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Diagnostic and Predictive Role of DLL3 Expression in Gastroenteropancreatic Neuroendocrine Neoplasms

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Abstract

Gastroenteropancreatic neuroendocrine neoplasms (GEP-NENs) are a rare and heterogeneous subgroup of tumors with a challenging management because of their extremely variable biological and clinical behaviors. Due to their different prognosis, there is an urgent need to identify molecular markers which would enable to discriminate between grade 3 neuroendocrine tumors (NETs) and neuroendocrine carcinomas (NECs), despite both being diagnosed mainly on the basis of proliferation index and cell differentiation. DLL3, a negative Notch regulator, is a promising molecular target highly expressed in several tumors with neuroendocrine features. We conducted a retrospective analysis of DLL3, RB1, and PD-L1 expression by immunohistochemistry (IHC), in formalin-fixed, paraffin-embedded (FFPE) samples from 47 patients with GEP-NENs. Then, we correlated the results with patients' clinical features and outcome. The absence of DLL3 expression in 5 well-differentiated GEP-NETs with high-grade features (G3 NET), and the presence of DLL3 in 76.9% of poorly-differentiated NECs (G3 NEC), highlights DLL3 expression as a marker of G3 NECs ($p = 0.007$). DLL3 expression was correlated with RB1-loss ($p < 0.001$), negative ^{68} Ga-PET/CT scan ($p = 0.001$), and an unfavorable clinical outcome, with important implications for treatment response and patient's follow-up. Median progression-free survival (PFS) and overall survival (OS) were 22.7 months (95% CI 6.1–68.8) and 68.8 months (95% CI 26.0–78.1), respectively, in patients with DLL3-negative tumor compared with 5.2 months (95% CI 2.5–18.5) and 9.5 months (95% CI 2.5–25.2), respectively, in patients with DLL3-positive tumor (PFS $p = 0.0083$, OS $p = 0.0071$). Therefore, combined with morphological cell analysis, DLL3 could represent a valuable histological marker, for the diagnosis of poorly differentiated NECs. The high percentage of DLL3 expression in NEC patients also highlights a potential opportunity for a DLL3 targeted therapy in this tumor subset.

Keywords GEP-NEN · DLL3 · Prognostic marker · NEC

Introduction

Gastroenteropancreatic neuroendocrine neoplasms (GEP-NENs) are a rare subgroup of tumors with a heterogeneous natural history and extremely variable clinical behavior in terms of prognosis and response to treatment [1]. The estimated annual incidence is 3.56 cases/100,000 persons [2]. GEP-NENs are divided according to World Health Organization (WHO) classification into grade (G) 1 and G2 neuroendocrine tumors (NETs), which have well-differentiated morphology and a Ki-67 score ≤ 20% (< 3% for G1; 3–20% for G2), and G3 well-differentiated NETs or poorly differentiated neuroendocrine carcinomas (NECs) with Ki-67 > 20% [3, 4]. The recent acknowledgement that G3 NENs include two heterogeneous subtypes has oriented the focus of

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clinicians on the different management of these two tumor entities [5]. GEP-NEC patients often have multiple distant metastases at the time of diagnosis, progress rapidly, and show lower survival rates than those with GEP-NETs [6]. However, the differential diagnosis of G3 NETs and NECs, based on morphology, is challenging and it would be substantially aided by the availability of molecular biomarkers and objective criteria. Although surgery is still the most effective therapeutic modality for resectable NETs, several treatments have provided additional value on the basis of tumor clinical pathologic characteristics [7–14]. Furthermore, the best therapeutic approach to G3 NENs is still debate but response to first line chemotherapy is completely different in G3 NECs or G3 NETs patients [15]. Recently, several studies reported that NETs G3 are identified by the loss of DAXX or ATRX protein expression and *MEN1* mutation, while the deregulated expression of *p53*, *Rb*, and *SMAD4* identified G3-NECs [16, 17].

The discovery of novel candidate targets for drug development is an urgent clinical need in this setting. For example, Notch-1 signaling has shown a tumor suppressor function in neuroendocrine tumors [18, 19]. It has been seen that although neuroendocrine tumor cells lack basal Notch-1 activation, pathway components are intact and cells are sensitive to Notch-1 signaling [20]. Both transient and stable Notch-1 expression in BON1 neuroendocrine cells result in a significant reduced cell growth and downregulation of specific neuroendocrine markers as serotonin, chromogranin A, synaptophysin, neuron-specific enolase, and ASCL-1 [20, 21]. Thus, the identification of compounds capable of activating endogenous Notch-1 in NEN cells represents a promising therapeutic strategy. In small cell lung carcinoma where Notch plays a tumor suppressor role, low Notch-1 levels have been correlated with high DLL3 expression, an inhibitory ligand of the Notch receptor [22, 23]. Conversely, large-cell neuroendocrine carcinomas (LCNEC) with low DLL3 levels exhibit active Notch-1 signaling [22]. Moreover, in prostate cancer, the neuroendocrine feature of DLL3 expression correlates with disease aggressiveness. Indeed, neuroendocrine morphology is correlated with poor overall survival (OS) and DLL3's high expression levels are indicative of *RB1* deletion [24].

Little is known about DLL3 expression in gastroenteropancreatic NEN cells, nor about its potential theranostic role. We previously showed that loss of *RB1* and negative ^{68}Gallium-positron emission tomography/computerized tomography (^{68} Ga-PET/CT) have a prognostic value in GEP-NEN patients and could help to discriminate between poorly differentiated NECs and G3 well-differentiated NETs [25]. Here, we provide evidence of DLL3 expression in GEP-NENs and its correlation with disease grade and differentiation, loss of *RB1*, ^{68} Ga-PET/CT scan response, immune status, and clinical outcome.

Materials and Methods

Study Design

We conducted a retrospective study on a case series of 47 patients enrolled at IRST IRCCS, in Meldola, Italy between 2010 and 2019. All patients were required to have GEP-NENs histologically confirmed by an expert pathologist. For each patient, at least one specimen from the primary tumor and/or one from a metastasis had to be available. Physical examination, brain-chest-abdominal CT, or ^{68} Ga- and ^{18}F-fluorodeoxyglucose (FDG)-PET/CT were included as staging procedures.

Immunohistochemical Analysis

Paraffin-embedded or bioptic NEN specimens were sliced into 5-μM-thick sections with a rotating microtome (Leica Biosystems, Wetzlar, Germany) and were mounted on positive-charged microslides (Thermo Fisher Scientific, Waltman, MA, USA). Immunostaining was performed using the VENTANA BenchMark Ultra (Ventana Medical Systems Inc, Tucson, AZ, USA), and the following antibodies were used: DLL3 (SP347) Ventana Assay (Ventana Medical Systems Inc.), prediluted by the supplier; RB1 (Cell Signaling Technology, Beverly, Massachusetts, USA) diluted 1:1000; and PD-L1 (SP142) Ventana Assay, (Ventana Medical Systems Inc.), prediluted by the supplier. All reactions were carried out for 1 h at room temperature, and sections were counterstained with hematoxylin II (Ventana Medical Systems Inc). Then, stained sections were evaluated by an expert pathologist in a blind fashion. For each section, immunohistochemical (IHC) staining was analyzed if there was a percentage of tumor cells sufficient for a suitable evaluation. Expression values of both DLL3 and RB1 were considered as dichotomous variables (positive/negative). PD-L1 analysis was performed in a subgroup of 42 patients by evaluating the percentage of tumor cells with a positive membranous staining. IHC was scored as positive if more than 1% of the tumor cells showed cytoplasmic or membranous localization of DLL3 and nuclear localization of RB1 (Fig. 1a, b). Stromal cells were used as a positive control for RB1 immunostaining.

Statistical Analysis

Median overall survival (OS) was estimated using the Kaplan-Meier method (two-sided 95% confidence intervals [CIs]). Continuous variables were presented as median and minimum-maximum values, while categorical variables were reported as frequency. In order to evaluate the

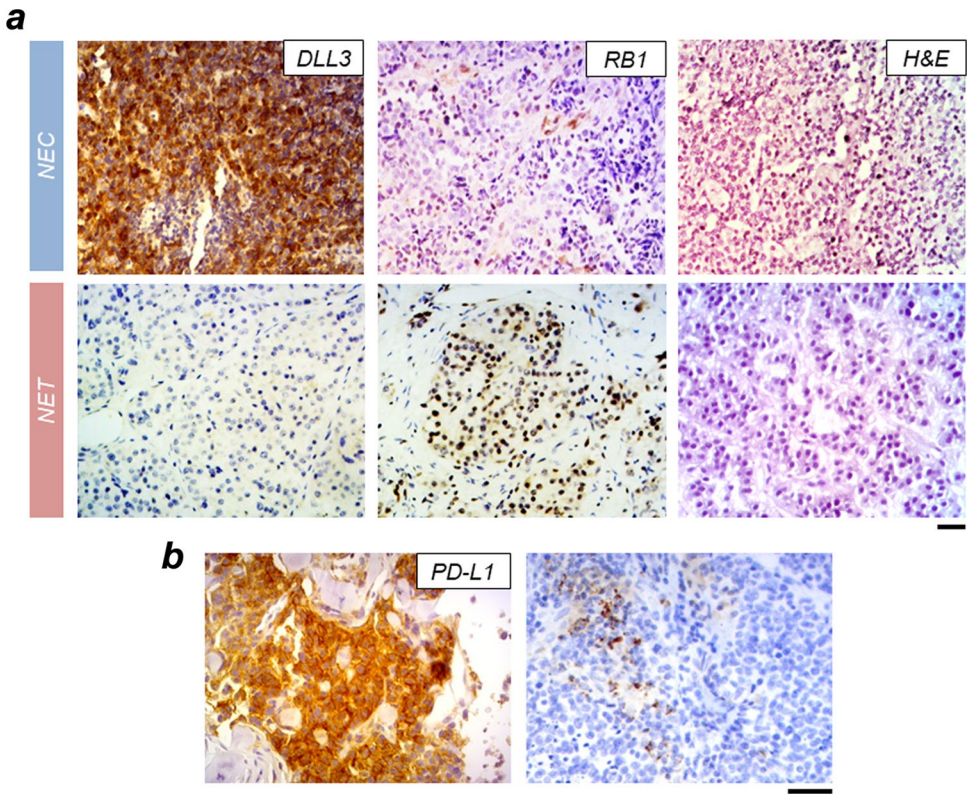


Fig. 1 **a** NEN tissue immunostained for DLL3 and RB1 with matching hematoxylin and eosin (H&E)-stained sections. Patients with NECs showed positive immunostaining for DLL3 and loss of RB1 expression, while those with NETs showed RB1 expression but no expression of DLL3. Scale bar 50 µm. **b** NEN tissue positive for PD-L1 (left) or negative for PD-L1 with positive immune infiltrate (right). Scale bar 50 µm

relationship between categorical variables, we used a Fisher's exact test and $P < 0.05$ were considered statistically significant. Statistical analyses were performed with STATA/MP 10.1 for Windows (StataCorp LP, College Station, TX, USA).

Results

Descriptive Characteristics

The main clinical and histological characteristics of the 47 patients analyzed in this study are shown in Table 1. Twenty-seven (57.4%) patients were males and 20 (42.6%) females. Median age at the time of diagnosis was 64.3 years (range 34.3–82.3 years). The origin site of primary tumor was pancreas in 16 (34%) patients, stomach in 7 (14.9%) patients, and colorectum in 24 (51.1%) patients. Twenty-two (46.8%) patients had G1 well-differentiated NETs, 7 (14.9%) G2 well-differentiated NETs, and 18 (38.3%) G3 NENs. Within these patients, 5 (27.8%) displayed well differentiated G3 NETs and 13 (72.2%) poorly differentiated G3 NECs. Eight (34.8%) patients who received first-line chemotherapy obtained a partial response, 8 (34.8%) showed stable disease (SD), and 7 progressed (PD). Nineteen (63.3%) patients showed a positive ^{68} Ga-PET/CT and 11 (36.7%) a negative ^{68} Ga-PET/CT, while for the remaining patients this data was not available. Twenty-one (87.5%) patients showed a positive ^{18}F-FDG PET/CT and 3 (12.5%) a negative ^{18}F-FDG PET/CT, while the referral for the remaining 23 patients was not available. Fourteen (31.1%) patients developed a second malignancy.

DLL3, RB1, and PD-L1 Immunohistochemical Expression

Expression levels of DLL3, RB1, and PD-L1 in neuroendocrine tumor tissue are reported in Table 2. DLL3 was expressed in 21.7% of tumor samples (10/46 patients; DLL3 was not evaluable in one case). RB1 was expressed in 79.1% of tumor tissues (34/43 patients; RB1 was not evaluable in 4 cases). PD-L1 was expressed in 19.5% of tumor samples (8/41 patients; PD-L1 was not evaluable in one case and not assessed in 5). The correlation between DLL3, RB1, and PD-L1 expression is reported in Table 3. Of the 10/46 DLL3-positive patients, 7 (70.0%) showed loss of RB1, while only 2/36 (6.3%) with negative DLL3 tumors revealed loss of RB1 expression ($p < 0.001$). No correlation was found between DLL3 and PD-L1 expression. Among the patients positive for DLL3 expression, 6 (85.7%) were negative for PD-L1 expression and one (14.3%) was positive, while in patients negative for DLL3 expression, 27 (79.4%)

Table 1 Clinicopathological characteristics ($n = 47$)

Clinicopathological characteristics	*n* (%)
Age at diagnosis, years (range)	64.3 (34.3–82.3)
Gender	
Male	27 (57.4)
Female	20 (42.6)
Site of disease	
Stomach	7 (14.9)
Colorectum	24 (51.1)
Pancreas	16 (34.0)
Grading	
G1	22 (46.8)
G2	7 (14.9)
G3	18 (38.3)
Histological classification of G3 neoplasms	
Well differentiated NET	5 (27.8)
Poorly differentiated NEC	13 (72.2)
Presence of a second malignant neoplasm	
No	31 (68.9)
Yes	14 (31.1)
ND	*2*
^{18}F-FDG PET/CT	
Negative	3 (12.5)
Positive	21 (87.5)
ND	*23*
^{68} Ga-PET/CT Octreoscan	
Negative	11 (36.7)
Positive	19 (63.3)
ND	*17*
Best response to first-line therapy	
PD	7 (30.4)
SD	8 (34.8)
PR	8 (34.8)
ND	*3*

G grade, *NET* neuroendocrine tumor, *NEC* neuroendocrine carcinoma, *ND* not defined, ^{18}*F-FDG PET/CT* fluorodeoxyglucose F 18-positron emission tomography/computerized tomography, ^{68} *Ga* Gallium-68, *PD* progressive disease, *SD* stable disease, *PR* partial response

were negative for PD-L1 and 7 (20.6%) were positive. PD-L1 was not associated with any clinical characteristic (Supplementary Table 1).

Table 2 DLL3, RB1, and PDL-1 expression

DLL3, RB1, and PDL-1 expression *n* (%)					
DLL3		RB1		PDL-1	
Positive	10 (21.7)	Positive	34 (79.1)	Positive	8 (19.5)
Negative	36 (78.3)	Negative	9 (20.9)	Negative	33 (80.5)
ND	1	ND	4	ND	1
				NE	5

ND not defined, *NE* not evaluated

Table 3 Correlation of the expression of DLL3 with RB1 and PD-L1

	DLL3			*p value*
	Positive (%)	Negative (%)	Total (%)	
Overall	10 (21.7)	36 (78.3)	46*	
RB1**				
Negative	7 (70.0)	2 (6.3)	9	< 0.001
Positive	3 (30.0)	30 (93.7)	33	
PDL1***				
Negative	6 (85.7)	27 (79.4)	33	1.000
Positive	1 (14.3)	7 (20.6)	8	

*1 patient was not evaluable for DLL3

**4 patients were not evaluable for RB1

***6 patients were not evaluable for PDL1

Clinical Significance of DLL3 Expression

The correlation between DLL3 expression and patient clinical features is shown in Table 4. All DLL3-positive patients showed a high-grade tumor, while none of

Table 4 Correlation of DLL3 expression with clinical characteristics

	DLL3			*p value*
	Positive (%)	Negative (%)	Total (%)	
Overall	10 (23.9)	36 (76.1)	46	
Grading				
G1	0 (0.0)	21 (100.0)	21	< 0.001
G2	0 (0.0)	7 (100.0)	7	
G3 NET	0 (0.0)	5 (100.0)	5	
G3 NEC	10 (76.9)	3 (23.1)	13	
^{68} Ga-PET/CT				
Negative	8 (72.7)	3 (27.3)	11	0.001
Positive	2 (10.5)	17 (89.5)	19	
^{18}F-FDG PET/CT				
Negative	0 (0.0)	3 (100.0)	3	0.239
Positive	10 (47.6)	11 (52.4)	21	
Site of disease				
Stomach	2 (28.6)	5 (71.4)	7	0.895
Intestine	5 (21.7)	18 (78.3)	23	
Pancreas	3 (18.7)	13 (81.3)	16	
Best response				
PR	3 (37.5)	5 (62.5)	8	1.000
SD	3 (37.5)	5 (62.5)	8	
PD	3 (42.8)	4 (57.2)	7	
Presence of secondary disease				
Yes	3 (23.1)	10 (76.9)	13	1.000
No	7 (22.6)	24 (77.4)	31	

G grade, ^{68} *Ga PET/CT* Gallium-68-positron emission tomography/computerized tomography, ^{18}*F-FDG* F-18-fluorodeoxyglucose, *PD* progressive disease, *SD* stable disease, *PR* partial response

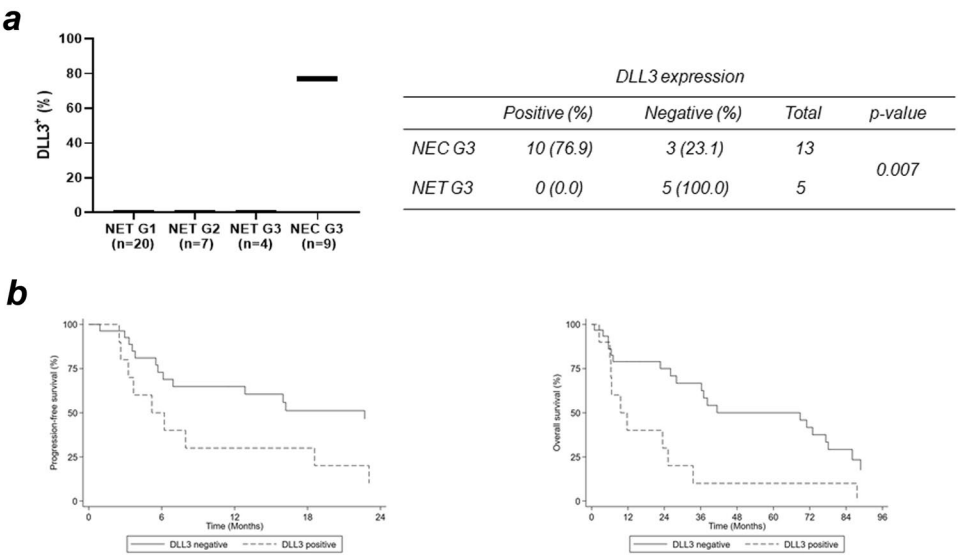
the patients with G1 or G2 GEP-NETs showed DLL3 expression. The DLL3 expression discriminated between poorly-differentiated G3 NECs and well-differentiated G3 NETs. About 76.9% of NEC patients (10/13) showed DLL3 positivity, while none of the 5 patients with G3 NETs were positive DLL3-positive ($p = 0.007$) (Fig. 2a). Within the NEC subgroup, 5 patients had large-cell NECs of whom all were positive for DLL3, while 7 had small-cell NECs of whom 4 were DLL3-positive (Supplementary Table 2). Conversely, RB1 expression did not show a statistical correlation with poorly-differentiated NECs (Table 5). DLL3 expression correlated with a negative ^{68} Ga-PET/CT. Within the 11 patients with a negative ^{68} Ga-PET/CT, 8 (72.7%) tumors showed DLL3-positive expression and 3 (27.3%) no expression. Within the 19 patients with a positive ^{68} Ga-PET/CT, 2 (10.5%) were DLL3-positive and 17 (89.5%) DLL3-negative ($p = 0.001$). No correlation was found between DLL3 expression and ^{18}F-FDG PET/CT. The 3 patients with negative ^{18}F-FDG PET/CT were negative for DLL3 expression, while of the 21 patients with positive ^{18}F-FDG PET/CT scan, 10 (47.6%) were DLL3-positive and 11 (52.4%) DLL3-negative. No correlation was found between DLL3 expression and site of disease. The site of origin of DLL3-expressing tumors was the stomach for 2 patients, intestine for 5 and pancreas for 3. No correlation was found between DLL3 expression and the best response to first-line therapy or the presence of second malignancies.

Prognostic and Predictive Value of DLL3 Expression

The median follow-up was 25.6 months (range 0.9–165). Median progression-free survival (PFS) was 16.2 months (95% CI 5.7–27.9). Median OS was 36.3 months (95% CI 22.7–72.9). No survival difference was observed according to gender or age (data not shown). Then, median PFS and OS analysis was performed for the different patients' groups according to DLL3 expression (Fig. 2b). Expression of DLL3 was negatively correlated with PFS and OS. mPFS was 22.7 months (95% CI 6.1–68.8) in the group with DLL3-negative tumors with respect to 5.2 months (95% CI 2.5–18.5) in the group with DLL3-positive disease ($p = 0.0083$). mOS was 68.8 months (95% CI 26.0–78.1) in the group with DLL3-negative tumors and 9.5 months (95% CI 2.5–25.2) in the DLL3-positive group ($p = 0.0071$).

Discussion

High-priority unmet needs in neuroendocrine neoplasia include a better molecular characterization of G3 GEP-NEN subgroups (NETs vs NECs) and the identification of molecular drivers of the disease that can be used as therapeutic targets [26, 27]. The negative Notch regulator DLL3 has aroused the interest of researchers for its potential as both a prognostic marker and candidate therapeutic target in neuroendocrine tumors, in particular, small-cell lung



DLL3 expression

	Positive (%)	Negative (%)	Total	p-value
NEC G3	10 (76.9)	3 (23.1)	13	0.007
NET G3	0 (0.0)	5 (100.0)	5	

	N° of pts	N° of events	mPFS (95% CI)	p-value
DLL3 neg	27	22	22.7 (6.1-68.8)	0.0083
DLL3 pos	10	10	5.2 (2.5-18.5)	

	N° of pts	N° of events	mOS (95% CI)	p-value
DLL3 neg	31	23	68.8 (26.0-78.1)	0.0071
DLL3 pos	7	7	9.5 (2.5-25.2)	

Fig. 2 **a** Percentages of tumors expressing DLL3 in G1, G2, and G3 NETs and G3 NECs. **b** Kaplan-Meier survival curves for progression-free survival (PFS) and overall survival (OS) of NEN patients ($n = 46$) according to DLL3 status

Table 5 Correlation of RB1 expression with histological classification

	RB1			*p value*
	Positive (%)	Negative (%)	Total	
Overall	10 (55.6)	8 (44.4)	18	
Histological classification				
G3 NEC	6 (46.2)	7 (53.8)	13	0.314
G3 NET	4 (80.0)	1 (20.0)	5	

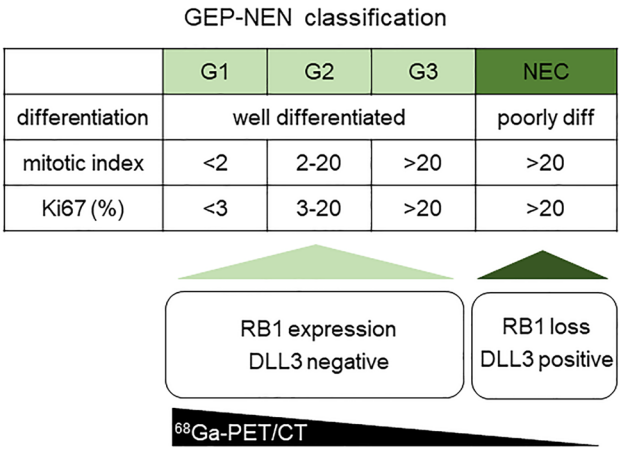


Fig. 3 Schematic representation of the current classification of GEP-NENs according to differentiation, mitotic index, and Ki-67 values, and of potential histological biomarkers to refine the differential diagnosis of NETs and NECs

cancer, LCNEC, and neuroendocrine prostate cancer [22, 24, 28–30]. In lung neuroendocrine neoplasms, DLL3 is highly expressed in a molecular subgroup of carcinoids with high dendritic cell infiltration, making it a plausible candidate for targeted therapeutic intervention [31]. In this study, for the first time, we demonstrated that DLL3 is expressed in GEP-NENs and shows clinical and prognostic significance. Our results revealed that DLL3 expression could distinguish poorly-differentiated NECs from well-differentiated tumors, thus increasing the arsenal of available diagnostic tools. None of the G1, G2, or G3 NET patients showed expression of this marker, whereas 76.9% of NEC patients had DLL3-positive disease. The identification of accurate biomarkers for the differential diagnosis of G3 NETs and NECs is urgently needed as the 2 subgroups have completely different clinical behavior and sensitivity to chemotherapy [6, 32]. However, the diagnostic process is currently based on the evaluation of subjective histopathological factors, such as proliferation rate and degree of differentiation, limiting the accuracy of prognosis prediction and treatment tailoring [32–34]. The mutation of *DAXX* and *ATRX* is frequently observed in well-differentiated pancreatic NETs, and it has a prognostic significance, especially in pancreatic NETs with alpha cell features [35–37]. Conversely, poorly differentiated NECs frequently harbor *RB1* mutations [31, 38]. DLL3 could thus represent an unbiased and well-defined histological marker to ensure an accurate identification of NECs (Fig. 3).

We also demonstrated that DLL3 was significantly correlated with the loss of RB1 expression. It has been found that a classic pathway driving the tumorigenesis of SCLC is the inactivation of Notch signaling within the context of bi-allelic mutation of *RB1* and *TP53* [39, 40]. Further studies are warranted to understand whether the DLL3/Notch pathway and loss of RB1 play a role as drivers of the development of GEP-NECs. The analysis of *TP53* expression could also shed light on this genetic landscape. The identification of molecular alterations responsible for NEC tumorigenesis could substantially increase our biological knowledge of this tumor. Moreover, we observed that DLL3 was correlated with negative ^{68} Ga-PET/CT scan. Previously, we demonstrated that ^{68} Ga-PET/CT scan in GEP-NEC patients treated with first-line platinum-based chemotherapy has a prognostic value; indeed, positive ^{68} Ga-PET/CT patients showed a better clinical outcome than those with a negative scan [13]. In the present study, DLL3 expression was correlated with an unfavorable prognosis. Patients with DLL3-positive tumors had a significantly shorter mPFS and mOS than those with DLL3-negative disease. The analysis of DLL3 and RB1 expression in tissue specimens, together with the results of ^{68} Ga-PET/CT imaging, could represent a diagnostic algorithm of risk factors associated with poorer clinical outcome. This could facilitate patient stratification and the definition of appropriate follow-up and treatment strategies given that GEP-NEC patients have a worse prognosis but more likely they could respond to chemotherapy [41]. Confirmation in larger case series, ideally in a prospective setting, is warranted.

The poor prognosis for the majority of patients diagnosed with NECs highlights the importance of having clinically useful information for treatment purposes [5]. Furthermore, the fact that there are still no clearly effective second-line chemotherapy strategies available for G3 NENs underlines the urgent need to identify novel treatment options to improve the current therapeutic landscape [33]. Along this line, the new antibody-drug conjugate SC16LD6.5 (rovalpituzumab tesirine) can efficiently target DLL3 protein on tumor cells [42]. Although rovalpituzumab has demonstrated modest clinical activity in phase II trials for SCLC [43], several other studies of DLL3-targeting strategies are currently ongoing, such as the use of chimeric antigen receptor (CAR) T cell therapy (AMG 119) [44]. Our finding that DLL3 was highly expressed

in NEC patients could potentially open up a new avenue of treatment for these orphan tumors. The combination of DLL3-targeting agents and immune therapy is also being explored in SCLC [44]. Immuno-oncology (IO) has modified the therapeutic approach in several cancers by positively affecting prognosis, especially in tumors characterized by poor outcome [45]. Great efforts have been made to guarantee the efficacy and safety of immune-oncology in patients with both well- and poorly differentiated neuroendocrine neoplasia [46]. PD-L1 expression, tumor mutational burden with neoantigen load, lymphocyte infiltration, and mismatch repair deficiency have been identified as predictive factors of response to IO [47–49]. A correlation between DLL3 expression and tumor immune profile was recently found in SCLC [22, 31], and aggressive poorly differentiated GEP-NENs have been shown to have moderate-to-strong PD-L1 membrane localization [50]. Based on this premise, we evaluated the correlation between PD-L1 and DLL3 expression, observing that PD-L1 was poorly expressed in neuroendocrine tumors (only 1/41 cases showed a > 50% percentage of positive tumor cells) and no clear correlation was observed between the status of this marker, DLL3 expression and clinical characteristics. Furthermore, no correlation was found between PD-L1 expression and histological tumor grading. Additional tumor inflammatory features [51] should probably be evaluated together with DLL3 status to better understand the applicability of treatment protocols involving immunotherapy and DLL3-targeting agents in GEP-NEC patients.

In conclusion, DLL3 is expressed in high-grade GEP-NECs and its expression is associated with RB1-loss, negative ^{68} Ga-PET/CT scan, and unfavorable clinical outcome. The expression of DLL3 can distinguish poorly differentiated NECs from NETs. DLL3 could thus prove useful as a prognostic factor to facilitate patient stratification and as a candidate therapeutic target in NEC patients to improve the management of this subset of tumors. Future research efforts should focus on understanding the connection between DLL3 expression and tumor immune status.

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Authors' Contributions CL, AB, and TI designed the study. CL, AB, LM, FP, CS, GM, GDM, SR, ADV, CC, GR, and FR collected and analyzed the clinical and biological data. FF carried out the statistical analyses. CL, AB, and TI interpreted the data. CL and AB drafted the manuscript. LM and TI revised the manuscript for important intellectual content. All authors approved the final version of manuscript for submission.

Data Availability All data is available upon reasonable request to the corresponding author

Compliance with Ethical Standards

Conflicts of Interest The authors declare that they have no conflict of interest.

Ethics Approval The study was reviewed and approved by IRST IRCCS Medical Scientific Committee and Ethics Committee. The study was carried out in accordance with the principles laid down in the 1964 Helsinki declaration.

Consent to Participate Informed consent was obtained from all participants in the study.

Authors' Disclosure The authors declare that preliminary data of this research were previously presented as poster presentation in the XXII AIOM 2020 National Congress and in the 17th Annual ENETS Conference 2020.

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