Am J Nucl Med Mol Imaging 2025;15(5):215-218
www.ajnmmi.us /ISSN:2160-8407/ajnmmi0166917

Editorial

DLL3-targeted immunoPET and radioimmunotherapy ligands

Steven H. Liang

Department of Radiology and Imaging Sciences, Emory University, Atlanta, Georgia 30322, The United States

Received June 18, 2025; Accepted June 21, 2025; Epub October 25, 2025; Published October 30, 2025

Abstract: DLL3 is overexpressed on the cell surface of NENs, such as SCLC and NEPC, but notably restricted to cytoplasm with low expression levels in normal adult human tissues. Several radioligands have been developed by targeting DLL3 for immunoPET or radioimmunotherapy use. These ligands hold great promise for mapping the heterogeneous DLL3 expression in neuroendocrine tumors and guiding the DLL3-directed therapeutic strategies.

Keywords: DLL3, immunopet, notch signaling, SCLC, in vivo imaging

Introduction

The Notch signaling pathway regulates critical cellular processes, including intercellular communication, proliferation, apoptosis, and differentiation, through interactions between Notch receptors (Notch1-4) and their ligands [1]. Among the five Notch ligands-Jagged1 (JAG1), JAG2, Delta-like ligand 1 (DLL1), DLL3, and DLL4-DLL3 is a non-canonical inhibitory ligand composed of 619 amino acids, featuring a Delta/Serrate/LAG-2 (DSL) domain, six epidermal growth factor (EGF)-like repeats, and a single-pass transmembrane domain (**Figure 1**) [1, 2]. Among these, the DSL domain, located in the extracellular *N*-terminal region, is highly conserved across this ligand family and functions as an essential binding interface for cognate Notch receptors. In normal tissues, DLL3 is expressed at low levels and primarily localized to the Golgi apparatus within the cytoplasm [3, 4]. Mechanistically, DLL3 inhibits Notch signaling by sequestering Notch receptors in endosomes for degradation, thereby preventing their cell-surface localization (**Figure 1**) [5]. In contrast, neuroendocrine neoplasms (NENs), particularly small-cell lung cancer (SCLC), exhibit aberrant DLL3 overexpression on the cell surface, driven by nuclear upregulation of achaete-scute complex homolog 1 (ASCL1, **Figure 1**). DLL3 exerts context-dependent pro- or anti-tumor effects, modulated by the specific Notch receptor activated and local Notch protein concentration. Due to their heterogeneity and limited treatment options, NENs pose significant diagnostic and therapeutic challenges. Consequently, DLL3 has emerged as a promising target for novel diagnostic and therapeutic approaches, particularly in SCLC where it is overexpressed in over 80% of patients. This overexpression correlates with tumor dedifferentiation, disease progression, and poor survival outcomes [3]. Furthermore, DLL3 enhances SCLC cell migration and invasion by modulating Notch1 signaling [5]. Given its aberrant overexpression on the cell surface of NENs, particularly in SCLC, and its minimal or absent expression on normal adult tissues, DLL3 represents an ideal target for tumor diagnosis and therapy.

DLL3 immunoPET and radioimmunotherapy ligands development

Positron emission tomography (PET) imaging has garnered growing attention as a non-invasive modality for cancer diagnosis, staging, and therapeutic response assessment [6-8]. Given the critical role of DLL3 in NENs, particularly SCLC, several DLL3-targeted therapies are under development, including antibody-drug conjugates (ADCs), chimeric antigen receptor (CAR) therapies, T-cell engager (TCE) molecules, and radioimmunotherapy ligands [9]. However, challenges persist in the efficacy and tolerability of these therapies, and there is a lack of standardized methods for assessing DLL3 expression in vivo. This highlights the urgent need for PET probes capable of visualizing DLL3 expression levels and heterogeneity in vivo. Initial efforts have produced two PET ligands, the antibody-based [^{89}Zr]Zr-DFO-SC16 [10] and smaller single-chain variable fragment-based [^{89}Zr]Zr-DFO-DLL3-scFv [11]. In H660 (DLL3-positive) xenografts models of neuroendocrine prostate cancer (NEPC), [^{89}Zr]Zr-DFO-SC16 demonstrated high tumor specificity, with uptake progressively increasing to 17.50 ± 3.19% injected dose per gram (% ID/g) at 120 hours post-injection (tumor-to-muscle ratio of 33.91), which was failed to be detected by [^{68}Ga]PSMA-11 (PSMA-targeting agent) and [^{68}Ga]DOTATATE (somatostatin receptor subtype 2-targeting agent). Meanwhile, the blood uptake was decreased slowly by 2-fold from 6.60 ± 0.90% ID/g at 24 h to 3.07 ± 0.45% ID/g at 120 h. As expected, low and stable uptake was observed in DLL3-negative DU145 xenografts models. These results highlighted the potential of [^{89}Zr]Zr-DFO-SC16 for detecting NEPC lesions, especially the ones lacking conventional biomarkers such as PSMA and somatostatin receptor subtype 2 [10]. Specificity was further confirmed by a significant signal reduction with unla-

https://doi.org/10.62347/MRZS8767

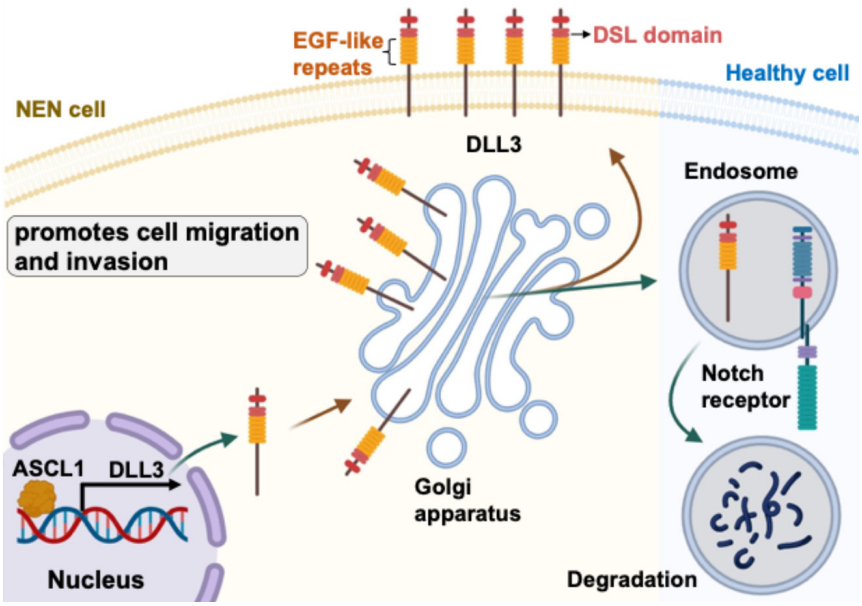

Figure 1. Notch inhibition pathway induced by DLL3. In healthy cells, DLL3 is predominantly localized in the Golgi apparatus, where it prevents Notch receptors from localization to the cell surface by redirecting them to degradation by endosome. In contrast, DLL3 has high expression on cell surface of NEN cells. ASCL1, achaete-scute complex homolog 1; DLL3, Delta-like ligand 3; DSL, Delta/Serrate/LAG-2; EGF, epidermal growth factor; NEN, neuroendocrine neoplasm.

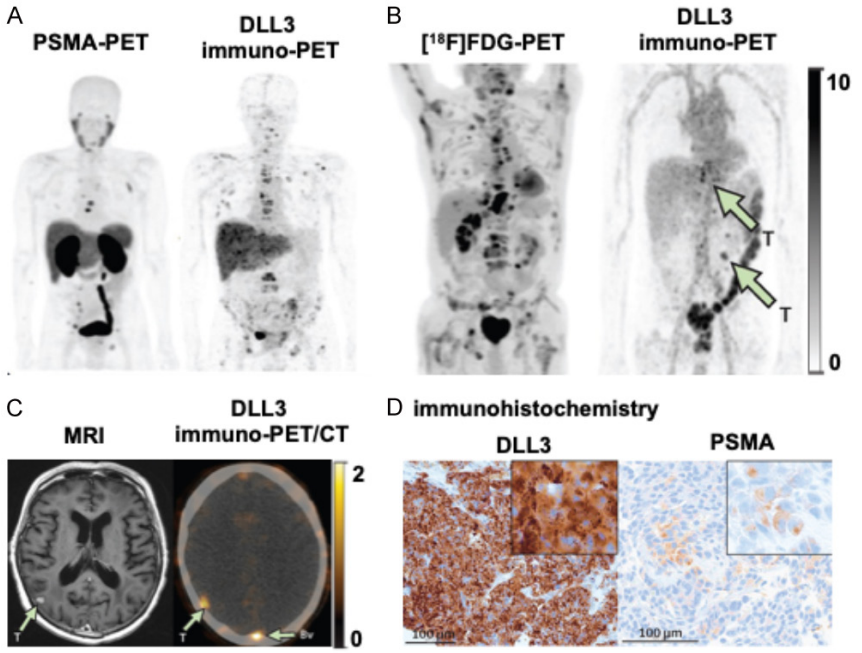

Figure 2. Tumor uptake of [^{89}Zr]Zr-DFO-SC16.56. DLL3 immunoPET showed more liver metastases than PSMA-PET in an NEPC patient (A), which was confirmed by immunohistochemistry results that exhibited high DLL3 expression but low of PSMA (D). Conversely, another NEPC patient had much less DLL3 immunoPET signals that of FDG-PET (B). Furthermore, in a patient with SCLC, a DLL3-avid brain metastasis was observed with DLL3 immunoPET, which corresponded to the MRI (C), with blood uptake of the tracer also observed in the posterior occipital fossa (Bv). DLL3, Delta-like ligand 3; NEPC, neuroendocrine prostate cancer; PSMA, prostate-specific membrane antigen; SCLC, small-cell lung cancer; T, tumor.

beled SC16 co-administration. Despite promising preclinical data, clinical validation of [^{89}Zr]Zr-DFO-SC16 remains pending. In contrast, [^{89}Zr]Zr-DFO-DLL3-scFv had rapid renal clearance with tumor uptake around 1.28-1.48% ID/g within 2-24 hours in mouse models of small-cell/neuroendocrine prostate cancer (SCNC) [11], suggesting faster pharmacokinetics but much lower tumor accumulation compared to full-length antibodies. Further clinical studies are necessary to assess its utility as a companion diagnostic for DLL3-targeted therapies, such as TCEs. Parallel advances in radioimmunotherapy have led to the development of [^{177}Lu]DTPA-SC16, a therapeutic agent derived from the SC16 antibody [12]. In H660 xenografts, it achieved high tumor uptake (reached 32.0 ± 4.7% ID/g at 120 h post-injection) and specificity, inducing complete tumor regression at doses of 9.25 and 27.75 MBq/mouse, demonstrating its therapeutic potential for NEPC. Similar to [^{89}Zr]Zr-DFO-SC16, [^{177}Lu]DTPA-SC16 displayed low uptake in DU145 xenografts models with tumor uptake of ~4.5% ID/g at 120 h. Recently, the DLL3-targeting macrocyclic peptide MC339, which has very high DLL3 affinity (K_D = 160 pM) and no binding to DLL1 or DLL4, was radiolabeled with ^{177}Lu and ^{225}Ac to give [^{177}Lu]MC339 and [^{225}Ac]MC339, respectively [13]. In DLL3-positive SHP-77 cells, [^{177}Lu]MC339 displayed high cell uptake (67%) and internalization (34%), while minimal uptake was observed in DLL3-negative CT26.WT cells, indicating its high specificity binding to DLL3. Furthermore, in SHP-77 xenograft model mice, [^{177}Lu]MC339 displayed rapid tumor accumulation at 2 h postinjection (12.4% IA/g, tumor/kidney = 2.8) and persistent high retention at 24 h (12.2% IA/g, tumor/kidney = 4.7). In addition, [^{225}Ac]MC339 could induce robust tumor regression and prolonged survival in SHP-77 model mice, highlighting its promise as an alpha-particle therapy.

Literature highlight: [^{89}Zr]Zr-DFO-SC16.56

[^{89}Zr]Zr-DFO-SC16.56, which incorporates the anti-DLL3 antibody SC16.56, represents the first clinical-stage DLL3 PET ligand evaluated in patients with NENs, including SCLC and NEPC [14]. The tracer exhibited strong tumor-specific uptake in DLL3-expressing malig-

nancies, including SCLC, NEPC, atypical carcinoid tumors, and non-small-cell lung cancer (NSCLC). This enabled visualization of metastatic lesions across multiple anatomical sites, including the lungs, lymph nodes, liver, skeletal system, adrenal glands, and brain, even in typically high-background tissues such as liver parenchyma, in approximately 80% of patients within 3-6 days post-administration (**Figure 2A** and **2C**). Meanwhile, the maximum standardized uptake value (SUV_{max}) in tumors showed significant variability both between and within patients (SUV_{max}: 3.3-66.7), and the tumor uptake was notably in accordance with DLL3 protein expression as confirmed by immunohistochemistry in 94% (15/16) of patients with evaluable tissue (**Figure 2D**). In contrast, low tumor uptake was observed in patients with low DLL3 expression, confirming its specificity. Notably, in one NEPC patient, a striking discordance was observed between [^{18}F]FDG-PET and DLL3-immunoPET: while multiple metastases were [^{18}F]FDG-avid, only five lesions exhibited DLL3 avidity (**Figure 2B**). In addition, pharmacokinetic analysis showed biphasic serum clearance with a terminal half-life of 119 h, and a primary hepatobiliary excretion pathway with minimal renal excretion. These findings highlight the potential of [^{89}Zr]Zr-DFO-SC16.56 as a non-invasive tool for *in vivo* detection of DLL3-expressing tumors and patient stratification for DLL3-targeted therapies. This first-in-human trial successfully established the safety and feasibility of DLL3 PET-CT imaging, which paves the way for clinical translation. Furthermore, these results are expected to increase interest in DLL3 as a therapeutic target and promote the development of companion imaging diagnostics for emerging DLL3-directed therapies. Furthermore, the nature of the radioimmunoconjugate suggests a potential radiotheranostic approach, where ^{89}Zr could be substituted with a therapeutic radionuclide, such as ^{177}Lu, for direct treatment of DLL3-expressing tumors, akin to successful PSMA-targeted therapies.

Conclusion

DLL3 has emerged as a promising target for both imaging and therapeutic applications in NENs. Targeted PET ligands show considerable potential for improving the diagnosis and monitoring of NEN patients, as well as for guiding the application of emerging DLL3-directed therapeutic strategies. Furthermore, DLL3-targeted radioimmunotherapy ligands present strong opportunities for treating DLL3-expressing tumors; however, further ligand optimization and clinical validation are essential to fully realize their therapeutic and diagnostic potential.

Disclosure of conflict of interest

None.

Address correspondence to: Steven H. Liang, Department of Radiology and Imaging Sciences, Emory University, No. 1364 Clifton Road, Atlanta, Georgia 30322, The United States. E-mail: steven.liang@emory.edu

References

[1] Leong KG and Karsan A. Recent insights into the role of Notch signaling in tumorigenesis. Blood 2006; 107: 2223-2233.

[2] Leonetti A, Facchinetti F, Minari R, Cortellini A, Rolfo CD, Giovannetti E and Tiseo M. Notch pathway in small-cell lung cancer: from preclinical evidence to therapeutic challenges. Cell Oncol 2019; 42: 261-273.

[3] Saunders LR, Bankovich AJ, Anderson WC, Aujay MA, Bheddah S, Black K, Desai R, Escarpe PA, Hampl J, Laysang A, Liu D, Lopez-Molina J, Milton M, Park A, Pysz MA, Shao H, Slingerland B, Torgov M, Williams SA, Foord O, Howard P, Jassem J, Badzio A, Czapiewski P, Harpole DH, Dowlati A, Massion PP, Travis WD, Pietanza MC, Poirier JT, Rudin CM, Stull RA and Dylla SJ. A DLL3-targeted antibody-drug conjugate eradicates high-grade pulmonary neuroendocrine tumor-initiating cells in vivo. Sci Transl Med 2015; 7: 302ra136.

[4] Sharma SK, Pourat J, Abdel-Atti D, Carlin SD, Piersigilli A, Bankovich AJ, Gardner EE, Hamdy O, Isse K, Bheddah S, Sandoval J, Cunanan KM, Johansen EB, Allaj V, Sisodiya V, Liu D, Zeglis BM, Rudin CM, Dylla SJ, Poirier JT and Lewis JS. Noninvasive interrogation of DLL3 expression in metastatic small cell lung cancer. Cancer Res 2017; 77: 3931-3941.

[5] Peddio A, Pietroluongo E, Lamia MR, Luciano A, Caltavituro A, Buonaiuto R, Pecoraro G, De Placido P, Palmieri G, Bianco R, Giuliano M and Servetto A. DLL3 as a potential diagnostic and therapeutic target in neuroendocrine neoplasms: a narrative review. Crit Rev Oncol Hematol 2024; 204: 104524.

[6] Rong J, Haider A, Jeppesen TE, Josephson L and Liang SH. Radiochemistry for positron emission tomography. Nat Commun 2023; 14: 3257.

[7] Deng X, Rong J, Wang L, Vasdev N, Zhang L, Josephson L and Liang SH. Chemistry for positron emission tomography: recent advances in ^{11}C-, ^{18}F-, ^{13}N-, and ^{15}O-Labeling Reactions. Angew Chem Int Ed Engl 2019; 58: 2580-2605.

[8] Krishnan HS, Ma L, Vasdev N and Liang SH. 18F-Labeling of sensitive biomolecules for positron emission tomography. Chemistry 2017; 23: 15553-15577.

[9] Rudin CM, Reck M, Johnson ML, Blackhall F, Hann CL, Yang JC, Bailis JM, Bebb G, Goldrick A, Umejiego J and Paz-Ares L. Emerging therapies targeting the delta-like ligand 3 (DLL3) in small cell lung cancer. J Hematol Oncol 2023; 16: 66.

[10] Korsen JA, Kalidindi TM, Khitrov S, Samuels ZV, Chakraborty G, Gutierrez JA, Poirier JT, Rudin CM, Chen Y, Morris MJ, Pillarsetty N and Lewis JS. Molecular imaging of neuroendocrine prostate cancer by targeting Delta-Like Ligand 3. J Nucl Med 2022; 63: 1401-1407.

[11] Chou J, Egusa EA, Wang S, Badura ML, Lee F, Bidkar AP, Zhu J, Shenoy T, Trepka K, Robinson TM, Steri V, Huang J, Wang Y, Small EJ, Chan E, Stohr BA, Ashworth A, Delafontaine B, Rottey S, Cooke KS, Hashemi Sadraei N, Yu B, Salvati M, Bailis JM, Feng FY, Flavell RR and Aggarwal R. Immunotherapeutic targeting and PET imaging of DLL3

in small-cell neuroendocrine prostate cancer. Cancer Res 2023; 83: 301-315.
[12] Korsen JA, Gutierrez JA, Tully KM, Carter LM, Samuels ZV, Khitrov S, Poirier JT, Rudin CM, Chen Y, Morris MJ, Bodei L, Pillarsetty N and Lewis JS. Delta-like ligand 3-targeted radioimmunotherapy for neuroendocrine prostate cancer. Proc Natl Acad Sci U S A 2022; 119: e2203820119.
[13] Savinainen A, Wu L, Bruton T, Chonkar A, Cupido J, Dearling J, Huang C, Lee K, Ma Z, Mok H, Nabi K, Salih A, Sutton A, Tavera L, Wan M, Wang Z, Xu X, Xu J, Zidell A and Ricardo A. 1153P Preclinical characterization of MC339: a novel radiotherapeutic agent for DLL3 expressing cancers. Ann Oncol 2024; 35: S755.
[14] Tendler S, Dunphy MP, Agee M, O'Donoghue J, Aly RG, Choudhury NJ, Kesner A, Kirov A, Mauguen A, Baine MK, Schoder H, Weber WA, Rekhtman N, Lyashchenko SK, Bodei L, Morris MJ, Lewis JS, Rudin CM and Poirier JT. Imaging with [^{89}Zr]Zr-DFO-SC16.56 anti-DLL3 antibody in patients with high-grade neuroendocrine tumours of the lung and prostate: a phase 1/2, first-in-human trial. Lancet Oncol 2024; 25: 1015-1024.