Radiotherapy and Oncology 141 (2019) 108–115

Contents lists available at ScienceDirect

Radiotherapy and Oncology

journal homepage: www.thegreenjournal.com

Original Article

^{68}Ga-DOTATATE PET/CT parameters predict response to peptide receptor radionuclide therapy in neuroendocrine tumours

Rohini Sharma [a,*], Wai Meng Wang [a], Siraj Yusuf [b], Joanne Evans [a], Ramya Ramaswami [a], Florian Wernig [c], Andrea Frilling [a], Francesco Mauri [a], Adil Al-Nahhas [b], Eric O. Aboagye [a], Tara D. Barwick [a,b]

[a] Department of Surgery and Cancer, Imperial College London; [b] Department of Nuclear Medicine, Imperial College NHS Trust; and [c] Department of Endocrinology, Imperial College London, UK

ARTICLE INFO

Article history:
Received 3 March 2019
Received in revised form 25 August 2019
Accepted 2 September 2019
Available online 18 September 2019

Keywords:
[^{68}Ga]-DOTATATE
SUV_{max}
Response assessment
Neuroendocrine tumours
Peptide receptor radiotherapy

ABSTRACT

Purpose: [^{177}Lu]DOTATATE prolongs progression free survival (PFS) in metastatic neuroendocrine tumours (NETs). However, objective response rate is low. This, coupled with long duration of therapy and expense suggest need for better selection. We aim to assess whether baseline [^{68}Ga]DOTATATE-PET/CT parameters, and whether response assessment by PET accurately predicts clinical outcome to [^{177}Lu]DOTATATE.

Experimental design: Retrospective study of patients receiving [^{177}Lu]DOTATATE was conducted. Patients were followed 3-monthly until disease progression. Four [^{68}Ga]DOTATATE-PET parameters (single lesion SUV_{max}, tumour to spleen and liver SUV ratios, and $SUV_{max\text{-}av}$ using up to five target lesions in multiple organ sites) were determined at baseline and follow-up. The association between these PET parameters either at baseline, or any changes following treatment, and PET response criteria (PERCIST and modified PERCIST) to predict PFS were determined. Patients were followed 3-monthly until disease progression. Response was determined using RECIST 1.1. Baseline SSTR2 expression was assessed and compared with PET parameters.

Results: 55 patients with metastatic NETs were identified predominantly small bowel ($N = 18$) and pancreatic ($N = 8$) in origin. 16 were low grade, 15 intermediate and 3 high grade. Response to PRRT ($N = 47$): partial response (PR) 28%, stable disease (SD) 60% progressive disease (PD) 13%. Response to PRRT predicted PFS: PR 71.8 months (95%CI: not achieved), SD 29.1 months (95%CI: 15.2–43.1), and PD 9.7 months (95%CI: 0–21.02). Baseline, single lesion SUV_{max} predicted both response and PFS with SUV cut-off of 13.0 giving high sensitivity and specificity. Tumoural SUV_{max} correlated with SSTR2 expression, Spearman's rho – 0.69, $p < 0.01$.

Conclusions: Baseline single lesion SUV_{max} and $SUV_{max\text{-}av}$ predicts response to [^{177}Lu]DOTATATE. Objective response following PRRT defines a subset of patients with markedly improved PFSBaseline SUV_{max} 13.0 defines a threshold below which patients have poor response to PRRT and worse PFS. SUV threshold analysis should be taken forward into prospective studies.

The introduction of peptide receptor radionuclide therapy (PRRT) represents a step change in the management of neuroendocrine tumours (NETs). The recently published NETTER-1 trial reported a significant improvement in progression free (PFS) and overall survival in those patients with small bowel NETs receiving lutetium oxodotreotide ([^{177}Lu]DOTATATE) compared to single agent octreotide, giving confirmation to a theranostic field that has been active for the past 20 years but lacked a definitive study [1]. PRRT exploits the presence of somatostatin receptors (SSTR) on the tumour surface, as indicated by imaging using radiolabeled somatostatin analogues (e.g. [^{68}Ga]DOTA(0)-Phe(1)-Tyr(3)-octreotide (DOTATOC) or -DOTA-(Tyr^3)-octreotate (DOTATATE)) [2]. PRRT consists of the administration of somatostatin analogues radiolabelled with long acting beta emitting radioisotopes, either [^{90}Y] or [^{177}Lu] that binds specifically to SSTR on the tumour surface delivering targeted, high dose radiation.

However, despite the improved survival rates, the goal of PRRT remains that of palliation. The objective response rate, both complete and partial, is only 30% [3–5]. Due to potential toxicities including renal toxicity and myelodysplasia coupled with the long

* Corresponding author at: Department of Surgery and Cancer, Imperial College London, Hammersmith Campus, Du Cane Road, W12 0HS London, UK.
E-mail address: r.sharma@imperial.ac.uk (R. Sharma).

https://doi.org/10.1016/j.radonc.2019.09.003
0167-8140/

duration of treatment, up to 12 months in some centres, and cost there is a need for baseline predictive response biomarkers in order to stratify those patients who will benefit clinically and those who will be exposed to a prolonged therapy without clinical utility [3,4].

The relationship between SSTR density and tumour response to PRRT has been previously considered, the premise being that the higher the tissue receptor density, the greater the binding of radiolabelled analogue, resulting in greater tumour kill [6,7]. Brunner and co-workers demonstrated that the expression of SSTR2 on tumour samples as delineated by immunohistochemistry (IHS) was an independent prognostic marker in NETs [6]. They also showed that SSTR2-IHS correlates with [^{68}Ga]DOTANOC-PET/CT uptake in the single "hottest" lesion. Kaemmerer and co-workers reported a relationship between SSTR2A and SSTR5 expression and SUV_{max} in a small number of NET patients ($n = 14$) imaged with [^{68}Ga]DOTANOC, indicating that tumour uptake on PET imaging is a surrogate biomarker of SSTR expression within the tumours [8]. Correlation with treatment response was not investigated. However, tumour biopsies are inherently limited in that only small aspect of the tumour is assessed and given that these tumours are heterogeneous, biopsies may not be representative of the receptor expression of the entire tumour. Given the association between SSTR tissue expression and uptake on [^{68}Ga]DOTANOC-PET, there is a move to define a minimum SUV threshold on baseline PET images that will define a good prognostic group.

Kratochwil and co-workers assessed 60 liver metastases in 30 patients with NETs, receiving PRRT, and defined a baseline threshold baseline SUV_{max} of >16.3 on [^{68}Ga]DOTANOC-PET that predicted lesion response, on contrast CT following 3 cycles of [^{177}Lu]DOTATATE, with a sensitivity of 90% and specificity of 60%. They proposed that this SUV cut off could be used to select patients for [^{177}Lu]DOTATATE [9]. However, the study is limited in that it only considers per lesion analysis of liver metastases whilst these tumours often spread widely, and no correlation was made with tissue expression of SSTR. Similarly, Oksuz and co-workers defined SUV_{max} of 17.9 as a discriminator of response to PRRT but the authors only considered a single lesion for response assessment [10]. Haug and co-workers, investigated whether [^{68}Ga]DOTATATE-PET could be used an early response marker to PRRT, reported that a reduction in tumour-to-spleen SUV ratio (in up to 3 target lesions/ patient) following 1 cycle of PRRT predicted PFS whilst reduction in SUV_{max} did not ($n = 33$) [11]. Whilst provocative, these small studies use differing PET response criteria and it is unclear which parameter to take forward in clinical trials, and some studies lack confirmation of SSTR2 in corresponding tissue samples, a step necessary in assessing true surrogacy of PET imaging. Furthermore, despite lacking sufficient evidence, the use of receptor PET imaging for assessing treatment response and surveillance is a practice that is gaining increasing traction.

Therefore, we explored the relationship between baseline PET parameters and PFS based on RECIST 1.1. [12]. The ability of best response to PRRT to predict PFS was also determined. In a subgroup of patients, we then evaluated the value of Positron Emission Tomography (PET) Response Criteria in Solid Tumours (PERCIST 1.0) and a modified PERCIST response assessment in predicting PFS. In an effort to identify a PET parameter predictive of clinical outcome, we compared the utility of the three studied PET uptake parameters, single lesion SUV_{max} of the "hottest" lesion, tumour to liver SUV ratio ($SUV_{T/H}$) and tumour to spleen SUV ratio $SUV_{T/S}$, as well investigating $SUV_{max\text{-}av,}$ where SUV uptake in up to 5 lesions was assessed, in determining a minimum threshold of [^{68}Ga]DOTATATE-PET uptake that would delineate PFS to [^{177}Lu]DOTATATE. The relationship between PET uptake parameters and tissue expression of SSTR2 was also determined.

Materials and methods

Study population

Consecutive patients who underwent as least one cycle of [^{177}Lu]DOTATATE for the management of histologically proven, metastatic NET were included (Imperial College Healthcare NHS Trust (ICHNT), London). All patients had undergone both CT chest, abdomen and pelvis and SSR functional imaging prior to and following 4 cycles of [^{177}Lu]DOTATATE. Functional imaging was ideally performed in the week prior to monthly SSA injection. Patients were reviewed clinically 2 weeks prior to and 2 weeks after each cycle of [^{177}Lu]DOTATATE where routine blood tests including chromogranin A and B (CgA and CgB) were performed. Complete clinical and follow up information including patient's demographics, tumour size and stage at diagnosis, and site of presentation was collected by review of medical records. Tumour blocks were retrieved. All patients gave fully informed consent prior to administration of [^{177}Lu]DOTATATE. The study was approved by the Imperial College Healthcare Tissue Bank, project R14014, (ICHTB HTA license: 12275; REC Wales approval: 12/WA/0196) and was study was conducted in accordance with the Declaration of Helsinki (2000)

PET/CT protocol

All patients were scanned on a Siemens Biograph 64-slice PET/CT scanner PET/CT scanner following injection of 102 MBq (±29.6%) of [^{68}Ga]DOTATATE (Mallinkrodt, London). The uptake period was 45 minutes. CT scan was performed from thighs to skull vertex (5 mm thickness with 3 mm spacing, 120 kVp, 50 mAs, 0.8 spiral pitch). The PET scan parameters were 5–6 overlapping bed positions or 3 min. Images were reconstructed in 3 dimensions using ordered subset expectation maximisation iterative algorithm (4 iterations, 8 subsets). The images were attenuation-corrected using the CT data. In the cohort with both baseline and follow-up PET/CT scans, these were all performed on the same PET scanner. The [^{68}Ga]DOTATATE radioactivity concentration was normalised for injected radioactivity and body weight in order to obtain the standardised uptake value (SUV) on baseline and post-treatment studies.

Image analysis

Lesions were defined as target lesions by RECIST 1.1 on the baseline contrast enhanced CT (CECT). The diameter of the target lesions was measured using electronic calipers on the PACS workstation (Carestream) using unidimensional measurement of longest axis (short axis for lymph nodes) by a radiologist with 6 years of experience blinded to the PET data. Response of the target lesions was assessed using RECIST 1.1. PET analysis was performed on a dedicated PET workstation (Hermes Medical Solutions, Sweden) by two experienced nuclear medicine radiologists (SY and TB). Physicians were blinded to patient Ki-67, patient response and survival outcome at the time of analysis.

Either a single lesion with maximal uptake on PET, "PERCIST" or up to five lesions (maximum 2 target lesions per organ) on the [^{68}Ga]DOTATATE-PET/CT, showing an increased visual uptake were considered as target lesions, "modified PERCIST". The lesion on PET at baseline with the highest uptake was always selected as a target. Some targets, such as bone metastases that were occult on the CECT were included as targets lesions for PET analysis. The same target lesions were used for analyses before and after treatment. In each PET scan, the SUV_{max} was measured by choosing the regions of interest (ROIs) in the tumours with the highest tracer uptake. For large tumours, the ROI was moved over the tumour

to ensure the region with the highest SUV_{max} was obtained. For background liver and spleen the SUV mean in a 3 cm diameter spherical ROI was measured taking care to avoid any sites of disease [13].

Where tumour blocks were available, the baseline scans were reviewed and the lesion that had been biopsied or removed, identified. The SUV of the resected/biopsied lesion was then determined (SUV_T).

PET response assessment was determined by consideration of SUV data. Standard PERCIST and a modified PERCIST criteria were used. Standard PERCIST identifies a single target lesion by which response is defined [13]. As these tumours metastasize widely, we considered the mean percentage reduction in SUV_{max} of up to 5 lesions ($SUV_{max\text{-}av}$), with no more than 2 lesions per organ site, analogous to recommendations for CT assessment in RECIST 1.1. As with PERCIST, the mean percentage reduction in average SUV_{max} was calculated as: $(SUV_{post} - SUV_{pre})/SUV_{pre}$). Changes in $SUV_{max\text{-}tumour}/SUV_{mean\ background\ spleen}$ ($SUV_{T/S}$) and $SUV_{max\ tumour}/SUV_{mean\ background\ liver}$ ($SUV_{T/H}$) in the same selected target lesions were also assessed in response to treatment. As per standard of care at ICHNT, patients were scanned with conventional CT imaging every 3 months until disease progression. Standard RECIST 1.1 assessment was performed on subsequent imaging until disease progression.

Histopathology

Formalin fixed, paraffin embedded specimens and matching haematoxylin and eosin (H&E) slides were retrieved from the local pathology archive. Five μm thick sections were de-paraffinized in xylene and rehydrated in graded alcohols. Optimal heat mediated antigen retrieval conditions were applied according to manufacturer's recommendations in relation to the primary antibody, using a water bath heated to 100 °C. Incubation in citrate buffer at pH 6.0 for 20 min. Before immunostaining, slides were cooled at room temperature and endogenous peroxidase activity was suppressed by incubation with CAS-Block (Invitrogen, Camarillo, California, USA for 5 min. The primary antibody against SSTR2A (UMB1, Abcam, Cambridge, UK) was used at a 1:250 dilution overnight. Slides were washed with buffered TRIS solution and blocked with Novolink polymer (Leica, Milton Keynes, UK) for 30 min, and subsequently developed with diaminobenzidine and Mayer's Haematoxylin counterstaining. Appropriately, selected tissue sections were used according to the manufacturer's instruction as external positive control during each reaction. Negative control reactions were performed omitting the primary antibodies from the dilution buffer. This resulted in a complete absence of staining in all cases. A trained histopathologist (FM) blinded to the clinical data scored all the cases and a consensus score was reached in any case of discrepancy, with a second pathologist if necessary. Tissue samples were scored manually using the immunohistochemical score. Briefly, each sample can be assigned an IHS ranging between 0 to 300, based on the multiplication of the percentage of cells showing immunohistochemical expression (0–100) by the intensity of the signal (graded 1–3).

Statistics

The relationship between best overall response to PRRT by RECIST 1.1 and four PET parameters, single lesion SUV_{max}, $SUV_{max\text{-}av}$, $SUV_{T/H}$ and $SUV_{T/H}$, was determined by one-way ANOVA with Bonferroni correction. We sought to determine an optimum cut-off value of $SUV_{max\text{-}av}$ on baseline PET imaging that would predict a response to PRRT as determined by receiver operating characteristic (ROC) analysis. The accuracy of single lesion SUV_{max} and $SUV_{max\text{-}av}$ was estimated from the area under the curve (AUC) in the ROC analysis. Any association of response status using PERCIST, modified PERCIST and RECIST 1.1 criteria was tested using Cohen's kappa statistic. Spearman's rank correlation coefficient test was used to assess the correlation between SSTR2 IHS, SUV and tumour response using PERCIST type criteria and RECIST 1.1 criteria. Mean values with standard deviation (SD) or median values with interquartile ranges (IQR) were reported for continuous variables, whereas for categorical variables absolute frequency and corresponding percentage were reported. PFS was defined as the interval from first [^{177}Lu]DOTATATE dose to the time of disease progression. The ability of the cut-off identified and RECIST 1.1 response to predict PFS was determined using Kaplan-Meier analyses and compared between groups using log-rank test. If the survival rate was significantly different, hazard ratio (HR) was reported using Cox regression analysis with the assumption of proportional hazards. Survival measures were calculated from date of diagnosis to date of death or date of last follow-up. A $P \leq 0.05$ was considered significant. Statistical analyses were performed using SPSS statistical package version 22 (SPSS Inc., Chicago, IL, USA).

Results

Fifty five consecutive patients (29 men, 26 women) were identified as having received at least one cycle of [^{177}Lu]DOTATATE with a total of 182 doses administered. The mean cycle activity of [^{177}Lu]DOTATATE administered was 7.2 ± 0.08 GBq. Treatment was well tolerated with no grade 3/4 adverse events noted. All patients had stage IV disease with the most common metastatic sites being liver and bone. The most prevalent primary site was small bowel, 31% ($n = 18$) followed by pancreatic (pNET) 14% ($n = 8$). Demographic data is presented in Table 1. The median interval between baseline PET/CT and cycle 1 of [^{177}Lu]DOTATATE was 3.9 ± 8 months. Thirty eight patients (69%) completed 4 cycles of PRRT. Nine patients did not complete four cycles of PRRT due to disease progression during therapy, four patients had unsatisfac-

Table 1
Summary of patient baseline characteristic ($n = 55$).

Characteristics	N (%)
Age	
Median age (years)	56 (range 16–78)
Gender	
Male	29 (53)
Female	26 (47)
Type of NET	
Small bowel	20 (36)
Pancreas	8 (14)
Paraganglioma	6 (11)
Bronchial	6 (11)
Gastric	2 (4)
Large bowel	2 (4)
Medullary thyroid carcinoma	2 (4)
Ovarian	1 (2)
Unknown origin	8 (14)
Grade	
Low	16 (29)
Intermediate	15 (27)
High	3 (5)
Unknown	21 (38)
Cycles of PRRT received	
1	8 (15)
2	5 (9)
3	4 (7)
4	38 (69)
Baseline chromogranins (median (IQR))	
Chromogranin A, pmol/L (<60 pmol/L)	180.0 (46.0–561.0)
Chromogranin B, pmol/L (<150 pmol/L)	142.0 (84–267)

tory tracer uptake in post-therapy SPECT imaging despite good uptake on baseline [^{68}Ga]DOTATATE imaging and a multidisciplinary meeting decision was taken not to continue treatment; two patients were lost to follow-up and two patients were deemed medically unfit to complete 4 cycles of PRRT (supplementary Fig. 1). No patients received therapy between date of last PRRT treatment and date of progression or last follow-up.

Forty-seven patients (86%) were evaluable for response assessment using RECIST 1.1 criteria. Four patients had unsatisfactory tracer uptake in post-therapy SPECT imaging; two patients were lost to follow-up and two patients were deemed medically unfit to complete 4 cycles of PRRT. The median interval between the final PRRT treatment and restaging imaging was 2.5 months (range 0.1–14.1). When considering the duration between final PRRT treatment and restaging imaging, none of the patient who were scanned more than 3 months after the final PRRT dose had progressive disease on imaging. Based on RECIST 1.1, partial response (PR) was reported in 13 (28%) patients, stable disease (SD) in 28 (60%) and six patients (13%) had progressive disease. The median follow-up period was 24 months. The median PFS was 39.0 months (95% CI: 30.67–47.38). On univariate analysis best response to PRRT as determined by CT imaging at the completion of therapy was predictive of PFS with patients experiencing a treatment response according to RECIST 1.1 having a median PFS of 71.8 months (95%CI: not achieved), 2.5× that of those with SD, 29.1 months (95%CI: 15.2–43.1), and almost 20x as long as those patients experiencing PD, 9.7 months (95%CI: 0–21.02), P < 0.01 (Fig. 1).

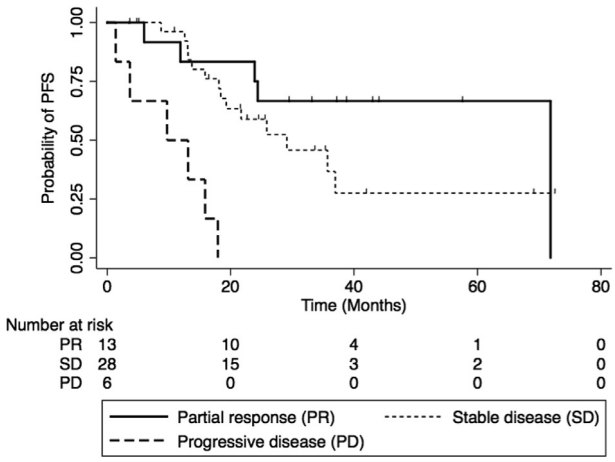

Fig. 1. Kaplan Meier curve analysis indicating progression free survival (PFS) in patients with NETs according to best response to PRRT at the completion of treatment (RECIST 1.1), (PD – progressive disease, SD – stable disease, PR – partial response).

First, we considered whether baseline [^{68}Ga]DOTATATE-PET parameters were predictive of response to PRRT (RECIST 1.1). Forty-two patients (76%) had baseline PET images available for assessment. The remaining patients had undergone baseline scintigraphy scans with [^{111}In]-diethylenetriamine pentaacetic acid-octreotide and were not included. Given that patients with PR and SD are clinically regarded as being similar in terms of subsequent therapy, patients were classified based on subsequent treatment pathways into two groups, responders (PR and SD) and non-responders (PD), based on evaluation of best overall response using RECIST 1.1, for subsequent statistical analyses. Considering single lesion uptake, the median SUV_{max} at baseline was 21.5 (IQR range 12.6–39.1). Single lesion SUV_{max} was predictive of response to PRRT such that responding patients had higher baseline uptake compared to non-responders ($p = 0.031$) (Fig. 2A). We explored the relationship between single lesion SUV_{max} and lesion size. No correlation between lesion size and SUV_{max} was observed. The median $SUV_{max\text{-}av}$ at baseline was 15.03 (IQR range 9.7–32.1), and $SUV_{max\text{-}av}$ was predictive of response to PRRT such that responding patients had higher baseline uptake compared to non-responders ($p = 0.039$) (Fig. 2B). The median $SUV_{T/H}$ at baseline

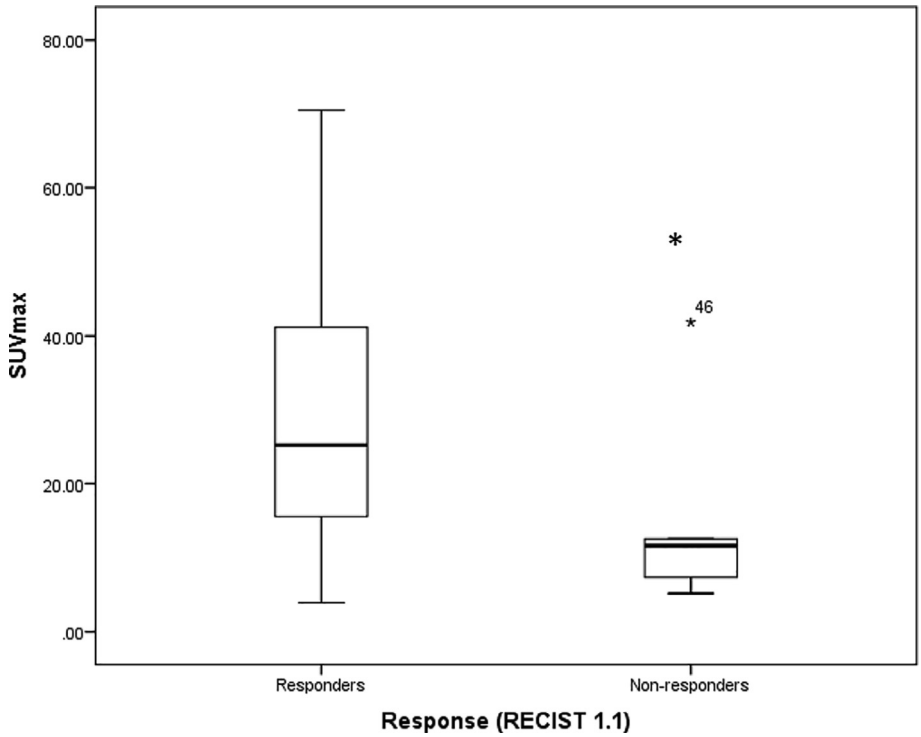

Fig. 2A. Box plots showing association between response (RECIST 1.1) and baseline SUVmax of a single lesion (*p < 0.05, ANOVA).

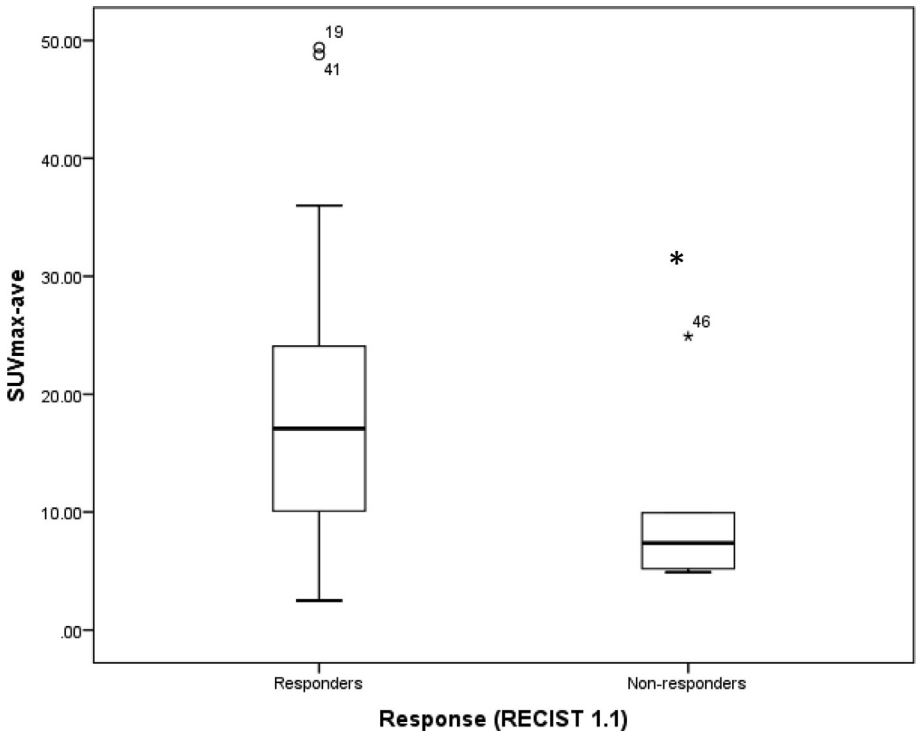

Fig. 2B. Box plots showing association between response (RECIST 1.1) and baseline SUVmax-av (*p < 0.05, ANOVA).

was 4.9 (IQR 12.6–39.1) and the median $SUV_{T/S}$ at baseline was 1.8 (IQR 9.3–22.8). Neither $SUV_{T/S}$ or $SUV_{T/H}$ were predictive of response to PRRT.

ROC curve analysis was used to establish a baseline SUV_{max} and $SUV_{max\text{-}av}$ below which patients were unlikely to respond to [^{177}Lu]DOTATATE. When considering single lesion SUV_{max}, the optimal cut-off was 13.0, giving a sensitivity and specificity of 0.83 (95% CI: 0.36–1.0) and 0.84 (95% CI: 0.67–0.95) ($p = 0.031$), respectively, AUC 0.78. The positive likelihood ratio was 5.3. The optimal cut-off point for $SUV_{max\text{-}av}$ that predicted treatment response was 10.2, and using the threshold value of 10.2 for $SUV_{max\text{-}av}$, the sensitivity and specificity in prognosticating a favourable treatment response was 0.80 (95% CI 0.67–0.96) and 0.83 (95% CI 0.45–1.15), respectively, AUC 0.78. The positive likelihood ratio was 4.08 (Fig. 3A–D).

In terms of PFS, a SUV_{max} of 13.0 was predictive of PFS, HR 2.5 (95%CI: 1.06–6.09; $p = 0.03$), such that patients with SUV_{max} of 13 or greater had a median survival of 45.1 months compared to 19.9 months in those with a maximal lesional uptake of less than 13. $SUV_{max\text{-}av}$ of less than 10.2 was not predictive for PFS (Fig. 4).

A subgroup of 25 patients (46%) had both baseline and follow-up [^{68}Ga]DOTATATE imaging and were evaluable for PET response. The main reason for patients not having baseline or a follow-up [^{68}Ga]DOTATATE-PET/CT was the use of baseline [^{111}In]-octreotide scintigraphy in 8 patients (15%) from external referral centres where [^{68}Ga]DOTATATE was not available, no follow-up scans conducted by the treating physician ($n = 7$, 13%) and progressive disease seen on routine CECT imaging ($n = 8$, 15%). Patients who did not have baseline [^{68}Ga]DOTATATE-PET imaging were excluded from this analysis. The median interval between the final PRRT treatment and restaging imaging using PET was 17.1 months (range 7.2–25.9). The main reason for the long duration was the lack of availability of PET tracer. Changes in PET uptake parameters were derived and correlated with clinical outcome (supplementary Figs. 2A–D). In terms of PERCIST, change in SUV_{max} did not predict for PFS. Change in $SUV_{max\text{-}av}$, modified PERCIST, predicted for PFS (HR 1.01, 95% CI: 1.0–1.02, $p = 0.024$). Neither change in $SUV_{T/H}$ or $SUV_{T/S}$ predicted PFS. No concordance was observed between PERCIST or PERCIST type response assessment and RECIST 1.1.

Tumour blocks from 27 subjects were retrieved. Fifteen were primary tumours and 12 were metastatic deposits. SSTR2 IHS was obtained in 23 patients; mean IHS score 177.2 ± 90.26. Matched SUV from the tumour (SUV_{TUM}) and $SUV_{T/S}$ were available in 21 patients. A positive correlation was observed between the SSTR IHS score and SUV_{TUM}, Spearman's rho 0.69, $p < 0.01$. A trend was observed between and $SUV_{T/S}$ and SSTR2 HIS, Spearman's rho 0.59, $p = 0.05$.

Discussion

Our study shows that baseline [^{68}Ga]DOTATATE-PET/CT has the potential to stratify patients likely to benefit from [^{177}Lu]DOTATATE therapy both in terms of response and PFS. In particular, we considered three PET uptake parameters that have previously been explored as predictors of response; SUV_{max} of a single lesion, $SUV_{T/H}$ and $SUV_{T/S}$. We also investigated $SUV_{max\text{-}av}$ where up to five lesions, maximum 2 target lesions per organ, are considered. We have shown that both baseline SUV_{max} of a single lesion and $SUV_{max\text{-}av}$ predict response to PRRT. However, only single lesion SUV_{max} predicted for PFS, whereby patients with SUV ≥13.0 had a PFS twice that of patients with SUV <13.0. Importantly, we have shown that SUV correlates with SSTR2 expression in tumoural tissue, and finally that response assessment – change in lesion uptake over time – using PET does not predict response or PFS.

The NETTER-1 trial has established that [^{177}Lu]DOTATATE therapy prolongs PFS compared to high dose octreotide LAR bringing together a decade of early phase and retrospective trials [1,14–16]. However, the reported objective response rate, in both NETTER-1 and our study, was only 20–30%, indicating capacity to improve patient selection [1]. What is clear from both our results, and those of others, is that there is a marked difference in PFS in those patients experiencing radiologic response to therapy, up to

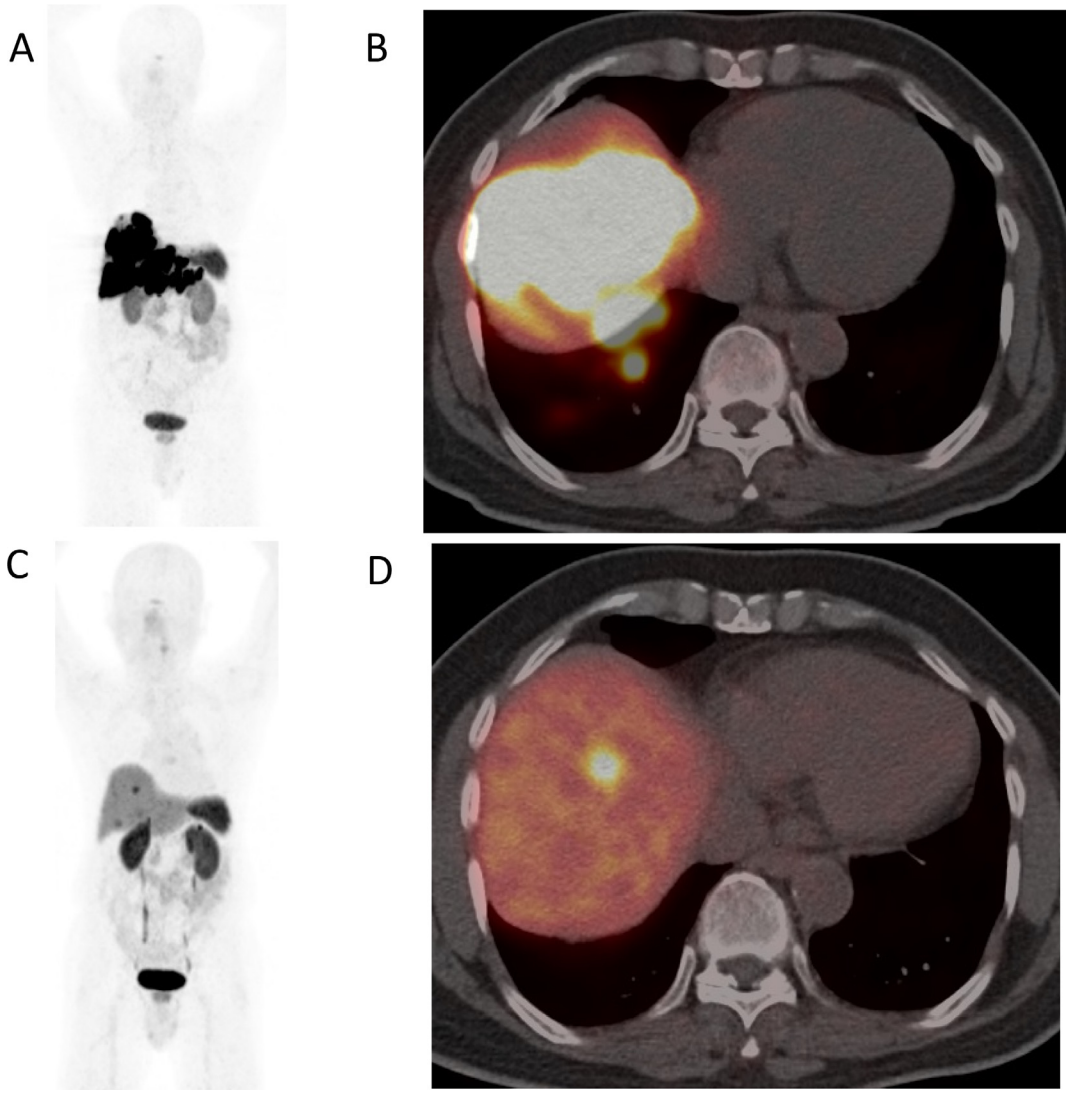

Fig. 3. (A and B) Baseline [^{68}Ga]DOTATATE-PET/CT scan indicating uptake >13.0 and following 4 cycles of PRRT (C and D) post treatment [^{68}Ga]DOTATATE-PET/CT illustrating good response to therapy.

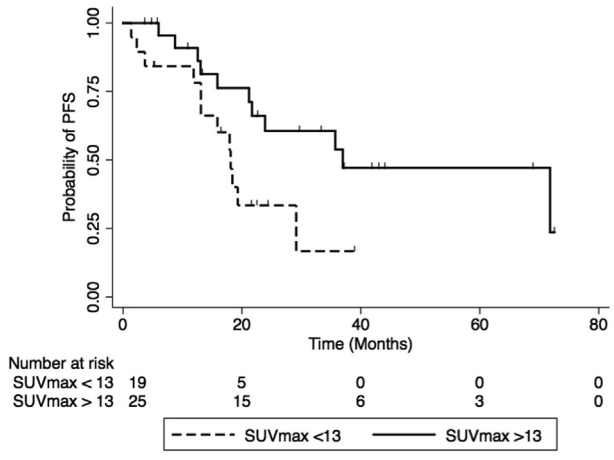

Fig. 4. Kaplan Meier curve analysis indicating progression free survival (PFS) in patients with NETs according to single "hottest" SUV_{max}<or ≥13.0.

20 fold difference [14,17]. There is a strong rationale therefore, to attempt to identify potential biomarkers of radiologic response particularly as patients undergoing [^{177}Lu]DOTATATE therapy are committed to up 12 months of therapy, a long time frame in the palliative setting. Moreover, given the high cost of a single dose of [^{177}Lu]DOTATATE, currently £10,000 in the United Kingdom, the need to delineate a patient population more likely to respond will have significant economic implications to healthcare authorities. Whilst [^{68}Ga]DOTATATE-PET is widely used to assess the presence of SSTR2 on the tumour surface and therefore suitability for [^{177}Lu]DOTATATE, little work has been done to define objective biomarkers of treatment response. The primary aim of this study was to investigate previously suggested PET parameters as well as a novel parameter $SUV_{max\text{-}av}$ to delineate a minimum threshold of PET uptake that stratifies patients for response to [^{177}Lu]DOTATATE therapy. We then sought to correlate PET uptake parameters with tumoural SSTR2 expression, and finally, we sought to explore whether response on [^{68}Ga]DOTATATE-PET could predict outcome compared to conventional imaging. Whilst there have been a number of studies investigating these concepts, to our knowledge our study is the first study to comprehensively bring these concepts together in a consecutive series of patients receiving [^{177}Lu]DOTATATE.

The premise of our hypothesis is that [^{177}Lu]DOTATATE will be more effective in those patients with greater phenotypic expression of SSTRs. This concept is supported by the work of Campana

and co-workers who determined that single lesion SUV_{max} of 19.3 on [^{68}Ga]DOTANOC-PET/CT imaging, differentiated responders versus non-responders to cold octreotide (17). The authors also reported a correlation between tumoural SSTR2 expression and SUV_{max}, supporting our findings and those of others [8,18,19]. In support of our findings, Ambrosini and co-workers, defined a minimum SUV_{max} of 37.8, again on the single "hottest" lesion, that differentiated responders and non-responders to a variety of therapies [20]. Moreover, the SUV_{max} cut-off defined by the authors was also predictive of PFS. Ambrosini only considered pancreatic NETs which are recognised to have higher uptake on [^{68}Ga] DOTANOC-PET imaging which may explain the difference between identified SUV_{max} [17].

We had surmised that there are inherent limitations in assessing liver metastases only in this disease setting where patients often present with extensive hepatic and extra-hepatic disease, where the presence of extrahepatic disease has been shown to be an independent predictor of PFS [14]. In addition, using per lesion response analysis as opposed to a 'per patient' analysis is not clinically useful when making therapeutic decisions a concept supported by Gabriel and co-workers who reported significant variation in SUV_{max} of single lesions and no correlation with outcome [21]. However, we found that both a single lesion and five lesion analysis resulted in baseline SUV_{max} cut-off values that predicted a greater likelihood of response to [^{177}Lu]DOTATATE. Moreover, SUV_{max} of the "hottest" lesion on PET was predictive of PFS such that patients with $SUV_{max} \geq 13.0$ had survival twice that of those with $SUV_{max} < 13.0$.

The "hottest" lesion on [^{68}Ga]DOTATATE-PET corresponds to the lesion with the greatest density of SSTR receptors. As [^{177}Lu] DOTATATE binds to specifically to SSTRs, it follows that these patients will have a better response and therefore improved PFS. However, radiobiology of [^{177}Lu]DOTATATE will depend on a number of other tumoural factors in particular tumour size. [^{177}Lu] is a β emitter and as such will only penetrate a depth of 0.23 mm and consequently be less efficacious in larger tumours; a concept behind the use of both [^{177}Lu]- and [^{90}Y]DOTATATE for the management of these tumours [22]. Furthermore, larger tumours will have reduced blood supply and a degree of hypoxia, a well-established radioresistance mechanism in radiotherapy. This is the basis of our rationale for considering per patient analysis, however this approach was not borne out by the results of this study.

Tumour to organ ratios are felt to be less variable between different scanners/institutions than SUV and hence we investigated the role of both SUV tumour normalised to liver and spleen [23]. Neither parameter was found to be predictive of response, contrary to the findings of Kratochowil and co-workers who reported that both $SUV_{T/H}$ and $SUV_{T/S}$ significantly predicted per lesional response but found SUV_{max} to be a better metric of response [9]. They suggested if no reliable SUV cut offs have been established in a particular institution that $SUV_{T/H}$ ratio should be used to predict lesional response for liver metastases.

The other concept explored in this study is the use of PET imaging to follow response to PRRT. This has been assessed by both Haug and Gabriel [11,21]. Haug and co-workers repeated PET imaging following one cycle of PRRT, and reported that a reduction in $SUV_{T/S}$ but not SUV_{max} was a significant predictor of PFS in a small group of patients ($n = 33$). However, Gabriel and co-workers reported no utility in using PET imaging for response assessment following PRRT. Whilst true PERCIST response assessment has only been utilised in FDG-PET, we have applied the criteria to our dataset, not only in terms of lesion selection but for assessing reduction in receptor uptake. This is an important issue as [^{68}Ga]DOTATATE-PET/CT is increasingly being used to monitor treatment response in the clinical setting. We found that there was no correlation between PERCIST and RECIST 1.1 response assessment and furthermore, PERCIST-type response assessment did not predict for PFS. It is likely that uptake on receptor scanning may not change significantly in the presence of residual disease, a finding we corroborated in terms of a lack of concordance between the two imaging modalities, thereby suggesting that [^{68}Ga] DOTATATE-PET should not be used to monitor treatment response, a position consistent with ENETs guidelines [13,21].

There are a number of limitations of our study including the retrospective nature, the small sample size and missing data. There is a large amount of missing data, heterogeneity in the primary tumour type and in the timing of the scans consistent with the retrospective, real-world nature of the study, all which may contribute to inclusion bias and may confound the results. There were a number of reasons why patients did not complete four cycles of PRRT as outlined, which resulted in 14% of patients being excluded from the response analysis, and this should be considered when interpreting the results. Nonetheless, our results are in line with the recently published NETTER-1 trial and are provocative in suggesting that baseline [^{68}Ga]DOTATATE-PET/CT uptake parameters can be used to identify a subset of patients who will have good clinical outcome to PRRT. Given the association between SUV of the tumour sample and SSTR2 expression, we hypothesise that those patients with a SUV >13.0 will have a greater density of receptors on the tumour surface available for binding to [^{177}Lu]DOTATATE. However, it should be noted that SUV_{max} measurement is dependent on a number of variables including scanner acquisition parameters, reconstructions parameters and biological factors [24]. The SUV_{max} cut off delineated in our study may not be directly transferable to different scanners with different acquisition and reconstruction parameters.

The latest PET/CT scanners can permit whole body dynamic imaging (4D PET) which has the potential to assess rate of tracer accumulation radiotracer absorption coefficient (Ki) for a lesion which may be better than static SUV parameters. Dynamic 4D PET has not yet been explored in NETs however there is a prospective trial in France currently recruiting which aims to evaluate the prognostic value of the tumour absorption coefficient Ki resulting from a 4D whole-body dynamic acquisition in PET/CT at [^{68}Ga] DOTATOC in patients with well-differentiated NETs [25]. In addition, a recent study has reported total volume of tumour on [^{68}Ga]DOTATATE-PET/CT correlated with tumours markers and was associated with metastatic disease[26]. However, the method for total tumour outlining has not been established and requires further work. Radiomic assessment of the total tumour volume is also of interest

More work need to be conducted to delineate further mechanisms to enhance the cytotoxicity of [^{177}Lu]DOTATATE. In a recent study by Torrasani et al., they highlight the role of epigenetic silencing of SSTR2, which can be reversed using demethylating agents, thereby enhancing SUV uptake on [^{68}Ga]DOTA-PET imaging [27]. Graf and co-workers also suggest that the presence of ATM mutations within the tumour may impact on sensitivity to [^{177}Lu]DOTATATE, a mechanism that may be reversed with the use of PARP inhibitors prior to the administration of [^{177}Lu]DOTATATE [28]. The recent development of radiolabeled somatostatin antagonists may also hold therapeutic promise. Unlike, the agonist [^{177}Lu]DOTATATE, antagonists bind with greater affinity to the SSTR2, delivering a greater therapeutic radiation dose to the tumour. The role of on-treatment dosimetry in patients has not been sufficiently explored with [^{177}Lu]DOTATATE, an important concept particularly in determining the actual dose of radiation delivered to the tumour. Correlating dosimetry with tissue biopsy may aid in understanding mechanism impacting on [^{177}Lu]DOTATATE delivery.

We have shown that baseline SUV_{max} of a single lesion, of 13 or greater, predicts both treatment response and PFS following [^{177}Lu]

DOTATATE suggesting that SUV threshold should be taken into consideration, rather than just visual assessment, when selecting patients for PRRT. Moreover, we have shown a correlation between SSTR2 expression and PET uptake. In terms of PFS, both baseline SUV_{max} and RECIST 1.1 were accurate predictors of outcome. Whilst of clinical interest, these findings need confirmation in a prospective study to further delineate the SUV cut-off as a response biomarker.

Funding

Study funded by an Imperial NIHR Biomedical Research Centre award to RS. The authors would like to acknowledge support from Imperial Experimental Cancer Medicines Centre, Cancer Research UK (C2536/A16584) and the UK Medical Research Council (MR/J007986/1).

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.radonc.2019.09.003.

References

[1] Strosberg J, El-Haddad G, Wolin E, Hendifar A, Yao J, Chasen B, et al. Phase 3 trial of 177Lu-dotatate for midgut neuroendocrine tumors. N Engl J Med 2017;376:125–35.
[2] Kam BL, Teunissen JJ, Krenning EP, de Herder WW, Khan S, van Vliet EI, et al. Lutetium-labelled peptides for therapy of neuroendocrine tumours. Eur J Nucl Med Mol Imaging 2012;39:S103–12.
[3] Strosberg J, Krenning E. 177Lu-dotatate for midgut neuroendocrine tumors. N Engl J Med. 2017;376:1391–2.
[4] Muros MA, Varsavsky M, Iglesias Rozas P, Valdivia J, Delgado JR, Forrer F, et al. Outcome of treating advanced neuroendocrine tumours with radiolabelled somatostatin analogues. Clin Transl Oncol 2009;11:48–53.
[5] Grozinsky-Glasberg S, Barak D, Fraenkel M, Walter MA, Mueller-Brand J, Eckstein J, et al. Peptide receptor radioligand therapy is an effective treatment for the long-term stabilization of malignant gastrinomas. Cancer 2011;117:1377–85.
[6] Brunner P, Jorg AC, Glatz K, Bubendorf L, Radojewski P, Umlauft M, et al. The prognostic and predictive value of sstr2-immunohistochemistry and sstr2-targeted imaging in neuroendocrine tumors. Eur J Nucl Med Mol Imaging 2017;44:468–75.
[7] Lapa C, Hanscheid H, Wild V, Pelzer T, Schirbel A, Werner RA, et al. Somatostatin receptor expression in small cell lung cancer as a prognostic marker and a target for peptide receptor radionuclide therapy. Oncotarget 2016;7:20033–40.
[8] Kaemmerer D, Peter L, Lupp A, Schulz S, Sanger J, Prasad V, et al. Molecular imaging with Ga-68-SSTR PET/CT and correlation to immunohistochemistry of somatostatin receptors in neuroendocrine tumours. Eur J Nucl Med Mol Imaging 2011;38:1659–68.
[9] Kratochwil C, Stefanova M, Mavriopoulou E, Holland-Letz T, Dimitrakopoulou-Strauss A, Afshar-Oromieh A, et al. SUV of [68Ga]DOTATOC-PET/CT predicts response probability of PRRT in neuroendocrine tumors. Mol Imaging Biol 2015;17:313–8.
[10] Oksuz MO, Winter L, Pfannenberg C, Reischl G, Mussig K, Bares R, et al. Peptide receptor radionuclide therapy of neuroendocrine tumors with (90)Y-DOTATOC: is treatment response predictable by pre-therapeutic uptake of (68)Ga-DOTATOC?. Diagn Interv Imaging 2014;95:289–300.
[11] Haug AR, Auernhammer CJ, Wangler B, Schmidt GP, Uebleis C, Goke B, et al. 68Ga-DOTATATE PET/CT for the early prediction of response to somatostatin receptor-mediated radionuclide therapy in patients with well-differentiated neuroendocrine tumors. J Nucl Med 2010;51:1349–56.
[12] Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D, Verweij J. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009;45:228–47.
[13] Wahl RL, Jacene H, Kasamon Y, Lodge MA. From RECIST to PERCIST: evolving considerations for PET response criteria in solid tumors. J Nucl Med 2009;50:122S–50S.
[14] Kwekkeboom DJ, de Herder WW, Kam BL, van Eijck CH, van Essen M, Kooij PP, et al. Treatment with the radiolabeled somatostatin analog [177 Lu-DOTA 0, Tyr3]octreotate: toxicity, efficacy, and survival. J Clin Oncol 2008;26:2124–30.
[15] Delpassand ES, Samarghandi A, Zamanian S, Wolin EM, Hamiditabar M, Espenan GD, et al. Peptide receptor radionuclide therapy with 177Lu-DOTATATE for patients with somatostatin receptor-expressing neuroendocrine tumors: the first US phase 2 experience. Pancreas 2014;43:518–25.
[16] Ezziddin S, Khalaf F, Vanezi M, Haslerud T, Mayer K, Al Zreiqat A, et al. Outcome of peptide receptor radionuclide therapy with 177Lu-octreotate in advanced grade 1/2 pancreatic neuroendocrine tumours. Eur J Nucl Med Mol Imaging 2014;41:925–33.
[17] 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.
[18] Miederer M, Seidl S, Buck A, Scheidhauer K, Wester HJ, Schwaiger M, et al. Correlation of immunohistopathological expression of somatostatin receptor 2 with standardised uptake values in 68Ga-DOTATOC PET/CT. Eur J Nucl Med Mol Imaging 2009;36:48–52.
[19] Leyton J, Iddon L, Perumal M, Indrevoll B, Glaser M, Robins E, et al. Targeting somatostatin receptors: preclinical evaluation of novel 18F-fluoroethyltriazole-Tyr3-octreotate analogs for PET. J Nucl Med 2011;52:1441–8.
[20] Ambrosini V, Campana D, Bodei L, Nanni C, Castellucci P, Allegri V, et al. 68Ga-DOTANOC PET/CT clinical impact in patients with neuroendocrine tumors. J Nucl Med 2010;51:669–73.
[21] 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.
[22] Gillmore R, Stuart S, Kirkwood A, Hameeduddin A, Woodward N, Burroughs AK, et al. EASL and mRECIST responses are independent prognostic factors for survival in hepatocellular cancer patients treated with transarterial embolization. J Hepatol 2011;55:1309–16.
[23] Kuyumcu S, Ozkan ZG, Sanli Y, Yilmaz E, Mudun A, Adalet I, et al. Physiological and tumoral uptake of (68)Ga-DOTATATE: standardized uptake values and challenges in interpretation. Ann Nucl Med 2013;27:538–45.
[24] Adams MC, Turkington TG, Wilson JM, Wong TZ. A systematic review of the factors affecting accuracy of SUV measurements. AJR Am J Roentgenol 2010;195:310–20.
[25] Trials.gov C. 2019 [cited 2019 August 23]. Available from: https://clinicaltrials.gov/ct2/show/NCT03576040.
[26] Tirosh 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(4):998 e1–1008 e1.
[27] Chamberlain RS, Canes D, Brown KT, Saltz L, Jarnagin W, Fong Y, et al. Hepatic neuroendocrine metastases: does intervention alter outcomes?. J Am Coll Surg 2000;190:432–45.
[28] Graf F, Fahrer J, Maus S, Morgenstern A, Bruchertseifer F, Venkatachalam S, et al. DNA double strand breaks as predictor of efficacy of the alpha-particle emitter Ac-225 and the electron emitter Lu-177 for somatostatin receptor targeted radiotherapy. PLoS ONE 2014;9:e88239.