Journal of Nuclear Medicine, published on February 12, 2021 as doi:10.2967/jnumed.120.256727

Quantitative ^{68}Ga-DOTATATE PET/CT parameters for the prediction of therapy response in patients with progressive metastatic neuroendocrine tumors treated with ^{177}Lu-DOTATATE

Manuscript type – Original research

Claudia Ortega[1], Rebecca KS Wong[2], Josh Schaefferkoetter[3], Patrick Veit-Haibach[1], Sten Myrehaug[4], Rosalyn Juergens[5], David Laidley[6], Reut Anconina[1], Amy Liu[7], Ur Metser[1]

[1] Joint Department of Medical Imaging, University Health Network, Mount Sinai Hospital & Women's College Hospital; University of Toronto, Toronto, ON, Canada

[2] Department of Radiation Oncology, Princess Margaret Cancer Center, University Health Network & University of Toronto, Toronto, ON, Canada

Princess Margaret Cancer Centre, Toronto, ON, Canada

[3] Siemens Medical Solutions USA, Inc. 810 Innovation Drive, Knoxville, TN 37932, USA

[4] Division of Radiation Oncology, Odette Cancer Centre, Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada.

[5] Department of Oncology, Juravinski Cancer Centre, McMaster University, Hamilton, ON, Canada.

[6] St. Joseph's Health Care London, Division of Nuclear Medicine, University of Western Ontario, London, ON, Canada

[7] Department of Biostatistics, Princess Margaret Cancer Centre, Toronto, ON, Canada

Corresponding author: Ur Metser, MD; 610 University Ave, Suite 3-920, Toronto, ON, M5G 2M9, Canada. Phone: 416-946 4501 ext 4394; Fax: 416-946 6564

Email: ur.metser@uhn.ca

First Author contact: Claudia Ortega, MD; Claudia.ortega@uhn.ca

Funding sources:

1. Ontario Health - Cancer Care Ontario (OH-CCO): Funding is made available to the OH-CCO neuroendocrine tumor consortium to run the trial at the local sites as well as central trial coordination.
2. Princess Margaret Hospital Foundation: Fund is made available to support the central trial coordination
3. Susan Leslie Clinic for Neuroendocrine Tumors; Odette Cancer Centre: Funding is made available to support the implementation of the trial at Odette Cancer Center.

Disclosure Statement:

No potential conflicts of interest relevant to this article exist.

Running title: ^{68}Ga-DOTATATE PET for therapy response

Keywords: ^{68}Ga-DOTATATE, PET/CT, quantitative, neuroendocrine tumors, PRRT, response, survival.

ABSTRACT

PURPOSE: To determine whether quantitative PET parameters on baseline ^{68}Ga-DOTATATE PET/CT (bPET) and interim PET (iPET) performed prior to second cycle of therapy are predictive of therapy response and progression free survival (PFS). **PATIENTS & METHODS:** Ninety-one patients with well-differentiated neuroendocrine tumors (mean Ki67, 8.3%) underwent ^{68}Ga-DOTATATE PET/CT (DT- PET) to determine suitability for peptide receptor radionuclide therapy (PRRT) as part of a prospective multicenter study. Mean follow-up was 12.2 months. Of them, 36 patients had iPET. Tumor metrics evaluated: 1. Marker lesion-based measures: mean SUVmax and ratio to liver/spleen; 2. Segmented DT tumor volume (DTTV) measures: DTTV; SUVmax and SUVmean using liver and spleen as thresholds; 3. Heterogeneity parameters (coefficient of variance, kurtosis, and skewness). Wilcoxon rank sum test was used for association between continuous variables and therapy response as determined by clinical response. Univariable and multivariable Cox proportional hazards model were used for association with PFS. **RESULTS:** There were 71 responders and 20 non-responders. Using marker lesions, higher mean SUVmax and mean SUVmax(Tumor/Liver) were predictors of therapy response (p=0.018 & 0.024, respectively). For DTTV, higher SUVmax and SUVmean using liver as threshold and lower kurtosis were predictors of favorable response (p=0.025, 0.0055 & 0.031, respectively. These also correlated with longer PFS. iPET DTTV SUVmean using liver threshold and ratio iPET mean SUVmax using target lesions correlated with therapy response (p=0.024 & 0.048, respectively) but not PFS. From the multivariable analysis adjusting for age, primary site and Ki67, mean SUVmax (p=0.019), SUVmax T/L (p=0.018), SUVmax T/S (p=0.041), DTTV SUV_{mean} Liver (p=0.0052) and skewness (p=0.048) remain significant predictors of PFS. **CONCLUSION:** Degree of somatostatin receptor expression and tumor heterogeneity as represented by several metrics in

our analysis are predictive of therapy response and/or PFS. Change in these parameters after first cycle of PRRT did not correlate with clinical outcomes.

INTRODUCTION

Neuroendocrine tumors (NET) are uncommon malignancies with a documented population incidence of 6-7 per 100,000 in 2012 (1). The most common primary tumor sites are gastroenteropancreatic, but they may arise in the lungs, adrenal glands, pituitary gland amongst other locations (2). The majority of NETs over-express somatostatin receptors (SSTR) on the cell surface, allowing for SSTR-based imaging with ^{68}Ga- (DOTA)-peptide PET, which in recent years has become the preferred imaging modality for diagnosis, staging and selection of patients for peptide receptor radionuclide therapy (PRRT) (3).

Coupling beta or gamma-emitting radionuclides (^{177}Lu or ^{90}Y) to somatostatin analogues allows radionuclide therapy for well differentiated unresectable or metastatic highly DOTA-somatostatin analog-avid tumors (4). Multiple studies, including a recent phase III randomized trial (NETTER-1) have shown favorable progression free survival (PFS) and overall survival (OS), with limited, acceptable side effects (5-11). In the latter study, treatment of patients with midgut NET with ^{177}Lu-DOTATATE and octreotide LAR resulted in longer PFS and OS than treatment with octreotide LAR alone (11).

In July 2016, a prospective, multicenter trial was launched in Ontario to assess the outcome of patients with progressive metastatic NETs treated with individualized dosimetry-guided ^{177}Lu-DOTATATE therapy while providing access to PRRT in Ontario with regulatory Health Canada oversight. A further aim of this trial is to evaluate prognostic value of various quantitative ^{68}Ga-DOTATATE PET/CT (DT-PET), performed for patient selection. The purpose of the current analysis is to determine whether semiquantitative, volumetric, and tumor heterogeneity parameters on baseline PET (bPET) and early interim PET (iPET) performed prior to second cycle of therapy are predictive of therapy response and PFS.

PATIENTS AND METHODS

Study Design

Patients included in this analysis were enrolled in a prospective, multicenter single arm institutional review board approved study (NCT02743741). All subjects signed a written informed consent. Eligibility criteria included biopsy proven NET, with no restriction on primary site of origin, Ki67 index of ≤30%, and progressive disease prior to study enrollment. All patients underwent screening with DT-PET to confirm adequate expression of SSTR type 2. In eligible patients most tumor sites exhibited high level of DOTATATE (DT) uptake (defined as Krenning score of 3 or 4) (12,13). An optional iPET scan was performed prior to cycle 2 of ^{177}Lu-DOTATATE to explore its prognostic value for clinical outcomes. An association between bPET and iPET parameters and the outcome measures, therapy response and PFS, was evaluated.

Study Procedure

DT PET Imaging Protocol. PET was performed on a Siemens mCT40 PET/CT scanner (Siemens Healthcare, Erlangen, Germany). Patients were positioned supine with arms outside of the region of interest. Images were obtained from the top of the skull to the upper thighs. Iodinated oral contrast material was administered for bowel opacification; no intravenous iodinated contrast was used. Overall, 5-9 bed positions were obtained as per patient height, with an acquisition time of 2-3 min per bed position as per injected dose. A mean dose of 131.8 MBq (± 29.7) of ^{68}Ga-DOTATATE was administered (range 54.5-205) with a mean uptake time of 65.8 min (range: 50-93). CT parameters were 120 kV; 5.0 mm slice width, 4.0 mm collimation; 0.8 sec rotation time; 8.4 mm feed/rotation; Kernel B30s medium smooth reconstruction. PET emission scan using time of flight with scatter correction was obtained covering the identical transverse field of view. Image size: 2.6 pix size; slice 3.27; 5mm FWHM Gaussian Filter type. PET/CT images were reviewed on a dedicated nuclear medicine PACS system with fusion

software (Mirada Medical, Denver, CO). SUV measurements were obtained using a region of interest delineating select tumor sites (Figure 1).

Tumor Volume Segmentation Software. A graphical user interface was developed in-house specifically to delineate whole body tumor burden. An initial mask was defined by setting a threshold equal to the maximal value in a 3D Volume of Interest drawn within normal reference tissue, initially with normal liver. From this general mask, normal physiological tracer uptake was manually removed – this was accomplished in 3D maximum intensity projection space. The PET volume, and mask overlay, was displayed at various radial angles, allowing the user to define and remove regions of normal uptake, (e.g. myocardium, spleen, bladder) at any views providing the clearest visible margins. Once the normal tissues were removed from the tumor mask, a final step allowed the user to manually add or subtract any other regions to/from the mask. Correlation with morphological imaging (contrast-enhanced CT or MR) was performed when needed to ensure accurate delineation of tumor sites. DT tumor volumes (DTTV) and SUVmax and mean were generated from the final tumor mask (Figure 2). The same process was repeated with threshold equal to the maximal value in 3D volume of interest drawn within normal spleen (a step-by-step demonstration of the segmentation process is provided in Supplemental Figure 1).

^{177}Lu-DOTATATE Therapy & Clinical Outcomes. Eligible patients were treated with 4 cycles of ^{177}Lu-DOTATATE, or fewer if limited by toxicity or disease progression. Individualized dosimetry was used for the provision of ^{177}Lu-DOTATATE therapy. The initial administered activity of ^{177}Lu-DOTATATE was standardized at 200 mCi. Whole body scintigraphy was performed at 4, 24 and 72 hours after each cycle to estimate a recommended activity that will result in an accumulated absorbed dose to the kidneys using the threshold of 23 Gy, with estimates of absorbed dose in bone marrow and tumor. Dose escalation was capped at a maximum of 300mCi per dose. Final prescribed activity was determined by the treating physician based on

the recommended activity and patient factors including renal and hematologic laboratory results. For this report, response to therapy was defined by the investigator based on CT chest, abdomen and pelvis performed 3 months after completion of therapy, or earlier if disease progression was observed (12). Responders were defined as patients with complete/ partial response or stable disease; and non-responders had progressive disease. Patients continued clinical and CT surveillance and time to progression was documented for all study participants.

PET Data Abstraction

Reference Normal Tissue Values. SUVmax in reference tissues, normal liver (lower threshold) and spleen (upper threshold) were recorded. In the liver SUVmax was measured in the posterior right lobe. For patients who underwent splenectomy, normal renal parenchyma was used instead of spleen for the upper threshold (12,13). For this report, both upper threshold references will be referred to collectively as “Spleen”.

Tumor parameters. Three types of tumor parameters were assessed including marker based- lesion, whole body DOTATATE Tumor Volume (DTTV), and first order heterogeneity parameters, for a total of 13 metrics were extracted from baseline and prior to cycle 2 scans where available.

For lesion-based parameters, reference background tracer uptakes in normal liver and spleen tissue (or left renal cortex in case of previous splenectomy) were measured (volume of interest placed in the right hepatic lobe and spleen). Up to 5 well-defined, reproducible markers lesions > 1 cm in size were chosen, with no more than 2 lesions per disease site or organ following RECIST 1.1 recommendations (14,15). SUVmax of each lesion and mean SUVmax of all marker lesions was recorded. In addition, ratio of lesion SUVmax to SUVmax of liver and spleen were calculated (=SUVmax T/L, SUVmax T/S, respectively).

For DTTV parameters, the maximum SUVs within volume of interest drawn over the liver and spleen were used as thresholds for measurement of DTTV (=$DTTV_{liver}$; $DTTV_{spleen}$). Total DTTV ($DTTV_{liver}$; $DTTV_{spleen}$ in cc), SUVmax generated from the contoured volumes and SUVmean from the generated volumes for each threshold (=DTTV SUV_{mean} Liver or Spleen) were documented.

Heterogeneity of SSTR type 2 expression at the various tumor sites was assessed by using the segmented 3-dimensional tumor volumes. For this purpose, 3 different first order heterogeneity radiomics parameters were evaluated within the generated volumes: 1. Coefficient of variation defined as the standard deviation divided by the mean value of the activity concentration in the tumor volume; 2. Skewness, the third standardized moment and measure of the asymmetry of the activity distribution at tumor sites; and 3. Kurtosis, the fourth standardized moment and measure of the tailedness of the probability distribution. For both the skewness and kurtosis, the metrics were calculated with and without sample size bias correction and no significant differences were observed between these.

Statistical Analysis

Summary statistics were used to describe patient and disease characteristics, with mean and range for continuous variables, and frequency and percentage for categorical variables. Box plots were used to visualize the distribution of continuous variables between responders and non-responders. Two-sided Wilcoxon's rank sum test was used to test for association between continuous variables and the response. All markers for progression-free survival (PFS, defined as survival without progression or death) were assessed using univariable Cox proportional hazards model. Each statistically significant marker from the univariable analysis was further assessed in multivariable Cox proportional hazards model while adjusting for patient factors including age, primary disease site and Ki 67 index. A p-value < 0.05 was considered statistically significant, without adjustment for multiple comparisons.

RESULTS

Patient Demographics

At time of data lock for our current analysis (December 2019), there were 96 consecutive subjects referred between August 2016 and January 2019 for consideration of PRRT. Five subjects who were not treated were excluded (two withdrew from the study and three had inadequate tracer uptake in tumor sites). There were 91 patients who fulfilled the inclusion criteria, received therapy, and had follow-up data available and comprise the study cohort. Thirty-six of them underwent an iPET. Patient characteristics are presented in Table .1 Patients received up to 4 cycles of PRRT (mean, 3.67: range 1-4) with an average dose of 7375.1 MBq (range: 972-12,659). Mean clinical follow-up was 12.2 months (range 1.4 - 38 months). There were 71 responders (51 with stable disease; 20 with partial response) and 20 nonresponders. Mean PFS was 18.9 months (95% CI, 15.6-22.8).

bPET Parameters

Reference Tissues. SUVmax in 2 reference tissues, normal liver and spleen were compared in responders and non-responders. There were 9 patients who had prior splenectomy for whom normal renal parenchyma was used instead of spleen as the upper threshold reference. For the lesion-based analysis, there was a mean of 4.7 target lesions evaluated for each patient (median, 5; range 2-5). Baseline reference tissue parameters, lesion-based DT PET measures, DTTV and heterogeneity parameters for responders and non-responders are summarized in Table 2.

Tumor Parameters. For marked lesions, higher mean SUVmax and SUVmax T/L were predictive of therapy response (p=0.018 and 0.024, respectively; Figure 3). Similarly, higher SUVmax measured in DTTV and tumor SUVmean of segmented tumor volumes with liver used as threshold (DDTV SUV_{mean} Liver) were associated with therapy response (p=0.025 and

0.0055, respectively; Figure 4). An association was demonstrated between mean SUVmax of target lesions, SUVmax T/L, SUVmax T/S, and DDTV SUV_{mean} Liver with progression or death (p=0.0023; p=0.028; p=0.047; p=0.0053, respectively).

Of the three analyzed first order heterogeneity parameters, only kurtosis emerged as a significant predictor of outcome with higher values in non-responders than responders (Figure 5). Both skewness (HR, 1.49 [95%CI, 1.07- 2.07], p=0.017) and kurtosis (HR, 1.06 [95%CI, 1.01-1.11], p=0.022) were associated with progression or death.

iPET Response Assessment Parameters. iPET lesion based, DTTV parameters, heterogeneity parameters on iPET are presented in Table 3. Changes in the various parameters between bPET and iPET are presented Supplemental Table 1.

Progression-free Survival (PFS)

There was a correlation between higher mean SUVmax, SUVmax T/L, SUVmax T/S and DTTV SUV_{mean} Liver and a longer PFS (p=0.023, p=0.028, p= 0.047 and p=0.0053, respectively). Conversely, higher kurtosis and skewness were correlated with shorter PFS (p=0.0022 and 0.017, respectively).

From the multivariable analysis adjusting for age, primary site, and Ki 67 index, mean SUVmax (p=0.019), SUVmax T/L (p=0.018), SUVmax T/S (p=0.041), DTTV SUV_{mean} Liver (p=0.0052) and skewness (p=0.048) remain statistically significant predictors of PFS; however variable kurtosis was not significant in multivariable analysis. None of analyzed parameters at iPET showed statistical correlation with PFS in the univariable analysis.

Results for univariable analysis of PFS to demographic data, tumor Ki 67 index, lesion-based measures, DTTV, and heterogeneity parameters on bPET as well as multivariable analysis results obtained from statistical significant parameters in univariable analysis when

correlated with age, primary disease site and Ki67 are presented in Table 4. Complete univariable analysis is presented in Supplemental Table 2.

DISCUSSION

Identification of predictive biomarkers for therapy response and prognosis are essential for personalized care. PRRT is an effective mode of therapy in patients with metastatic NET, but it is only appropriate for patients whose tumors highly overexpress somatostatin receptors (16). As would be expected and as previously reported, higher tumor SUVmax on DT PET correlates with treatment response (17-20). A few SUV based parameters including mean SUVmax of target lesions and SUVmean generated from the DTTV with liver as threshold were predictive of response to PRRT and both parameters (mean SUVmax; DDTV SUV_{mean} Liver) also correlated with progression free survival (HR of 1.04 and 1.19, respectively). A further interesting observation from our study was the there was generally a higher SUVmax in reference tissues (normal liver and spleen) at iPET in patients who responded to PRRT. In the normal liver SSTRs are predominantly found in bile ducts, whereas in the normal spleen, autoradiography and immunohistochemistry studies have shown that they are found predominantly in red pulp (21,22). This reason for the presumed flare phenomenon in tracer uptake in normal tissues with high expression of SSTR is uncertain; however, it may reflect an inflammatory response with increase in activated macrophages that overexpress SSTR-1 and 2 on their cell surface (23,24). This has also been the basis of imaging of inflammation in atherosclerosis and myocarditis with DT-PET (25,26).

Early prediction of treatment failure can be a powerful clinical tool. For certain disease entities, early biomarkers have been validated and are clinically used to tailor therapy and reduce toxicity. Although generally well tolerated, PRRT can be associated with significant hematological, renal and hepatic toxicities (in approx. 10%, 0.4% and 0.4% of patients, respectively) (27). Despite high radiation dose delivered, disease progression is observed in 20-30% of patients, and most patients achieve stable disease as best response (28). Biomarkers

that accurately predict outcome to PRRT may identify those patients who are unlikely to benefit from it and limit unnecessary toxicity. Although SUVmean generated from DTTV with liver as threshold on iPET was significantly higher in responders than non-responders (18.1± 9.1 and 11.2 ± 3.3, respectively), the overlap may limit the clinical utility of this observation.

NETs often exhibit intra-tumoral heterogeneity. Skewness and kurtosis, first-order features of heterogeneity depict the asymmetry within the grey-level distribution observed within a volume of interest and spreading of the expected gaussian curve, respectively. We have shown an inverse relationship between baseline measurement of kurtosis and response to PRRT. Both measured parameters correlate inversely with PFS (HR: 0.54 and 0.9, respectively). These findings are in line with previous studies showing predictive value of subjective assessment of tumor heterogeneity and response to PRRT (29-31). Graf et al recently reported that visual assessment of SSTR heterogeneity had both predictive and prognostic value in progressive grade 1 or grade 2 NET patients undergoing PRRT, exceeding the prognostic value of Ki-67 index (31).

There are several limitations to this study. First, we included patients with multiple different NET sites, as the study was designed to enable broad access to PRRT for patients with metastatic NETs. However, patient selection criteria including Ki 67index and SSTR type 2 expression on PET were standardized. Second, iPET was optional and was only performed for a subset of patients. However, iPET parameters added little to patient outcome and were not predictive of progression free survival. Third, DTTV and the quantitative heterogeneity parameters require use of segmentation tools, some of which are time consuming and may not be practical for routine clinical use. However, we have shown that manual SUVmax measurements of target lesion can be used. Furthermore, previous publication has suggested that subjective evaluation of heterogeneity has prognostic value and some of our objective measures of tumor heterogeneity confirm this observation (31). Fourth, there exists an overlap

between responders and nonresponders for all the predictive parameters, including parameters after first cycle of PRRT, limiting their clinical utility in isolation to guide patient management.

In conclusion, the degree of SSTR type 2 expression and tumor heterogeneity as represented by several metrics in our analysis are predictive of therapy response and/or PFS. Change in these parameters after first cycle of PRRT did not correlate with clinical outcomes. A model or scoring system, integrating combinations of the predictive parameters identified with other clinical prognostic factors should be developed to predict therapy response and patient outcomes more reliably.

KEY POINTS

Question:

Are there quantitative ^{68}Ga-DOTATATE PET parameters at baseline or interim PET after 1 cycle of peptide receptor radionuclide therapy that are predictive of response to therapy and/or progression free survival?

Pertinent Findings:

Higher SUVmax in marker tumor sites and SUVmean of segmented ^{68}Ga-DOTATATE tumor volume using liver as threshold at baseline and interim PET correlated with therapy response; and the baseline parameters were predictive of progression free survival. Skewness, a first-order feature of tumor heterogeneity was also associated with progression free survival. Changes observed at interim PET did not correlate with outcome.

Implications for patient care:

Although certain quantitative ^{68}Ga-DOTATATE PET parameters are predictive of response to peptide receptor radionuclide therapy and progression free survival, the overlap of these parameters in responders and non-responders limits their clinical utility in isolation to guide patient management**.**

REFERENCES

1. Dasari A, Shen C, Halperin D, et al. Trends in the incidence, prevalence, and survival outcomes in patients with neuroendocrine tumors in the United States. *JAMA Oncol*. 2017;3:1335–1342.
2. National Comprehensive Cancer Network. Clinical Practice Guidelines in Oncology. Neuroendocrine Tumors v.1.2013. Available at: www.nccn.org. Accessed on Aug 1, 2020.
3. Hope TA, Bergsland EK, Bozkurt MF, et al. Appropriate use criteria for somatostatin receptor PET imaging in neuroendocrine tumors. *J Nucl Med.* 2018;59:66-74.
4. Bodei L, Kidd M, Modlin IM, et al. Measurement of circulating transcripts and gene cluster analysis predicts and defines therapeutic efficacy of peptide receptor radionuclide therapy (PRRT) in neuroendocrine tumors. *Eur J Nucl Med Mol Imaging.* 2016;43:839-851.
5. Kwekkeboom DJ, Teunissen JJ, Bakker WH, et al. Radiolabeled somatostatin analog [177Lu-DOTA0, Tyr3] octreotate in patients with endocrine gastroenteropancreatic tumors. *J Clin Oncol.* 2005;23:2754–2762.
6. Kwekkeboom DJ, de Herder WW, Kam BL, et al. Treatment with the radiolabeled somatostatin analog [177 Lu-DOTA 0, Tyr3] octreotate: toxicity, efficacy, and survival. *J Clin Oncol.* 2008;26:2124-2130
7. Kwekkeboom DJ, Kam BL, van Essen M, et al. Somatostatin-receptor-based imaging and therapy of gastroenteropancreatic neuroendocrine tumors. *Endocr Relat Cancer.* 2010;17:R53-R73.
8. Imhof A, Brunner P, Marincek N, et al. Response, survival, and long-term toxicity after therapy with the radiolabeled somatostatin analogue [90Y-DOTA]-TOC in metastasized neuroendocrine cancers. *J Clin Oncol.* 2011;29:2416-2423

9. Baum RP, Kulkarni HR, Singh A, et al. Results and adverse events of personalized peptide receptor radionuclide therapy with ^{90}Yttrium and ^{177}Lutetium in 1048 patients with neuroendocrine neoplasms. *Oncotarget.* 2018;9:16932-16950.
10. Hörsch D, Ezziddin S, Haug A, et al. Effectiveness and side-effects of peptide receptor radionuclide therapy for neuroendocrine neoplasms in Germany: a multi-institutional registry study with prospective follow-up. *Eur J Cancer.* 2016;58:41-51.
11. Strosberg J, El-Haddad G, Wolin E, et al. Phase 3 trial of (177) Lu-Dotatate for midgut neuroendocrine tumors. *N Engl J Med.* 2017;376:125–135.
12. Krenning EP, Valkema R, Kooij PP, et al. Scintigraphy and radionuclide therapy with [indium- 111-labelled-diethyl triamine pentacetic acid-D-Phe1]-octreotide. *Ital J Gastroenterol Hepatol*. 1999;31:219–223.
13. 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;60:1266-1269.
14. 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(suppl 1):122S-150S.
15. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: Revised RECIST guideline (Version 1.1)**.** *Eur J Cancer*. 2009;45:228-247.
16. De Jong M, Breeman WA, Kwekkeboom DJ, Valkema R, Krenning EP. Tumor imaging and therapy using radiolabeled somatostatin analogues. *Acc Chem Res*. 2009;42:873-880.
17. Haug AR, Auernhammer CJ, Wängler 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;5:1349-1356.

18. Miederer M, Seidl S, Buck A, 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. Campana D, Ambrosini V, Pezzilli R, et al. Standardized uptake values of (68) Ga-DOTANOC PET: a promising prognostic tool in neuroendocrine tumors. *J Nucl Med.* 2010;51:353-359.
20. Kratochwil C, Stefanova M, Mavriopoulou E, et al. SUV of [68Ga] DOTATOC-PET/CT predicts response probability of PRRT in neuroendocrine tumors. *Mol Imaging Biol.* 2015;17:313-318.
21. Reynaert H, Rombouts K, Vandermonde A, et al. Expression of somatostatin receptors in normal and cirrhotic human liver and in hepatocellular carcinoma. *Gut*. 2004;53:1180–1189.
22. Sarikaya I, Sarikaya A, Alnafisi N, Alenezi S. Significance of splenic uptake on somatostatin receptor imaging studies. *Nucl Med Review.* 2018;21:66-70.
23. Dalm VA, van Hagen PM, van Koetsveld PM, et al. Expression of somatostatin, cortistatin, and somatostatin receptors in human monocytes, macrophages, and dendritic cells. *Am J Physiol Endocrinol Metab.* 2003;285:E344-E353.
24. Armani C, Catalani E, Balbarini A, Bagnoli P, Cervia D. Expression, pharmacology, and functional role of somatostatin receptor subtypes 1 and 2 in human macrophages. *J Leukoc Biol.* 2007;81:845-855.
25. Li X, Bauer W, Kreissl MC, et al. Specific somatostatin receptor II expression in arterial plaque: (68)Ga-DOTATATE autoradiographic, immunohistochemical and flow cytometric studies in apoE-deficient mice. *Atherosclerosis.* 2013;230:33-39
26. Lapa C, Reiter T, Li X, et al. Imaging of myocardial inflammation with somatostatin receptor based PET/CT – A comparison to cardiac MRI. *Int J J Cardiol.* 2015;194:44-49.

27. Lin e, Chen, T, Little A, et al. Safety and outcomes of ^{177} Lu-DOTATATE for neuroendocrine tumours: Experience in New South Wales, Australia. *Intern Med J*. 2019;(49):1268-1277.
28. Marusyk A, Polyak K. Tumor heterogeneity: causes and consequences. *Biochim Biophys Acta*. 2010;1805:105–17.
29. Werner RA, Ilhan H, Lehner S, et al. Pre-therapy somatostatin receptor-based heterogeneity predicts overall survival in pancreatic neuroendocrine tumor patients undergoing peptide receptor radionuclide therapy. *Mol Imaging Biol.* 2019;21:582–590.
30. Wetz C, Apostolova I, Steffen IG, et al. Predictive value of asphericity in pretherapeutic [(111) In] DTPA-Octreotide SPECT/CT for response to peptide receptor radionuclide therapy with [(177) Lu] DOTATATE. *Mol Imaging Biol*. 2017;19:437–45.
31. Graf, J, Pape, U, Jann, H et al. Prognostic significance of somatostatin receptor heterogeneity in progressive neuroendocrine tumor treated with Lu-177 DOTATOC or Lu-177 DOTATATE. *Eur J Nucl Med Mol Imaging.* 2020;47:881–894.

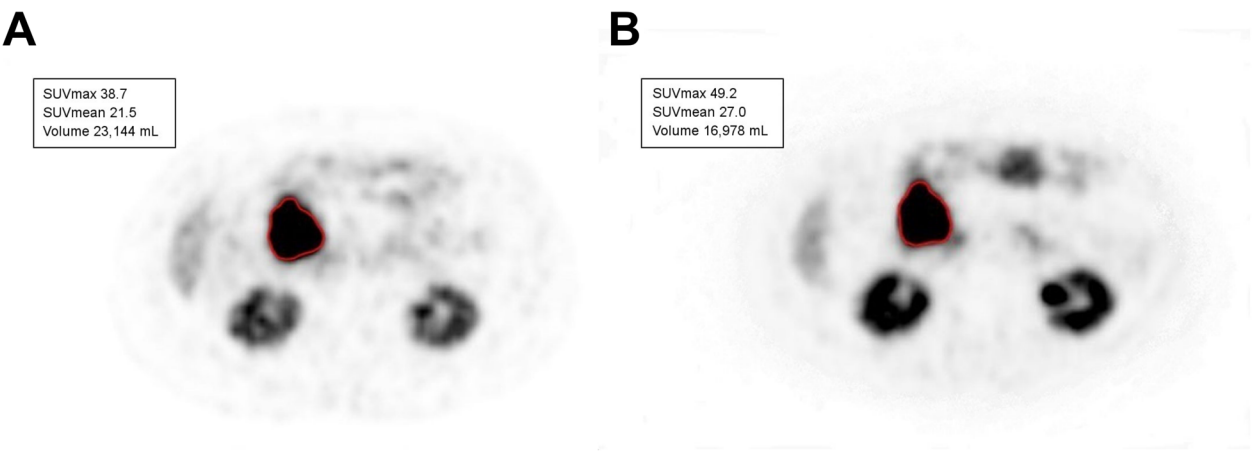


Figure 1. 60-year old woman with metastatic well-differentiated small bowel neuroendocrine tumor to liver and retroperitoneal lymph nodes (G1, Ki67 index =6%). Lesion-based assessment on bPET and iPET prior to cycle 2 of ^{177}Lu-DOTATATE therapy. A. Metastatic nodal mass chosen as marker lesion at bPET (SUVmax, 38.7); B. Same lesion prior to 2nd cycle of therapy (SUVmax 49.2).

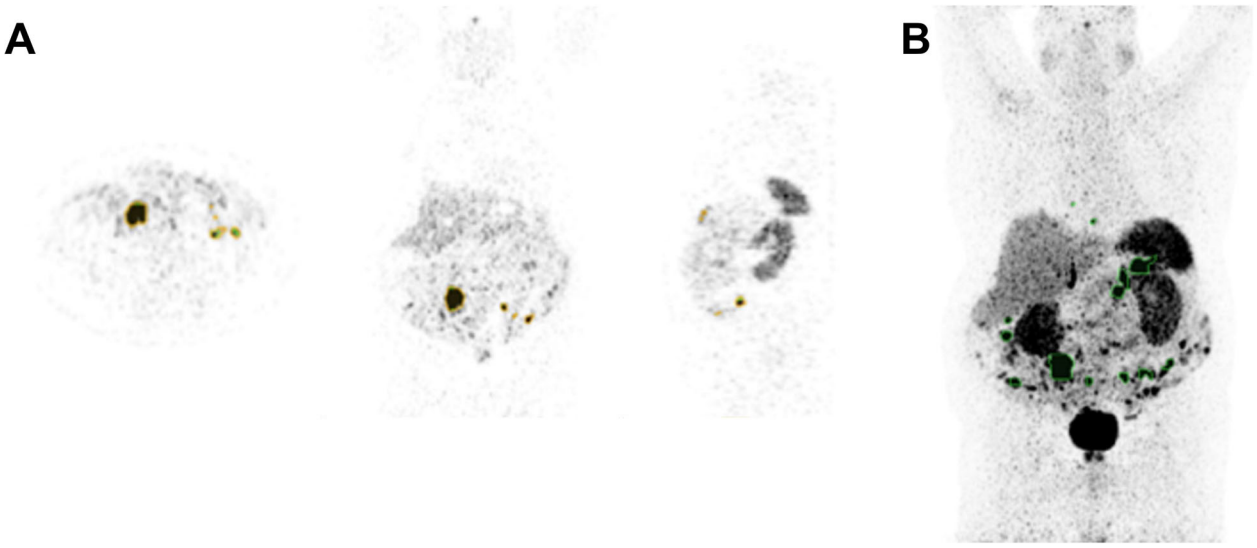


Figure 2. ^{68}Ga-DOTATATE tumor volume analysis using in-house automated segmentation software. A. multiplanar segmentation tool (to identify and confirm tumor sites). B. generated mask using tracer uptake in spleen as threshold (tumor lesions with SUVmax above spleen are outlined in green).

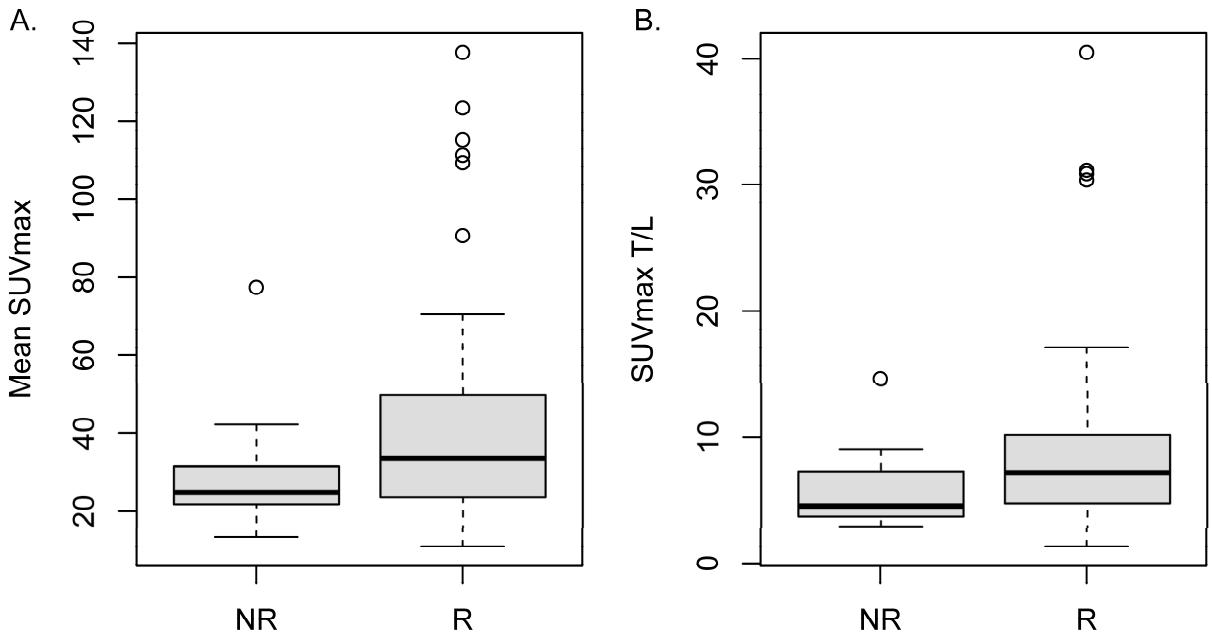


Figure 3. Lesion -based measures. Distribution of median SUVmax (A., p=0.018) and SUVmaxT/L (B., p=0.024). Box plots represent the median, upper and lower quartiles of each distribution with whiskers showing the limits of the distribution (1.5 times the interquartile range).

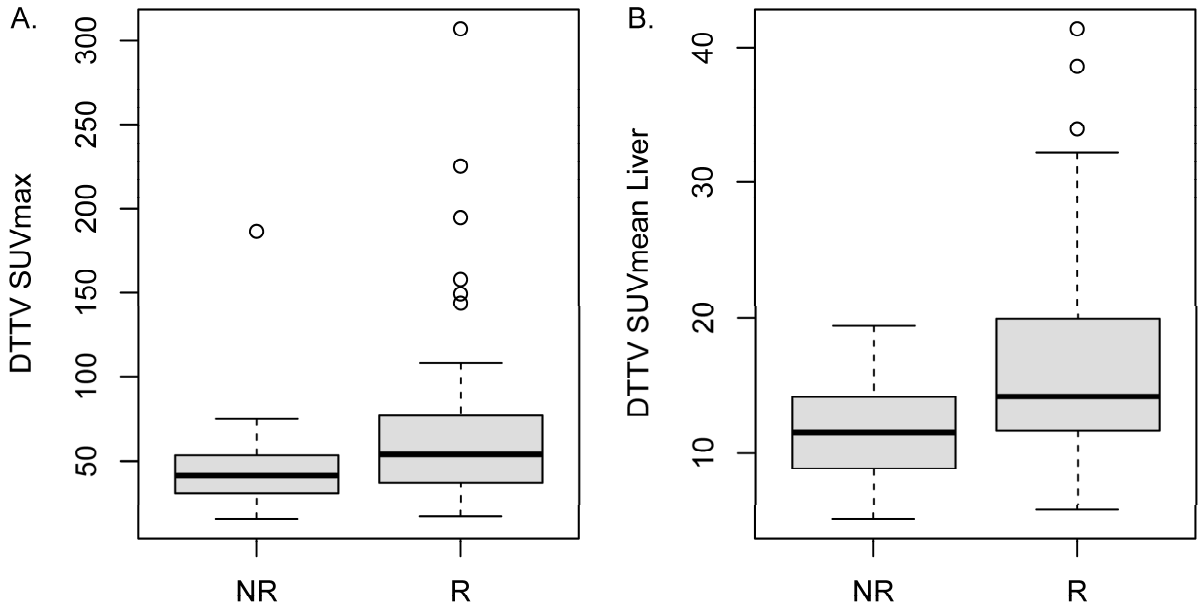


Figure 4. ^{68}Ga-DOTATATE Tumor volume (DTTV) parameters. Distribution of meanDTTV SUVmax (A, p=0.025) and SUVmaxLiver (B, p=0.0055). Box plots represent the median, upper and lower quartiles of each distribution with whiskers showing the limits of the distribution (1.5 times the interquartile range).

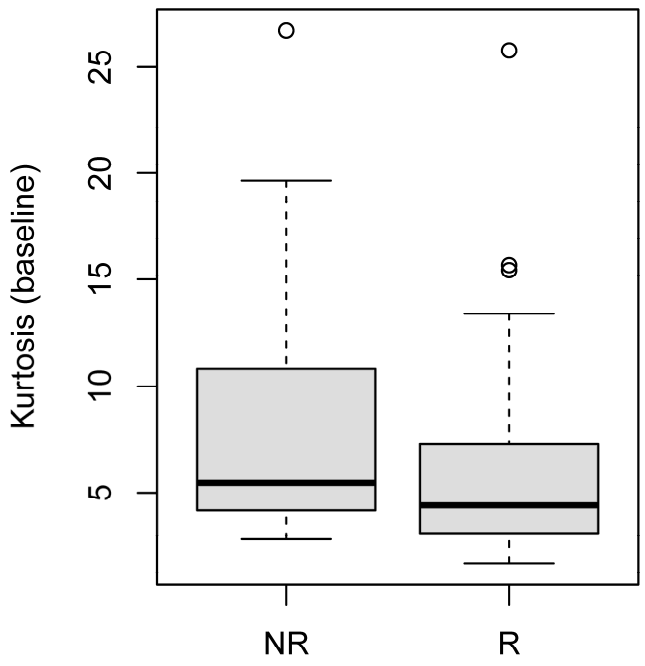


Figure 5. **Kurtosis on baseline ^{68}Ga-DOTATATE PET/CT.** Distribution of kurtosis estimated from ^{68}Ga-DOTATATE tumor volumes (p=0.031). Box plot represents the median, upper and lower quartiles of each distribution with whiskers showing the limits of the distribution (1.5 times the interquartile range).

TABLES

	Entire Cohort	**iPET Cohort**
N	96	36
Gender (M:F)	53:38	20:16
Age mean (SD)	62.5(±12.9)	59.7(±14.2)
Primary site (N)		
GI	48	19
Pancreas	19	8
Unknown primary	9	2
Bronchopulmonary	6	3
Adrenal	4	2
Other	5	2
Tumor Ki67 index mean (SD)%	8.3(± 7.8)	10.5(± 8.3)
Response status		
Non responders	20	6
Other	71	30
Follow up mean (SD) months	12.2(±7.2)	20.1(± 9.7)

Table 1. Patient characteristics for the entire cohort and the subgroup with iPET.

M=male, F=female, SD=Standard Deviation, GI= gastrointestinal

bPET Parameter		**All** (n=91)	**R** (n=71)	**NR** (n=20)	**p-value**
Reference Tissue Mean±SD (min,max)	**SUVmaxLiver**	5.7±2.1 (2,13.1)	5.8±2.3 (3.2,8.2)	5.3±1.6 (2,13.1)	0.49
	SUVmaxSpleen*	13.8±6.9 (4.4,45.8)	14.1±6.9 (4.4,26.7)	12.6±6.7 (5,45.8)	0.30
Lesion-based Mean±SD (min,max)	**MeanSUVmax**	38.7±25.1 (11,137.7)	41.7±26.8 (13.4,77.4)	28.2±14.2 (11,137.7)	**0.018**
	MeanSUVmaxT/L	8.3±6.4 (1.4,40.5)	9±7 (1.4,40.5)	5.8±2.9 (2.9,14.6)	**0.024**
	MeanSUVmaxT/S*	3.5±2.7 (0.2,14.6)	3.7±2.9 (0.2,14.6)	2.8±1.8 (1,8.3)	0.13
DTTV Mean±SD (min,max)	**$DTTV_{liver}$[cc]**	554.1±853.8 (4.7,4891.8)	611.5±921.7 (4.7,4891.8)	350.4±516.8 (8.1,2200.9)	0.12
	$DTTV_{spleen}$[cc]	317.1±616.3 (0,3825.3)	363.9±677.6 (0,3825.3)	150.9±265.4 (0,1049.6)	0.06
	DTTV SUV_{max}	62.8±46.4 (15.6,307)	66.8±48.4 (17.1,307)	48.4±36.2 (15.6,186.6)	**0.025**
	DTTV SUV_{mean}Liver	15.6±7.3 (5.1,41.4)	16.7±7.7 (5.8,41.4)	11.6±3.6 (5.1,19.4)	**0.0055**
	DTTV SUV_{mean}Spleen	23.5±10.3 (0,50.4)	24.5±10.4 (0,50.4)	20±9.3 (7.7,45.3)	0.06
Heterogeneity Mean±SD (min,max)	**CoV**	0.6±0.2 (0.2,1.6)	0.6±0.2 (0.3,1.5)	0.6±0.3 (0.2,1.6)	0.17
	Skewness	1.5±0.8 (0.1,4)	1.4±0.8 (0.1,4)	1.9±1.0 (0.6,4)	0.055
	Kurtosis	6.4±4.8 (1.7,26.7)	5.8±4.1 (1.7,25.8)	8.6±6.4 (2.8,26.7)	**0.031**

Table 2. bPET reference parameters, lesion-based tumor parameters, DTTV parameters and first order heterogeneity parameters.

Wilcoxon's rank sum test p-values are shown. R= responders; NR = non-responders; DTTV = DOTATATE tumor volume; SUVmaxT/L = meanSUVmax/SUVmax liver; SUVmaxT/S = mean SUVmax/SUVmax spleen. SUV_{mean}Liver / spleen = mean SUV in segmented volume using liver or spleen as threshold. SD= standard deviation; CoV= coefficient of variance.

iPET		All (n=36)	R (n=30)	NR (n=6)	p-value
Reference Tissue Mean±SD (min, max)	**SUVmaxLiver**	5.8±1.8 (2.8,11.2)	6.1±1.8 (3.6,11.2)	4.2±1.2 (2.8,6.4)	**0.011**
	SUVmaxSpleen*	19.4±10.6 (4.3,49.1)	21.2±10.7 (7.3,49.1)	10.6±3.8 (4.3,14.6)	**0.0085**
Lesion-based Mean±SD (min, max)	**MeanSUVmax**	34.3±19.4 (6.8,93.1)	37±20.2 (6.8,93.1)	21.2±6 (12.4,29.9)	**0.048**
	MeanSUVmaxT/L	6.1± 3.2 (0.9,17.4	6.3±3.5 (0.9,17.4)	5.2±1.4 (2.9,6.8)	0.57
	MeanSUVmaxT/S*	2.1±1.2 (0.1,6.1)	2.1±1.3 (0.1,6.1)	2.2±0.9 (1.5,3.9)	0.92
DTTV Mean±SD (min, max)	**$DTTV_{liver}$[cc]**	332.7±424.6 (6,1897.1)	343.9±438.8 (6,1897.1)	276.9±375.1 (11.6,979.8)	0.66
	$DTTV_{spleen}$[cc]	158.7±256.3 (0,1182.8)	163±270.1 (0,1182.80)	137.1±189.9 (7.2,452.9)	0.85
	DTTV SUV_{max}	58.8±37.2 (16.9,197.5)	63±39.3 (16.9,197.5)	38.1±11.1 (25.8,55.1)	0.11
	DTTV SUV_{mean}Liver	16.9±8.7 (5.7,50.7)	18.1±9.1 (5.7,50.7)	11.2±3.3 (6.3,16.6)	**0.024**
	DTTV SUV_{mean}Spleen	25.1±12.6 (0,50.7)	26.6±13 (0,50.7)	17.5±7.9 (8,31.5)	0.071
Heterogeneity Mean±SD (min,max)	**CoV**	0.6±0.2 (0.3,0.9)	0.6±0.2 (0.3,0.9)	0.6±0.1 (0.5,0.7)	0.47
	Skewness	1.4±0.8 (0.2,3.3)	1.4±0.8 (0.2,3.3)	1.4±0.8 (0.6,2.5)	0.95
	Kurtosis	6.1±4.1 (1.8,18.3)	6.1±4 (1.8,18.3)	6.3±5 (2.6,14.3)	0.82

Table 3. Interim PET (iPET) parameters for reference tissues, lesion-based tumor parameters, DTTV parameters and first order heterogeneity measures.

P-values are shown. R=responders; NR=non-responders; DTTV=DOTATATE tumor volume; SUVmaxT/L = meanSUVmax/ SUVmax liver; SUVmaxT/S= meanSUVmax/ SUVmax spleen. SUV_{mean}Liver/ spleen=meanSUV in segmented volume using liver/spleen as threshold. SD=standard deviation; CoV=coefficient of variance.

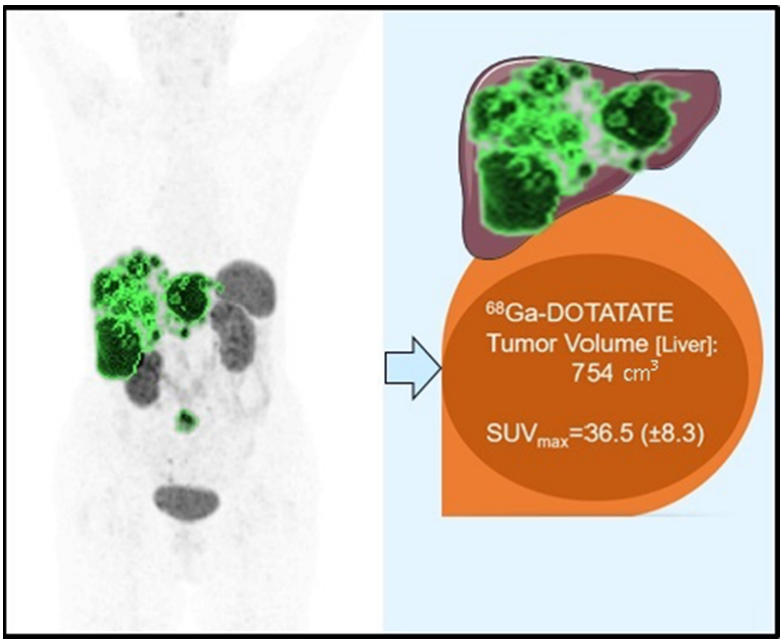
bPET Covariate		UVA		MVA	
		HR (95%CI)	**p-value**	**HR (95%CI)**	**p-value**
Lesion-based	MeanSUVmax	0.98 (0.97,1)	**0.023**	0.98 (0.96,1)	**0.019**
	SUVmaxT/L	0.92 (0.85,0.99)	**0.028**	0.89 (0.8,0.98)	**0.018**
	SUVmaxT/S	0.86 (0.75,1)	**0.047**	0.83 (0.69,0.99)	**0.041**
Tumor Volume	$DTTVSUV_{mean}$ Liver	0.92 (0.87,0.98)	**0.0053**	0.9 (0.83,0.97)	**0.0052**
Heterogeneity	Skewness	1.49 (1.07,2.07)	**0.017**	1.48 (1,2.18)	**0.048**
	Kurtosis	1.06 (1.01,1.11)	**0.022**	1.05 (0.99,1.12)	0.085

Table 4. Univariable and multivariable analysis of lesions-based, TV based and heterogeneity parameters as predictors of PFS.

P-values of Cox proportional hazards model are shown. UVA= univariable analysis; MVA= multivariable analysis; HR=hazard ratio; 95% CI=95% confidence interval; SUVmax T/L=mean SUVmax/ SUVmax liver; SUVmax T/S=mean SUVmax/SUVmax spleen;

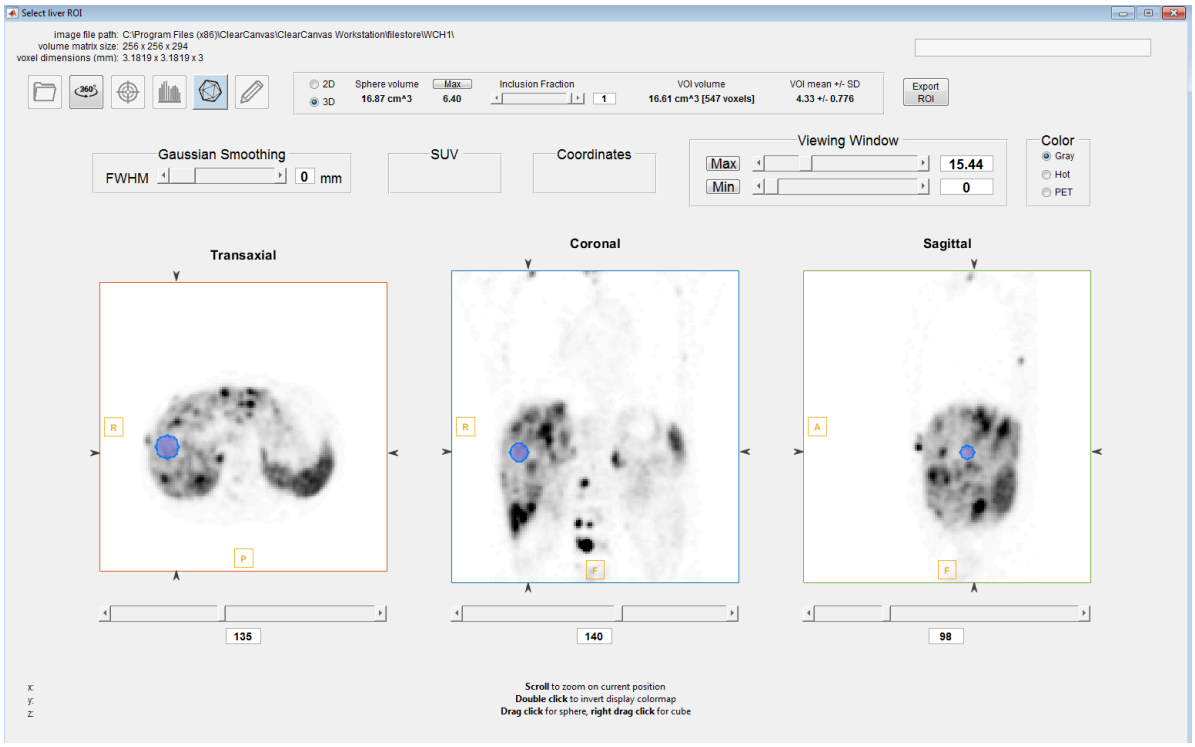
$DTTV_{Liver/spleen}$= ^{68}Ga-DOTATATE tumor volume with liver or spleen as threshold; DTTV SUVmean Liver/Spleen=meanSUV from tumor volume obtained with liver or spleen as threshold. CoV=coefficient of variance. PFS=progression free survival.

GRAPHICAL ABSTRACT

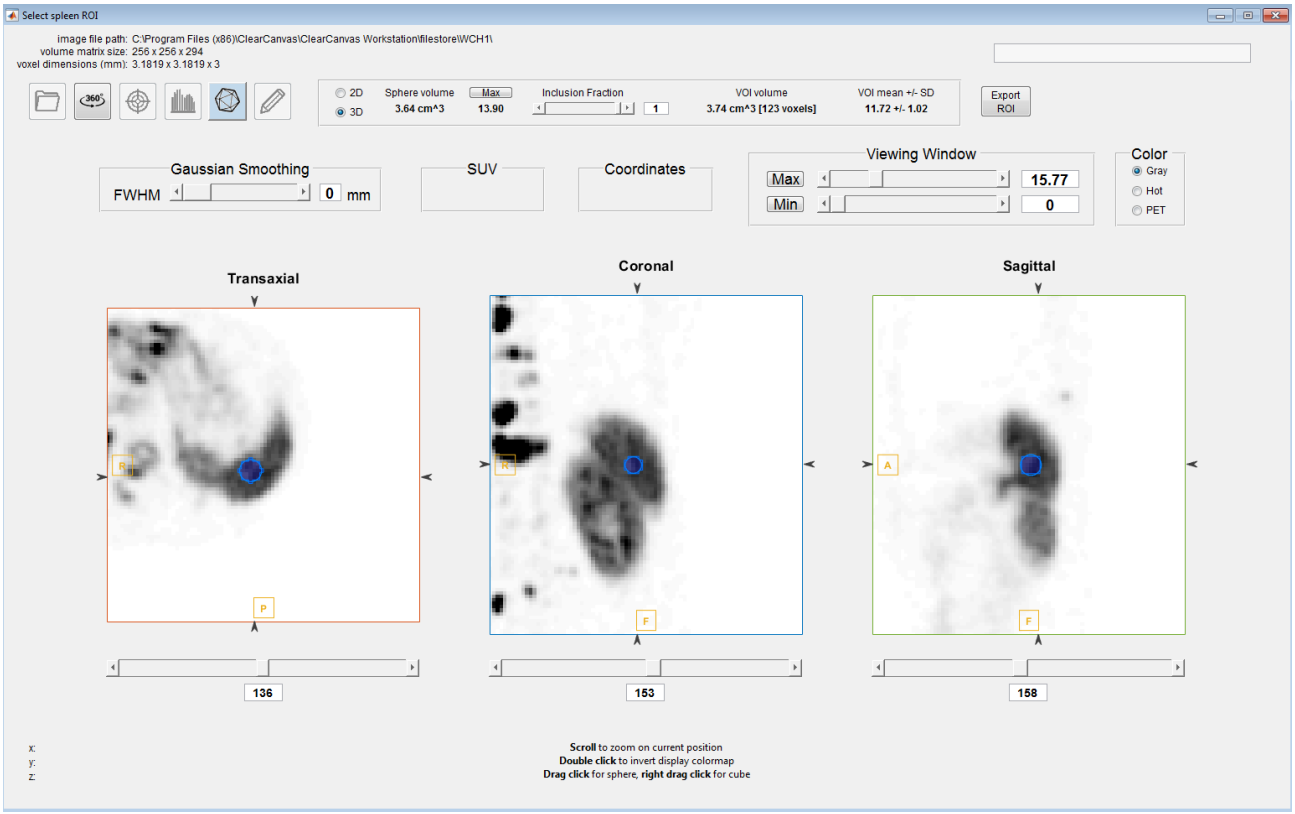


SUPPLEMEMNTAL FIGURE 1. Step-by-step demonstration of tumor segmentation method using a semi-automated whole-body tumor volume contouring software

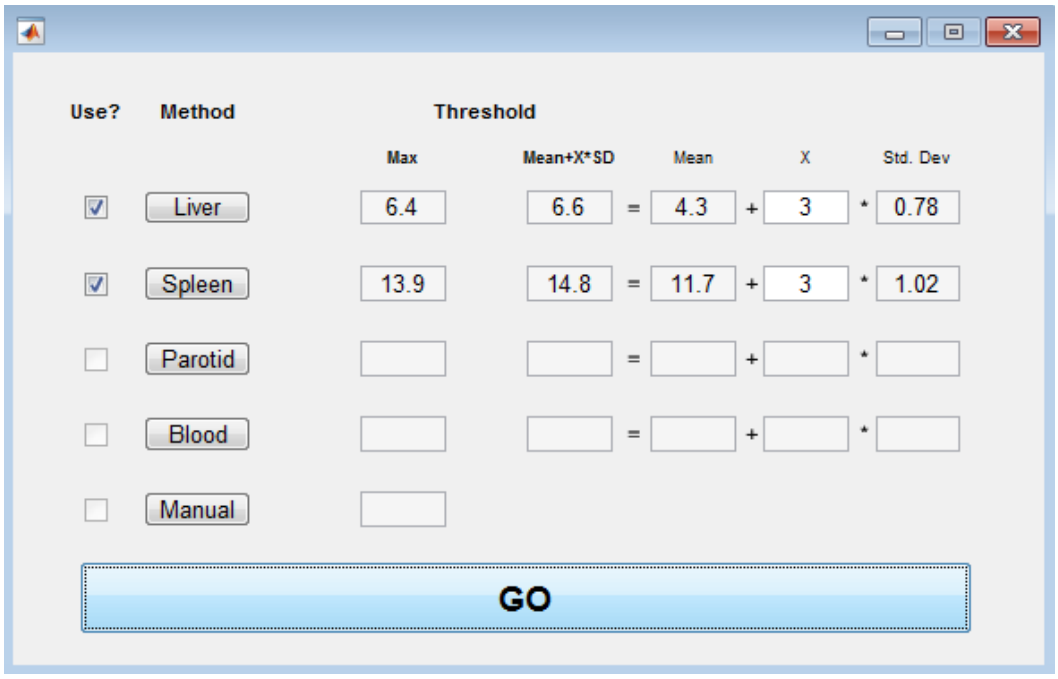
Whole body DTTV was generated using the in house developed automated segmentation tool using reference tissues as threshold. For each patient, PET masks were defined by all somatic voxels having values above a defined threshold, and then regions of normal uptake were manually removed leaving only those corresponding to the tumors.



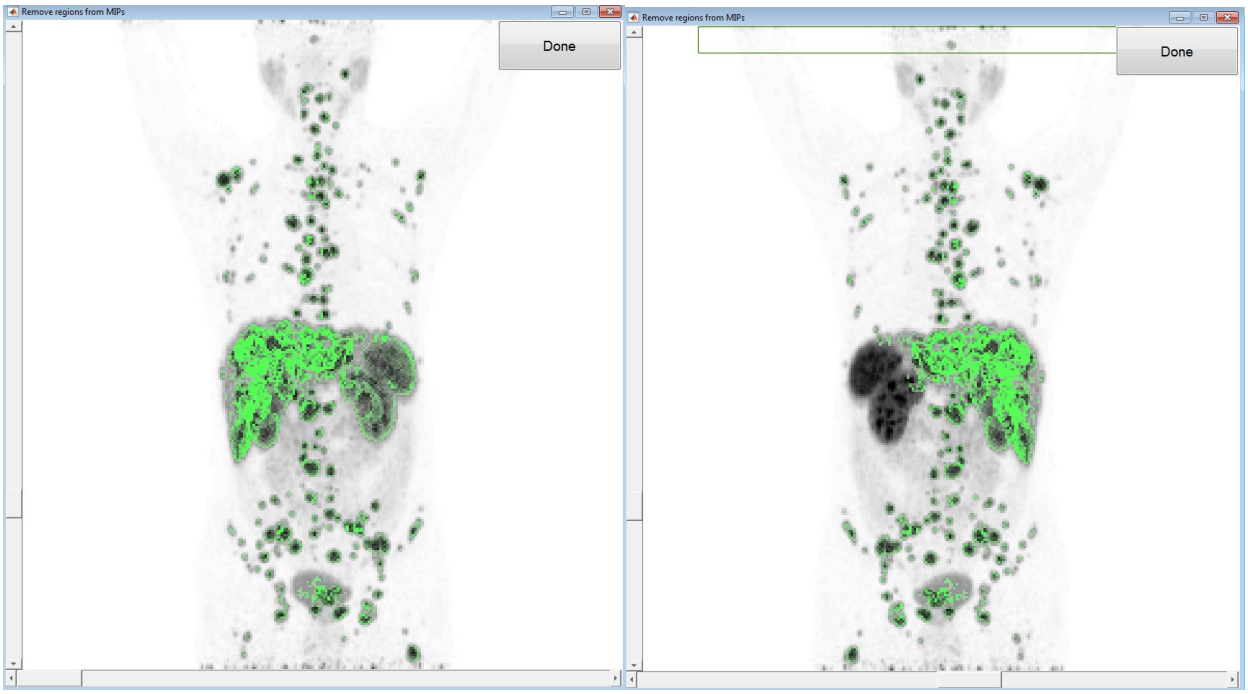
Supplemental Figure 1A: Step 1. A VOI is placed in normal liver parenchyma (avoiding metastases if present) to define the lower threshold used for segmentation



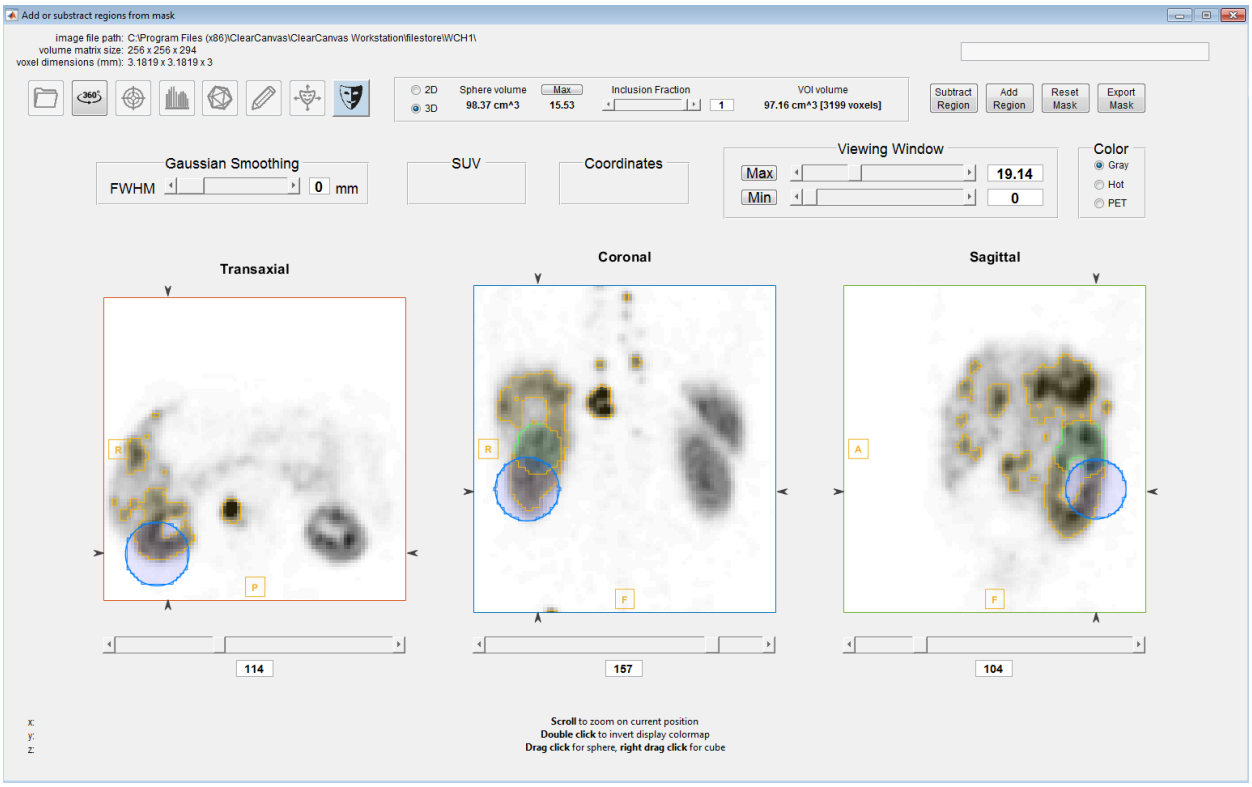
Supplemental Figure 1B: Step 2. A VOI is placed in normal spleen parenchyma to defined upper threshold for volume segmentation.



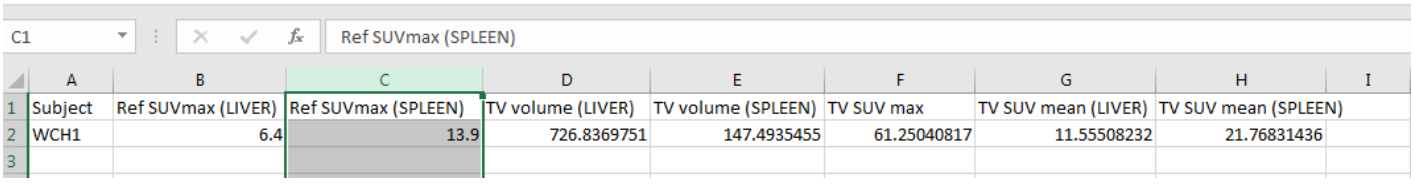
Supplemental Figure 1C: Step 3. After the thresholds are defined, the software will automatically generate a mask with all the somatic voxels containing values above the defined threshold.



Supplemental Figure 1D: Step 4. From the generated mask, all areas of physiologic tracer uptake are manually removed (in 3D mode).



Supplemental Figure 1E: Step 5. Further correction of the segmentation using 2D images.



C1 | Ref SUVmax (SPLEEN)

	A	B	C	D	E	F	G	H	I
1	Subject	Ref SUVmax (LIVER)	Ref SUVmax (SPLEEN)	TV volume (LIVER)	TV volume (SPLEEN)	TV SUV max	TV SUV mean (LIVER)	TV SUV mean (SPLEEN)	
2	WCH1	6.4	13.9	726.8369751	147.4935455	61.25040817	11.55508232	21.76831436	
3									

Supplemental Figure 1F: Step 6. The obtained results from the generated volumes are presented in a spreadsheet. These includes: The references values of the defined thresholds, the volume of the tumor (in cc), and the SUVmax and SUVmean within the tumor volume using liver and spleen as thresholds.

Supplemental Table 1. iPET / bPET ratios

iPET / bPET ratios					
		All (n=36)	**R** (n=30)	**NR** (n=6)	**p-value**
Reference Tissue Median±SD (min max)	**SUVmaxLiver**	1.1 ± 0.2 (0.7, 1.8)	0.9 ± 0.2 (0.8,1.2)	1.1 ± 0.4 (0.6, 2.2)	**0.046**
	SUVmaxSpleen*	1.2 ± 0.4 (0.6, 2.2)	1.2 ±0.4 (0.6, 2.2)	1.1 ± 0.3 (0.8, 1.5)	0.28
Lesion-based Median±SD (min,max)	**MeanSUVmax**	0.9±0.3 (0.3,1.9)	0.9±0.3 (0.3,1.9)	0.8±0.1 (0.6,1)	0.24
	MeanSUVmaxT/L	0.8±0.3 (0.2,1.4)	0.8±0.3 (0.2,1.4)	0.8±0.2 (0.6,1.1)	0.85
	MeanSUVmaxT/S*	0.8±0.2 (0.2,1.4)	0.8±0.3 (0.2,1.4)	0.7±0.2 (0.5,1)	0.82
DTTV Median±SD (min,max)	**$DTTV_{liver}$(cc)**	0.8 ±0.6 (0.2,3.3)	0.8 ±0.7 (0.2,3.3)	0.8 ±0.5 (0.5,1.7)	0.45
	$DTTV_{spleen}$(cc)	0.7 ± 0.8 (0,4.1)	0.8± 0.8 (0,4.1)	0.8± 0.3 (0,4.1.1)	0.81
	$DTTVSUV_{max}$	0±0.3 (-0.9,1)	0±0.4 (-0.9,1)	0.2±0.2 (-0.1,0.3)	0.22
	DTTV SUV_{mean}Liver	0.7±0.3 (0,1.9)	0.6±0.1 (0,1.9)	0.7±0.3 (0.6,0.8)	0.45

	DTTV SUV_{mean}Spleen	1.1±0.4 (0,1.9)	1.1±0.5 (0,1.9)	1.1±0.2 (0.8,1.3)	0.56
Heterogeneity Median±SD (min,max)	**CoV**	0.9±0.5 (0.3,1.9)	0.9±0.5 (0.3,1.9)	0.9±0.3 (0.6,1,2)	0.6
	Skewness	0.9±1.5 (0.1,8.8)	1±1.6 (0.1,8.8)	0.7±0.8 (0.2,2.5)	0.29
	Kurtosis	0.9±1.3 (0.1,4.9)	0.7±1.3 (0.1,4.9)	0.9±1.4 (0.2,3.8)	0.31

Supplemental Table 1. Interim PET (iPET)/bPET ratios for reference tissues, lesion-based tumor parameters, DTTV parameters and first order heterogeneity measures.

P-values are shown. R=responders; NR=non-responders; DTTV=DOTATATE tumor volume; SUVmaxT/L = meanSUVmax/ SUVmax liver; SUVmaxT/S= meanSUVmax/ SUVmax spleen. SUV_{mean}Liver/ spleen=meanSUV in segmented volume using liver/spleen as threshold. SD=standard deviation; CoV=coefficient of variance.

UVA		HR (95%CI)	p-value
bPET Parameter			
Demographic	Age	0.97 (0.93,1.02)	0.24
	Gender		0.4
	F	Reference	
	M	1.54 (0.57,4.16)	
	Ki 67	0.99 (0.93,1.05)	0.67
Reference tissues	Reference SUVmax Liver	1.13 (0.78,1.63)	0.52
	Reference SUVmax Spleen*	0.99 (0.92,1.06)	0.72
Lesion-based	Mean SUVmax	0.98 (0.97,1)	**0.023**
	SUVmax T/L	0.92 (0.85,0.99)	**0.028**
	SUVmax T/S	0.86 (0.75,1)	**0.047**
Tumor volume	$DTTV_{Liver}$	1 (1,1)	0.99
	$DTTV_{Spleen}$	1 (1,1)	0.61
	DTTV SUV max	0.99 (0.99,1)	0.13
	$DTTV\ SUV_{mean}$ Liver	0.92 (0.87,0.98)	**0.0053**
	$DTTV\ SUV_{mean}$ Spleen	0.98 (0.95,1.01)	0.13
Heterogeneity	CoV	0.62 (0.14,2.67)	0.52
	Skewness	1.49 (1.07,2.07)	**0.017**
	Kurtosis	1.06 (1.01,1.11)	**0.022**

iPET Parameter			
Reference tissues	Reference SUVmax Liver	0.89 (0.61,1.28)	0.53
	Reference SUVmax Spleen*	0.98 (0.91,1.05)	0.55
Lesion-based	Mean SUVmax	1 (0.97,1.03)	0.96
	SUVmax T/L	0.99 (0.87,1.13)	0.88
	SUVmax T/S	0.9 (0.6,1.33)	0.59
Tumor volume	$DTTV_{Liver}$	1 (1,1)	0.83
	$DTTV_{Spleen}$	1 (1,1)	0.5
	DTTV SUVmax	1 (0.99, 1.01)	0.96
	$DTTV\ SUV_{mean}$ Liver	0.95 (0.86, 1.06)	0.36
	$DTTV\ SUV_{mean}$ Spleen	0.99 (0.95, 1.03)	0.69
Heterogeneity	CoV	1.57 (0.07, 38.08)	0.78
	Skewness	1.41 (0.72,2.77)	0.32
	Kurtosis	1.07 (0.95,1.2)	0.27
iPET/bPET ratios			
Reference tissues ratio	Reference SUVmax Liver	0.01 (2.1e-04,0.5)	**0.021**
	Reference SUVmax Spleen*	0.42 (0.05,3.45)	0.42
Lesion-based ratio	Mean SUVmax	0.49 (0.09,2.59)	0.4
	SUVmax T/L	1.31 (0.21,8.04)	0.77

	SUVmax T/S	0.66 (0.09,5.09)	0.69
Tumor volume ratio	DTTV $_{Liver}$	1.29 (0.62,2.7)	0.49
	DTTV $_{Spleen}$	0.79 (0.29,2.11)	0.64
	DTTV SUVmax	0.48 (0.07,3.27)	0.46
	DTTV SUV $_{mean}$ Liver	0.97 (0.11,8.24)	0.98
	DTTV SUV $_{mean}$ Spleen	1.24 (0.3,5.15)	0.63
Heterogeneity ratio	CoV	0.75 (0.2,2.77)	0.66
	Skewness	1 (0.57,1.75)	0.99
	Kurtosis	1.15 (0.75,1.76)	0.53

Supplemental Table 2. Complete univariate analysis of lesions-based, TV based and heterogeneity parameters including at baseline (bPET), interim PET (iPET), and iPET/bPET ratios as predictors of PFS.

P-values of Cox proportional hazards model are shown. HR=hazard ratio; 95% CI=95% confidence interval; SUVmax T/L=mean SUVmax/ SUVmax liver; SUVmax T/S=mean SUVmax/SUVmax spleen;

$DTTV_{Liver/spleen}$= ^{68}Ga-DOTATATE tumor volume with liver or spleen as threshold; DTTV SUVmean Liver/Spleen=meanSUV from tumor volume obtained with liver or spleen as threshold. CoV=coefficient of variance. PFS=progression free survival.