 (4.3) | 0 (0.0) | |
| IVA | 3 (2.0) | 3 (8.6) | 0 (0.0) | 0 (0.0) | |
| IVB | 5 (3.4) | 0 (0.0) | 5 (10.9) | 0 (0.0) | |
| Cannot be assessed[a] | 9 | 9 | 0 | 0 | |
| Additional treatment, N (%) | | | | | |
| None | 108 (68.8) | 9 (20.5) | 34 (73.9) | 65 (97.0) | |
| Neoadjuvant radiation | 1 (0.6) | 0 (0.0) | 1 (2.2) | 0 (0.0) | |
| Adjuvant radiation + etoposide + cisplatin | 4 (2.5) | 2 (4.5) | 2 (4.3) | 0 (0.0) | |
| Salvage cisplatin + etoposide | 2 (1.3) | 0 (0.0) | 2 (4.3) | 0 (0.0) | |
| Salvage sandostatin | 7 (4.5) | 0 (0.0) | 5 (10.9) | 2 (3.0) | |
| Adjuvant chemotherapy | 15 (9.6) | 15 (34.1) | 0 (0.0) | 0 (0.0) | |
| Adjuvant chemotherapy and radiation | 6 (3.8) | 6 (13.6) | 0 (0.0) | 0 (0.0) | |
| Unknown | 14 (8.9) | 12 (27.3) | 2 (4.3) | 0 (0.0) | |
| Follow up, median in months[b] | 50.3 | 30.8 | 50.8 | 82.4 | |
| (range) | (0.1-243.4) | (0.6-188.5) | (0.4-211.4) | (0.1-243.4) | |
| Recurrence/metastasis, N[c] | 29 | 14 | 13 | 2 | |
| 5-year event-free, % (95% CI) | 77.9 (70.4, 85.5) | 60.1 (43.6, 76.6) | 66.1 (49.2, 83.0) | 96.1 (90.7, 100.0) | |
| Alive without disease, N | 82 | 5 | 24 | 53 | |
| Alive with disease, N | 5 | 1 | 3 | 1 | |
| Died due to disease, N | 19 | 11 | 8 | 0 | |
| Died due to other cause, N | 14 | 8 | 2 | 4 | |
| Died of unknown cause, N | 37 | 19 | 9 | 9 | |
| 5-year survival % (95% CI)[d] | 66.0 (58.0, 74.1) | 26.1 (13.0, 39.3) | 74.6 (60.9, 88.3) | 93.0 (86.3, 99.6) | |

[a]These patients are removed from the denominator; [b]Includes 6 patients who had < 30 days of follow-up after surgery; [c]Kaplan-Meier estimate and 95% confidence interval, 5-year recurrence or metastasis-free survival; [d]Kaplan-Meier estimate and 95% confidence interval, 5-year overall survival.

### *3.2. High DLL3 expression was associated with small cell lung carcinoma morphology*

High DLL3 expression (≥50%) was observed in 74 (of 157, 47.1%) cases. High expression was most likely among those with SCLC morphology (35/44, 79.5%) (Fig. 1), as compared to atypical (17/46, 37.0%) and typical (22/67, 32.8%) carcinoid tumors ($p < 0.0001$).

In most cases with less than 90% of tumor cells staining there was heterogeneity in distribution of DLL3-staining; in some cases heterogeneity in intensity of staining was also noted.

In typical carcinoid tumors, those with high DLL3 expression tended to be older than those with low DLL3 expression (median age 67.9 years versus 52.5 years, $p = 0.02$, Table 2). High DLL3 expression was associated with female sex in SCLC (65.7% female among high DLL3 versus 22.2% among low DLL3, $p = 0.03$) and atypical carcinoid tumors (88.2% female among high DLL3 versus 55.2% among low DLL3, $p = 0.03$), with a similar, though non-significant, finding among typical carcinoid tumors ($p = 0.29$). High DLL3 expression was also associated with smaller tumor size in typical carcinoid tumors (median 1.5 cm versus 2.5 cm for high versus low DLL3, respectively, $p = 0.002$) and with similar, though non-significant, findings among atypical carcinoid tumors ($p = 0.16$). There were no significant differences in staging between low and high DLL3 expression.

### *3.3. High DLL3 expression was associated with better overall survival in small cell lung carcinoma*

Patient follow up is summarized in Table 1. High DLL3 expression was associated with better OS in SCLC (5-year survival = 33.1% vs 0% for high vs low DLL expression, HR = 0.43 [95% CI: 0.20-0.997], $p = 0.049$); however, only 9 cases had low DLL3 expression in that group (Fig. 2). Moreover, after adjusting for age, tumor size, and stage, DLL3 expression was no longer associated with OS among SCLC (HR for high vs low expression = 1.0 [95% CI: 0.985–1.008, $p = 0.49$]). DLL3 expression was not associated with DFS in SCLC ($p = 0.27$). DLL3 expression was not associated with OS or DFS among typical ($p = 0.64$, $p = 0.68$, respectively) or atypical carcinoid tumors ($p = 0.13$, $p = 0.84$, respectively).

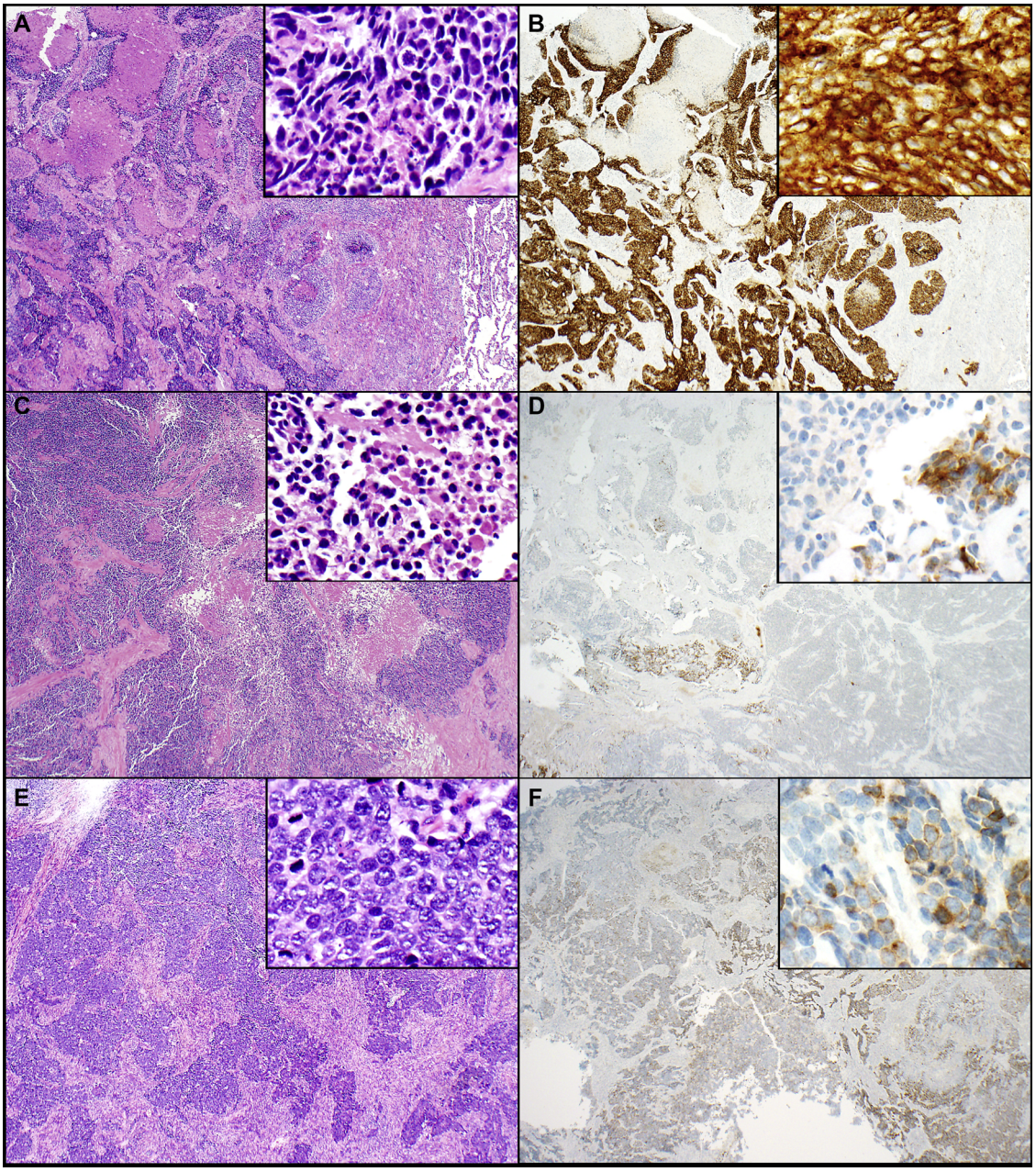


**Fig. 1.** Expression of DLL3 in various small cell lung carcinomas. All 3 cases show classic morphologic features of small cell carcinoma (A, C, E, inserts A, C, E). B. All reviewers agreed upon 50% or more of tumor cells expressing DLL3 (range, 90–100%) at 3+ intensity. D. All reviewers agreed upon less than 50% of tumor cells expressing DLL3 (range, 5–10%) at 2+ or 3+ intensity. F. Three reviewers reported DLL3 expression in less than 50% of tumor cells (range, 20–45%), two reviewers noted expression in 50% or more tumor cells (range, 50–70%) at 1+ (N = 1) or 2+ (N = 4) intensity. Magnification x 40 (A–C, E), x 20 (D, F), x 400 (all inserts).

### *3.4. Interobserver agreement for expression of DLL3 was substantial*

Interobserver agreement was assessed in 70 cases including 44 SCLC, 19 atypical and 7 typical carcinoid tumors. Data are summarized in Table 3 and Fig. 3. Using a high (≥ 50%) vs low expression score for DLL3, interobserver agreement was 82.9% with a Krippendorff α of 0.79 (substantial agreement). Among the 70 cases, 59 (82.9%) were in perfect agreement, 7 (10.0%) were in agreement among 4 reviewers (4-1 split, 6 SCLC, 1 atypical carcinoid tumor) and 5 (7.1%) were at a 3-2 split in agreement (2 SCLC, 2 atypical and 1 typical carcinoid tumor). Among the 4-1 split agreements, most of the cases (5 SCLC, 1 atypical carcinoid tumor) were scored as high expression by the majority of the reviewers, only 1 SCLC was scored as low expression. Among the 3-2 split in agreement, again, the majority of reviewers scored most cases (1 SCLC, 2 atypical carcinoid tumors) as high expression and only 1 SCLC and 1 typical carcinoid tumor were scored as low expression by the majority of the reviewers.

## 4. Discussion

Most SCLC showed high DLL3 expression (expression in 50 percent or more of tumor cells) in our series. Furthermore, we identified high DLL3 expression in about one third of typical and atypical carcinoid tumors. In addition we found that DLL3 expression in resected pulmonary neuroendocrine tumors can be assessed with substantial reproducibility by thoracic pathologists. Expression of DLL3 in a high percent of SCLC and a subset of carcinoid tumors together with substantial reproducibility of expression would make DLL3 an attractive target for therapy in these tumors. In our study, high DLL3 expression was associated with better OS in SCLC in univariate analysis although

**Table 2**
Demographics and clinical and pathologic features of neuroendocrine tumors based on DLL3 expression.

| Feature | Small cell lung carcinomas (N = 44) | | P-Value | Atypical carcinoid tumors (N = 46) | | P-Value | Typical carcinoid tumors (N = 67) | | P-Value |
|---|---|---|---|---|---|---|---|---|---|
| DLL3 Expression | Low | High | | Low | High | | Low | High | |
| N (%) | 9 (20.5) | 35 (79.5) | | 29 (63.0) | 17 (37.0) | | 45 (67.2) | 22 (32.8) | |
| Age, median (range) | 71.7 (41.9-88.1) | 69.5 (53.2-81.8) | 0.49 | 60.2 (24.3-83.2) | 66.5 (49.5-81.1) | 0.20 | 52.5 (23.2-87.0) | 67.9 (35.2-86.3) | 0.02 |
| Male gender, N (%) | 7 (77.8) | 12 (34.3) | 0.03 | 13 (44.8) | 2 (11.8) | 0.03 | 19 (42.2) | 6 (27.3) | 0.29 |
| Tumor size, median in cm (range) | 3.0 (1.5-4.2)[a] | 2.3 (0.9-6.8)[b] | 0.24 | 3.5 (0.9-8.3) | 2.5 (0.9-7.0) | 0.16 | 2.5 (0.8-5.5) | 1.5 (0.9-5.5) | 0.002 |
| Complete resection, N (%) | 7 (100)[a] | 33 (100)[b] | N/A | 29 (100) | 16 (94.1) | 0.37 | 44 (97.8) | 21 (95.5) | 1.0 |
| Pathologic AJCC Stage, N (%) | | | 0.73 | | | 0.16 | | | 0.76 |
| I | 2 (28.6)[a] | 13 (46.4)[c] | | 12 (41.4) | 7 (41.2) | | 34 (75.6) | 15 (68.2) | |
| II | 1 (14.3) | 3 (10.7) | | 10 (34.5) | 2 (11.8) | | 7 (15.6) | 5 (22.7) | |
| III/IV | 4 (57.1) | 12 (42.9) | | 7 (24.1) | 8 (47.1) | | 4 (8.9) | 2 (9.1) | |

Data available in [a]7, [b]33, [c]28 patients, respectively; N/A, not applicable.

this association was no longer present after adjusting for age, tumor size, and stage.

While pulmonary carcinoid tumors are usually treated surgically, high grade neuroendocrine carcinomas such as SCLC and LCNEC in general present at advanced stages and therefore are not suitable for surgical treatment. For decades there have been few changes in the systemic management of SCLC.

Literature suggests that Notch activation suppresses growth in neuroendocrine tumors [7]. The Notch signaling pathway plays a role in cell proliferation, stem cell maintenance, and differentiation during both embryonic and adult development [17]. Five ligands [DLL1, DLL3, DLL4, Jagged 1 (JAG1), and JAG2] and 4 receptors (NOTCH 1–4) are involved in Notch signaling [18]. While many of the Notch family ligands activate Notch receptor signaling, evidence suggests that DLL3 is unable to active Notch signaling and in fact might inhibit signaling by retaining Notch receptors in the cytoplasm or by *cis*-inhibition [17,19]. Furthermore, DLL3 is usually not expressed in normal adult human tissue making it an appealing target for tumor therapy [9].

As DLL3 might be a potential target for the treatment of SCLC and possibly even other neuroendocrine tumors, further studies of the expression of DLL3 on these tumors are warranted. Therefore, we investigated the expression of DLL3 on pulmonary neuroendocrine tumors. Using a cutoff of 50% of tumor cell expression as previously suggested [10], we confirmed that the vast majority (almost 80%) of SCLC highly express DLL3. In addition about one third of carcinoid tumors express DLL3. Our results are similar to another study that found high DLL3 expression in 74% of 39 biopsies of SCLC and LCNEC [10]. Our findings are in contrast to a study by Tanaka et al in which only 32% of 63 SCLC showed high DLL3 expression [20]. The cause of this discrepancy is not entirely clear, but could be, at least in part, due to technical differences in staining since the clone of DLL3 antibody used in that study was not provided. It is also uncertain how many of the specimens in that study were derived from resections vs biopsies. DLL3 expression has not yet been described in carcinoid tumors, so our data regarding DLL3 expression in roughly one third of typical and atypical carcinoid tumors is a novel finding.

The association of DLL3 expression with clinical features has not been thoroughly explored and is still largely uncertain. In our study high DLL3 expression was associated with better OS in SCLC but not in carcinoid tumors. However, the association with OS did not maintain significance in SCLC when adjusted for age, tumor size, and stage. We also identified that high DLL3 expression is associated with smaller tumor size in typical carcinoid tumors and trended towards smaller tumors among atypical carcinoid tumors. Interestingly, high DLL3 expression was associated with female sex, at least in SCLC and atypical carcinoid tumors and trended towards female sex in typical carcinoid tumors. The findings of better OS and smaller tumor size were intriguing as both suggest that high DLL3 expression might be associated with less aggressive pulmonary neuroendocrine tumors, even though the better survival in SCLC was not confirmed in multivariate analysis. In the study by Tanaka et al, no association between high DLL3 expression and clinical features was identified [20]. While we used exclusively resection specimens, it is not clear whether only biopsies or biopsies and resection specimens were used in the study by Tanaka. However, we found that in cases with less than 90% of tumor cell expression by DLL3, expression is often patchy which might lead to a sampling bias in biopsies. It appears somewhat contradictory that high DLL3 expression is associated with better outcome and less aggressive tumors given its alleged function to inhibit Notch signaling leading to growth of neuroendocrine tumors. However, literature suggests that

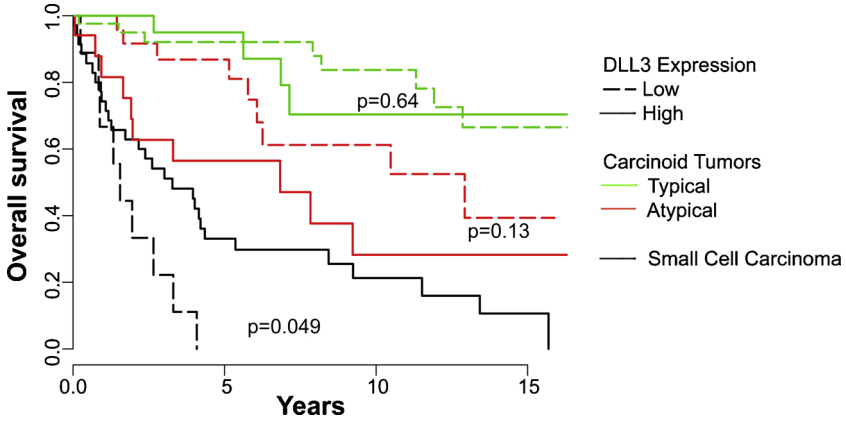


**Fig. 2.** Kaplan Meier curves for overall survival of patients with pulmonary neuroendocrine tumors based on low and high expression of DLL3, stratified by diagnosis.

**Table 3**
Summary of DLL3 expression of all 5 reviewers (n = 70).

| Reviewer<br>DLL3 expression | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Median (IQR[a]) | 90 (45-100) | 80 (40-90) | 80 (40-90) | 80 (40-90) | 80 (50-90) |
| # (%) Cases with expression ≥ 50% | 52 (74.3) | 51 (72.9) | 52 (74.3) | 49 (70.0) | 54 (77.1) |
| DLL3 intensity, N (%) | | | | | |
| 0 | 8 (11.4) | 11 (15.7) | 9 (12.9) | 10 (14.3) | 10 (14.3) |
| 1+ | 3 (4.3) | 10 (14.3) | 6 (8.6) | 4 (5.7) | 6 (8.6) |
| 2+ | 15 (21.4) | 22 (31.4) | 21 (30.0) | 23 (32.9) | 30 (42.9) |
| 3+ | 44 (62.9) | 27 (38.6) | 34 (48.6) | 33 (47.1) | 24 (34.3) |

[a] Range for observers 1–4 is 0–100 and 0–95 for observer 5.

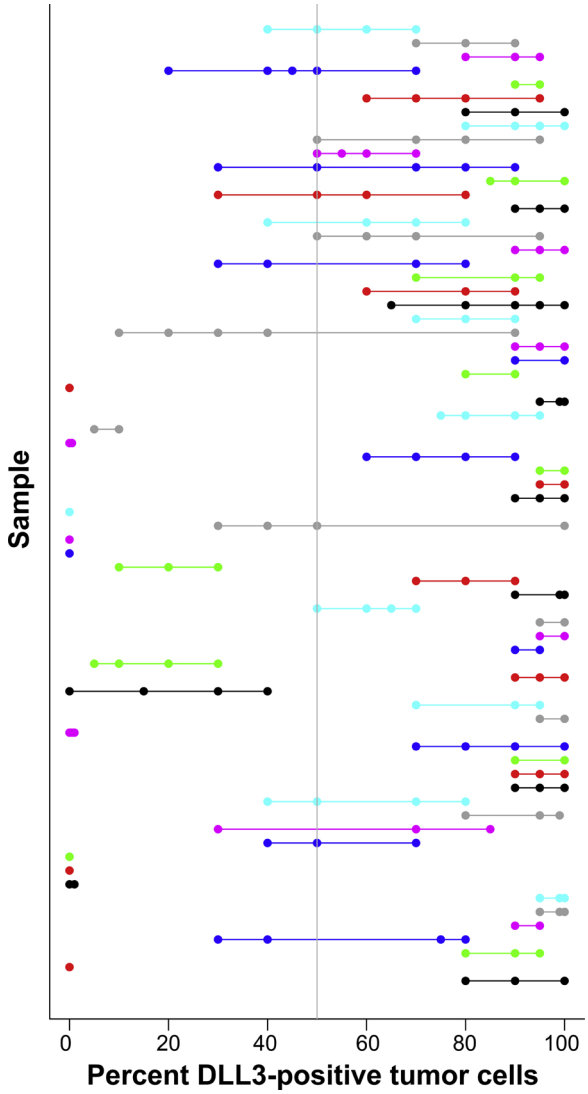


**Fig. 3.** Reproducibility of DLL3 expression, evaluated in 70 pulmonary neuroendocrine tumors. Continuous percent DLL3-positive tumor cells as recorded by 5 thoracic pathologists for each sample (N = 70). Each sample is depicted by a horizontal color line. Vertical grey line at 50% marks the cutoff between low and high DLL3 expression.

while some DLL3 can be expressed on the cell membrane, DLL3 is mainly localized inside the cell including in the Golgi apparatus, and its function at that site might be different [19]. Furthermore, our data need to be validated in larger studies as only a relatively small percentage of SCLC had low expression of DLL3 and therefore survival data have to be interpreted with caution. Conceivably, given that only resection specimens were included in this study, a bias towards SCLC that have a better outcome may have also been introduced. Interestingly, in other tumors, DLL3 expression was found to be largely associated with more aggressive tumor behavior. For instance, in small cell bladder cancer, DLL3 protein expression in more than 10% of tumor cells was associated with shorter overall and progression free survival. In endometrial cancer DLL3 expression by mRNA was associated with older patients, advanced stage, myometrial invasion, pelvic involvement, para-aortic lymph node metastasis, and shorter OS and was found to be an independent poor prognostic parameter [21]. High DLL3 mRNA expression also correlated with poor OS in lung adenocarcinoma [22]. While in contrast to our study most studies showed that DLL3 expression is associated with more aggressive tumor behavior, many of these studies were performed using mRNA expression instead of protein expression or used different cutoffs of DLL3 expression. Furthermore, evidence suggests that different Notch components may have opposing roles in some tumors [23].

Reproducibility of expression of a marker is always of concern, especially if a specific cutoff is used for therapy. We found that the interpretation of DLL3 expression on pulmonary neuroendocrine tumors was easy and reproducible. In fact, in nearly 83% of cases, all 5 pathologists agreed upon high or low expression of DLL3 which would have led to the same treatment if 50% of tumor cell expression would have been used as a cutoff for therapy. Moreover, in the majority of cases of disagreement, most reviewers favored high expression of DLL3. Therefore, expression of DLL3, if proven clinically useful targetable treatment, would be a reliable measurement for treatment.

Our study has some limitations. First, only resection specimens were included to avoid bias due to potential heterogeneity of expression of DLL3. However, in practice, the vast majority of SCLC is diagnosed on biopsies without subsequent resection. This introduced a selection bias likely towards better outcome of SCLC in our study, since usually only limited stage SCLC undergo resection. Conceivably, this selection bias might have led to a bias in DLL3 expression although our results are similar to the study by Rudin et al [10] that used biopsies of SCLC to assess for DLL3 expression. However, follow up studies to evaluate DLL3 expression in biopsies and compare expression with outcome in a more representative population of SCLC are warranted. Second, while our series is large in regards to resected SCLC, the overall number of cases is relatively small further warranting larger validation studies.

In conclusion, DLL3 is highly expressed in SCLC and is expressed in about one third of pulmonary carcinoid tumors. DLL3 expression can be reliably assessed. These findings suggest that DLL3 could potentially be a useful biomarker for targeted therapy with Rova-T in SCLC and a subset of pulmonary carcinoid tumors, if the drug is proven to be beneficial and safe in these patient populations. Our results also suggest that at least a subset of carcinoid tumors have a different pathogenesis than SCLC which might not involve the Notch pathway altered by DLL3.

## Disclosures

None

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.lungcan.2019.07.016.

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