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REVIEW

Delta-like ligand 3 (DLL3): an attractive actionable target in tumors with neuroendocrine origin

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ABSTRACT

Introduction: Neuroendocrine carcinomas are very aggressive tumors with few treatment options. DLL3 seems to be an optimal target for therapeutic intervention, as it is expressed mainly on the membrane of tumor cells with neuroendocrine origin.

Areas covered: In this article, we outline the preclinical and clinical studies published in the last years on DLL3 in neuroendocrine neoplasm, above all of lung origin. Furthermore, we review the current literature on the interaction between DLL3 and the tumor microenvironment.

Expert opinion: Several DLL3-targeting strategies have been proposed in the last years with mixed results. Understanding the influence of DLL3 on the tumor (immune) microenvironment and developing adoptive therapies directed against this optimal target might represent the key strategy. Building on the clinical data obtained so far, future trials on in vivo diagnostic tools for predictive purpose and new specific therapies are needed.

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1. Introduction

Neuroendocrine neoplasms (NENs) are a heterogeneous group of tumors that arise most commonly in the lung, pancreas, and gastrointestinal tract [1]. According to the WHO classification lung NENs are divided into low grade typical carcinoid (TC) or atypical carcinoid (AC), and high grade large cell neuroendocrine carcinoma (LCNEC) or small cell lung carcinoma (SCLC) on the basis of mitotic rate, presence of necrosis and cytomorphological features [2]. Gastroenteropancreatic (GEP) NENs are divided into grade (G) 1 and G2 well-differentiated NETs neuroendocrine tumors (NETs), and G3 well-differentiated NETs or poorly differentiated neuroendocrine carcinomas (NECs) according to morphology and Ki-67 [3,4]. In this latter subgroup, the prognosis is very poor and the discrimination between the two G3 categories is challenging [5].

Treatment of metastatic NECs beyond the first line is still associated with low response rates despite the trials conducted in the last years [6,7]. Immune-therapy produced encouraging data in SCLC and LCNEC, but conclusive results are lacking for all other neuroendocrine tumors [8]. Therefore, identifying new treatment options for NEC patients, especially in the second-line setting and beyond, is an area of unmet clinical need.

The Notch pathway is a cell–cell signaling interplay involved in several physiological and pathological processes [9,10]. In tumors with neuroendocrine origins, it has been demonstrated that Notch signaling plays a suppressor function. Moreover, the inhibitory ligand of Notch 1 Delta-like ligand 3 (DLL3) has been found highly upregulated and aberrantly expressed on the cell surface of some aggressive NENs [11,12]. As an example, in prostate cancer with neuroendocrine features high levels of DLL3 have been associated with greater neuroendocrine differentiation, deletion of RB1 and a more aggressive disease with worse overall survival (OS) [13].

The cell surface expression of DLL3 makes it an optimal target for directed therapies. For all these reasons, several DLL3-targeted therapy strategies for the treatment of tumors with neuroendocrine features have been proposed and are currently in pre- or clinical trials. DLL3 targeting holds great potential especially in SCLC and GEP-NEC, which are characterized by a very poor prognosis and by the lack of therapeutic options.

The aim of this review is to summarize the biological role of DLL3 and the correlation with the tumor (immune) microenvironment in NENs and to present the state of the art and the future perspectives of DLL3-related clinical trials, especially as a therapeutic target.

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Article highlights

- Neuroendocrine carcinoma are tumors with a poor prognosis and limited therapeutic options
- Notch pathway and in particular DLL3 seems to play an important role in these tumors
- DLL3 is highly expressed in tumor cells and could be involved in the modification of tumor (immune) microenvironment
- In recent years, several adoptive therapies and immune radioligand tracers targeting DLL3 in SCLC and other neuroendocrine neoplasia have been developed.

2. The role of DLL3 and the notch pathway in NENs

2.1. DLL3 function and expression

The Notch pathway is a cell–cell signaling pathway involved in several pathophysiological processes. Four Notch receptors (Notch1–4) and five Notch ligands (Delta-like 1, 3, and Jagged 1–2) are known in mammals. In cancer, Notch signaling can have both an oncosuppressor or an oncogenic role [14]. As an example, in lung neoplasms Notch signaling either enhances or inhibits cell growth depending on the tumor types: in non-small cell lung cancer, Notch1 activation promotes tumor growth, while it inhibits the growth of neuroendocrine SCLC [15,16]. Overall, signaling has been found to play a suppressor function in tumors with neuroendocrine origins [17,18]. In particular, it seems that the loss of Notch1 allows neuroendocrine cells to acquire and/or maintain a differentiated neuroendocrine phenotype while retaining the ability to proliferate [17,19]. Moreover, preclinical evidence has shown that despite the fact that neuroendocrine tumor cells might lack basal Notch-1 activation, the cells are able to respond to Notch-1 signaling as the pathway components are intact. Re-expression of Notch-1 in BON1 cell line results in a significant inhibition of cell growth and in the downregulation of several neuroendocrine tumor markers, such as serotonin, Chromogranin A, synaptophysin, neuron-specific enolase, and ASCL-1 [19,20]. Thus, the identification of compounds able to activate endogenous Notch-1 in neuroendocrine tumor cells represent a promising therapeutic strategy.

DLL3, unlike other ligands, is considered an inhibitor of the Notch receptors that can negatively regulate Notch signaling through a variety of mechanisms [21]. It can localize to the Golgi apparatus and bind to DLL1 and to the full-length Notch1 receptor promoting their degradation [22]. Alternatively, membrane DLL3 can bind to Notch receptors and prevent their activation [23]. DLL3 is found in the Golgi apparatus, but it can localize at the cell surface if overexpressed [24,25]. In particular, DLL3 is not expressed on the surface of normal cells, but is highly expressed on the surface of several tumor cells, particularly those with a neuro- or neuro-endocrine origin. Specifically, DLL3 overexpression has been found in glioblastoma multiforme [26], neuroendocrine lung cancer [27], neuroendocrine prostate cancer [13],

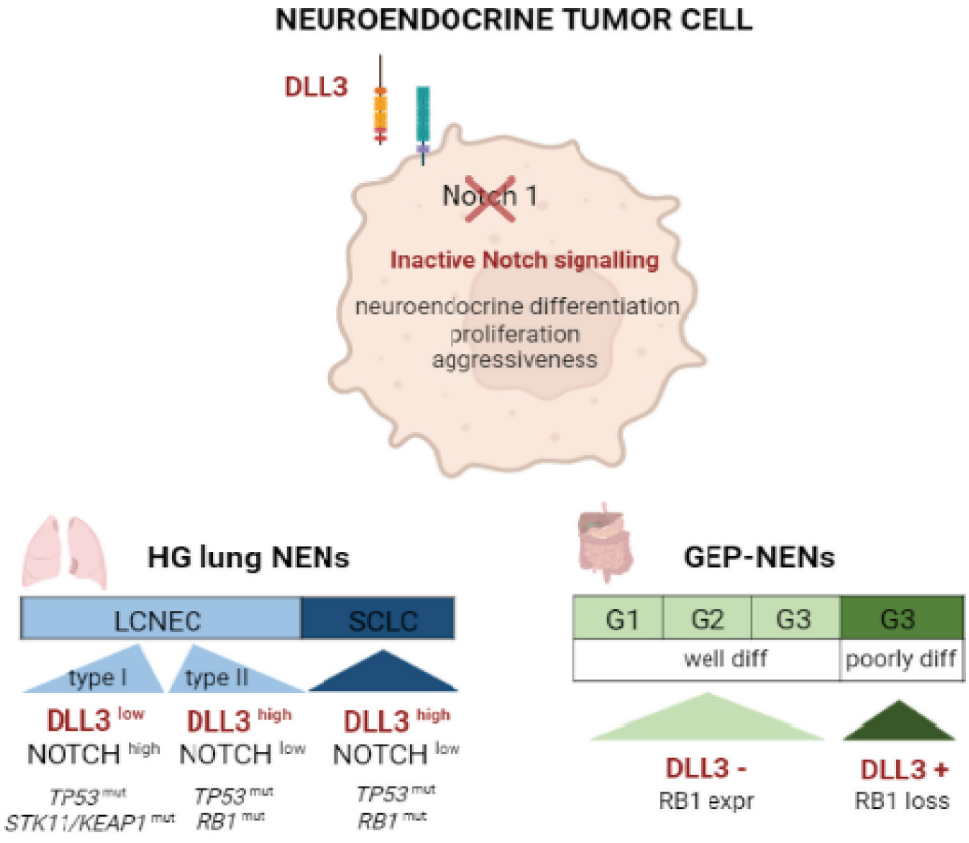

Figure 1. Schematic representation of the putative role and expression pattern of DLL3 in NENs. In neuroendocrine tumor cells DLL3 has been shown to promote neuroendocrine differentiation, proliferation and aggressiveness through the inhibition of Notch signaling. Expression of DLL3 has been extensively found on high grade (HG) neuroendocrine neoplasms of the lung (SCLC and type II LCNEC). In GEP-Nen expression of DLL3 has been found on poorly differentiated G3 carcinoma cells.

gastrointestinal neuroendocrine cancer [28] and small cell bladder cancer [29]. In neuroendocrine tumors, DLL3 transcription is upregulated by the oncogenic driver ASCL1, in particular in advanced and aggressive disease [8].

Regarding lung neuroendocrine neoplasms, DLL3 expression has been robustly found in SCLC [30]. In these tumors, Notch plays a tumor suppressor function and low Notch-1 levels correlate with high expression of DLL3 [27,31]. Preclinical studies have shown that in human SCLC cell lines, DLL3 promotes cell proliferation, migration, and invasion through a Snail-dependent mechanism [32]. Conversely, LCNEC that displays active Notch-1 signaling shows a low expression of DLL3. However, a genomic profiling of lung neoplasms have revealed the existence of a distinct subtype of LCNEC that exhibits a high neuroendocrine profile, high expression of ASCL1 and DLL3, a low Notch level and a mutation in TP53 and RB1 [27]. Moreover, DLL3 has been detected in circulating tumor cells (CTCs) from SCLC patients: patients with DLL3-positive CTCs show reduced overall survival (OS) and progression-free survival (PFS) compared to DLL3-negative patients [33,34]. The percentage of DLL3-positive CTCs has been proposed as a circulating biomarker to predict cancer progression.

Recently, it has been shown that DLL3 is expressed also on the membrane of GEP neuroendocrine tumor cells and that its expression could distinguish between low grade and high-grade disease [35]. In particular, DLL3 seems to be frequently expressed in poorly differentiated GEP-NECs, where it correlates with loss of RB1, while it is almost absent in well-differentiated GEP-NETs. For this reason, the development of tailored treatments for DLL3-expressing tumors has recently gained increasing attention (Figure 1).

2.2. DLL3 and tumor microenvironment (TME) remodeling

The overexpression of DLL3 has been found to significantly affect the proportion of B and T cells as well as neutrophils in Invasive Breast Cancer (IBC), and found to be associated with high levels of immune infiltration [36]. DLL3 may have a potential regulatory effect on B and T cells in IBC and affect the survival of tumor cells. Several studies indicate Notch signaling as a driving effector of T helper cell activation. Indeed, Delta-like ligands of Notch, are expressed by antigen presenting cells (APCs) and promote activation and differentiation of naïve CD4 + T cells to a Th1 phenotype, while JAG1/2 expressing APCs lead to Treg/Th2/Th17 polarization [37]. Overexpression of DLL-3 in APCs surface results in Notch inhibition, which impairs T cell functions. In addition, DLL3 was positively correlated with the expression of PD-1 and CTLA4, suggesting that DLL3 may be used as an immunoadjuvant checkpoint or a marker of immunotherapeutic effect [36].

2.3. DLL3 as an immune- therapeutic target

Moieties of ligands can be engineered to induce immune responses improving antitumor immunity [38,39]. Murine models of solid tumors indicate great potential of the DLL-family as immunomodulatory agents for the management of malignant cancers [40]. Seventy percent of SCLC overexpress DLL3 with high specificity when compared to normal adult lung tissue [27]. Type I LCNECs with STK11 and KEAP1 alterations exhibit a high degree of similarity with SCLC, as well as high expression of neuroendocrine genes and a profile of $ASCL1^{high}/DLL3^{high}/NOTCH^{low}$ [27]. On the other hand, type II LCNECs with RB1 alterations revealed reduced expression of neuroendocrine genes and a pattern of $ASCL1^{low}/DLL3^{low}/NOTCH^{high}$. The efficacy of DLL3-targeted T cell-based immunotherapy has been proven in these tumor types. Indeed, both T cell-engaging bispecific antibodies and CAR-based therapies on the mAb SC 16.15, a specific antibody directed against the tumor-associated antigen DLL3 protein, have demonstrated to selectively kill DLL3-positive cancer cells *in vitro* and to inhibit tumor growth *in vivo* [41]. Of clinical relevance, combination with PD-1 inhibitors can improve the activity and the efficacy of the treatment with the anti-DLL3 bispecific antibodies but not with CAR-T cells [42]. Optimizing the DLL3 bispecific antibodies, can represent a key step toward DLL3-targeted and combined immunotherapy.

Gene expression profiling revealed that the type II LCNECs show an upregulation of immune related pathways with a positive impact on immunotherapy response [27]. With these premises, the distinction between poorly differentiated neuroendocrine carcinoma representing type I LCNECs and as RB1-mutated SCLC or type II LCNECs, may be the key to understanding the differences observed in immunotherapy efficacy.

3. Therapeutic implications

3.1. Antibody-drug conjugates

Antibody-drug conjugates (ADCs) represent novel antibodies binding to specific antigens on the tumor cell surface to deliver cytotoxic agents directly into the tumor cells [43]. Recently, Rovalpituzumab tesirine (SC16LD6.5/Rova-T), a DLL3-targeted ADC consisting of an anti-hDLL3 IgG1 antibody, a linker, and a pyrrolobenzodiazepine dimer toxin, has been studied in several clinical trials [44–47,45].

In the phase I clinical study (NCT01901653) 72 SCLC and 8 LCNEC patients were treated with Rova-T obtaining an objective response rate (ORR) of 38% in DLL3-high patients (DLL3 expression superior to 50%). No responses were seen in patients with DLL3 expression <50% [44].

In another phase I clinical study (NCT03086239) on the Japanese population, 17% of SCLC patients with DLL3-high expression (≥75%) treated with ROVA-T obtained an objective response, and 56% achieved disease control. The median overall survival was higher in patients with highly expressed DLL3 than in patients with DLL3-low expression (7.4 vs 5.1 months) [46].

Despite these promising results, in the single-arm phase II TRINITY trial (NCT02674568) Rova-T showed modest responses with an ORR of 16% in the DLL3-high expression (≥75%) in patients with a large percentage of severe side effects [47].

The TAHOE Phase III trial NCT03061812 comparing Rova-T to topotecan as second-line therapy for SCLC was stopped due to a shorter OS in the Rova-T arm [48].

Also in the MERU phase III trial NCT03033511, the addition of Rova-T as a first-line maintenance therapy following first-line platinum-based chemotherapy for advanced SCLC demonstrates no survival benefit at the preplanned interim analysis [45].

Similarly to previous phase 1 and phase 2 trials published [44–47,45], Rova-T side effects were characterized by pleural and pericardial effusions, cutaneous reaction, edema, photosensitivity reaction, pneumonitis, hypoalbuminemia, and thrombocytopenia. These adverse events were attributed to the Pyrrolobenzodiazepine (PBD) payload, with some of the characteristic toxicities also observed with other PBD-based ADCs [49].

Despite the initial encouraging activity of Rova-T in SCLC the enthusiasm was reduced due to unexpected negative results. Table 1 resumes all the clinical trials published on ROVA-T.

3.2. Adoptive cell therapy

DLL3-targeted agents include also AMG 119 (HLE BiTE® antibody) and AMG 757, a chimeric antigen receptor (CAR), built to activate and redirect CD3-positive T cells to DLL3-expressing tumors to induce cell apoptosis [51].

The CAR AMG 757 is a half-life extended BiTE® immunotherapy, binding both DLL3 on tumor cells and CD3 on T cells, resulting in a T cell-dependent killing of tumor cells administered intravenously every two weeks at doses of 0.003–3.0 mg in patients with metastatic SCLC that progressed or recurred following ≥1 platinum-based regimen. In 52 patients enrolled, with a median treatment duration of 6.1 (0.1–59.4) weeks, a confirmed partial response was reported in 7 (14.0%) patients and stable disease in 12 (24.0%) of all treated patients. Most responses occurred after 8 weeks of treatment. AEs were reported in 41 (79.0%) patients, including 12 (23.0%) who were grade ≥3 and one grade 5 (pneumonitis in DL5 [0.3 mg]). Three AEs (dyspnea, pneumonitis, and fatigue) led to treatment discontinuation. The most common AE was grade 1–2 cytokine release syndrome (CRS), which was reported in 44.0% patients within 24 hours of the first or second dose of AMG 757. CRS events were reversible [52].

A phase 1b study on De Novo or Treatment Emergent Neuroendocrine Prostate Cancer (NCT04702737) and a phase II study in SCLC (NCT05060016) are ongoing.

DLL3-targeted CAR T cell based therapy, AMG 119, is being studied in an ongoing phase I trial of patients with relapsed/refractory SCLC (NCT03392064). Another DLL3-targeting bispecific T-cell engager, BI 764532, consisting of an anti-CD3 scFv and an anti-DLL3 scFV linked by an engineered Fc region demonstrates target specificity, T-cell redirection and a half-life of 10 days [53]. A Phase 1 study (NCT04429087) is currently enrolling patients with small cell lung cancer and other neuroendocrine tumors.

3.3. Radionuclide therapy

In recent years, a great interest for nuclear medicine has been rising for two main reasons: the ability to display a radionuclide distribution in the organism for diagnostic purposes, and the opportunity to irradiate malignant cells for therapeutic ones. A potential role of the positron-emitting zirconium-89 (89Zr) (t1/2 = 78.4 h) has been recently described for immune positron emission tomography [54]. This is a radioimmunoconjugate composed of SC16.56, the chelator deferoxamine (DFO), and the radioisotope zirconium (Zr-89). Upon administration of zirconium Zr-89-DFO-SC16.56, the monoclonal antibody can be specifically targeted and bound to DLL3 and can be detected using a PET in order to visualize and to quantify DLL3-expressing tumor cells [55].

The alpha-emitter ^{225}Ac and beta-emitter ^{177}Lu have been explored as radioimmunoconjugates (RICs) and compared with the PBD-based ADCs in preclinical studies involving DLL3-expressing and non-expressing cell lines and SCLC patient derived xenografts (PDX) onto NOD SCIDmice. RICs have some benefits if compared with small ADC: the latter group requires cellular internalization to be effective and the Van der Waals radius distances to their drug target [56].

Table 1. Clinical trials on DLL3-targeted therapies.

TREATMENT	SETTING	PHASE	PRIMARY OBJECTIVE	NUMBER OF PATIENTS	RESULTS	Refereces
Rovalpituzumab Tesirine	SCLC, LCNEC	Phase I	Safety	74 (SCLC) and 8 (LCNEC)	ORR 18% (11/60); ORR 38% (10/26 in DLL3-high patients (≥50%)	[38]
Rovalpituzumab Tesirine	SCLC	Phase I	Safety	29 Japanese	ORR 17%	[39]
Rovalpituzumab Tesirine	Solid tumors	Phase I/II	Safety	201 (101 NEC and NET; 99 other solid tumors)	ORR 10%; NEC and NET: ORR 13% (9/69)	[50]
Rovalpituzumab Tesirine (TRINITY)	SCLC	Phase II	ORR, OS	339	ORR 12.4, OS 5.6 months	[40]
Rova-T alone vs Rova-T + Platinum and Etoposide	SCLC	Phase I	Safety	26	ORR 50% (7/14)	[60]
Rova-T maintenance therapy following first-line platinum-based cht (MERU)	SCLC	Phase III	PFS	748	ORR 9%; futility criteria were met	[42]
Rova-T plus Nivo vs Rova-T plus Nivo+Ipi	SCLC	Phase I/II	Safety	42	ORR 30%	[61]
Rovalpituzumab Tesirine (R) vs Topotecan (T) (THAOE)	SCLC (DLL3 high)	Phase III	OS	Median OS 6.3	Median OS 6.3 (R) vs 8.6 months (T)	[41]

Abbreviations: Rova-T = *Rovalpituzumab Tesirine; SCLC*:small cell lung cancer; LCNEC = large cell neuroendocrine carcinoma; ORR = Objective response rate; OS = Overall survival; NET: neuroendocrine tumors

Despite ^{225}Ac radioimmunoconjugates seem to perform better than ^{177}Lu with extended life expectancy and tumor growth suppression, the PBD conjugates showed a superior anti-tumor efficacy. Furthermore, an undesired release and deposition of ^{225}Ac was seen in non-targeted tissues [57].

4. Conclusion

DLL3 and Notch pathways are involved in several pathological mechanisms in tumors with neuroendocrine origins and in particular in the relationship between the tumor and the (immune)-microenvironment. Given the data presented DLL3 is the optimal theranostic maker for NEN patients, which can represent both a prognostic factor and an attractive therapeutic target. Despite the fact that the development of Rova-T has been withdrawn, new technologies, such as BiTE therapies and nuclear medicine compounds seem to be promising.

5. Expert opinion

Neuroendocrine neoplasia are rare tumors with different biological and clinical behaviors related to the tumor grade [1].

Some important issues need to be improved for NEN patients: the distinction between G3 NET and NEC, based only on morphology and ki67 evaluation, and the lack of therapeutic options above all in the poor prognosis group of neuroendocrine carcinomas [2,3]. Immunotherapy has been explored in this latter group with unsatisfactory results. Recent evidence has highlighted that tumors with neuroendocrine histopathological features often present a high expression of DLL3 on the cell surface. Expression of DLL3 seems to be correlated with tumor progression and to be a discriminant between low grade and high-grade diseases [35].

In the preclinical studies presented, DLL3 is involved in the specific molecular crosstalk between cancer and stromal cells within the TME impacting on tumor invasion, intra- and extravasation and the ability to establish distant metastasis and finally in the immune response [36,37].

PD-1/PD-L1 and PDL-2 overexpression is reported in metastatic GEP-NENs, including 97% of pancreatic (P)-NEN and 82% of small intestinal (SI)-NEN, with even increased expression in NECs. The expression of immune checkpoint proteins PD-1 and PD-L1 is correlated to shorter survival (PFS and OS) and increased tumor grade [40].

Preclinical studies on a mouse model indicate that targeting PD-1/PD-L1 alone or in combination with Tumor Associated Macrophages (TAMs), regulatory T-lymphocytes (Tregs), and CD73 represent a promising therapeutic strategy for high-grade GEP-NENs and NECs [58–60].

Moreover, CTLA-4 can promote T-cells, which will undergo activation or anergy [61,62]. A single study reported extended survival for patients with metastatic functional G2 gastric NEN after a combination of PD-1 and CTLA-4 inhibitors, indicating that CTLA-4 blockade could represent a therapeutic strategy for GEP-NEN. Recently, novel therapeutic targets and potential biomarkers of immune response have been described for GEP-NENs and other cancers [7–9]. Indeed, targeting the immune TME (TIME) can represent a novel therapeutic option for GEP-NENs. In recent years, several clinical trials with agents able to target DLL3 have been developed. Rova-T was one of the first compounds clinically studied in DLL3 expressing SCLC. Despite some initial encouraging data from the phase I and II clinical trials, preliminary results of phase III TAHOE study were negative and the enrollment was discontinued. Similarly, the MERU trial on ROVA-T maintenance therapy didn't meet the primary aim and the study was withdrawn. Thus, the development of ROVA-T was suspended in August 2019 [63,64].

With these premises, a therapeutic strategy on combination of ROVA-T with nivolumab alone or plus showed encouraging antitumor activity in heavily pretreated SCLC patients but burdened by drug-related serious side effects occurred in 43% of patients [65].

However, in patients affected by platinum refractory SCLC the ORR reported in preliminary studies seems valuable. We believe that a better selection of patients based on the biological tumor characteristics could help repurposing this drug in the therapeutic armamentarium.

Furthermore, despite the fact that development of ROVA-T was interrupted, new technologies targeting DLL3 are currently being explored, like near-infrared photoimmunotherapy, radionuclide therapy, and bispecific antibodies (BiTE). In the first example, ROVA-T is conjugated with a photosensitizer realizing the drug upon near-infrared light exposure in order to increase the activity and to reduce the side effects [66]. Bite therapies such as BI 764532, AMG 757, and AMG 119 seem to be more promising and are currently being investigated in first-in-human studies (NCT04429087, NCT03319940, and NCT03392064). One of the remaining issues is to understand if the expression of DLL3 at the cell surface or in the cytoplasm influences the efficacy and side effects of DLL3-directed therapies.

In our opinion, research efforts should also be focused on defining the role of DLL3 in the immune-microenvironment of NENs and understanding how the interplay between DLL3 and immune cells influence disease progression and responsiveness to immunotherapy. These findings might guide the development of novel inhibitory strategies targeting DLL3, with the development of chimeric antigen receptors (CAR) therapies representing the optimal approach. Furthermore, we believe that future clinical trials would benefit from corollary endpoint analyses on the effects of DLL3 targeting on tumor immune infiltration and NOTCH signaling. Further understanding of these mechanisms might help identify candidate biomarkers to predict therapy efficacy and select the patient population that would benefit more from specific DLL3-targeted treatments.

Finally, results of in-vivo detection of DLL3 expression through a Zr-89 PET/CT, could help screening patients for DLL3 expression, especially in cases where histological material is scarce or unavailable.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer disclosures

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