Pre-therapy extrahepatic $^{68}$Ga-DOTATATE avid tumor burden is associated with short-term clinical outcomes of $^{177}$Lu-DOTATATE in advanced metastatic gastroenteropancreatic neuroendocrine tumors

Hong Song $^1$ · Pamela L. Kunz $^2$ · Benjamin L. Franc $^1$ · Farshad Moradi $^1$ · Judy Nguyen $^1$ · George Fisher $^2$ · Carina Mari Aparici $^1$ · Andrei Iagaru $^1$ · Guido A. Davidzon $^1$

$^1$ Division of Nuclear Medicine and Molecular Imaging, Department of Radiology, Stanford University Medical Center, 300 Pasteur Drive, Stanford, CA 94305-5281, USA, $^2$ Division of Oncology, Department of Medicine, Stanford University Medical Center, 875 Blake Wilbur Drive, Stanford, CA 94305-6562, USA.

Corresponding author: Guido A. Davidzon MD SM, gdavidzon@stanford.edu
Abstract:

Lutetium-177 (\(^{177}\text{Lu}\))-DOTATATE is an effective systemic therapy for metastatic somatostatin receptor positive neuroendocrine tumors (NETs). Here we report our experience with the use of pre-therapy \(^{68}\text{Ga}\)-DOTATATE PET as prognostic marker for short-term clinical outcomes of \(^{177}\text{Lu}\)-DOTATATE therapy in patients with advanced NETs.

Materials and methods: We retrospectively reviewed patients who received at least one dose of \(^{177}\text{Lu}\)-DOTATATE between Dec. 2016 and July 2019 at our institution. 50 patients (63.6 ± 10.0 years) with advanced gastroenteropancreatic neuroendocrine tumors (GEP-NETs) who had pre-therapy \(^{68}\text{Ga}\)-DOTATATE PET were included in the analysis. \(^{68}\text{Ga}\)-DOTATATE avid tumor volumes were determined automatically using an SUV thresholding approach. Total and extrahepatic \(^{68}\text{Ga}\)-DOTATATE avid tumor volumes were measured and dichotomized into large and small tumor volume groups. Association with progression free survival (PFS) and overall survival (OS) were determined at median follow up of 32 months by Kaplan-Meier survival analysis with Log-Rank test.

Results: During follow up, 38 patients (76%) had disease progression and 15 patients (30%) died. Kaplan-Meier analysis of PFS in GEP-NETs patients showed that smaller extrahepatic \(^{68}\text{Ga}\)-DOTATATE avid tumor volume (<140 mL) is associated with significantly longer PFS (Median PFS 29.0 ± 6.7 months vs 9.0 ± 1.7 months, \(P = 0.0001\)). This trend in PFS is less prominent when total \(^{68}\text{Ga}\)-DOTATATE avid tumor volume is analyzed. Similarly, Kaplan-Meier analysis of OS found that GEP-NETs patients with smaller extrahepatic \(^{68}\text{Ga}\)-DOTATATE avid tumor volume (<150 mL) is associated with significantly longer OS (Median OS not reached vs 44.0 ± 12.3 months, \(P = 0.002\)). This association with OS is not statistically significant when total \(^{68}\text{Ga}\)-DOTATATE avid tumor volume is analyzed. When \(^{68}\text{Ga}\)-DOTATATE avid hepatic tumor volume is grouped into low (<500 mL), medium (500-1000mL) and large (> 1000 mL) tumor volumes, no statistically significant difference in PFS is observed, \(P = 0.19\). The accuracy
of extrahepatic $^{68}$Ga-DOTATATE avid tumor volume as prognostic marker for PFS and OS at 32 months are moderate at 58% and 72%.

**Conclusions:** Smaller extrahepatic $^{68}$Ga-DOTATATE avid tumor volumes are associated with longer PFS and OS following $^{177}$Lu-DOTATATE treatment in patients with advanced GEP-NETs. The accuracy of extrahepatic $^{68}$Ga-DOTATATE avid tumor volume as prognostic marker for PFS and OS at 32 months are moderate, which may limit its clinical application.

**Keywords** $^{177}$Lu-DOTATATE · Neuroendocrine tumors · $^{68}$Ga-DOTATATE PET · PET avid tumor volume · progression free survival · overall survival
Declarations

**Funding:** 'Not applicable'

**Conflicts of interest/Competing interests (include appropriate disclosures):** 'Not applicable'

**Availability of data and material (data transparency):** available upon request

**Code availability (software application or custom code):** 'Not applicable'

**Authors' contributions (optional):** Hong Song and Guido A. Davidzon: study design, data collection, image interpretation, image analysis, manuscript preparation; Pamela L. Kunz, Benjamin L. Franc, Farshad Moradi, Judy Nguyen, George Fisher, Carina Mari Aparici, Andrei Iagaru: study design, image interpretation, manuscript review.

**Ethics approval (include appropriate approvals or waivers):** retrospective study, approved by IRB review

**Consent to participate:** waived after IRB review, retrospective study

**Consent for publication:** waived after IRB review, retrospective study
Introduction

Neuroendocrine tumors (NETs) are a group of heterogeneous neoplasms arising from the neuroendocrine system which most commonly includes endocrine islet cells of the pancreas and endocrine cells of the gastrointestinal and respiratory tracts [1-3]. The most common sites of primary NETs are the gastrointestinal tract and about 12-22% of the patients had metastatic disease at presentation [4]. In the past decade, innovations in imaging and the development of novel therapeutics such as somatostatin analogues [5, 6], Everolimus [7, 8], Sunitinib in pancreatic NET[9], Temodar/Capecitabine in pNET [10] and $^{177}$Lu-DOTATATE [11] have significantly improved PFS in patients with metastatic disease [12-14].

Lutetium-177 ($^{177}$Lu) DOTATATE (Lutathera®) was approved by the US FDA in January 2018 for the treatment of somatostatin-receptor positive gastroenteropancreatic neuroendocrine tumors (GEP-NETs) after a phase III clinical trial (NETTER-1) showed significantly longer progression-free survival in patients with advanced midgut NETs compared to patients receiving high dose octreotide long-acting release (LAR) [11]. Although it is still debatable at what stage in the course of the disease patients with advanced NETs will benefit the most from $^{177}$Lu-DOTATATE therapy, this new approach of targeted peptide receptor radionuclide therapy (PRRT) is most commonly used to treat advanced stage NETs refractory to somatostatin analogues. Often enough, $^{177}$Lu-DOTATATE becomes a last resort as palliative therapy in patients with advanced NETs and widespread metastases. It remains to be determined which subgroup of patients with advanced NETs might realize the greatest progression free survival benefit from PRRT.

Prior to treatment with $^{177}$Lu-DOTATATE, patients undergo imaging with somatostatin receptor positron emission tomography (PET), such as $^{68}$Ga-DOTATATE or $^{68}$Ga-DOTATOC PET, to confirm the presence of somatostatin receptor overexpression. Prior studies have established that $^{68}$Ga-DOTATATE PET and $^{68}$Ga-DOTATOC PET showed the extent of disease more accurately compared to $^{111}$In-pentetreotide scintigraphy (Octreoscan) and better
reflects SSTR2 expression in small lesions [15, 16], for which reason $^{68}$Ga-DOTATATE PET has replaced scintigraphy in clinical practice. Given that the NETTER-1 trial used pre-therapy $^{111}$In-pentetreotide scintigraphy for patient selection, $^{68}$Ga-DOTATATE PET imaging is likely to increase the eligible patient population that could potentially receive $^{177}$Lu-DOTATATE therapy.

$^{68}$Ga-DOTATATE PET is now widely used in the diagnosis and clinical follow up of patients with well differentiated NETs [17-19]. Furthermore, $^{68}$Ga-DOTATATE PET has been studied to predict prognosis of NETs patients [20, 21]. Tirosch et al. has demonstrated that higher total $^{68}$Ga-DOTATATE avid tumor volume is associated with worse PFS and increased risk of disease specific mortality [22]. Similarly, our group [23] has shown that total somatostatin receptor expressing tumor volume and other derived metrics have prognostic value for progression free survival (PFS) in patients with well-differentiated NETs. However, only a small fraction of the patients in these studies received PRRT. Here we evaluated the use of pre-therapy $^{68}$Ga-DOTATATE as a prognostic marker for short-term clinical outcomes following $^{177}$Lu-DOTATATE therapy.
Materials and methods

Patient population

We retrospectively reviewed the images and medical records of 63 patients who received at least one dose of $^{177}$Lu-DOTATATE treatment from December 2016 to July 2019 at our institution. 50 patients (37-80 years old, mean ± SD: 63.6 ± 10.0; 21 male and 29 female) with advanced GEP-NETs who had pre-therapy $^{68}$Ga-DOTATATE PET were included in the study. Among them, 11 patients were in the NETTER-1 trial expanded access protocol. Institutional Review Board approved this retrospective study and waived the requirement for obtaining written informed consent.

Patients were screened and had clinical laboratory workup prior to each PRRT administration, which was withheld according to the following key exclusion criteria: 1. Serum creatinine > 1.7 mg/dL or GFR < 50 mL/min; 2. Hgb < 8.0 g/dL, WBC < 2000/mm$^3$, platelets < 75 x 10$^3$/mm$^3$; 3. Total bilirubin > 3 x upper limit of normal; 4. Serum albumin < 3.0 g/dL unless prothrombin time is within the normal range; 5. Pregnancy or lactation. 6. Any surgery, radioembolization, chemoembolization, chemotherapy and radiofrequency ablation within 12 weeks prior to treatment. 7. Interferons, everolimus or other systemic therapies within 4 weeks prior to treatment; 8. Known brain metastases, unless these metastases have been treated and stabilized. 9. Uncontrolled congestive heart failure or diabetes mellitus.

$^{177}$Lu-DOTATATE treatment

Patients received one fixed dose of 7.4 GBq $^{177}$Lu-DOTATATE intravenously once every 8 weeks (± 1 week) if they meet the screening criteria prior to each infusion according to package insert. 11 patients in the NETTER-1 trial expanded access program were treated based on trial protocol.
$^{68}$Ga-DOTATATE PET and quantitative image analysis

All 50 patients included in the study had pre-therapy $^{68}$Ga-DOTATATE PET/CT or PET/MR scans. Images were acquired from the vertex of the skull to mid-thigh approximately 60 mins following IV administration of the radiopharmaceutical. For PET/CT, a low-dose CT scan was performed for attenuation correction and anatomic correlation. For PET/MRI, a T1-weighted two-point Dixon axial 3D-spoiled gradient echo (SPGR) sequence was used for MR-based attenuation correction.

$^{68}$Ga-DOTATATE PET scans were visually evaluated by two board-certified nuclear medicine physicians by consensus. Quantitative image analysis of $^{68}$Ga-DOTATATE PET/CT or PET/MR were performed with MIMvista version 6.9.2 (MIMvista, Cleveland, Ohio) as previously reported [22, 23]. Briefly, using the whole-body automatic contour tool, a SUV$_{bw}$ (SUV body weight) based threshold approach was applied to delineate every $^{68}$Ga-DOTATATE avid tumors. The PET avid tumors were then compared to the anatomic images to optimize the SUV$_{bw}$ threshold that maximizes the overlap between PET avid and anatomic tumor volumes by visual inspection. For PET/CTs, a SUV$_{bw}$ threshold of 4.0-4.5 were applied to delineate tumor volumes that are most consistent with anatomic tumor volumes. Background non-specific and physiologic uptake, most commonly in the spleen, kidneys, pancreas, stomach, pituitary and adrenal glands were visually inspected and manually removed using the 3D brush tool. The total $^{68}$Ga-DOTATATE avid tumor volume and maximal SUV (SUV$_{max}$) measurements were performed automatically. The hepatic uptake was then removed manually, and the remaining extrahepatic tumor volumes were measured automatically. The non-osseous uptake was then removed manually, and the osseous metastases tumor volume were measured automatically.

**Overall survival and progression free survival**
PFS was defined as the time from the first dose of treatment to documented disease progression based on post-therapy imagings (\(^{68}\)Ga-DOTATATE PET, CT, MR), or death by any cause. Overall survival (OS) was defined as time from first dose of treatment to death by any cause. During median follow-up of 32 months, 38 patients had disease progression and 15 patients died. Pre-therapy total and extrahepatic \(^{68}\)Ga-DOTATATE avid tumor volumes were measured and dichotomized into large and smaller tumor volume groups. Similarly, SUV\(_{\text{max}}\) was dichotomized into higher and lower level groups. As illustrated in Supplement Tables 1-3, a range of cut-off tumor volumes were used to dichotomize the groups and ranges of tumor volume with \(p < 0.05\) were identified. PFS and OS were then correlated with total and extrahepatic \(^{68}\)Ga-DOTATATE avid tumor volume and SUV\(_{\text{max}}\). In addition, extrahepatic tumor volume was visually inspected and dichotomized into small and large tumor volume groups for PFS analysis.

**Statistical analysis**

Statistical analysis was performed with SPSS Statistics 27 (IBM, Armonk, NY). The Kaplan–Meier survival analysis with Log-Rank test was performed for PFS and OS based on a range of cut-off values that dichotomize pre-therapy total and extrahepatic \(^{68}\)Ga-DOTATATE avid tumor volumes and SUV\(_{\text{max}}\). A \(p\)-value less than 0.05 (\(p \leq 0.05\)) is considered statistically significant. Furthermore, the sensitivity, specificity and accuracy of using extrahepatic \(^{68}\)Ga-DOTATATE tumor volume in predicting PFS and OS at 32 months follow up were calculated.
Results

\textbf{\textsuperscript{177}Lu-DOTATATE treatment, toxicity and safety}

Among the 50 patients who had pre-therapy \textsuperscript{68}Ga-DOTATATE PET, 24 patients had midgut primary and 21 patients had pancreatic primary NETs. 5 patients had unknown site of primary but most likely gastroenteropancreatic origin given based on symptoms and metastatic patterns. The majority of these patients (46 out of 50 patients) had low grade GEP-NETs (Grade 1 and 2) tumors based on Ki-67 labeling index and mitotic count \cite{24}. Two patients have well differentiated grade 3 NETs. Tumor grades were based on the last available biopsy samples prior to \textsuperscript{177}Lu-DOTATATE therapy. All slides were reviewed by pathologists at our institution. The 13 patients excluded from the analysis included five patients with other types of neuroendocrine tumors include 3 paragangliomas, 1 pheochromocytoma and 1 pulmonary neuroendocrine tumor, two patients with pancreatic neuroendocrine tumor who had MEN-I syndrome with prolonged and complicated disease course and high-grade disease (mixed higher Grade 1-3), and six patients who did not have pre-therapy \textsuperscript{68}Ga-DOTATATE PET (Figure 1). The patient’s demographic and clinical characteristics are summarized in Table 1.

Most patients tolerated \textsuperscript{177}Lu-DOTATATE treatment well. 36 out of 50 patients (72\%) completed all four therapy cycles of \textsuperscript{177}Lu-DOTATATE (Table 2), which is comparable to 77\% observed in the NETTER-1 trial \cite{11}. Eight patients developed adverse events: thrombocytopenia (n=5, 10\%), neutropenia and thrombocytopenia (n=1, 2\%), low albumin (n=1, 2\%), elevated total bilirubin (n=1, 2\%). These led to delays or treatment termination (Table 2), which is comparable to the toxicity profile observed in the NETTER-1 trial as well. Two patients recovered from mild thrombocytopenia and completed all four infusions. A few patients had mild nausea and vomiting that were controlled with medication and occurred at the time when a
standard amino acid solution was used before it was replaced with two amino acid (arginine and lysine) only compound solution.

**68Ga-DOTATATE PET imaging**

Among the pre-therapy 68Ga-DOTATATE PETs, 44 PET scans were performed at our institution including 40 PET/CT using GE Discovery 600 (n=1), 690 (n=8), 710 (n=1) or MI (n =30) scanners (GE Healthcare, Waukesha, WI), and 4 PET/MR with GE SIGNA scanner. 68Ga-DOTATATE dosage ranged from 122.1 to 222.0 MBq (mean ± SD: 180.7 ± 26.3 MBq). Six PET/CT were done at outside institutions (Philips Gemini TF 64 x 2, GE Discovery 690, GE Discovery STE x 2, Siemens Biograph 40_mCT).

On pre-therapy 68Ga-DOTATATE PET scans, all patients had metastatic disease. Most of the patients (47 patients, 94%) had extrahepatic metastases including 27 patients (54%) with peritoneal and retroperitoneal metastases, 29 patients (58%) with osseous metastases and 33 patients with metastases to other sites (Table 1). Patient with varied degree of extrahepatic metastases on pretherapy 68Ga-DOTATATE PET are shown on Fig. 2.

**PFS and OS analysis**

The median follow-up after first dose of 177Lu-DOTATATE treatment is 32 months (range 21 to 52 months). Kaplan-Meier analysis of PFS found a range of cut-off extrahepatic 68Ga-DOTATATE avid tumor volumes (range from 130 mL to 180 mL), when used to dichotomize GEP-NETS patients into two groups with large and small extrahepatic tumor volumes, that are associated with significantly longer PFS in the smaller tumor volume group (Table S1). For example, when cut-off tumor volume 140 mL is used, smaller extrahepatic 68Ga-DOTATATE avid tumor volume (<140 mL, 29 out of 50 patients) is associated with significantly longer PFS
(Median PFS 29.0 ± 6.7 months vs 9.0 ± 1.7 months, \( P = 0.0001 \)) (Fig. 3a). This trend in PFS is less prominent when total \(^{68}\text{Ga-DOTATATE}\) avid tumor volume is analyzed (Table S1), for example, for cut-off total tumor volume 1000 mL, patients with smaller total tumor volume had median PFS 24.0 ± 5.4 months vs 10.0 ± 3.4 months in group with larger total tumor volume, \( P = 0.04 \) (Fig. 3b).

Similarly, Kaplan-Meier analysis of OS found a range of cut-off extrahepatic \(^{68}\text{Ga-DOTATATE}\) avid tumor volumes (130 mL to 190 mL), when used to dichotomize patients into large and small tumor volume groups, are associated with significantly longer PFS in the smaller tumor volume group (Table S1). Kaplan-Meier curves were shown for cut-off value 150 mL in Fig. 3c. For total \(^{68}\text{Ga-DOTATATE}\) avid tumor volume, however, no statistically significant association with OS was found for any cut-off values (Table S2 and Fig. 3d). Extrahepatic \(^{68}\text{Ga-DOTATATE}\) avid tumor volume (cut-off 140 mL) has sensitivity of 50%, specificity of 83% and accuracy of 58% when predicting PFS. For OS, extrahepatic \(^{68}\text{Ga-DOTATATE}\) avid tumor volume (cut-off 150 mL) has sensitivity of 67%, specificity of 74% and accuracy of 72%.

Further analysis of specific site extrahepatic metastasis such as bone showed that smaller \(^{68}\text{Ga-DOTATATE}\) avid osseous tumor volume is associated with longer PFS (Median PFS 22.0 ± 2.4 months vs 7.0 ± 1.1 months, \( P = 0.006 \)) although such statistically significant association was only found at cut-off tumor volume of 50 mL (Table S3, Fig. 4a). Apparent decrease in \( P \) value at large cut-off values of 125 mL is considered artifactual. We then analyzed hepatic metastatic volume and its association with PFS. Several cut-off \(^{68}\text{Ga-DOTATATE}\) avid hepatic tumor volumes were found to dichotomize patients into large and small tumor volume groups where an association with statistically different PFS was present, however, such trend is less prominent compared to extrahepatic tumor volume (Table S3, Fig. 4b). However, when \(^{68}\text{Ga-DOTATATE}\) avid hepatic tumor volume is grouped into low (<500 mL), medium (500-1000mL) and large (> 1000 mL) tumor volumes, no statistically significant difference in PFS is observed, \( P = 0.19 \) (Fig. 4C). Our finding that larger \(^{68}\text{Ga-DOTATATE-avid}\)
hepatic tumor volume is not associated with worse PFS after $^{177}$Lu-DOTATATE therapy is consistent with recent analysis of liver tumor burden among patients enrolled in the $^{177}$Lu-Dotatate arm of NETTER-1 trial [25]. Similarly, no statistically significant difference is observed between groups with higher and lower SUV$_{\text{max}}$ values (Fig. 4D). In addition, when extrahepatic tumor volume was visually inspected and dichotomized into small and large tumor volume groups (Supplement Fig. 1), Kaplan-Meier survival analysis showed similar association where smaller extrahepatic tumor volume is associated with longer PFS, $P = 0.005$ (Fig. 5).

**Discussion**

In this single center retrospective study, we evaluated pre-therapy $^{68}$Ga-DOTATATE PET tumor volume as a prognostic marker for short-term clinical outcomes following $^{177}$Lu-DOTATATE therapy. Smaller extrahepatic $^{68}$Ga-DOTATATE-avid tumor volume is associated with longer PFS and OS. As comparison, the association with OS is not statistically significant when total $^{68}$Ga-DOTATATE avid tumor volume is analyzed. To our knowledge, this is the first study that evaluated extrahepatic tumor burden in GEP-NETs patients undergoing $^{177}$Lu-DOTATATE therapy.

Extrahepatic metastatic tumor burden had been recognized as an important prognostic factor for GEP-NETs [26-28]. It is not entirely known whether progression to extrahepatic metastases is spread of the low-grade indolent tumor or an indication of transition to higher grade more aggressive tumors. Systemic therapies such as PRRT are currently reserved for these patients. However, our findings suggest the PRRT become less effective in patients with extensive extrahepatic metastases, which is not unexpected. It is not completely clear why hepatic tumor volume alone does not predict PFS as well as extrahepatic metastatic tumor volume. It is likely that liver has large functional reserve and significant tumor involvement is needed to impair its function and leads to mortality.
Ga-DOTATATE PET/CT plays a central role in the care of patients with NETs and alters diagnosis and management in one third of the cases [29]. Several groups have assessed somatostatin receptor PET as prognostic factor in long term PFS of NETs patients [21-23, 30-32]. Tirosh et al. demonstrated in a prospective study that \(^{68}\)Ga-DOTATATE tumor volume correlates with PFS and disease specific survival in NETs patients [22]. Our group has shown that somatostatin receptor expressing tumor volume measured on \(^{68}\)Ga-DOTATATE PET/CT may have prognostic value of PFS in NETs patients [23]. Ambrosini et al. found that \(^{68}\)Ga-DOTANOC PET/CT SUV\(_{\text{max}}\) is independent prognostic factor in patients with G1 and G2 pancreatic NETs [30]. However, only a small percentage of patients in these studies were treated with \(^{177}\)Lu-DOTATATE. Our study directly assessed pre-therapy \(^{68}\)Ga-DOTATATE PET/CT is relation to short term clinical outcomes in patients with GEP-NETs treated with \(^{177}\)Lu-DOTATATE.

An early study of \(^{68}\)Ga-DOTANOC PET/CT showed that SUV\(_{\text{max}}\) is an independent prognostic factor in patients with low grade NETs [30, 33]. A recent study with \(^{64}\)Cu-DOTATATE PET/CT found association between tumor SSTR density and PFS but not OS [31]. We did not observe association PFS or OS with SUV\(_{\text{max}}\) on \(^{68}\)Ga-DOTATATE PET in this patient cohort. It is uncertain what is the cause of this difference and it is likely related to the use of different PET isotopes and somatostatin analogs, for example, DOTANOC is known to have higher affinity to SSTR-3 in addition to SSTR-2 and 5 [34]. Clinically, unlike SUV\(_{\text{max}}\) in \(^{18}\)FDG PET, it is clear whether a higher SUV\(_{\text{max}}\) observed in GEP-NETs is correlated with well-differentiated NETs that has higher SSTR expression or higher tumor cell density and therefore a sign of tumor aggressiveness, nor is it certain what is the significance of changes in SUV\(_{\text{max}}\) values after \(^{177}\)Lu-DOTATATE treatment. Does a decrease in SUV\(_{\text{max}}\) during short-term follow-up PET imaging suggest that tumor cells are eradicated leading to decreased receptor density or is tumor dedifferentiation occurring because of selective pressure from \(^{177}\)Lu-DOTATATE? Longer
term follow-up with $^{68}$Ga-DOTATATE PET or combined with $^{18}$FDG-PET may help us better understand DOTATATE PET SUV$_{\text{max}}$ measurements and incorporate them into clinical decision making [35].

There are a few limitations to this study. First, this is a retrospectively study with a relatively small patient population. Although a statistically significant differences were observed for PFS and OS in patients with large vs small extrahepatic tumor volumes, the accuracy of extrahepatic $^{68}$Ga-DOTATATE avid tumor volume as prognostic marker for PFS and OS at 32 months are moderate, limiting its clinical utility. Furthermore, the small patient number limits the subgroup analysis, for example, in patients receiving different number of therapy cycles, with different subtypes of NETs (GI vs pancreatic) or Grades [36], or in patients who received various other treatments which may impact the outcome of PRRT. Second, 11 out of the 50 patients included in the analysis were enrolled in the NETTER-1 trial expanded access protocol may have had more advanced metastatic burden as $^{177}$Lu-DOTATATE was not offered outside of this protocol in the US. Third, pre-therapy $^{68}$Ga-DOTATATE PET were performed on different PET/CT and PET/MR scanners and image qualitative and quantitative analysis were not normalized for scanners and reconstruction methods. A whole-body SUV (SUV$_{\text{bw}}$) threshold segmentation approach was used to ensure reproducibility and consistency between $^{68}$Ga-DOTATATE avid tumor volume measurement. However, due to relatively lower SUV of smaller extrahepatic metastases, SUV$_{\text{bw}}$ threshold adjusted to pick up these small metastatic lesions may over-estimate the large hepatic tumors. Alternatively, SUV$_{\text{bw}}$ threshold could be applied to each lesion, but would decrease the reproducibility of the segmentation method. Fourth, the cut-off $^{68}$Ga-DOTATATE avid tumor volume for PFS and OS analysis was generated arithmetically. Although such an approach avoids the subjective selection of cutoff tumor burden, the tumor volume generated is abstract and difficult for oncologist to relate clinically when quantified $^{68}$Ga-DOTATATE avid tumor volume is not readily available. Our findings that visual inspection and
grouping of extrahepatic tumor volume into small and large tumor volume groups had similar findings as quantitative tumor volume approach could help improve clinical utility of pretherapy $^{68}$Ga-DOTATATE PET.

Conclusions

Smaller extrahepatic $^{68}$Ga-DOTATATE avid tumor volume is associated with longer PFS and OS following $^{177}$Lu-DOTATATE treatment in patients with advanced GEP-NETs. The accuracy of extrahepatic $^{68}$Ga-DOTATATE avid tumor volume as prognostic marker for PFS and OS at 32 months are moderate, which may limit its clinical application.
References

1. Oronsky B, Ma PC, Morgensztern D, Carter CA. Nothing But NET: A Review of Neuroendocrine Tumors and Carcinomas. Neoplasia. 2017;19:991-1002.

2. Klimstra DS, Modlin IR, Coppola D, Lloyd RV, Suster S. The pathologic classification of neuroendocrine tumors: a review of nomenclature, grading, and staging systems. Pancreas. 2010;39:707-12.

3. Cai W, Tan Y, Ge W, Ding K, Hu H. Pattern and risk factors for distant metastases in gastrointestinal neuroendocrine neoplasms: a population-based study. Cancer Med. 2018;7:2699-709.

4. Taal BG, Visser O. Epidemiology of neuroendocrine tumours. Neuroendocrinology. 2004;80 Suppl 1:3-7.

5. Rinke A, Muller HH, Schade-Brittinger C, Klose KJ, Barth P, Wied M, et al. Placebo-controlled, double-blind, prospective, randomized study on the effect of octreotide LAR in the control of tumor growth in patients with metastatic neuroendocrine midgut tumors: a report from the PROMID Study Group. J Clin Oncol. 2009;27:4656-63.

6. Caplin ME, Pavel M, Cwikla JB, Phan AT, Raderer M, Sedlackova E, et al. Lanreotide in metastatic enteropancreatic neuroendocrine tumors. N Engl J Med. 2014;371:224-33.

7. Yao JC, Fazio N, Singh S, Buzzoni R, Carnaghi C, Wolin E, et al. Everolimus for the treatment of advanced, non-functional neuroendocrine tumours of the lung or gastrointestinal tract (RADIANT-4): a randomised, placebo-controlled, phase 3 study. Lancet. 2016;387:968-77.

8. Yao JC, Shah MH, Ito T, Bohas CL, Wolin EM, Van Cutsem E, et al. Everolimus for advanced pancreatic neuroendocrine tumors. N Engl J Med. 2011;364:514-23.

9. Raymond E, Dahan L, Raoul JL, Bang YJ, Borbath I, Lombard-Bohas C, et al. Sunitinib malate for the treatment of pancreatic neuroendocrine tumors. N Engl J Med. 2011;364:501-13.

10. Kunz PL, Catalano PJ, Nimeiri H, Fisher GA, Longacre TA, Suarez CJ, et al. A randomized study of temozolomide or temozolomide and capecitabine in patients with advanced pancreatic neuroendocrine tumors: A trial of the ECOG-ACRIN Cancer Research Group (E2211). Journal of Clinical Oncology. 2018;36:4004-.

11. Strosberg J, El-Haddad G, Wolin E, Hendifar A, Yao J, Chasen B, et al. Phase 3 Trial of (177)Lu-Dotatate for Midgut Neuroendocrine Tumors. N Engl J Med. 2017;376:125-35.
12. Strosberg JR, Halfdanarson TR, Bellizzi AM, Chan JA, Dillon JS, Heaney AP, et al. The North American Neuroendocrine Tumor Society Consensus Guidelines for Surveillance and Medical Management of Midgut Neuroendocrine Tumors. Pancreas. 2017;46:707-14.

13. Pavel M, O'Toole D, Costa F, Capdevila J, Gross D, Kianmanesh R, et al. ENETS Consensus Guidelines Update for the Management of Distant Metastatic Disease of Intestinal, Pancreatic, Bronchial Neuroendocrine Neoplasms (NEN) and NEN of Unknown Primary Site. Neuroendocrinology. 2016;103:172-85.

14. Howe JR, Cardona K, Fraker DL, Kebebew E, Untch BR, Wang YZ, et al. The Surgical Management of Small Bowel Neuroendocrine Tumors: Consensus Guidelines of the North American Neuroendocrine Tumor Society. Pancreas. 2017;46:715-31.

15. Hope TA, Calais J, Zhang L, Dieckmann W, Millo C. (111)In-Pentetreotide Scintigraphy Versus (68)Ga-DOTATATE PET: Impact on Krenning Scores and Effect of Tumor Burden. J Nucl Med. 2019;60:1266-9.

16. Gabriel M, Decristoforo C, Kendler D, Dobrozemsky G, Heute D, Uprimny C, et al. 68Ga-DOTA-Tyr3-octreotide PET in neuroendocrine tumors: comparison with somatostatin receptor scintigraphy and CT. J Nucl Med. 2007;48:508-18.

17. Srirajaskanthan R, Kayani I, Quigley AM, Soh J, Caplin ME, Bomanji J. The role of 68Ga-DOTATATE PET in patients with neuroendocrine tumors and negative or equivocal findings on 111In-DTPA-octreotide scintigraphy. J Nucl Med. 2010;51:875-82.

18. Haug AR, Cindea-Drimus R, Auernhammer CJ, Reincke M, Wangler B, Uebleis C, et al. The role of 68Ga-DOTATATE PET/CT in suspected neuroendocrine tumors. J Nucl Med. 2012;53:1686-92.

19. Hofman MS, Lau WF, Hicks RJ. Somatostatin receptor imaging with 68Ga DOTATATE PET/CT: clinical utility, normal patterns, pearls, and pitfalls in interpretation. Radiographics. 2015;35:500-16.

20. Koch W, Auernhammer CJ, Geisler J, Spitzweg C, Cyran CC, Ilhan H, et al. Treatment with octreotide in patients with well-differentiated neuroendocrine tumors of the ileum: prognostic stratification with Ga-68-DOTA-TATE positron emission tomography. Mol Imaging. 2014;13:1-10.

21. Campana D, Ambrosini V, Pezzilli R, Fanti S, Labate AM, Santini D, et al. Standardized uptake values of (68)Ga-DOTANOC PET: a promising prognostic tool in neuroendocrine tumors. J Nucl Med. 2010;51:353-9.
22. Tirossh A, Papadakis GZ, Millo C, Hammoud D, Sadowski SM, Herscovitch P, et al. Prognostic Utility of Total (68)Ga-DOTATATE-Avid Tumor Volume in Patients With Neuroendocrine Tumors. Gastroenterology. 2018;154:998-1008 e1.

23. Toriihara A, Baratto L, Nobashi T, Park S, Hatami N, Davidzon G, et al. Prognostic value of somatostatin receptor expressing tumor volume calculated from (68)Ga-DOTATATE PET/CT in patients with well-differentiated neuroendocrine tumors. Eur J Nucl Med Mol Imaging. 2019;46:2244-51.

24. Chai SM, Brown IS, Kumarasinghe MP. Gastroenteropancreatic neuroendocrine neoplasms: selected pathology review and molecular updates. Histopathology. 2018;72:153-67.

25. Strosberg J, Kunz PL, Hendifar A, Yao J, Bushnell D, Kulke MH, et al. Impact of liver tumour burden, alkaline phosphatase elevation, and target lesion size on treatment outcomes with (177)Lu-Dotatate: an analysis of the NETTER-1 study. Eur J Nucl Med Mol Imaging. 2020.

26. Panzuto F, Nasoni S, Falconi M, Corleto VD, Capurso G, Cassetta S, et al. Prognostic factors and survival in endocrine tumor patients: comparison between gastrointestinal and pancreatic localization. Endocr Relat Cancer. 2005;12:1083-92.

27. Massironi SC, Rossi, R.E.; Spampatti, M.P.; Zilli, A.; Casazza, G.; Conte, D.; Ciafardini, C.; Peracchi, M. Prognostic Factors In Patients with Gastroenteropancreatic Neuroendocrine Neoplasms and Hepatic Metastases. JOP J Pancreas 2018;S(3):371-9.

28. Cavalcoli F, Rausa E, Conte D, Nicolini AF, Massironi S. Is there still a role for the hepatic locoregional treatment of metastatic neuroendocrine tumors in the era of systemic targeted therapies? World J Gastroenterol. 2017;23:2640-50.

29. Crown A, Rocha FG, Raghu P, Lin B, Funk G, Alseidi A, et al. Impact of initial imaging with gallium-68 dotatate PET/CT on diagnosis and management of patients with neuroendocrine tumors. J Surg Oncol. 2020;121:480-5.

30. Ambrosini V, Campana D, Polverari G, Peterle C, Diodato S, Ricci C, et al. Prognostic Value of 68Ga-DOTANOC PET/CT SUVmax in Patients with Neuroendocrine Tumors of the Pancreas. J Nucl Med. 2015;56:1843-8.

31. Carlsen EA, Johnbeck CB, Binderup T, Loft M, Pfeifer A, Mortensen J, et al. (64)Cu-DOTATATE PET/CT and Prediction of Overall and Progression-Free Survival in Patients with Neuroendocrine Neoplasms. J Nucl Med. 2020;61:1491-7.
32. Deppen SA, Liu E, Blume JD, Clanton J, Shi C, Jones-Jackson LB, et al. Safety and Efficacy of 68Ga-DOTATATE PET/CT for Diagnosis, Staging, and Treatment Management of Neuroendocrine Tumors. J Nucl Med. 2016;57:708-14.

33. Sharma P, Naswa N, Kc SS, Alvarado LA, Dwivedi AK, Yadav Y, et al. Comparison of the prognostic values of 68Ga-DOTANOC PET/CT and 18F-FDG PET/CT in patients with well-differentiated neuroendocrine tumor. Eur J Nucl Med Mol Imaging. 2014;41:2194-202.

34. Pauwels E, Cleeren F, Bormans G, Deroose CM. Somatostatin receptor PET ligands - the next generation for clinical practice. Am J Nucl Med Mol Imaging. 2018;8:311-31.

35. Zhang J, Liu Q, Singh A, Schuchardt C, Kulkarni HR, Baum RP. Prognostic Value of (18)F-FDG PET/CT in a Large Cohort of 495 Patients with Advanced Metastatic Neuroendocrine Neoplasms (NEN) Treated with Peptide Receptor Radionuclide Therapy (PRRT). J Nucl Med. 2020.

36. Waseem N, Aparici CM, Kunz PL. Evaluating the Role of Theranostics in Grade 3 Neuroendocrine Neoplasms. J Nucl Med. 2019;60:882-91.
Figure Legends

**Fig. 1** Flowchart illustrating the patient selection process.

**Fig. 2** Pre-therapy $^{68}$Ga-DOTATATE PET/CT of GEP-NETs patients with large and small extrahepatic $^{68}$Ga-DOTATATE avid tumor volume. (a) 60-year-old female with Grade 1 small bowel NETs and extensive $^{68}$Ga-DOTATATE avid hepatic metastases and peritoneal carcinomatosis (arrow to thickened right anterolateral peritoneum with intense DOTATATE uptake). The patient expired in 2 months. (b) 79-year-old female with Grade 1 small bowel NETs and extensive peritoneal, retroperitoneal and osseous metastases (arrows to L2 vertebral body sclerotic lesion with intense DOTATATE uptake). The patient expired in 3 months. (c) 71-year-old female with Grade 2 pancreatic NETs and small hepatic metastatic volume and no extrahepatic tumor volume. Patient has progressed at 31 months and alive at 33 months post-therapy. (d) 60-year-old female with Grade 1 pancreatic NETs and extensive $^{68}$Ga-DOTATATE avid hepatic metastases but small extrahepatic tumor volume. Patient has progressed at 30 months and alive at 33 months post-therapy.

**Fig. 3** (a) Kaplan-Meier analysis of PFS in GEP-NETs patients showed that smaller extrahepatic $^{68}$Ga-DOTATATE avid tumor volume (<140 mL) is associated with significantly longer PFS (Median PFS 29.0 ± 6.7 months vs 9.0 ± 1.7 months, $P = 0.0001$). (b) This trend in PFS is less prominent when total $^{68}$Ga-DOTATATE avid tumor volume (< 1000 mL) is analyzed (Median PFS 24.0 ± 5.4 months vs 10.0 ± 3.4 months, $P = 0.04$). (c) Kaplan-Meier analysis of OS showed that GEP-NETs patients with smaller extrahepatic $^{68}$Ga-DOTATATE avid tumor volume (<150 mL) is associated with significantly longer OS (Median OS not reached vs 44.0 ± 12.3 months, $P = 0.002$). (d) This association with OS is not statistically significant for total $^{68}$Ga-DOTATATE avid tumor volume ($P = 0.08$).
Fig. 4 (a) Kaplan-Meier survival analysis of PFS showed that smaller $^{68}$Ga-DOTATATE avid osseous tumor volume (< 50 mL) is associated with longer PFS (Median PFS 22.0 ± 2.4 months vs 7.0 ± 1.1 months, $P = 0.006$). (b) Such trend of PFS is observed in patients with larger $^{68}$Ga-DOTATATE avid hepatic tumor volume but less prominent ($P = 0.03$). (c) However, when $^{68}$Ga-DOTATATE avid hepatic tumor volume is grouped into low (<500 mL), medium (500-1000mL) and large (> 1000 mL) tumor volumes, no statistically significant difference in PFS is observed ($P = 0.19$). (d) No statistically significant difference is observed in PFS between groups with higher and lower SUV$_{\text{max}}$ values.

Fig. 5 GEP-NETs patients were grouped into large and small extrahepatic tumor volumes by visual inspection. Kaplan-Meier survival analysis of PFS showed similar association where smaller extrahepatic tumor volume is associated with longer PFS ($P = 0.005$).
Table 1 Patient Demographics and Clinical Characteristics

| Characteristic                       | Tumor Grade | Number of patients | Primary | Number of patients | Metastatic sites          | Number of patients |
|--------------------------------------|-------------|--------------------|---------|--------------------|---------------------------|--------------------|
| Age (yr)                             | Grade 1     | 15                 | Midgut  | 24                 | Liver                     | 47 (94%)           |
|                                      | Grade 2     | 31                 | Pancreas| 21                 | Peritoneal/Retroperitoneal| 27 (54%)           |
| Sex                                  | Grade 3 †   | 2                  | Unknown*| 5                  | Bone                      | 29 (58%)           |
| Female                               | Grade 3 †   | 2                  | Unknown*| 5                  | Bone                      | 29 (58%)           |
| Male                                 | Grade 3 †   | 2                  | Unknown*| 5                  | Bone                      | 29 (58%)           |
| Median time since diagnosis (yr)     | 4.8         |                    |         |                    |                           |                    |

* Five patients with unknown site of primary are most likely GEP origin based on progression and metastatic pattern.
† Two patients have well differentiated grade 3 NETs, therefore, were treated with $^{177}$Lu-DOTATATE.
‡ One patient’s tumor grade was not documented when it was initially diagnosed 16 years ago. One tumor grade was not available since only FNA was performed.
### Table 2 Number of $^{177}$Lu-DOTATATE Treatment and Side Effects

| Number of treatments | Number of patients * | Significant side effects | Number of patients |
|----------------------|----------------------|--------------------------|--------------------|
| 4                    | 36                   | Thrombocytopenia          | 5 †                |
| 3                    | 4                    | neutropenia and thrombocytopenia | 1                 |
| 2                    | 5                    | Low albumin              | 1                  |
| 1                    | 5                    | Elevated total bilirubin. | 1                  |

* Among 14 patients who did not complete all 4 treatments, 4 patients had side effects that did not meet screening criteria, 7 patients expired before completion of all 4 treatments (one patient developed neutropenia and thrombocytopenia, and one patient had elevated total bilirubin); 2 patients progressed and were switched to chemotherapy and 1 patient opted not to proceed.
† 2 patients recovered from mild thrombocytopenia and completed all four infusions.
63 patients received at least one dose of $^{177}$Lu-DOTATATE from 12/2016-7/2019.

6 with no pre-therapy PET available
3 Paragangliomas
1 Pheochromocytoma
1 Pulmonary NET
2 MEN-I with pancreatic NETs

50 patients with metastatic GEP-NETs who had pre-therapy $^{68}$Ga-DOTATATE PET.

At 32 months median follow up, 38 patients (76%) had disease progression and 15 patients (30%) died.
Fig. 2
Fig. 4

![Graphs showing progression-free survival over time](image)

**a**
Log Rank Test $P = 0.006$

- **68Ga-DOTATATE avid osseous tumor volume**
  - < 50
  - > 50

**b**
Log Rank Test $P = 0.03$

- **68Ga-DOTATATE avid hepatic tumor volume**
  - < 1250 mL
  - > 1250 mL

**c**
Log Rank Test $P = 0.19$

- **68Ga-DOTATATE avid hepatic tumor volume**
  - < 500 mL
  - 500 mL-1000 mL
  - > 1000 mL

**d**
Log Rank Test $P = 0.10$

- **SUVmax**
  - < 40
  - > 40
Fig. 5

Log Rank Test \( P = 0.005 \)

Extrahepatic tumor volume by visual inspection
- Small
- Large

Progression free survival

Time (months)
Supplement Tables

**Table S1** Kaplan-Meier analysis of PFS in GEP-NETs

**Total $^{68}$Ga-DOTATATE avid tumor volume**

| Dichotomy value (mL) | 600 | 800 | 1000 | 1200 | 1400 | 1600 |
|----------------------|-----|-----|------|------|------|------|
| $p$ value            | 0.56| 0.09| 0.04 | 0.05 | 0.03 | 0.02 |

**Extrahepatic $^{68}$Ga-DOTATATE avid tumor volume**

| Dichotomy value (mL) | 110 | 120 | 130 | 140 | 150 | 160 | 170 | 180 |
|----------------------|-----|-----|-----|-----|-----|-----|-----|-----|
| $p$ value            | 0.33| 0.09| 0.002| 0.0001| 0.002| 0.002| 0.003 | 0.007 |

**SUV$_{\text{max}}$**

| Dichotomy value | 35 | 40 | 45 | 50 | 55 | 60 | 65 |
|-----------------|----|----|----|----|----|----|----|
| $p$ value       | 0.54| 0.10| 0.37| 0.63| 0.53| 0.42| 0.42 |

**Table S2** Kaplan-Meier analysis of OS in GEP-NETs

**Total $^{68}$Ga-DOTATATE avid tumor volume**

| Dichotomy value (mL) | 600 | 800 | 1000 | 1200 | 1400 | 1600 |
|----------------------|-----|-----|------|------|------|------|
| $p$ value            | 0.89| 0.10| 0.08 | 0.39 | 0.17 | 0.26 |

**Extrahepatic $^{68}$Ga-DOTATATE avid tumor volume**

| Dichotomy value (mL) | 110 | 130 | 150 | 170 | 190 | 210 | 230 |
|----------------------|-----|-----|-----|-----|-----|-----|-----|
| $p$ value            | 0.12| 0.012| 0.002| 0.008| 0.01| 0.05| 0.12 |

**SUV$_{\text{max}}$**

| Dichotomy value | 40 | 45 | 50 | 55 | 60 | 65 | 70 |
|-----------------|----|----|----|----|----|----|----|
| $p$ value       | 0.44| 0.39| 0.98| 0.45| 0.60| 0.60| 0.99 |
**Table S3** Kaplan-Meier analysis of PFS with hepatic and osseous tumor volumes

**68Ga-DOTATATE avid hepatic tumor volume**

| Dichotomy value (mL) | 500 | 750 | 1000 | 1250 | 1500 | 1750 |
|----------------------|-----|-----|------|------|------|------|
| *p* value            | 0.95| 0.20| 0.13 | 0.03 | 0.02 | 0.001|

**68Ga-DOTATATE avid osseous tumor volume**

| Dichotomy value (mL) | 10 | 25 | 50 | 75 | 100 | 125 |
|----------------------|----|----|----|----|-----|-----|
| *p* value            | 0.91| 0.15| 0.006| 0.05| 0.05| 0.004|
Supplement Fig. Legend

**Fig. S1** Visual inspection and dichotomize extrahepatic $^{68}$Ga-DOTATATE avid tumor volume into two groups with (a) large ($N = 14$) and (b) small ($N = 36$) extrahepatic tumor volumes.
Supplement Fig. S1a
Supplement Fig. S1b