Delta-like canonical Notch ligand 3 as a potential therapeutic target in malignancies: A brief overview

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Abbreviations:
BiTE, bispecific T-cell engager; CAR, chimeric antigen receptor; CI, confidence interval; CRPC-NE, castration-resistant small-cell neuroendocrine prostate cancer; DLL, delta-like canonical Notch ligand; DOR, duration of response; DSL, Delta/Serrate/Lag2; EGFL7, epidermal growth factor-like domain multiple 7; GHPA, growth hormone-secreting pituitary adenoma; GI, gastrointestinal; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; IDH, isocitrate dehydrogenase; IHC, immunohistochemistry; JAG, jagged; LCNEC, large cell neuroendocrine carcinoma; MCC, Merkel cell carcinoma; miRNA, microRNA; NEC, neuroendocrine carcinoma; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; ROVA-T, rovalpituzumab tesirine; SCBC, small-cell bladder cancer; SCLC, small-cell lung cancer.

Abstract
Delta-like canonical Notch ligand 3 (DLL3) is a member of the Delta/Serrate/Lag2 (DSL) Notch receptor ligand family and plays a crucial role in Notch signaling, which influences various cellular processes including differentiation, proliferation, survival, and apoptosis. DLL3 is expressed throughout the presomitic mesoderm and is localized to the rostral somatic compartments; mutations in DLL3 induce skeletal abnormalities such as spondylocostal dysostosis. Recently, DLL3 has attracted interest as a novel molecular target due to its high expression in neuroendocrine carcinoma of the lung. Moreover, a DLL3-targeting Ab-drug conjugate, rovalpituzumab tesirine (ROVA-T), has been developed as a new treatment with proven antitumor activity. However, the development of ROVA-T was suspended because of shorter overall survival compared to topotecan, the second-line standard treatment. Thus, several studies on the mechanism and function of DLL3 in several malignancies are underway to find a new strategy for targeting DLL3. In this review, we discuss the roles of DLL3 in various malignancies and the future perspectives of DLL3-related research, especially as a therapeutic target.

KEYWORDS
DLL3, malignancy, Notch signaling, ROVA-T, therapeutic target

1 | INTRODUCTION

Delta-like canonical Notch ligand 3 is a member of the DSL Notch receptor ligands, which include five ligands in mammals: DLL1, DLL3, DLL4, JAG1, and JAG2. Delta-like canonical Notch ligand 3 plays a crucial role in Notch signaling, which influences various cellular processes, including differentiation, proliferation, survival, and apoptosis. DLL3 is expressed throughout the presomitic mesoderm and is localized to the rostral somatic compartments; mutations in DLL3 are known to induce skeletal abnormalities such as spondylocostal dysostosis.

High DLL3 expression has been recently observed on the surface of SCLC and LCNEC cells. A DLL3-targeting Ab-drug conjugate, rovalpituzumab tesirine.
MATSUO et al.

ROVA-T, a promising targeted therapy, showed efficient regression in SCLC and LCNEC.\(^5\) Notably, our recent findings showed that GI neuroendocrine malignancies had high DLL3 expression, similar to neuroendocrine lung cancer, and that DLL3 silencing inhibited their cell growth through apoptosis induction.\(^6\) Thus, DLL3 is a potential target for novel lung cancer treatments and has attracted attention as a therapeutic gene for several malignancies. However, in lung cancer, ROVA-T development was suspended because of shorter OS compared with the control, topotecan, which is the current standard care.

On the contrary, our previous findings indicate that DLL3 expression is frequently silenced by epigenetic modifications such as aberrant DNA methylation and histone acetylation in HCC cells and that DLL3 overexpression induces apoptosis in HCC cells.\(^7,8\) Hepatitis B virus protein HBx also causes epigenetic modifications and suppresses DLL3 expression in HBV-associated HCC.\(^6,9\) Thus, despite being a therapeutic target due to its high expression in some carcinomas, DLL3 expression could demonstrate different tendencies in each malignancy.

Based on this background, despite the potential of DLL3 as a novel therapeutic target, and several ongoing studies on the mechanism and function of DLL3 in several malignancies, it is necessary to determine the diseases in which DLL3 can be targeted, and their characteristics. In this article, we summarize the characteristics of DLL3, discuss its roles in various malignancies, and elaborate on the future perspectives of DLL3-related research, especially as a therapeutic target.

2  |  STRUCTURE AND FUNCTION OF DLL3

Delta-like canonical Notch ligand 3 is a structurally divergent DSL family member. Unlike other DSL ligands, DLL3 localizes in the Golgi apparatus and emerges on the cell surface when overexpressed.\(^10\) Delta-like canonical Notch ligand 3 does not bind to Notch receptors, and inactivates Notch signaling in cis.\(^11\) Delta-like canonical Notch ligand 3 also prevents the localization of Notch and/or DLL1 on the cell surface through intracellular retention.\(^12\) Thus, DLL3 is regarded as a cell-autonomous inhibitor of Notch signaling.\(^13\) It is also one of several notch ligands that is a direct downstream target of ASCL1, a transcription factor associated with pulmonary neuroendocrine cell development.\(^14-17\) These findings suggest that DLL3 is related to neuroendocrine tumorigenesis, especially in lung cancer.

3  |  ASSOCIATIONS AND ROLES OF DLL3 IN LUNG CANCERS

The roles of DLL3 are being mostly investigated in lung cancer. Increased DLL3 expression was detected in SCLC by whole transcriptome RNA-sequencing.\(^7\) Further investigation indicated that DLL3 expression is detectable on the membrane of SCLC and LCNEC tumor cells. Thus, ROVA-T was developed and was proved to demonstrate antitumor activity.\(^5\) A phase I open-label study was undertaken in the USA, and the safety of ROVA-T, its tolerated dose, and dose-limiting toxic effects were determined.\(^4\) Serious adverse events, grade 3 or worse, included thrombocytopenia, pleural ef- fusion, and increased lipase levels. The maximum tolerated dose was 0.4 mg/kg every 3 weeks, whereas 0.3 mg/kg every 6 weeks was recommended as the appropriate dose and schedule in the phase II trial.\(^4\)

Significant clinical findings were obtained from TRINITY, an open-label, single-arm, phase II study including patients with DLL3-expressing SCLC showing relapsed or refractory disease, previously treated with at least two chemotherapy lines.\(^18\) The primary end-points in this trial were the ORR by central radiographic assessment according to RECIST version 1.1 and OS. The secondary end-points were DOR, disease control rate, and PFS. For all patients, the ORR was 12.4%, and the median OS was 5.6 months. The median DOR was 4.0 months, the median PFS was 3.5 months, and the disease control rate was 69.6%. In contrast, for patients with DLL3-high expression, the ORR was 14.3%, and the median OS was 5.7 months. The median DOR was 3.7 months, median PFS was 3.8 months, and disease control rate was 73.5%.\(^18\) These results were comparable for the DLL3-high and DLL3-positive groups. Furthermore, the response of DLL3-high patients to ROVA-T was significantly higher than that of DLL3-nonhigh patients, showing some DLL3 expression.

Two randomized phase III studies, the TAHOE and MERU studies, were also carried out. The TAHOE study was an open-label, two-to-one randomized study comparing ROVA-T with topotecan, the second-line standard treatment for DLL3-high SCLC with first disease progression during or after first-line platinum-based chemotherapy.\(^19\) The primary end-point was OS. The median OS of the ROVA-T group was 6.3 months (95% CI, 5.6-7.3), and that of the topotecan group was 8.6 months (95% CI, 7.7-10.1).\(^19\) The median PFS of the ROVA-T group (3.0 months; 95% CI, 2.9-3.6) was also inferior to that of the topotecan group (4.3 months; 95% CI, 3.8-5.4). ORR was 15% in the ROVA-T group compared to 21% in the topotecan group, and the median DOR was 3.5 months (95% CI, 2.8-4.2) in the ROVA-T treatment group, compared to 4.9 months with topotecan (95% CI, 3.9-7.9). Based on these results, enrollment in the TAHOE study was discontinued.\(^19\) The primary purpose of the MERU study was to evaluate the effect of ROVA-T, given as maintenance therapy following first-line chemotherapy compared to the placebo; the results could not meet their primary objective. Thus, the development of ROVA-T was suspended in August 2019.

Finally, in a phase I trial, the ORR was 38%, median PFS was 4.3 months, and DOR was 4.3 months in DLL3-high patients.\(^4\) Although these results are superior to the data from TRINITY and TAHOE trials, the phase I study could contain an exploratory aspect, and the number of DLL3-high patients was also small (n = 26).\(^4\) The recommended dose (0.3 mg/kg every 6 weeks) was applied in the TRINITY and TAHOE trials considering the results of the phase I study. These differences could be related to the unsuccessful results of the trials. The unique toxicity of ROVA-T due to pyrrolobenzodiazepine, which damages DNA, should also be considered. Indeed,
pleural effusion, pericardial effusion, edema, cutaneous reactions, and thrombocytopenia were observed in the TAHOE trial, and these side-effects interfered with the good clinical course in patients with SCLC. In particular, pleural effusion and pericardial effusion were not reported in preclinical experiments. These adverse events might have resulted in inadequate treatment with ROVA-T.

Regardless of these findings, DLL3 remains a pivotal target for SCLC and further investigations are required to gain a breakthrough in developing therapeutic strategies and mechanisms targeting DLL3 in other malignancies. A summary of DLL3 in lung cancer is shown in Table 1.

### 4 | NOVEL DLL3-TARGETING TREATMENTS EXPECTED FOR LUNG CANCER

#### 4.1 | Near-infrared photoimmunotherapy

A new cancer treatment technology, near-infrared phototherapy, which uses an Ab-photosensitizer conjugate followed by near-infrared light exposure and specifically damages cancer cells, was developed. Cells incubated with ROVA-IR700, wherein ROVA-T was conjugated with an IR700 photosensitizer, were remarkably lysed upon near-infrared light exposure. Furthermore, xenografts in mice treated with ROVA-IR700 were observed to shrink.

#### 4.2 | AMG 757: a half-life extended, DLL3-targeted BiTE

Bispecific T-cell engager is a novel immune treatment that redirects a patient’s T cells to kill tumor cells. AMG 757 is a first-in-class, half-life-extended, BiTE molecule that activates T cells to reduce DLL3-expressing tumor cells. AMG 757 was effective against SCLC cell lines in vitro and resulted in significant tumor regression through T cell activation in both patient-derived xenografts and orthotopic SCLC tumors in mouse models. These findings suggest that AMG 757 can be used as a DLL3-targeting immune therapeutic for SCLC. A phase I study on AMG 757 in patients with SCLC is ongoing (NCT03319940).

#### 4.3 | AMG 119: a CAR T cell therapy targeting DLL3

Chimeric antigen receptor T cell therapy involves genetic modification of a patient’s autologous T cells. It was developed to direct a patient’s T cells to express the chimeric receptor for a tumor antigen and reinfuse these cells into the patient to attack and kill the target cells.

AMG 119 is an adoptive cellular therapy comprising a patient’s autologous T cells modified to express a transmembrane CAR that targets DLL3 and attacks DLL3-positive cells. Treatment with AMG 119 CAR T cells results in decreased SCLC cells expressing DLL3 in vitro and inhibition of tumor growth in an SCLC xenograft model in vivo. A phase I study evaluating the safety, tolerability, and efficacy of AMG 119 for SCLC (NCT03392064) is also ongoing.

As these findings indicate, DLL3-targeting therapies still have great potential with the most advanced application in lung cancer (Table 2).

### 5 | ASSOCIATION AND ROLE OF DLL3 IN VARIOUS MALIGNANCIES

#### 5.1 | Liver cancer

In HCC, DLL3 was found to be an aberrantly methylated gene. DLL3 expression was not observed in HCC cell lines (HuH1, HuH2, ALEX, and Kim1) by real-time PCR, and methylation of DLL3 was detected in HuH2 and Kim1 cells, but not in ALEX analyzed by methylation-specific PCR. Expression of DLL3 in HuH2 and Kim1 cells was recovered after treatment with 5-aza-2′-deoxycytidine and trichostatin, which act as a demethylating agent and histone deacetylase inhibitor, respectively. Thus, DLL3 was downregulated in HCC cells through aberrant promoter methylation. Moreover,
DLL3 expression was found to be silenced in clinical specimens of HBV-associated HCC; HepG2.2.15 cells, which are transformed with the HBV gene, also showed low DLL3 expression by western blotting and real-time PCR, compared to the parental HepG2 cells. We then observed increased DLL3 expression in HepG2.2.15 cells after trichostatin treatment, but not with 5-aza-2′-deoxycytidine. Therefore, the HBV protein HBx could cause epigenetic modifications such as histone acetylation, and suppress DLL3 expression in HBV-associated HCC. Overall, the expression and function of DLL3 differed in HCC compared to other malignancies.

5.2 | Pancreatic cancer

Quantitative PCR for DLL3 gene expression in 22 pancreatic cancer cell lines showed that PANC-1 and SU86.86 cells showed a 3-fold or higher copy number than that in human pancreatic epithelial cells. Moreover, DLL3 knockdown in SU86.86 cells caused considerable growth inhibition. Similarly, high DLL3 expression was detected in SU86.86 cells by western blot analysis. These findings suggest that targeting DLL3 might be useful in pancreatic cancer; however, the location of DLL3 expression in pancreatic cancer is unclear. Thus, further experiments are needed to determine the possibility of widening the applications of targeting DLL3.

5.3 | Breast cancer

Wnt signaling in human mammary epithelial cells, as achieved by ectopic Wnt-1 expression, elicits a DNA damage response followed by Notch activation; furthermore, DLL3 expression is significantly increased in Wnt-1-expressing human mammary epithelial cells at both the protein and RNA levels. However, none of these cells exhibited high DLL3 expression by IHC. In contrast, low DLL3 expression was observed in two of 19 breast NEC cases. Considering these results, DLL3 could be a challenging therapeutic target in breast cancer at present.

5.4 | Gastrointestinal neuroendocrine carcinoma

Expression of DLL3 in GINEC has both similar and different characteristics, which are essential when considering DLL3 as a therapeutic target. Localization and expression levels of DLL3 were thus examined in GINEC cells. Expression of DLL3 in clinical specimens of the GI tract including the stomach, duodenum, jejunum, ileum, and rectum was examined by IHC. Delta-like canonical Notch ligand 3 was found to be expressed in the cytoplasm of cells in the deep layer of the GI tract mucosa. These tendencies were similar to chromogranin A, a representative neuroendocrine cell marker. Double-fluorescence IHC showed that the expression of DLL3 and chromogranin A was synchronized. Thus, DLL3 was expressed mainly in the neuroendocrine cells of the GI tract. For experiments on GINEC cell lines, only ECC4 (small cell carcinoma of the rectum), ECC10, and ECC12 (small cell carcinoma of the stomach) were available. Notably, DLL3 mRNA and protein expression was significantly (several thousand-fold compared to other cancer cell lines such as colon cancer cells) upregulated in these cell lines, similar to SCLC cells. These features suggest the usefulness of DLL3 as a novel therapeutic target. Moreover, silencing of DLL3 in these cells induced cell growth inhibition through internal apoptosis.

However, unlike SCLC, DLL3 is expressed in the cytoplasm, and not on the cell surface. Interestingly, electron microscopy analysis showed that DLL3 was expressed in neurosecretory granules,

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**TABLE 2** Summary of novel expected Delta-like canonical Notch ligand 3 (DLL3)-targeting treatments and new clinical trials for lung cancer

| DLL3-targeting treatments | Functions and associations | Clinical trial number | References |
|---------------------------|---------------------------|-----------------------|------------|
| NIR-PIT                   | A new cancer treatment technology: an Ab-photosensitizer conjugate followed by near-infrared light exposure that specifically damages cancer cells | – | 20 |
| AMG 757                   | A half-life extended, DLL3-targeted BiTE: AMG 757 was effective against SCLC cell lines in vitro and led to significant tumor regression through T cell activation | NCT03319940 | 22,23 |
| AMG 119                   | A CAR T cell therapy targeting DLL3: AMG 119 shows potent killing of SCLC cells expressing DLL3 in vitro and inhibits tumor growth in an SCLC xenograft model in vivo | NCT03392064 | 26,27 |

Abbreviations: BiTE, bispecific T cell engager; CAR, chimeric antigen receptor; NIR-PIT, near-infrared photoimmunotherapy SCLC, small cell lung cancer.
specific to GINEC cell lines. Although further investigation is required to determine whether DLL3-targeting agents are useful for GINEC, DLL3 does show potential as a novel therapeutic target.

5.5 | Small-cell bladder cancer

Small-cell bladder cancer, which accounts for 2%-5% of all bladder tumors, has a poor prognosis. There is no standard therapy for advanced SCBC, and early relapses result in poor overall outcomes. Given this situation, more effective treatments and biomarkers are required for SCBC. In a study including 46 samples, unsupervised hierarchical clustering of gene expression patterns and gene expression correlated with clinical phenotypes indicated that the "normal-like" type had a superior OS than the "metastasis-like" type. Notably, more than 10% of DLL3 expression was associated with shorter OS and PFS, and more than 30% of CD56 (neuroendocrine marker) expression was also associated with shorter PFS and OS in SCBC. High expression of DLL3 protein was associated with shorter OS and PFS. The efficacy of a DLL3-targeting agent has also been observed in a patient-derived xenograft model of SCBC. This was the first study to report increased DLL3 expression as a poor prognostic biomarker for SCBC, and that DLL3 has potential as a new therapeutic target in SCBC.

5.6 | Neuroendocrine prostate cancer

A subset of patients with advanced prostate cancer show histologic transformation to small-cell neuroendocrine prostate cancer. Castration-resistant small cell neuroendocrine prostate cancer is typically associated with poor outcomes, and patients are treated with platinum-based chemotherapy regimens. Because the clinical behavior of CRPC-NE shares similarities with SCLC, the association of DLL3 expression with the CRPC-NE phenotype in prostate cancer was investigated and the antitumor activity of SC16LD6.5 (humanized Ab against DLL3) was evaluated in DLL3-expressing prostate cancer models. Delta-like canonical Notch ligand 3 was found to be expressed in most CRPC-NE and some castration-resistant prostate adenocarcinoma cases, but not in the localized benign prostate cancer. Moreover, a single dose of SC16LD6.5 induced a complete and durable response in DLL3-expressing prostate cancer xenografts. Overall, these findings indicate that DLL3 is a potential therapeutic target in neuroendocrine prostate cancer.

5.7 | Gynecological cancer

Endometrial cancer is a common female neoplasm, and its incidence and/or mortality have been increasing recently. Despite its detection at an early stage and a 5-year survival rate of more than 90%, advanced stages of the disease or high-risk histopathology lead to worse survival rates in endometrial cancer. Expression of DLL3 was found to be significantly upregulated in endometrial cancer; DLL3 overexpression, advanced tumor stage grades, and lymph node metastasis were all found to be independent prognostic predictors for endometrial cancer. In ovarian cancers, the expression of Notch components (NOTCH2, NOTCH3, DLL3, MAML1, and ADAM17), which are the top five most relevant genes, is associated with poor OS; furthermore, the expression of these genes is increased with progressing cancer stages. Thus, DLL3 plays a pivotal role in promoting gynecological cancer cell growth and causes poor outcomes in these cancer types. However, further investigation is needed to determine whether DLL3 can be considered a therapeutic target in gynecological cancer.

5.8 | Isocitrate dehydrogenase-mutant glioma

Isocitrate dehydrogenase-mutant glioma is a distinct molecular subtype of glioma with poor prognosis, and 80%-90% of low-grade gliomas are IDH-mutant. RNA sequencing analysis of more than 20 cancer types in The Cancer Genome Atlas dataset revealed that IDH-mutant glioma showed the highest DLL3 expression. Furthermore, DLL3 expression was extraordinarily high and homogeneous in IDH-mutant glioma by IHC, and an anti-DLL3 Ab-drug conjugate was effective for patient-derived IDH-mutant glioma tumorsphere cultures. MicroRNAs are key to glioma development and progression. Expression of DLL3 as a proneural marker was upregulated in secondary glioblastoma, compared with the primary tumor, and was downregulated by inhibiting miRNA-128a. These findings indicate a relationship between DLL3 expression and miRNA in glioma, and could contribute to a breakthrough in additional treatment for DLL3-expressing malignancies.

5.9 | Growth hormone-secreting pituitary adenoma

Growth hormone-secreting pituitary adenomas constitute approximately 20% of all pituitary adenomas, which are the third most common intracranial neoplasm. Approximately one-third of GHPAs show invasive and aggressive clinical courses, and invasion is a crucial factor in their treatment and clinical progress. Epidermal growth factor-like domain multiple 7 plays a pivotal role in physiologic and pathological angiogenesis. In fact, high EGFL7 expression is associated with a poor clinical course in several malignancies, resulting in enhanced tumor cell migration and invasion by promoting cell motility through the Notch signaling pathway. Moreover, EGFL7 interacts with all four Notch receptors, including DLL3, and modulates its signaling. The expression levels of EGFL7 and Notch2 are markedly higher in invasive GHPA and EGFL7 knockdown was found to downregulate Notch2 and DLL3. These findings suggest that EGFL7 could be a novel therapeutic target through the inactivation of DLL3 expression in malignancies.
Merkel cell carcinoma is a rare malignancy and a subset of neuroendocrine carcinomas of the skin. Considering the neuroendocrine features of MCC, the expression of DLL3 and ROVA-T treatment response was evaluated in MCC.\textsuperscript{54,55} High DLL3 expression was detected in MCC, and a partial response to ROVA-T therapy was observed in some patients enrolled in an open-label study (NCT02709889). However, DLL3 expression in patients with MCC was not associated with OS.\textsuperscript{55} Thus, extensive studies are required to confirm whether DLL3 might be a prognostic biomarker in MCC and whether ROVA-T is useful in large studies.

A summary of DLL3 in various neuroendocrine malignant cell types is shown in Table 3 and other malignant types in Table 4. Notably, in malignancies tending to have high DLL3 expression, treatment methods are not well established due to their relative rarity (Figure 1).
FUTURE PERSPECTIVES

Given the findings of high DLL3 expression in SCLC and LCNEC, DLL3 is an attractive therapeutic target, and ROVA-T has been developed as a treatment. However, the treatment effects did not overcome those of the current standard therapy. The impact of ROVA-IR700 application using near-infrared photoimmunotherapy has been investigated. Moreover, novel treatments such as AMG 757 and AMG 119 have been developed, and further clinical trials are ongoing. High expression of DLL3 was confirmed in other malignancies, especially in neuroendocrine-related tumors such as gastrointestinal neuroendocrine carcinoma. DLL3 may thus have potential for therapeutic breakthrough in rare tumors. CRPC-NE, castration-resistant small cell neuroendocrine prostate cancer; DLL3, Delta-like 3; GHPA, growth hormone-secreting pituitary adenoma; GINEC, gastrointestinal neuroendocrine carcinoma; IDH, isocitrate dehydrogenase; LCNEC, large cell neuroendocrine carcinoma; MCC, Merkel cell carcinoma; NEC, neuroendocrine carcinoma; SCBC, small-cell-bladder cancer; SCLC, small cell lung cancer.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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