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Prospective phase II trial of [^{68}Ga]Ga-NODAGA-E[c(RGDyK)]$_2$ PET/CT imaging of integrin $\alpha_v\beta_3$ for prognostication in patients with neuroendocrine neoplasms

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Running title

Integrin $\alpha_v\beta_3$ PET in NEN

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ABSTRACT

Integrin $\alpha_v\beta_3$, a subtype of the arginine-glycine-aspartate (RGD) recognizing cell surface integrins, is upregulated on endothelial cells during angiogenesis and on tumor cells. Due to involvement in tumor growth, invasiveness/metastases, and angiogenesis, integrin $\alpha_v\beta_3$ is an attractive target in cancers. In this study we applied ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$ for imaging of integrin $\alpha_v\beta_3$ in patients with neuroendocrine neoplasms (NEN) and its potential use for prognostication. We hypothesized that ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$ PET/CT would show tumor lesion uptake and that higher tumor lesion uptake was associated with a poorer prognosis.

Methods

Between December 2017-November 2020 we prospectively enrolled 113 patients with NEN of all grades (2019 World Health Organization classification) for ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$ PET/CT. The scan was acquired 45 minutes after injection of 200 MBq of ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$. Board certified specialists in nuclear medicine and radiology analyzed the PET/CT measuring SUV_{max} in tumor lesions. Positive tumor lesions were defined as those with tumor-to-liver background ≥ 2. Maximal tumor SUV_{max} for each patient was used as predictor of outcome. Patients were followed for at least one year to assess progression-free survival (PFS) and overall survival (OS).

Results

Of 113 patients enrolled in the trial, 99 had a ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$ PET/CT performed, hereof 97 patients having evaluable lesions. The patients predominantly had small intestinal (64%) or pancreatic (20%) NEN and most had metastatic disease (93%). The majority of patients had low-grade tumors (78%), while (22%) had high-grade tumors. During follow-up of median 31 months (interquartile range: 26-38), 62 patients (64%) experienced disease progression and 26 (27%) patients died. In total, 76% of patients had positive tumor lesions, and of patients with high-grade tumors, 91% had positive tumor lesions. High integrin $\alpha_v\beta_3$ expression, defined as SUV_{max} at least 5.25 had a hazard ratio (95% confidence interval) of 2.11 (1.18-3.78) and 6.95 (1.64-29.51) for PFS and OS, respectively (p = 0.01 for both).

Conclusion

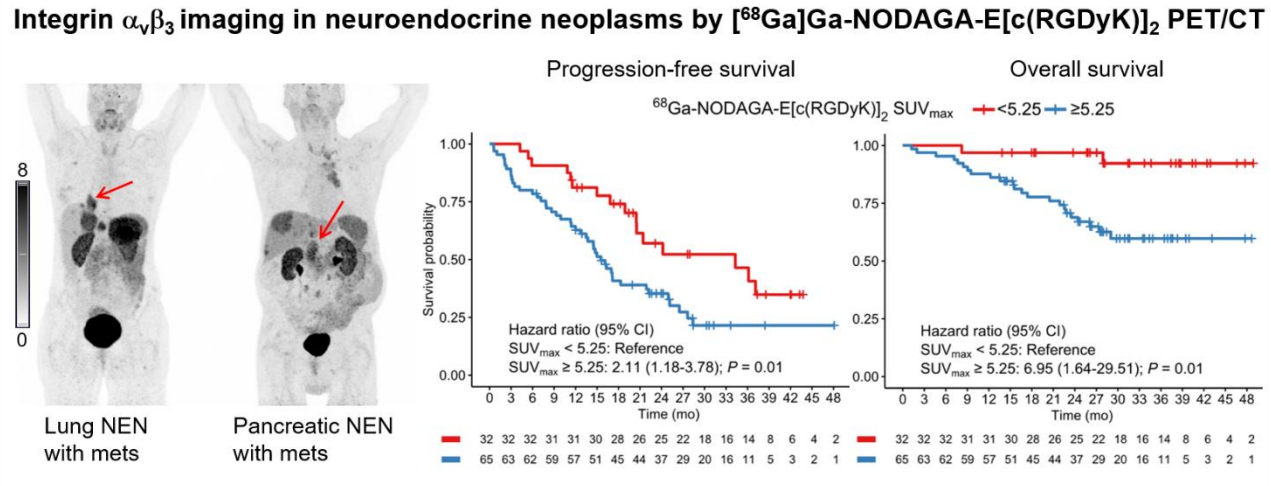
Tumor lesion uptake of ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$ was evident in patients with all grades of NEN. High uptake was associated with a poorer prognosis. Further studies are warranted to

establish if ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$ PET/CT may become a prediction tool for identification of patients eligible for treatments targeting integrin $\alpha_v\beta_3$.

KEYWORDS

Integrin alphavbeta3; neuroendocrine neoplasms; PET; prognosis; molecular imaging

GRAPHICAL ABSTRACT



INTRODUCTION

Neuroendocrine neoplasms (NEN) represent a heterogeneous group of tumors originating from the neuroendocrine cells. NEN are primarily found in the gastro-intestinal tract, pancreas, and lungs. Patients with NEN are often diagnosed when the disease has metastasized, yet the clinical course for these patients varies greatly. Origin of primary tumor, presence of metastases as well as tumor morphology and proliferation activity (i.e. Ki67) are known prognostic factors (*1*). The 2019 World Health Organization (WHO) classification stratifies NEN into neuroendocrine tumor (NET) G1 (Ki67 <3%), NET G2 (Ki67 3-20%), NET G3 (Ki67 > 20% and well-differentiated) and neuroendocrine carcinoma (NEC) (Ki67 > 20% and poorly differentiated) (*2*). Furthermore, imaging modalities aids in diagnosing, staging, treatment selection as well as follow-up for patients with NEN. In particular, PET radiotracers reflecting somatostatin receptor expression (e.g. ^{64}Cu-DOTATATE or ^{68}Ga-DOTATATE) and glucose metabolism (^{18}F-FDG) are used for these purposes in addition to providing prognostic information (*3,4*). Finally, targeting the somatostatin receptors with peptide receptor radionuclide therapy (PRRT), e.g. ^{177}Lu-DOTATATE, has been approved for patients with NEN.

Additional tumor markers may be useful for further improvement in prognostication and ultimately identifying novel treatment targets in patients with NEN. Cell-surface adhesion receptors of the integrin superfamily have been extensively investigated due to their role in physiological as well as in pathophysiological processes, and especially in cancers (*5*). The subfamily of Arginine - Glycine - Aspartate (RGD) recognizing integrins have implications on several of hallmarks of cancer; tumor growth, invasiveness and metastases and angiogenesis. Integrin $\alpha_v\beta_3$ is significantly up-regulated on activated endothelial cells during angiogenesis, but absent on quiescent endothelial cells, as well as overexpressed on tumor cells in several cancers (*6*). NEN are generally characterized as highly vascularized tumors with overexpression of various pro-angiogenetic factors such as vascular endothelial growth factor (*7*). Previously we found, using quantitative gene expression, that the expression of integrin $\alpha_v\beta_3$ shows high variability between NEN (*8*). Due to its integral role in cancer, our group therefore developed and clinically translated the PET radiotracer ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$ targeting integrin $\alpha_v\beta_3$ with high affinity (*9,10*).

The aim of this phase II clinical trial of ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$ PET/CT in patients with NEN of all grades was to further assess tumor uptake and prognostic value. We hypothesized that PET/CT with ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$ would show accumulation in tumor lesions in patients with NEN of all grades and that the uptake of the radiotracer would be associated with progression-free survival (PFS) and overall survival (OS).

METHODS

Patients

Patients with histologically confirmed NEN were included from the Dept. of Endocrinology (managing low-grade NEN, Ki67 ≤20%) and Dept. of Oncology (managing high-grade NEN, Ki67 >20%), Copenhagen University Hospital – Rigshospitalet, Denmark between December 4th 2017 – November 26th 2020. Rigshospitalet is a Neuroendocrine Tumor Center of Excellence accredited by the European Neuroendocrine Tumor Society. The study was conducted in accordance with the Helsinki Declaration and Good Clinical Practice. The study was approved by the Danish Medicines Agency (EudraCT 2017-002512-14), Scientific Ethics Committee (H-17019542) and the Danish Data Protection Agency (2012-58-0004), and registered on clinicaltrials.gov (NCT03271281).

Eligible patients were aged above 18 years, capable of reading and understanding the patient information in Danish and giving informed consent, diagnosed with gastro-entero-pancreatic NEN of all grades or broncho-pulmonary NEN, and have a WHO performance status of 0-2. Patients were excluded if pregnant/breast-feeding, had a body mass > 140 kg, a history of allergic reaction attributable to compounds of similar chemical or biologic composition to ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$ or in case of broncho-pulmonary NEN if the subtype was small cell lung cancer. After obtaining written informed consent, the patients were referred to a ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$ PET/CT at first given opportunity.

Image acquisition

Data acquisition was performed using a Biograph 128 mCT PET/CT (Siemens Medical Solutions, Erlangen, Germany) with an axial field of view of 216 mm. Based on the previous phase I trial, the scans were acquired 45 minutes after intravenous administration of approximately 200 MBq of ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$ ([^{68}Ga]Ga-NODAGA-Glu[cyclo(-Arg-Gly-Asp-D-Tyr-Lys-)]$_2$) equaling 4.4 mSv. Radiotracer production was performed as

previously described (*9*). Whole-body PET scans (mid of orbita to mid of thigh) were acquired with an acquisition time of 4 min per bed position. Attenuation- and scatter- corrected PET data were reconstructed iteratively using a 3D ordinary Poisson ordered-subset expectation-maximization algorithm including point-spread function and time-of-flight information using the TrueX algorithm (Siemens Medical Solutions); the settings were 2 iterations, 21 subsets, 2-mm Gaussian filter. A diagnostic CT scan was obtained before the PET scan with a 2-mm slice thickness, 120 kV, and a quality reference of 225 mAs modulated by the Care Dose 4D automatic exposure control system (Siemens Medical Solutions). An automatic injection system was used to administer 75 mL of an iodine containing contrast agent (Optiray 300; Covidien) for arterial and venous phase CT.

Patients were observed for adverse events after injection of ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$, and following discharge, patients were asked to record any adverse events occurring within the first 24 hours of injection. Adverse events were categorized according to Common Terminology Criteria for Adverse Events v. 5.0.

Image analysis

An experienced board-certified nuclear medicine physician together with an experienced board-certified radiologist analyzed side-by-side the ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$ PET/CT scans. The readers had access to previous imaging studies, but were blinded to other patient data and follow-up data. Lesions were identified on CT and/or PET. SUV was calculated as decay-corrected measured radioactivity concentration/(injected activity/body weight). If more than one lesion was present in an organ, the lesion with the highest SUV_{max} was noted. If no lesions were identified on PET, but lesions were visible on CT, the largest lesion (based on viable tissue within the lesion) on CT was used as guide for lesion delineation on the PET scan from which SUV_{max} was determined. As background reference of uptake in tumor lesions, the radiotracer uptake in normal liver tissue was measured as SUV_{mean}. A tumor lesion to liver ratio (TLR) was calculated as tumor lesion SUV_{max}/normal liver SUV_{mean}. A cutoff of TLR ≥ 2 was used to define "positive" lesions.

Follow-up

The patients were followed at the Rigshospitalet Neuroendocrine Tumor Center of Excellence with regular visits including clinical examination, blood samples, and imaging (CT, magnetic resonance, ultrasound and/or PET/CT). The frequency was in accordance with European

Neuroendocrine Tumor Society guidelines, typically every 3-6 months (*11*). The ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$ PET/CT was not made available for the treating physicians and thus not used to guide clinical decisions regarding treatment or follow-up. End of follow-up was December 31st 2021 for the current study. Routine CT and or magnetic resonance imaging were used for evaluation of PFS in accordance with Response Evaluation Criteria in Solid Tumors v. 1.1 (*12*). PFS was defined as time from ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$ PET/CT to, if any, progression or death from any cause. If no progression or death from any cause occurred within the follow-up, the patient was censored at the time of last available diagnostic imaging. OS was defined as time from ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$ PET/CT to death by any cause. As all death but two were directly related to NEN, we refrained from analyzing disease specific survival. Patients alive at follow-up were censored to the day of end of follow-up, i.e. December 31st 2021.

Statistics

Continuous variables are reported as mean ± standard deviation (SD) or median with range unless otherwise noted. Kaplan Meier analyses were used for estimation of time to outcome (PFS and OS) and using reverse Kaplan Meier analysis to estimate median follow-up time. Univariate and multivariate Cox regression analyses for OS and PFS with predictor variables being SUV_{max} and WHO grade were performed to determine hazard ratio (HR) and 95% confidence interval (CI). We used the Cutoff Finder application to determine the optimal cutoff for SUV_{max} (*13*). A p-value <0.05 was considered statistically significant. R version 3.6.0 (R Foundation for Statistical Computing, Vienna, Austria) was used for the analyses.

RESULTS

Patients and image acquisition

We prospectively included 113 patients, whereof 14 patients did not undergo ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$ PET/CT due to worsening of disease (n=4), withdrawal of consent (n=2), died before PET/CT (n=3), logistically not possible to perform PET/CT (n=2), and not possible to perform PET/CT due to COVID-19 restrictions (n=3). Of the 99 patients scanned with PET/CT, 97 patients had evaluable lesions. The patients predominately had small intestinal (n=62, 64%) or pancreatic (n=19, 20%) NEN and metastatic disease (n=90, 93%), see Table 1. Most

patients had low-grade tumors (Ki67≤20%) (n=76, 78%), while 21 (22%) had high-grade tumors (Ki67 >20%). No patients were treatment naïve before the PET/CT scan.

Patients undergoing ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$ PET/CT (n=99) received a median (range) mass dose of 18.9 (7.7-49.3) µg of ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$ and the activity dose was 193 (104-226) MBq. Median time from injection to PET scan start was 47 minutes (range: 35-86). Three patients experienced an adverse event - dizziness (grade 1), fall (grade 1), infusion related reaction in relation to injection of CT contrast (grade 2) within 24 hours of injection of ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$. All were deemed unrelated to ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$ injection. No grade 3-5 adverse events occurred.

Image analysis

The median maximal tumor lesion SUV_{max} was 6.1 (range: 1.4-14.1). The mean ± SD of tumor lesion SUV_{max} was 6.36±2.49 and the mean ± SD normal liver SUV_{mean} was 2.41±0.55. Examples of ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$ PET/CT are shown in Figure 1-3. By applying the cutoff of TLR ≥ 2 to determine "positive" lesions approximately two thirds of patients with NET G1 had positive lesions and gradually increasing to nearly all patients with NET G3/NEC (91%) having positive lesions (Table 2). In total, 76% of patients had positive tumor lesions.

Follow-up

During follow-up of median 31 months (interquartile range: 26-38), 62 patients (64%) experienced disease progression and 26 (27%) patients died. Overall median PFS was 18.9 months (15.5-25.1). No patients were lost to follow-up. The patients' treatments after ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$ PET/CT are given in Table 3. Treatment with somatostatin analogue was the most frequent (80%, 78/97), and 32% (31/97) of all patients underwent PRRT during the follow-up period.

Progression-free survival and overall survival

In univariate analyses, the maximal tumor SUV_{max} as a continuous variable was significantly associated with PFS and OS with a HR (95% CI) of 1.17 (1.07-1.28), p<0.001 and 1.19 (1.03-1.38), p =0.02 per one unit increase, respectively. High integrin $\alpha_v\beta_3$ expression, defined as maximal tumor SUV_{max} above median (SUV_{max} 6.10) had a HR (95% CI) of 1.96 (1.17-3.29) and 2.66 (1.14-6.16) for PFS and OS, respectively (p<0.05 for both), see Figure 4 and Tables 4 and 5. Optimal cutoffs for dichotomizing maximal tumor SUV_{max} were assessed by Cutoff Finder for either PFS or OS as outcome (Supplemental Figure S1). By using a lower cutoff of SUV_{max}

(5.25) a smaller group of patients (n=32) with a very low risk of death could be identified, Figure 5 and Tables 6 and 7. Patients with SUV_{max} above 5.25 had a HR (95% CI) of 2.11 (1.18-3.78) and 6.95 (1.64-29.51) for PFS and OS, respectively (p = 0.01 for both). With the cutoff of 5.25, median OS was not reached in neither groups with low nor high SUV_{max} and median PFS was 34.3 months (20.5; upper limit not reached) for patients with low SUV_{max} vs. 15.5 months (13.5-22.2) for patients with high SUV_{max}. Furthermore, by using a higher cutoff of SUV_{max} of 7.45, the dichotomization was optimized for prediction of disease progression with a HR (95% CI) of 2.57 (1.52-4.34), p<0.001 (Supplemental Figure 2 and Supplemental Tables 1 and 2). Patients with NET G3 and NEC had significantly worse PFS and OS as compared to patients with NET G1, whereas no difference was seen between NET G2 and NET G1 (Tables 4 and 5). In multivariate analyses including SUV_{max} and WHO classification (NET G3 and NEC vs NET G1), both remained significantly associated with PFS and OS, see Table 4-7.

DISCUSSION

The major finding of our phase II prospective study of ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$ PET/CT for integrin $\alpha_v\beta_3$ imaging in patients with NEN was that integrin $\alpha_v\beta_3$ expression was seen in both low and high-grade NEN. Furthermore, we found a significant association between radiotracer uptake and both PFS and OS. When dichotomized at SUV_{max} 5.25, patients with higher radiotracer uptake in tumor lesion had a 2-fold higher risk of progressive disease and a 7-fold higher risk of death. This highlights integrin $\alpha_v\beta_3$ as an important prognostic marker in patients with NEN.

A great number of radiotracers using the RGD motif have been tested preclinically with only some being further translated into clinical trials (*14*). In our phase I trial on ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$ PET/CT imaging we included patients with NEN or breast cancer and demonstrated that administration of the radiotracer was safe, had low radiation burden and high tumor lesion uptake (*9*). Beside our phase I trial, no specific PET imaging studies with a RGD-based radiotracer in patients with NEN has so far been conducted, although combined integrin $\alpha_v\beta_3$ and somatostatin receptor targeting has been examined with ^{68}Ga-NOTA-3P-TATE-RGD PET/CT (*15*). To the best of our knowledge, the current study is the largest to be conducted with an RGD-based PET radiotracer. Other clinical trials have used RGD-based radiotracers to examine patients with e.g. breast cancer and head and neck cancers, as

well as several other non-oncological applications, e.g. atherosclerosis and rheumatoid arthritis (*16-19*).

Integrin $\alpha_v\beta_3$ is a cell-surface adhesion receptor and a member of the integrin superfamily. The subfamily of integrins recognized by RGD also includes $\alpha_v\beta_1$, $\alpha_v\beta_5$, $\alpha_v\beta_6$, $\alpha_v\beta_8$, $\alpha_5\beta_1$, $\alpha_8\beta_1$ and $\alpha_{IIb}\beta_3$ (*20*). Integrins are involved in several physiologic and pathophysiological pathways, e.g. embryogenesis, wound healing, angiogenesis as well as tumor growth, invasion/metastasis, and angiogenesis related to cancer. The natural ligands of integrin $\alpha_v\beta_3$ are extracellular matrix proteins such as fibronectin and vitronectin. Additionally, integrins interact with several other factors also involved in angiogenesis and invasive growth, e.g. vascular endothelial growth factor and urokinase plasminogen activator receptor (*5,21*). An indication that integrin expression is involved in promoting the metastatic process in patients with NEN is supported by gene expression analysis in patients with pulmonary NEN. Upregulation of fibrogenic genes, including ITGAV (the gene encoding integrin α_v), was related to poor differentiation and increased risk of metastases (*22*). However, conflicting data in regards to the relation between poorer prognosis and integrin $\alpha_v\beta_3$ expression has been reported for immunohistochemical staining of gastric cancer (*23*) and non-small cell lung cancer (*24*).

Spurred on by the upregulation of $\alpha_v\beta_3$ during angiogenesis, early-phase clinical trials with the $\alpha_v\beta_3/\alpha_v\beta_5$ targeting ligand cilengitide were performed showing modest effect on tumor growth (*25,26*). However, later phase II/phase III trials failed to meet expectations due to an unintended pro-angiogenic effect at lower concentrations, while anti-angiogenetic effect was seen only at higher concentration (*27*). Recently, new promising pure $\alpha_v\beta_3$ ligands (TDI-4161 and TDI-3761) have been shown to circumvent the pro-angiogenetic effect previously seen with cilengitide (*28*), hence reinforcing the need for development of methods as companion diagnostics to assess *in vivo* the level of integrin $\alpha_v\beta_3$ expression for selection of patients for such targeted therapies.

Another possible avenue for integrin $\alpha_v\beta_3$ targeted treatments is peptide receptor radionuclide therapy (PRRT). In patients with NEN, PRRT with ^{177}Lu-DOTATATE, exploiting somatostatin receptor overexpression, has become an integrated part of treatment of patients with NEN (*29,30*). In two preclinical studies, the potential of extending the use of the RGD sequence by coupling it with a radionuclide for therapy has been examined. One

combined $\alpha_v\beta_3$-targeting PRRT with immune checkpoint inhibitor programmed death ligand 1 (*31*) and the other $\alpha_v\beta_3$-targeting PRRT with temozolomide (*32*). Both demonstrated additional effect of the combined therapy. Further studies of coupling imaging and PRRT in the setting of integrin $\alpha_v\beta_3$ are needed. Concerns over physiological uptake reported in RGD-based imaging have been raised in regards to PRRT (*20*). However, compared with dosimetry data from somatostatin based PET radiotracers ^{68}Ga-DOTATATE and ^{68}Ga-DOTATOC (*33*), we found similar kidney, liver, spleen, and intestinal absorbed doses in our phase I trial study assessing the dosimetry of ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$ (*9*). Finally, a potential advantage of $\alpha_v\beta_3$-targeting PRRT over somatostatin receptor-targeting PRRT in NEN is the fact that in particular in high grade tumors, somatostatin receptor expression is low or absent and therefore somatostatin receptor PRRT cannot be used. In contrast, we found a high uptake of ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$ also in high grade tumors (91% of patients with NET G3/NEC).

In our study, included patients predominately had small intestinal or pancreatic primary tumors and nearly all had metastatic disease with liver involvement. Hence use of ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$ PET/CT in other settings, e.g. for assessment of newly diagnosed patients with localized disease remains to be elucidated.

CONCLUSION

Tumor uptake of ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$ was evident in both patients with low and high-grade NEN, although more pronounced with increasing WHO grade. High tumor uptake of ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$ was associated with a poorer prognosis in patients with NEN with a 2-fold higher risk of progression and 7-fold higher risk of death. Further studies are warranted to establish if ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$ PET/CT may become a tool for risk stratification and for identification of patients eligible for treatments targeting integrin $\alpha_v\beta_3$.

DISCLOSURE

This project received funding from the European Union's Horizon 2020 research and innovation programme under grant agreements no. 670261 (ERC Advanced Grant) and 668532 (Click-It), the Lundbeck Foundation, the Novo Nordisk Foundation, the Innovation Fund Denmark, IPSEN Nordic, the Danish Cancer Society, Arvid Nilsson Foundation, the Neye Foundation, Novartis Healthcare, the Research Foundation of Rigshospitalet, the Danish National Research Foundation (grant 126), the Research Council of the Capital Region of

Denmark, the Danish Health Authority, the John and Birthe Meyer Foundation, the Research Council for Independent Research and the Neuroendocrine Tumor Research Foundation. Andreas Kjaer is a Lundbeck Foundation Professor.

Andreas Kjaer is inventor/holds intellectual property rights on a patent application: "68Ga- and 64Cu -NODAGA-E[c(RGDyK)]2 for use as pet tracers in the imaging of angiogenesis in humans" (WO2019091534A1). No other potential conflicts of interest relevant to this article exist.

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KEY POINTS

Question

Is integrin $\alpha_v\beta_3$ expression assessed by PET evident in tumor lesions of patients with neuroendocrine neoplasms and associated with prognosis?

Pertinent Findings

Using ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$ for integrin $\alpha_v\beta_3$ PET imaging, integrin $\alpha_v\beta_3$ was evident in tumor lesions from both patients with low and high-grade tumors. High tumor uptake of ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$ was associated with a poorer prognosis in regards to both disease progression and death.

Implications for Patient Care

Integrin $\alpha_v\beta_3$ is a prognostic marker and a potential treatment target in patients with neuroendocrine neoplasms. ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$ PET/CT may become a tool for risk stratification and for identification of patients eligible for treatments targeting integrin $\alpha_v\beta_3$.

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FIGURES

Figure 1: Example of ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$ PET/CT. Transaxial PET and fused PET/CT and maximum intensity projection with color bars (unit: SUV). Patient with lung neuroendocrine tumor grade 2 (Ki67 15 %) with liver and bone metastases. Arrow at primary tumor.

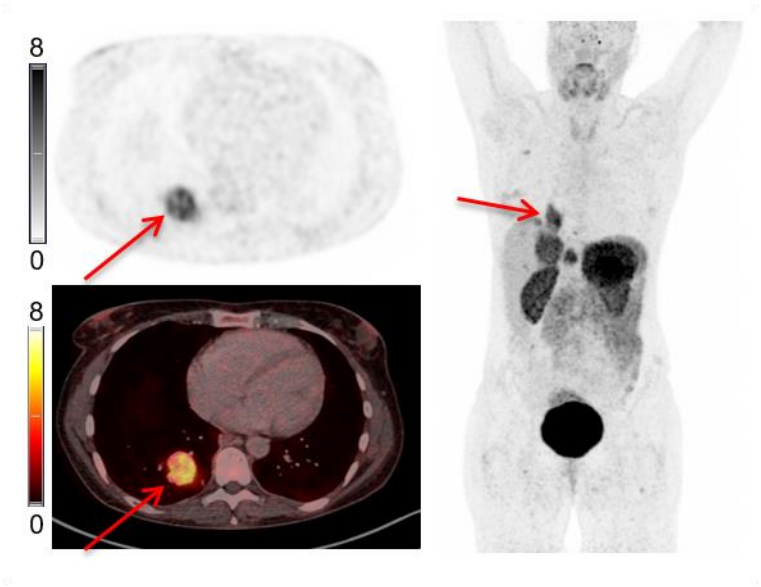


Figure 2: Example of ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$ PET/CT. Transaxial PET and fused PET/CT and maximum intensity projection with color bars (unit: SUV). Patient with gastric neuroendocrine tumor grade 2 (Ki67 8 %) with liver metastases. Arrow at liver metastasis.

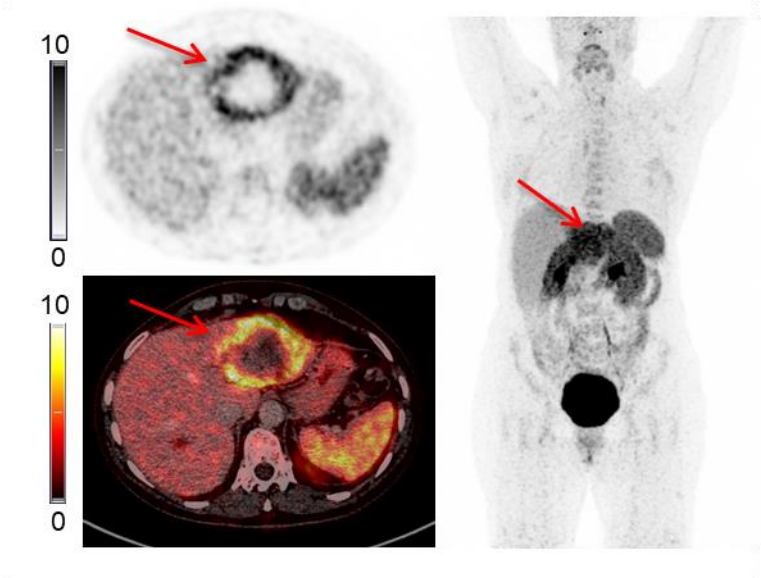


Figure 3: Example of ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$ PET/CT. Transaxial PET and fused PET/CT and maximum intensity projection with color bars (unit: SUV). Patient with pancreatic neuroendocrine tumor grade 2 (Ki67 11 %) with liver and lymph node metastases. Arrow at primary tumor.

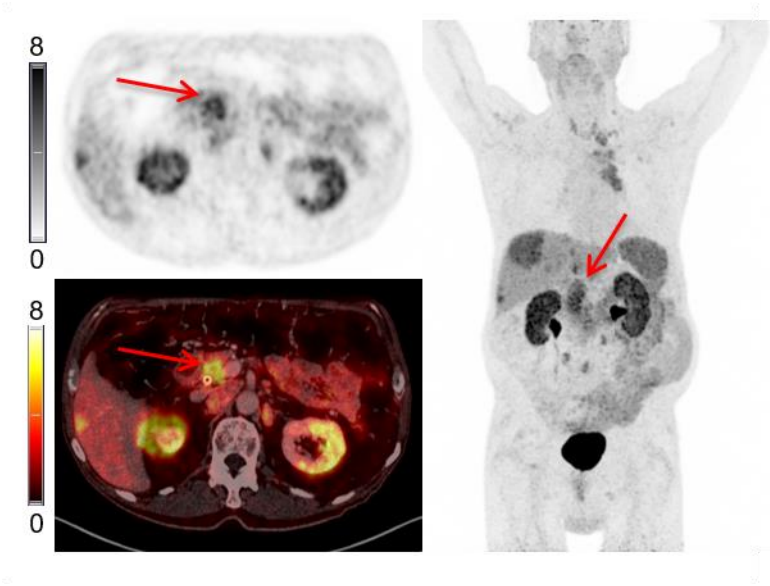


Figure 4. Kaplan Meir plots of ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$ PET SUV$_{max}$ dichotomized at 6.10 (median) for prediction of progression-free survival and overall survival.

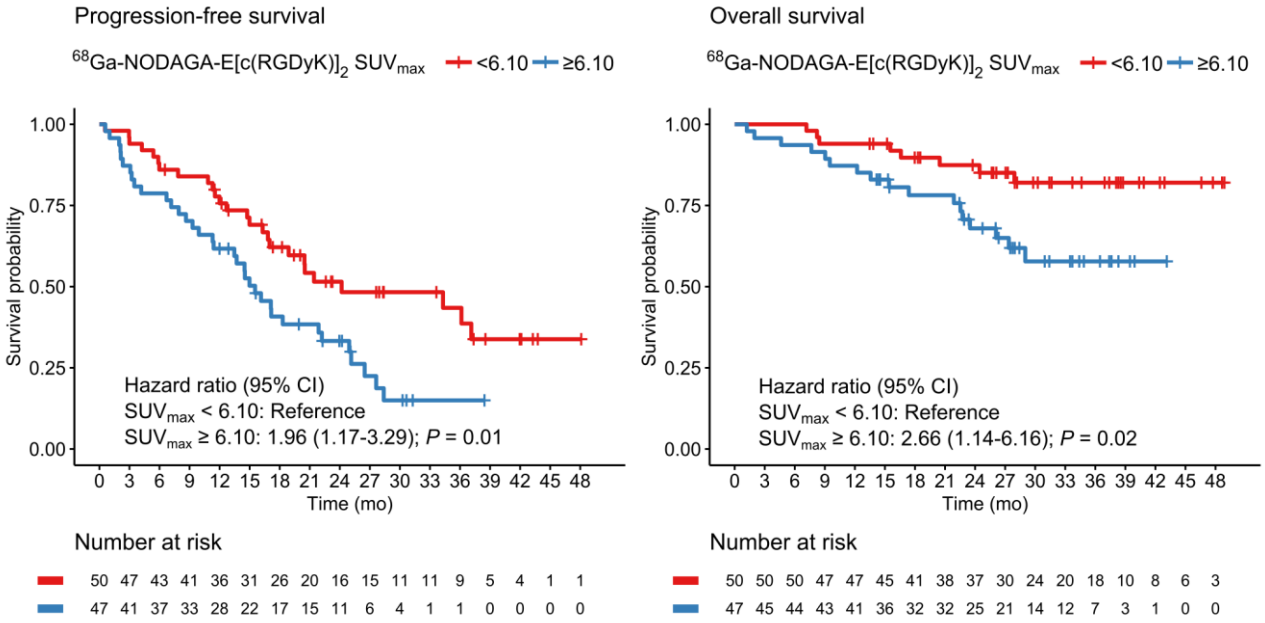
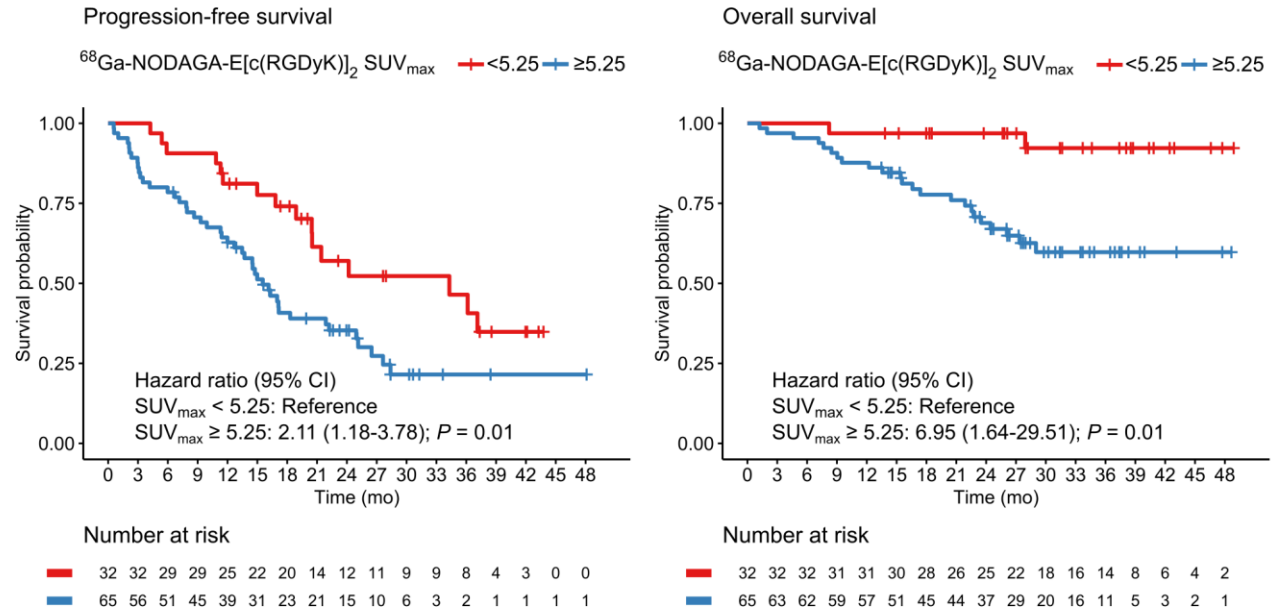


Figure 5 Kaplan Meir plots of ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$ PET SUV$_{max}$ dichotomized at 5.25 for prediction of progression-free survival and overall survival.



TABLES

Table 1: Baseline characteristics of 97 patients with neuroendocrine neoplasms

Baseline characteristics		**(N=97)**
Median age (y)		67 (range, 44-83)
Gender		
	Female	43 (44%)
	Male	54 (56%)
Site of primary tumor		
	Small intestine	62 (64%)
	Pancreas	19 (20%)
	Lung	6 (6%)
	Colon	6 (6%)
	Stomach	2 (2%)
	Esophagus	1 (1%)
	Rectum	1 (1%)
Metastatic disease		90 (93%)
Liver metastases		76 (79%)
Median Ki67 (%)		6 (range, 1-100)
2019 World Health Organization grade		
	NET G1	21 (22%)
	NET G2	55 (57%)
	NET G3	14 (14%)
	NEC	7 (7%)
Median time from diagnose to PET/CT (mo.)		27 (range, 2-265)
Primary tumor resected		37 (38%)
Ongoing treatment(s) at PET/CT scan time		
	Somatostatin analogue	75 (77%)
	Interferon	8 (8%)
	Capecitabine/5FU	5 (5%)
	Etoposide +/- carboplatin	4 (4%)
	Streptozotocin	4 (4%)
	Everolimus	3 (3%)
	Temozolomide	2 (2%)
Completed treatment(s) before PET/CT		
	On first line of therapy	45 (46%)
	Peptide receptor radionuclide therapy	30 (31%)
	Etoposide +/- carboplatin	16 (16%)
	Capecitabine/5-fluorouracil	12 (12%)
	Temozolomide	7 (7%)
	Streptozotocin	7 (7%)
	Interferon	6 (6%)
	External radiation therapy	5 (5%)
	Liver radiofrequency ablation or embolization	5 (5%)
	Resection of liver metastases	4 (4%)
	Everolimus or sunitinib	2 (2%)

Table 2: Patients with ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$ PET positive lesions (TLR ≥2) according to WHO classification of neuroendocrine neoplasms

	NET G1 (n=21)	**NET G2 (n=55)**	**NET G3/NEC (n=21)**	**All (n=97)**
TLR ≥ 2	13 (62%)	42 (76%)	19 (91%)	74 (76%)
TLR < 2	8 (38%)	13 (24%)	2 (10%)	23 (24%)

A tumor was defined as positive when the TLR (tumor-to-liver ratio), measured as lesion SUV_{max} to normal liver SUV_{mean}, was ≥2. Of the patients with NET G3/NEC tumors, 13/14 (93%) patients with NET G3 were positive and 6/7 (86%) patients with NEC were positive.

Table 3: Treatments given to patients with neuroendocrine neoplasms (n=97) during follow-up

Treatment(s) after PET/CT	**(N=97)**
Somatostatin analog	78 (80%)
Peptide receptor radionuclide therapy	31 (32%)
Capecitabine/5-fluorouracil	13 (13%)
Everolimus or sunitinib	12 (12%)
Surgery	11 (11%)
Temozolomide	9 (9%)
Liver radiofrequency ablation or embolization	9 (9%)
External radiation therapy	7 (7%)
Etoposide +/- carboplatin	5 (5%)
Interferon	5 (5%)
Streptozotocin	3 (3%)
Docetaxel	3 (3%)

Table 4: Uni- and multivariate Cox regression analyses for progression-free survival (SUV_{max} cut-off at 6.10)

Progression-free survival		Univariate Cox		Multivariate Cox	
		HR (95% CI)	**P-value**	**HR (95% CI)**	**P-value**
SUV_{max}					
	< 6.10	*Reference*	-	*Reference*	-
	≥ 6.10	1.96 (1.17-3.29)	0.01	1.82 (1.07-3.08)	0.03
WHO grades					
	NET G1	*Reference*		*Reference*	
	NET G2	1.25 (0.63-2.49)	0.52	1.25 (0.63-2.49)	0.52
	NET G3	4.01 (1.68-9.54)	<0.01	4.08 (1.70-9.77)	<0.01
	NEC	7.01 (2.65-18.50)	<0.001	5.87 (2.21-15.61)	<0.001

The median SUV_{max} was 6.10

Table 5: Uni- and multivariate Cox regression analyses for overall survival (SUV_{max} cut-off at 6.10)

Overall survival		Univariate Cox		Multivariate Cox	
		HR (95% CI)	**P-value**	**HR (95% CI)**	**P-value**
SUV_{max}					
	< 6.10	*Reference*	-	*Reference*	-
	≥ 6.10	2.66 (1.14-6.16)	0.02	2.59 (1.08-6.24)	0.03
WHO grades					
	NET G1	*Reference*		*Reference*	
	NET G2	1.84 (0.40-8.50)	0.44	1.80 (0.39-8.35)	0.45
	NET G3	15.99 (3.26-78.50)	<0.01	18.04 (3.59-90.63)	<0.001
	NEC	28.46 (5.62-144.24)	<0.001	22.55 (4.45-114.27)	<0.001

The median SUV_{max} was 6.10

Table 6: Uni- and multivariate Cox regression analyses for progression-free survival (SUV_{max} cut-off at 5.25)

Progression-free survival		Univariate Cox		Multivariate Cox	
		HR (95% CI)	**P-value**	**HR (95% CI)**	**P-value**
SUV_{max}					
	< 5.25	*Reference*	-	*Reference*	-
	≥ 5.25	2.11 (1.18-3.78)	0.01	1.92 (1.06-3.47)	0.03
WHO grades					
	NET G1	*Reference*		*Reference*	
	NET G2	1.25 (0.63-2.49)	0.52	1.22 (0.62-2.44)	0.56
	NET G3	4.01 (1.68-9.54)	<0.01	3.94 (1.65-9.44)	<0.01
	NEC	7.01 (2.65-18.50)	<0.001	5.84 (2.20-15.55)	<0.001

SUV_{max} cut-off optimized for prediction of overall survival was 5.25.

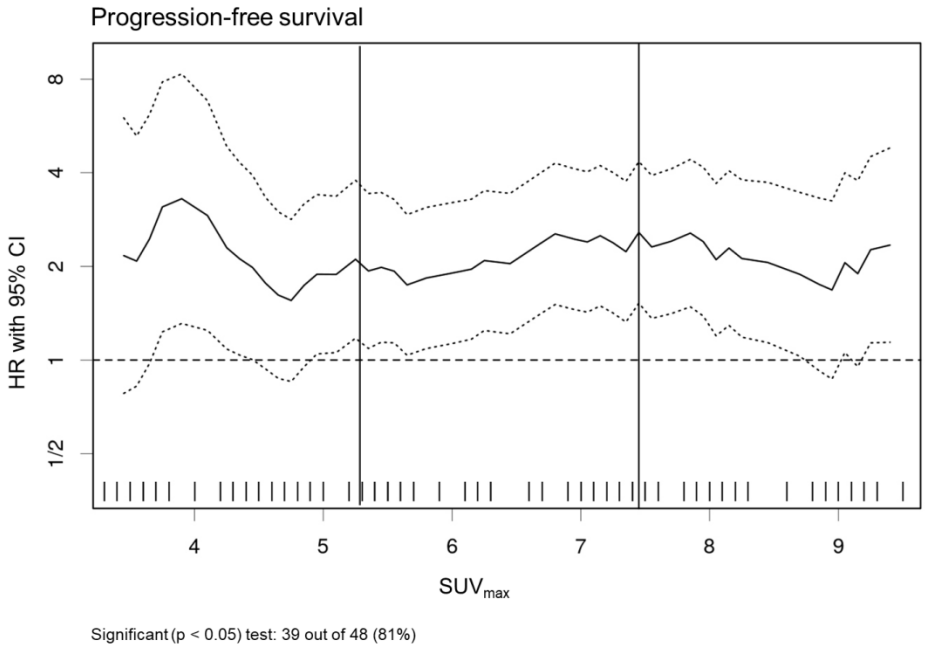
Table 7: Uni- and multivariate Cox regression analyses for overall survival. (SUV_{max} cut-off at 5.25)

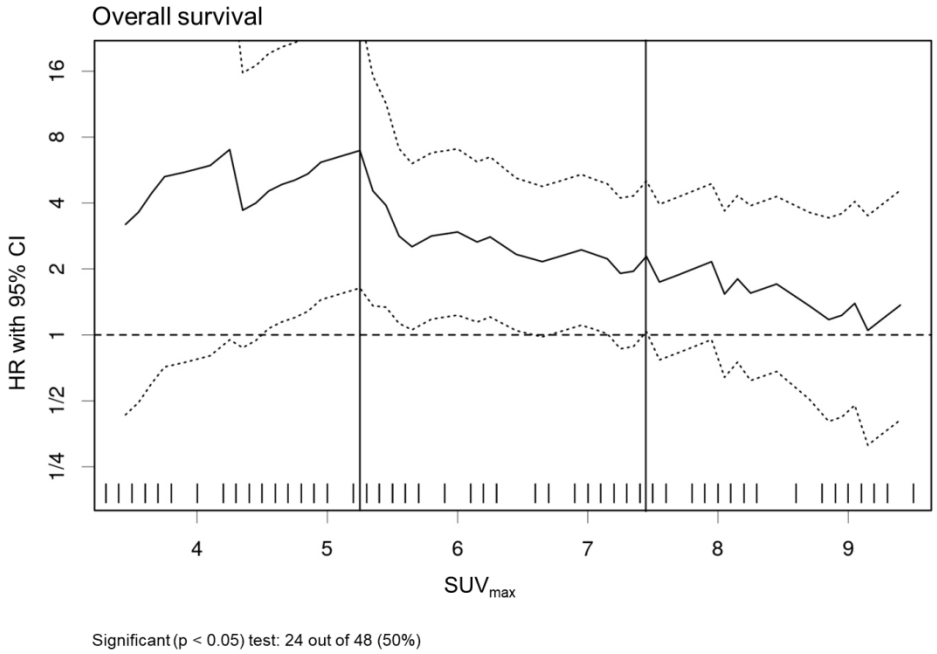
Overall survival		Univariate Cox		Multivariate Cox	
		HR (95% CI)	**P-value**	**HR (95% CI)**	**P-value**
SUV_{max}					
	< 5.25	*Reference*	-	*Reference*	-
	≥ 5.25	6.95 (1.64-29.51)	0.01	5.45 (1.26-23.60)	0.02
WHO grades					
	NET G1	*Reference*		*Reference*	
	NET G2	1.84 (0.40-8.50)	0.44	1.78 (0.38-8.26)	0.46
	NET G3	15.99 (3.26-78.50)	<0.01	15.67 (3.13-78.47)	<0.001
	NEC	28.46 (5.62-144.24)	<0.001	20.23 (3.98-102.87)	<0.001

SUV_{max} cut-off optimized for prediction of overall survival was 5.25.

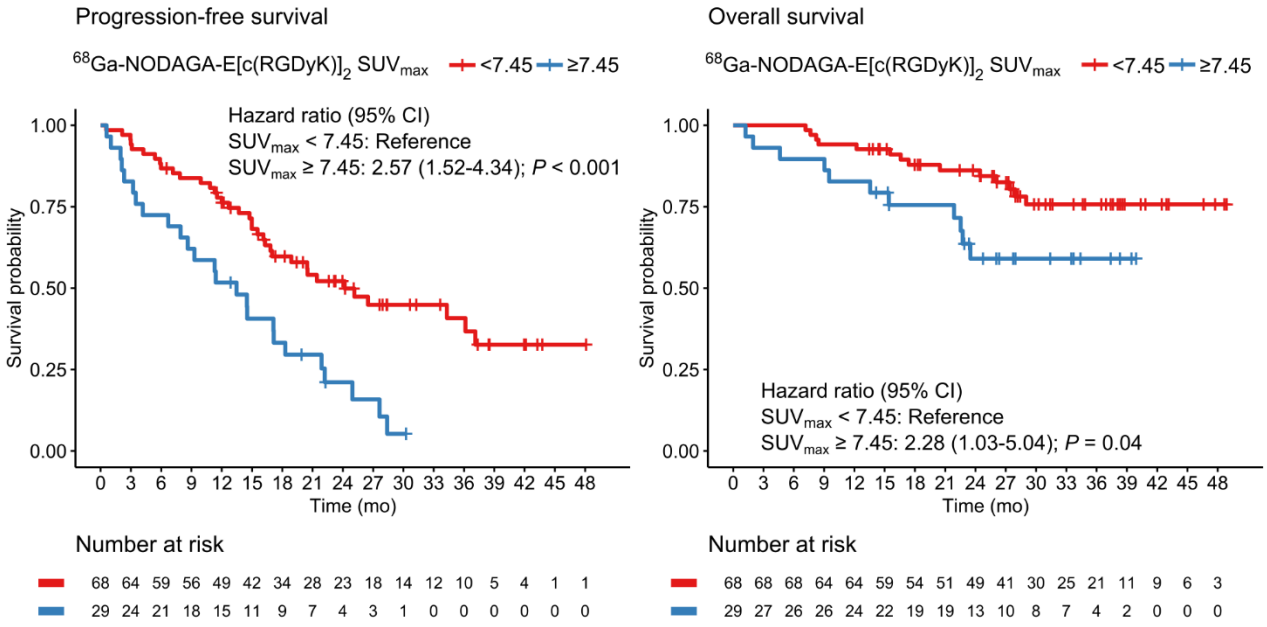
SUPPLEMENTAL DATA

Supplemental Figure 1. Hazard ratio with 95% confidence interval plotted vs cut-off of SUV_{max} for prediction of progression-free survival and overall survival. Solid lines at 5.25 (optimized cut-off for overall survival) and 7.45 (optimized cut-off for progression-free survival).





Supplemental Figure 2 Kaplan Meir plots of ^{68}Ga-NODAGA-E[c(RGDyK)]$_2$ PET SUV_{max} dichotomized at 7.45 for prediction of progression-free survival and overall survival.



Supplemental Table 1: Uni- and multivariate Cox regression analyses for progression-free survival (SUV_{max} cut-off at 7.45)

Progression-free survival		Univariate Cox		Multivariate Cox	
		HR (95% CI)	**P-value**	**HR (95% CI)**	**P-value**
SUV_{max}					
	< 7.45	*Reference*	-	*Reference*	-
	≥ 7.45	2.57 (1.52-4.34)	<0.001	3.08 (1.79-5.29)	<0.001
WHO grades					
	NET G1	*Reference*		*Reference*	
	NET G2	1.25 (0.63-2.49)	0.52	1.12 (0.56-2.24)	0.75
	NET G3	4.01 (1.68-9.54)	<0.01	4.32 (1.80-10.36)	<0.01
	NEC	7.01 (2.65-18.50)	<0.001	8.42 (3.13-22.69)	<0.001

SUV_{max} cut-off optimized for prediction of progression-free survival was 7.45.

Supplemental Table 2: Uni- and multivariate Cox regression analyses for overall survival (SUV_{max} cut at 7.45)

Overall survival		Univariate Cox		Multivariate Cox	
		HR (95% CI)	**P-value**	**HR (95% CI)**	**P-value**
SUV_{max}					
	< 7.45	*Reference*	-	*Reference*	-
	≥ 7.45	2.28 (1.03-5.04)	0.04	3.57 (1.50-8.46)	<0.01
WHO grades					
	NET G1	*Reference*		*Reference*	
	NET G2	1.84 (0.40-8.50)	0.44	1.68 (0.36-7.79)	0.51
	NET G3	15.99 (3.26-78.50)	<0.01	21.42 (4.17-110.13)	<0.001
	NEC	28.46 (5.62-144.24)	<0.001	35.14 (6.65-185.56)	<0.001

SUV_{max} cut-off optimized for prediction of progression-free survival was 7.45.