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ORIGINAL ARTICLE

Long-term follow-up and role of FDG PET in advanced pancreatic neuroendocrine patients treated with ^{177}Lu-D OTATATE

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Abstract
Purpose Lu-DOTATATE (Lu-PRRT) is a valid therapeutic option in differentiated pancreatic neuroendocrine tumors (P-NETs). FDG PET seems to be an important prognostic factor in P-NETs. We evaluated the efficacy of Lu-PRRT and the role of FDG PET in 60 patients with advanced P-NETs.
Methods From March 2008 to June 2011, 60 consecutive patients with P-NETs were enrolled in the study. Follow-up lasted until March 2016. Eligible patients were treated with two different total cumulative activities (18.5 or 27.8 GBq in 5 cycles every 6–8 weeks), according to kidney and bone marrow parameters.
Results Twenty-eight patients received a mean full activity (FA) of 25.9 GBq and 32 a mean reduced activity (RA) of 18.5 GBq. The disease control rate (DCR), defined as the sum of CR+PR+SD was 85.7 % in the FA group and 78.1 % in the RA group. Median progression-free survival (mPFS) was 53.4 months in the FA group and 21.7 months in the RA group ($P = 0.353$). Median overall survival (mOS) was not reached (nr) in FA patients and was 63.8 months in the RA group ($P = 0.007$). Fifty-five patients underwent an FDG PET scan before Lu-PRRT, 32 (58 %) showing an increased FDG uptake in tumor sites. mPFS was 21.1 months in FDG PET-positive patients and 68.7 months in the FDG PET-negative group ($P < 0.0002$), regardless of the total activity administered.
Conclusion Both FA and RA are active in patients undergoing Lu-PRRT. However, an FA of 27.8 GBq of Lu-PRRT prolongs PFS and OS compared to an RA of 18.5 GBq. Our results indicate that FDG PET is an independent prognostic factor in this patient setting.

Keywords ^{177}Lu-DOTATATE · P-NET · PRRT · Long-term follow-up · Toxicity · FDG PET

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Introduction

Pancreatic neuroendocrine tumors (P-NETs) are increasing in frequency and represent 7 % of the total SEER database of more than 49,000 patients with NETs [1]. A consistent percentage of P-NETs are metastatic at diagnosis because of frequent delays in correct diagnosis. The diagnosis of a P-NET is based on the conventional histology and the immunohistochemical characterization of neuroendocrine markers, such as CgA and synaptophysin, or hormones. Despite limitations, due to lack of uniformity and consistency in quantification,

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the proliferation marker Ki-67 is currently used to determine tumor grade and to give prognostic indications [2]. The WHO 2010 grading system classifies well-differentiated P-NETs according to the Ki67 or mitotic index in: G1 (Ki67 ≤2 %, MI <2/10 HPF) and G2 (Ki67 = 3–20 %; MI = 2–20/10 HPF) [3]. Prognosis is influenced by the presence of distant metastases, liver involvement and the degree of tumor differentiation [4]. Binderup et al. in 2010 [5], reported that ^{18}F-FDG positron emission tomography/computed tomography (FDG PET) was positive in 40 % and 70 % of naive G1 and G2 NETs, respectively, and has a prognostic value in gastroenteropancreatic NETs (GEP-NETs) representing an important tool for the determination of tumor aggressiveness. In this respect, it is well known that increased glucose metabolism is a characteristic of many tumors (the so-called "Warburg effect") [6] and that the activation of this metabolic pathway correlates with a higher degree of malignancy. In a retrospective analysis, FDG PET was positive in 57 % of G1 and 66 % of G2 GEP-NETs and patients with a negative FDG PET responded significantly better to peptide receptor radionuclide therapy (PRRT) than those with a positive scan [7]. PRRT is a valid, experimental, therapeutic option for NETs and it has been used with different schedules in several protocols [8–12]. However, the best clinical practice for PRRT has not been defined yet. Sandström et al. [13] reported in a dosimetric study on a large population of patients receiving 27–29 GBq of ^{177}Lu DOTATATE (Lu-PRRT) in four cycles that the high level of radioactivity in the circulation led to a higher acute dose for the kidneys and/or bone marrow in 20 % of cases. These levels of activity approached the toxic dose (TD50/5) in some patients after only 2 cycles of 7.4 GBq of Lu-PRRT. These considerations and other dosimetric data such as fractionation and patients characteristic [14] support our decision to divide the total activity into 5 cycles and reduce it, in patients at risk of toxicity for kidney and bone marrow, to 18.5 GBq. Preliminary data on a smaller cohort have been reported [15]. Herein, we report the outcomes and the long-term follow-up in 60 advanced P-NET patients treated with a personalized Lu-PRRT protocol based on the presence of risk factors for renal or hematological toxicity. We further investigate the role of FDG PET in P-NET patients treated with Lu-PRRT.

Materials and methods

Patients

Eligible patients were >18 years old and were consecutively enrolled with histologically confirmed of unresectable or metastatic G1-G2 P-NETs according to the WHO 2010 classification into this phase II study at our institute (IRST-IRCCS, Meldola Italy). Before therapy, OctreoScan scintigraphy and/or PET/CT with ^{68}Ga-Dota-peptide was performed in all patients; only those with tumor uptake at least as high as that in normal liver tissue were admitted to the therapeutic phase. Extension of disease was documented with CT scan or MRI a maximum of 1 month before Lu-PPRT started. Tumor progression at baseline was defined as radiologic documentation of disease progression (unequivocal increase in tumor size) in the 12 months before enrolment, according to Southwest Oncology Group (SWOG) criteria. Prior treatments were allowed, including octreotide/lanreotide (≥4 weeks from long-acting preparations or >72 h from short-acting ones) and cytotoxic chemotherapy or radiotherapy (>1 month before and 2 months after PRRT). Eastern Cooperative Oncology Group (ECOG) performance status ≤2, adequate bone marrow, renal and hepatic function (WBC >2.5·10 9 /l, hemoglobin >10 g/dl, platelets >100 * 10 9 /l, bilirubin <2.5 mg/dl and creatinine <2.0 mg/dl) were required. Pregnant and lactating females were excluded, as were patients with a life expectancy <6 months and those with known previous malignancies. All patients gave their written informed consent. The protocol was approved by the Ethics Committee of Area Vasta Romagna and by the competent Italian regulatory authorities. The study followed the Declaration of Helsinki and good clinical practice guidelines. Fifty-five of these patients also underwent an ^{18} F-FDG PET/CT scan which, however, was not a prerequisite for study inclusion.

All patients were scheduled to receive five cycles of therapy at intervals of 6 to 8 weeks. The planned activity per cycle was 3.7 or 5.5 GBq of [^{177}Lu-DOTA^{0},Tyr^{3}]-octreotate on the basis of the presence or absence of risk factors for kidney and bone marrow. Patients were allocated to the reduced dosage (RA) of 3.7 GBq/cycle when at least one of the following risk factors was present [14]: Previous ^{90} Y-PRRT with a cumulative activity ≥9.25 GBq (250 mCi) not less than 2 months before Lu-PRRT, creatinine in the range of 1.5–2 mg/dl, morphological renal abnormalities, severe hypertension not properly controlled by drugs, insulin-dependent diabetes not properly controlled by medications, previous platinum-based chemotherapy, and age >80 years.

Evaluation of prognostic factors

Extent of tumor burden was scored on the basis of a three-point scale, as previously reported [16]: 1. Limited: up to five sites in one part of the body (head/neck, chest, upper abdomen, lower abdomen); 2. Moderate: multiple lesions in up to two sites of the body; 3. Extensive: multiple tumor sites in more than two parts of the body.

FDG PET was considered positive when an arbitrary cut off >2.5 standardized uptake value (SUV) was present in a documented lesion [7]. Hepatic involvement was defined in a three-grade scale: 0. No lesions; 1. 1–6 lesions; 2. >6 lesions.

The follow up period started from the last cycle of therapy. Tumor evaluation (CT and/or MRI) was performed at 3, 6, 12,

18 and then every 6 months after the end of treatment until documented progression of disease.

Radiopeptide preparation

DOTA-Tyr^3,Thr^8-octreotide (DOTATATE) was purchased from piCHEM (Graz, Austria). The radioisotope ^{177}Lu was purchased from PerkinElmer (Waltham, MA, USA). Preparation was carried out following a published procedure [17].

Radiopeptide administration and amino acid co-infusion

The radiopharmaceutical was slowly infused intravenously over 30′ using a dedicated pump system (patent US 7,842,023 B2). In order to protect the kidneys during the excretion of the radiopeptide, patients were pre-treated with intravenous amino acids (lysine 70 MEq in 500 ml of saline: 250 cc in 30′ immediately before therapy, 250 cc during therapy, lysine 70 MEq in 500 ml of saline in the first 3 h after therapy, and lysine 60 MEq in 500 ml of saline over 1 h twice the following day) [14].

Imaging

The gamma emission of ^{177}Lu (113 and 208 KeV, relative abundance 6 % and 11 %, respectively) allowed us to monitor radiopharmaceutical biodistribution during the therapeutic phase. Twenty-four h after Lu-PRRT administration, anterior and posterior whole-body images were acquired on a 128×512 matrix using a double-headed gamma camera equipped with a low-energy high-resolution collimator (LEHR) with the energetic window set on ^{177}Lu peaks. A single-photon emission computed tomography (SPECT) study was acquired (64 projections, 360°) in selected patients to better document tumor uptake. Tomographic images were reconstructed in trans-axial, sagittal and coronal slices. Results were compared with clinical history (histology, previous conventional examinations).

Statistical analysis

The main objective of this phase II, prospective study was to evaluate the disease control rate (DCR) of Lu-PRRT treatment in P-NETs treated with two different total activity levels. Secondary objectives were safety, progression-free survival (PFS) and overall survival (OS).

DCR, defined as the percentage of patients who achieved a complete response (CR) rate, partial response (PR) or stable disease (SD) for at least 12 months, was evaluated according to SWOG criteria. PFS was defined as the time from the start of Lu-PRRT to the date of the first observation of documented disease progression or death due to any cause. Patients without tumor progression at the time of analysis were censored at their last date of tumor evaluation. OS was defined as the time from start of treatment to the time of death from any cause. Subjects who were no longer alive at the time of the final analysis or who had been lost to follow-up were censored at their last known alive date.

Toxicity was evaluated according to National Cancer Institute (NCI) "Common Terminology Criteria for Adverse Events" (CTCAE) criteria, version 3 [18]. Patients who stopped therapy before the 3rd cycle for any reason other than progressive disease (PD) were considered unevaluable for treatment activity but were included in the safety analysis. Safety analysis was performed on the safety population and included patients who received at least one cycle of therapy.

With regard to the recruited patients, descriptive statistics were reported as appropriate for demographic characteristics, baseline characteristics of the tumor, anamnesis, and physical examination. Mean, median, standard deviation, minimum and maximum values were reported for continuous variables, and counts and proportions were reported for non-continuous variables.

DCR was calculated with an exact 95 % two-sided confidence interval (95 % CI) using standard methods based on binomial distribution. Time to event data (PFS and OS) were described using Kaplan–Meier curves and compared with a log-rank test. The 95 % CI for median time was calculated with non-parametric methods. Hazard ratio (HR) and 95 % CI were estimated using a Cox regression model.

The sample size was based on previous preliminary published data [15].

Hypothesizing an expected proportion of 80 % of DCR, a two-sided 95 % CI using large sample normal approximation was extended 0.10 from the observed proportion when the sample size was 60.

All statistical analyses were performed with SAS statistical software (version 9.4, SAS Institute Inc., Cary, NC, USA).

Results

Patient population

From March 2008 to June 2011, 63 consecutive advanced P-NET patients were enrolled; among these, 3 withdrew consent after 1 cycle, and the other 60 completed the 5 therapy cycles. Of these 60 patients, 29 (48 %) patients underwent surgery before PRRT, five patients (8 %) became operable after PRRT, 29 patients (48 %) had received or were in the process of being treated with long-acting somatostatin analogs, 15 patients (25 %) had received chemotherapy with different regiments and 16 patients (27 %) had previously received PRRT with ^{90}Y-DOTATOC. Nine patients (15 %) had received other treatments such as interferon, everolimus, locoregional therapy for

liver metastases and palliative radiotherapy. Liver metastases were present in 46 (77 %) patients, lymph node involvement in 28 patients (47 %), bony lesions in 13 patients (22 %) patients, and 3 patients (5 %) presented lung metastases. Median follow-up was 59 months (range 6.5–97). Patient characteristics are summarized in Table 1.

Activity

Twenty-eight patients received a mean full activity (FA) of 25.9 GBq (range 22.2–29.2) and 32 patients a mean reduced activity (RA) of 18.5 GBq (range 11.1–21.4). Overall response was CR in 4 (6.6 %) cases, PR in 14 (23.3 %) and SD in 31 (51.7 %), with a DCR of 81.7 %. Median PFS was 28.7 months (95 % CI 20.2–53.8) and median OS has not been reached yet.

Analyzing outcomes according to the cumulative administered activity, 3 patients in the FA group were categorized as CR (10.7 %), 9 PR (23.7 %), and 12 (42.9 %) SD, while 4 (14.3 %) patients showed PD, resulting in an overall DCR of 85.7 %. In the RA group, we observed 1 (3.1 %) CR, 5 (15.6 %) PR and 19 (59.4 %) SD cases, while 7 (21.9 %) patients showed PD, with a DCR of 78.1 % (Table 2).

Table 1 Patient characteristics

Characteristic	No. of patients (%)
Male	35 (58.3)
Female	25 (42.7)
Median age, years (range)	62 (34–82)
Administered activity	
Full activity group (27.g8 GBq)	28 (46.7)
Reduced activity roup (18.5 GBq)	32 (53.3)
Histological grading (WHO)	
G1	15 (25.0)
G2	32 (53.3)
Grade missing	13 (21.7)
FDG PET	
Performed	55 (91.7)
Positive	32 (58.2)
Negative	23 (41.8)
Tumor burden[a]	
Score 1	13 (21.7)
Score 2	32 (53.3)
Score 3	15 (25.0)
Hepatic involvement[a]	
G0	12 (20.0)
G1	16 (26.7)
G2	32 (53.3)

[a] See text for details

Table 2 Objective response according to administered cumulative activity and the FDG PET outcomes

	No.	CR No. (%)	PR No. (%)	SD No. (%)	PD No. (%)	DCR No. (%)
Overall	60	4 (6.6)	14 (23.3)	31 (51.7)	11 (18.3)	49 (81.7)
FA	28	3 (10.7)	9 (23.7)	12 (42.9)	4 (14.3)	24 (85.7)
RA	32	1 (3.1)	5 (15.6)	19 (59.4)	7 (21.9)	25 (78.1)
FDG PET -	23	3 (13.0)	7 (30.4)	12 (52.2)	1 (4.3)	22 (95.7)
FDG PET +	32	0	8 (25.0)	17 (53.1)	7 (21.9)	25 (78.1)

CR complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease, *DCR* disease control rate, *FA* full activity, *RA* reduced activity

Median PFS (mPFS) in the FA group was 53.4 months (95 % CI 20.1–68.7), while in the RA group, it was 21.7 (95 % CI 18.1–48.2, $P = 0.353$). Median OS (mOS) was not reached in the FA group, while in the RA group, it was 63.8 (95 % CI 25.9-nr, $P = 0.007$).

Taking into account the role of glucose consumption at tumor level, mPFS in the FDG positive group was 21.2 months (95 % CI 18.1–28.7), while in the negative FDG group, it was 68.7 months (95 % CI 53.4-nr, $P < 0.0002$) regardless the total administered activity (Fig. 1).

Median OS was not reached (nr) in the negative FDG PET group and was 63.8 (95 % CI 28.2-nr) in the positive FDG PET group ($P = 0.006$).

The other prognostic factors that influenced PFS in a univariate analysis were: grade 2 hepatic lesion (21.1 months-95 % CI 18.1–31.0, $P = 0.003$) and tumor burden (score 3, 20.1 months-95 % CI 8.3–28.7, $P = 0.020$). Whereas grade 2 hepatic lesion (63.8 months-95 % CI 28.2-nr, $P = 0.012$), positive FDG PET and RA administered influenced OS by univariate analysis. Results are summarized in Tables 3 and 4.

In a multivariate analysis including FDG PET, tumor burden, hepatic lesions, and cumulative activity, FDG PET was found to be the only independent prognostic factors for PFS ($P = 0.013$). After a backward stepwise procedure, glucose consumption became statistically significant both for PFS ($P = 0.0005$) and OS ($P = 0.015$). Full cumulative activity of 27.8 had a statistically significant impact on OS ($P = 0.0361$) (Table 5).

Toxicity

Complete blood counts with differential and platelet counts, hemoglobin and creatinine, were performed each cycle after 2 and 4 weeks from the day of therapy, every 3 months for the first year of follow up and then every 6 months.

According to the CTCAE criteria [17], in the FA group, 11 (39.2 %) patients had anemia (9 patients were grade 1, 2 patients were grade 2); 3 (10.7 %) patients had

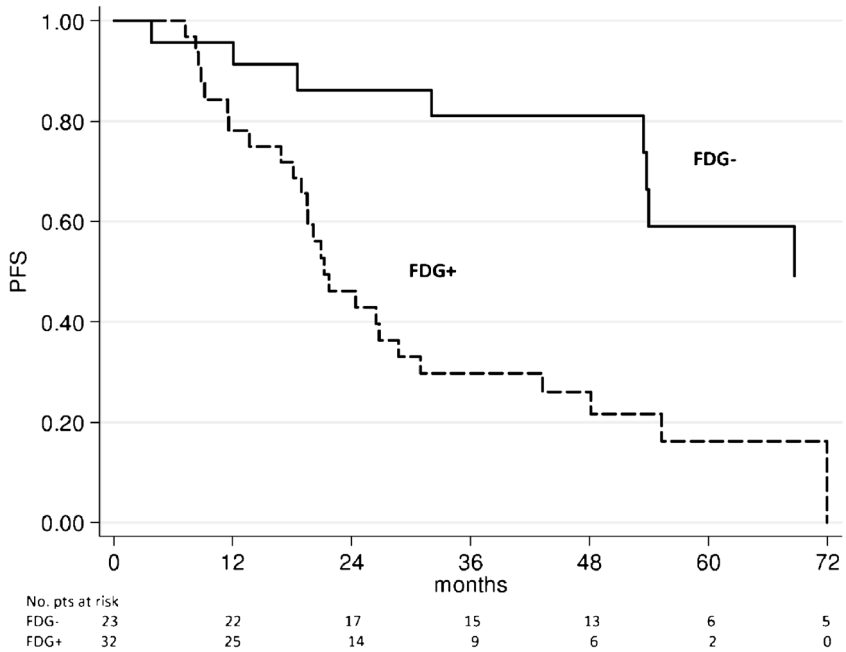

Fig 1 Role of glucose consumption related to mPFS. Notably, in the FDG positive group, it was 21.2 months (95 % CI 18.1–28.7), while in the negative FDG group, it was 68.7 months (95 % CI 53.4-nr, $P < 0.0002$), regardless of the total administered activity

thrombocytopenia (all grade 1); 6 (21.4 %) patients had leucopenia (2 patients were grade 1, 4 patients were grade 2).

In the RA group, 10 (31.3 %) patients had anemia (8 patients were grade 1, 2 were grade 2); 9 (28.1 %) patients had thrombocytopenia (8 patients were grade 1, 1 patient was grade 2); 7 (21.9 %) patients had leucopenia (all grade 1). Grade 3 or 4 hematological toxicity did not occur in any patient of both groups and the whole therapy course could be completed in all of them without delay.

Table 3 Univariate analysis related to progression-free survival

	No. patients	No. events (%)	Median PFS (months) (95 % CI)	*P*
Overall	60	40	28.7 (20.2–53.8)	–
Age, years				
<60	27	16	26.8 (18.1–72.0)	
≥60	33	24	31.0 (20.1–53.8)	0.739
Gender				
Males	35	26	20.2 (18.5–32.1)	
Female	25	14	54.0 (26.8–68.7)	0.075
Tumor burden				
Score 1	13	5	72.0 (26.8-nr)	
Score 2	32	22	26.3 (19.5–53.4)	
Score 3	15	13	20.1 (8.3–28.7)	0.020
Hepatic lesions				
Grade 0	12	4	54.0 (20.1-nr)	
Grade 1	16	7	57.7 (13.7-nr)	
Grade 2	32	29	21.1 (18.1–31.0)	0.003
FDG PET				
Negative (FDG-)	23	9	68.7 (53.4-nr)	
Positive (FDG+)	32	27	21.1 (18.1–28.7)	0.0002
Cumulative activity				
RA	32	22	21.7 (18.1–48.2)	
FA	28	18	53.4 (20.1–68.7)	0.353

RA reduced activity, *FA* full activity, *nr* not reached

Table 4 Univariate analysis related to Median OS

	No. patients	No. events (%)	Median OS (months) (95 % CI)	*P*
Overall	60	20	nr	–
Age, years				
<60	27	8	nr	
≥60	33	12	nr	0.646
Gender				
Males	35	14	nr	
Female	25	6	nr	0.153
Tumor burden				
Score 1	13	1	nr	
Score 2	32	12	nr	
Score 3	15	7	63.8 (16.2-nr)	0.058
Hepatic lesions				
Grade 0	12	1	nr	
Grade 1	16	3	nr	
Grade 2	32	16	63.8 (28.2-nr)	0.012
FDG PET				
Negative (FDG-)	23	3	nr	
Positive (FDG+)	32	15	63.8 (28.2-nr)	0.006
Cumulative activity				
RA	32	15	63.8 (25.9-nr)	
FA	28	5	nr	0.007

OS overall survival, *RA* reduced activity, *FA* full activity, *nr* not reached

Regarding nephrotoxicity, only two (6 %) patients in the group at risk had, respectively, grade 2 and grade 3 kidney toxicity. In particular, the latter patient, affected by a locally advanced non-resectable P-NET, had elevated creatinine at baseline, likely due to a previous treatment with ^{90}Y-based PRRT and a long history of hypertension. However, in consideration of disease progression, we decided to treat this patient with a reduced activity of 18.5 GBq. She responded to Lu-PRRT and 1 year after the treatment, the primary tumor lesion was amenable of surgery. She developed acute kidney failure shortly after surgery. Currently, she remains disease-free and recently started dialysis. None of the remaining patients to date developed late renal toxicity (Table 6).

Discussion

These results confirm previous data published by our group after a three additional years of follow up [15]. Using a

Table 5 Multivariate analysis related to PFS and Median OS

	PFS		mOS	
	HR (95 % CI)	*P*	HR (95 % CI)	*P*
FDG (positive vs. negative)	5.15 (1.42–18.75)	0.013	5.08 (0.85–30.42)	0.075
Tumor burden (score 2 vs. 1)	3.03 (0.92–9.99)	0.188	4.12 (0.41–40.96)	0.477
Tumor burden (score 3 vs. 1)	2.55 (0.75–8.71)		3.98 (0.38–41.95)	
Hepatic lesions (grade 1 vs. grade 0)	1.54 (0.30–7.80)	0.871	1.76 (0.13–22.92)	0.910
Hepatic lesions (grade 2 vs. grade 0)	1.31 (0.31–5.49)		1.54 (0.15–15.99)	
Cumulative activity (RA vs. FA)	0.85 (0.41–1.76)	0.658	2.32 (0.75–7.16)	0.144
After backward stepwise procedure				
FDG (positive vs. negative)	4.27 (1.88–9.69)	0.0005	4.89 (1.35–17.65)	0.015
Cumulative activity (RA vs. FA)	1.18 (0.60–2.34)	0.627	3.17 (1.08–9.34)	0.0361

PFS progression-free survival, *mOS* median overall survival, *HR* hazard ratio, *RA* reduced activity, *FA* full activity,

Table 6 Lu-PRRT-induced toxicities according to CTCAE v.3

	FA ($n = 28$)				RA ($n = 32$)			
	G0	G1	G2	G3	G0	G1	G2	G3
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
WBC	22 (78.6)	2 (7.1)	4 (14.3)	0	25 (78.1)	7 (21.9)	0	0
HgB	17 (60.7)	9 (32.2)	2 (7.1)	0	22 (68.7)	8 (25.0)	2 (6.3)	0
PLT	25 (89.3)	3 (10.7)	0	0	23 (71.9)	8 (25.0)	1 (3.1)	0
Creatinine	28 (100)	0	0	0	25 (78.1)	5 (15.7)	1 (3.1)	1 (3.1)

G0 grade O, *G1* grade 1, etc., *FA* full activity, *RA* reduced activity

personalized approach based on risk factors, the side effects of Lu-PRRT were substantially reduced with no grade 3–4 toxicity in all patients except one. This personalized protocol allowed us to treat patients otherwise excluded from PRRT, due to an increased risk to develop kidney or hematologic toxicity at the standard dosage of 27–29 GBq.

The use of "standard" activities of 7.4 GBq/cycle repeated for four cycles, as reported in literature, may lead to the impossibility to complete the treatment without exceeding renal or bone marrow adsorbed dose constraints in over 20 % of the cases or cause grade 3–4 hematotoxicity in over 10 % of cases [8, 10, 19–21].

The use of "customized" activity, especially in selected patients at risk of developing serious adverse sequelae, had limited side-effects and allowed us to reduce the interval between cycles from 10–12 weeks to 6–8 weeks. This probably resulted in good tumor control and may also explain the DCR obtained in the RA group as the intended total activity was administered in all patients within 6 to 8 months.

Other PRRT protocols are based on a fixed treatment dosage that does not take into consideration the possible detrimental effects in categories at risk. In this study, we used lower activity levels per cycle while increasing the number of therapy courses. In our opinion, this is the reason for the absence of grade 3–4 hematotoxicity in all our patients, including those at risk. An interval of 8–12 weeks is used in the majority of trials in order to check acute toxicity and allow recovery when needed. The nadir of acute toxicity with Lu-PRRT is generally at 3–4 weeks with recovery in the following 4–6 weeks. However, prolonging the time between cycles might allow for lesion regrowth and ultimately result in a lower tumor control probability (TCP). In absence of acute toxicity, it is logical to reach a minimal effective activity at an earlier time point, in order to increase the TCP and decrease the percentage of surviving cancer cells in the tumor lesions.

Regarding the activity, in terms of DCR in patients treated with a reduced dosage of Lu-PRRT, our data are consistent with those recently reported in a meta-analysis carried out by Kim S-J et al. [22]. The authors reported a DCR ranging from 73.9 to 89.1 % according to SWOG criteria.

However, it must be considered that in the present study, a cumulative activity of 27.8 GBq provided better outcome compared to 18.5 GBq, in terms of PSF and OS. Of note, all patients with a negative FDG scan treated with the FA were alive at their last follow-up (Fig. 2a, b). Interestingly, this was not the case in an identical protocol carried out in gastrointestinal-NETs where a reduced activity of 18.5 GBq turned out results comparable to the higher cumulative activity of 27.8 GBq [23]. Clearly, P-NETs and GI-NETs have to be considered two separate entities of the NET family [24].

Another important point to emerge from this study is the prognostic role of FDG PET in P-NETs. One of the most common biochemical phenotypes of cancer cells is their ability to metabolize glucose at high rates, even under aerobic conditions [25]. This altered metabolic pathway effect, also known as the Warburg effect, leads to the establishment of tumor-specific metabolic machinery that is sufficient for supporting the biosynthetic and energy requirements of the tumor cells to facilitate rapid tumor growth and adaptation to new metastatic niches. Several oncogenes have been implicated in altering tumor cell metabolism in order to facilitate tumor growth and metastasis. It is still unclear whether this phenomenon is either the consequence of altered genetic regulation or it is one of the causes of cancer development. In this study, among 55 patients who received an FDG PET scan, 32 (58 %) presented an increased glucose metabolism and 23 (42 %) were classified as negative, regardless of the Ki67 grading score.

Despite reservations on its efficacy (there is evidence that it regulates ribosomal expression rather than directly contributing to cell cycle progression) [26], Ki67 is currently used as the default tool in differentiating low- and high-grade NETs. However, it usually provides the evaluation of selected tumor areas within the primary or the metastases, therefore, not necessarily representing the actual situation of the entire tumor lesion and of other possible coexistent lesions [5, 27, 28]. As opposed to Ki67, FDG PET represents a real-time whole-body technique, and the accelerated glucose metabolism of many G1 tumors (57 %), as opposed to its absence in some G2 forms (34 %) [7], indicates the need to develop a more

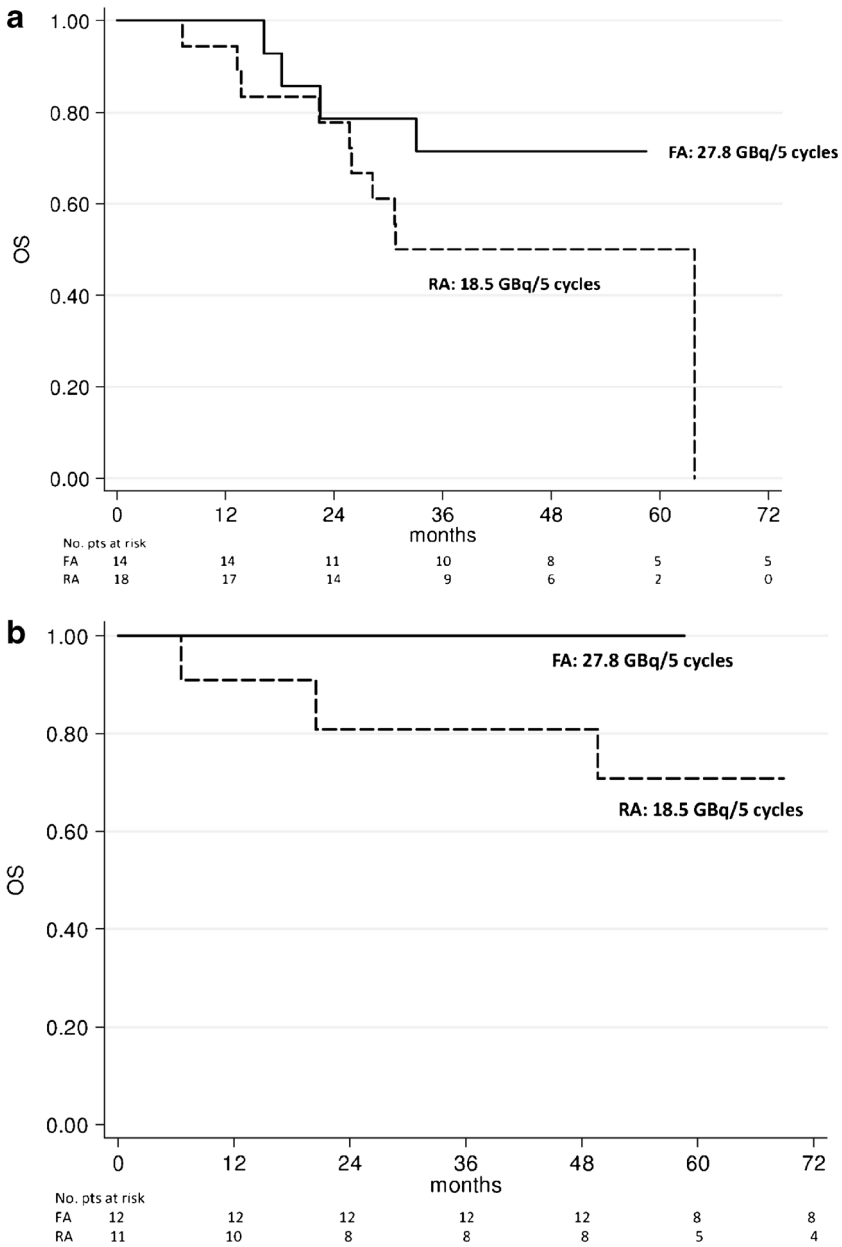

Fig 2 **a** OS related to total administered activity in FDG-positive patients: a cumulative activity of 27.8 GBq provided better outcome compared to 18.5 GB. **b** All patients with a negative FDG scan treated with the FA of 27.8 in five cycles were alive at their last follow-up of March 2016 (median 59 months, range 6.5–97). Median OS was nr also in those patients treated with a reduced activity of 18.5 GBq. FDG PET outcome is an independent prognostic factor in advanced patients treated with Lu-PRRT

comprehensive characterization of NET patients, in addition to the standard evaluation of Ki-67. In the present study, patients with a negative FDG PET showed a significantly better outcome after Lu-PRRT as opposed to those with positive scans. The strong link among the genetic factors, epigenetic modulation and the glucose metabolism is of relevant interest to develop novel biomarkers or integrated biomarkers (nomograms) as well as advanced cancer therapeutic drugs with the intent of a curative approach for P-NETs [29].

The results of the present study reinforce a study presently ongoing in our institute. The previous demonstration of a differential PFS according to the FDG status in patients with gastrointestinal-pancreatic NET treated with PRRT [7] constitutes the basis for an intensified protocol comprising the use of capecitabine plus Lu-PRRT in FDG-positive P-NETs. This protocol, named LuX (Lutethium+Xeloda) has the objective to increase the PFS and OS in those patients that may have a worse prognosis as predicted by a positive FDG scan at baseline. Similar protocols utilizing a combination of Lu-PRRT and 5-fluorouracil in progressive FDG-avid GEP-NETs demonstrated high response rates (98 % DCR) and 27 % complete metabolic responses, with low toxicity [30]. Protocols combining Lu-PRRT and capecitabine in GEP-NETs without FDG stratification have also been demonstrated to be effective [31] with modest hematotoxicity [32].

In conclusion, this prospective study suggests that Lu-PRRT is a valid and efficacious therapeutic option for advanced G1–G2 P-NETs that can be adapted to the patient'

characteristics. Patients unable to receive the full dosage, due to their comorbidities, can benefit from PRRT at a reduce dosage and should not be excluded from the treatment. However, based on the better outcome in terms of PFS and OS, the full activity of 27.8 GBq should be administered whenever feasible and preferably divided into five cycles. FDG PET is an independent prognostic factor in advanced P-NETs.

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Compliance with ethical standards

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Ethical approval The protocol was approved by the Ethics Committee of Area Vasta Romagna and by the competent Italian regulatory authorities. The study was performed in accordance with the principles of Good Clinical Practice and the Declaration of Helsinki.

Informed consent All patients gave their written informed consent.

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