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Neuroendocrine Differentiation and Response to PSMA-Targeted Radioligand Therapy in Advanced Metastatic Castration-Resistant Prostate Cancer: a Single-Center Retrospective Study

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ABSTRACT

Introduction. Neuroendocrine differentiation is associated with treatment failure and poor outcome in metastatic castration-resistant prostate cancer (mCRPC). We investigated the effect of circulating neuroendocrine biomarkers on the efficacy of PSMA-targeted radioligand therapy (RLT).

Methods. Neuroendocrine biomarker profiles (progastrin-releasing peptide, neuron-specific enolase, and chromogranin-A) were analyzed in 50 patients commencing ^{177}Lu-PSMA-617 RLT. The primary endpoint was PSA response in relation to baseline neuroendocrine marker profiles. Additional endpoints included progression-free survival. Tumor uptake on post-therapeutic scans, a known predictive marker for response, was used as control-variable.

Results. Neuroendocrine biomarker profiles were abnormal in the majority of patients. Neuroendocrine biomarker levels did not predict treatment failure or early progression ($P \geq 0.13$). By contrast, intense PSMA-ligand uptake in metastases predicted both treatment response ($P=0.0030$) and reduced risk of early progression ($P=0.0111$).

Conclusion. Neuroendocrine marker profiles do not predict adverse outcome of RLT. By contrast, high ligand uptake was confirmed to be crucial for achieving tumor-response.

Key words: Neuroendocrine differentiation; Chromogranin A; Prostate-specific membrane antigen; PSMA; PSMA-617; radioligand therapy

INTRODUCTION

Small molecule inhibitors targeting the prostate-specific membrane antigen (PSMA) such as PSMA-617 have recently emerged for therapy of advanced metastatic castration-resistant prostate cancer (mCRPC) (*1-3*). However, a fraction of patients will not respond to ^{177}Lu-PSMA-617 or eventually progress, underlining an unmet clinical need for response prediction and better understanding of the biological basis of treatment failure.

A late manifestation of advanced, pretreated prostate cancer is the development of treatment-related neuroendocrine prostate cancer (*4*), induced by a variety of therapeutic agents (*5*). Neuroendocrine-like cells secrete peptide hormones and growth factors to support the growth of surrounding tumor cells in a paracrine manner (*6*), and express high levels of survival genes (*7*), conferring resistance to treatments (*5*).

Recently, Rathke et al. suggested that elevated baseline CgA may have a moderate impact as a negative prognostic marker in PSMA-targeted RLT (*3*). In this study, we aimed to further investigate the significance of neuroendocrine differentiation. The primary endpoint was PSA response to ^{177}Lu-PSMA-617 in advanced mCRPC in relation to pre-treatment neuroendocrine marker profiles. Additional endpoints included progression-free survival. Tumor-uptake on post-therapeutic ^{177}Lu-PSMA-617 scans, a known predictive marker for response (*3*), was used as a control-variable.

MATERIALS AND METHODS

Study Cohort, Neuroendocrine Biomarkers and Non-oncologic Influencing Factors

50 consecutive mCRPC patients (**Supplemental Table 1**) were referred for ^{177}Lu-PSMA-617 RLT between December 2017 and July 2019. Laboratory parameters including standard hematology, PSA, progastrin-releasing peptide (ProGRP; upper limit of normal, 40 ng/l), neuron-specific enolase (NSE; upper limit of normal, 16 µg/l) and chromogranin-A (CgA; upper limit of normal, 76 µg/l) were obtained (*8*). PSMA expression was confirmed by ^{68}Ga-PSMA-ligand PET prior to RLT (PSMA-ligand uptake higher than background on PET). Non-oncologic factors with potential influence on serum neuroendocrine biomarker levels, i.e. medication with proton-pump inhibitors, reduced renal function, chronic heart failure and chronic atrophic gastritis, were documented (*8*). Such factors were identified in a subgroup of 21 patients (**Supplemental Figure 1**). ^{177}Lu-PSMA-617 was administered in compliance with the Declaration of Helsinki, §37 (“unproven interventions in clinical practice”) and the German Medicinal Products Act, AMG §13.2b, and all subjects provided written informed consent. The institutional review board approved this retrospective study (No. 8148_BO_K).

^{177}Lu-PSMA-617 RLT

The PSMA-targeting ligand ^{177}Lu-PSMA-617 was prepared in compliance with good manufacturing practice (**Supplemental Data**). Patients received 6.0-7.4 GBq of ^{177}Lu-PSMA-617 every 6-8 weeks by slow intravenous injection over 5 min. Treatment followed the national consensus recommendation for the use of PSMA-RLT (*9*).

PSMA-Ligand Uptake in Posttherapeutic Scintigraphy

For verification of regular post-therapeutic tracer distribution, ^{177}Lu-PSMA-617 scans were acquired 20-24 h p.i. (planar anterior and posterior whole-body scans), using a dual-head gamma camera (GE Discovery NM/CT 670; medium energy collimators, scan speed 20 cm/min, 208keV +/-10% photo peak window). PSMA-ligand uptake of the salivary glands was used as reference level on greyscale. Based on qualitative visual assessment, PSMA-ligand uptake in tumor lesions at Cycle 1 Day 2 was dichotomized into intense PSMA-ligand uptake (defined as semi-quantitative uptake > salivary gland uptake) and low PSMA-ligand uptake (defined as uptake ≤ salivary gland uptake) (*3*), and used for further analysis.

Treatment Response and Early Progression

Treatment response was determined according to *Prostate Cancer Clinical Trials Working Group 2* (PCWG2) (*10*) criteria, defined as a ≥50% serum PSA decline from baseline with confirmation 3–4 weeks apart, and absence of new metastases in a ^{68}Ga-PSMA-11 PET/CT scan performed after 2 cycles. Progression was defined as a PSA increase of 25% or more and absolute increase of 2 ng/mL or more from the nadir (*10*), or appearance of new metastases in PET/CT. PSA response rate and the percentage of PSA decline after 2 cycles (i.e., at Cycle 3 Day 1) were documented. In patients who discontinued RLT (*n*=4 after 1 cycle, and *n*=15 after 2 cycles), the PSA level at termination of therapy was used for further analyses. For calculation of progression-free survival (PFS), patients were followed for 125 days after the first RLT (i.e., for two full cycles plus PET restaging).

***Ex Vivo* Analysis of Prostate Cancer Tissue**

Three primary tumor samples and two specimens of metastases were available, and evaluated for marker protein expression (**Supplemental Data**).

Statistical Analysis

For group comparison, the Mann-Whitney test (continuous variables) and Fisher`s exact test (categorical variables) were used. Spearman correlation was used to assess the relation between percentage PSA change and neuroendocrine marker levels. Intra- and inter-rater reliability of assessment of PSMA-ligand uptake was determined using Cohen`s kappa. To this end, scans were evaluated by two independent raters blinded to all clinical information. One reader scored the images twice, two weeks apart. Univariate logistic regression analysis was performed to assess the relation between outcome and various laboratory and clinical parameters. Results were expressed as odds ratios (OR), with corresponding 95%-confidence intervals and *P*-values. Survival analysis (PFS) was performed using the Kaplan-Meier method, and data were compared using the logrank test. Statistical significance was established for *P*-values <0.05. Analysis was performed using GraphPad Prism® (version 8.3 for Windows; Graphpad Software).

RESULTS

Neuroendocrine Secretory Profiles are Abnormal in the Majority of mCRPC Patients

In the total study population, pre-treatment serum levels of ProGRP, NSE and CgA were abnormal in 80%, 88% and 74% of patients. In the subgroup of patients without potential influencing factors, pre-treatment serum levels of ProGRP, NSE and CgA were abnormal in 76%, 90% and 59% of patients, and demonstrated marked interindividual variability (**Supplemental Table 1** and **Supplemental Figure 2**).

Neuroendocrine markers were not associated with Gleason scores ($P \geq 0.65$ in all cases) or previous therapies ($P \geq 0.10$ in all cases) besides second line chemotherapy with cabazitaxel (NSE, 50 ± 44 µg/l vs 24 ± 9 µg/l; $P = 0.04$). Last, neuroendocrine marker levels were not significantly different in patients with or without lymph node metastases at ^{68}Ga-PSMA-ligand PET/CT prior to RLT ($P \geq 0.11$), in patients with or without bone metastases ($P \geq 0.59$), or in patients with or without hepatic metastases ($P \geq 0.29$ in all cases).

Serum Levels of Neuroendocrine Markers are Not Associated with Response to RLT

In the total study population, 19 (38%) of 50 patients demonstrated a treatment response following 2 cycles of ^{177}Lu-PSMA-617. In the subgroup, a similar fraction (9/29 patients; 31%) responded.

PSA change (%) after 2 cycles was not significantly associated with biomarker levels in the entire cohort ($P \geq 0.10$ in all cases) and in the subgroup ($P \geq 0.13$ in all cases) (**Figure 1** and **2**). Intense PSMA-ligand tumor uptake, but not neuroendocrine markers, predicted treatment response in both the total study population (OR 11.77 (95% CI, 2.743 to 82.81), $P = 0.0030$) and the subgroup (OR 6.500 (95% CI, 1.194 to 52.38), $P = 0.0439$) (**Supplemental Table 2**). In both the total study population and the subgroup, low PSMA-ligand accumulation was not associated with marker levels ($P \geq 0.2145$ in all cases.)

Cohen`s kappa for intra-rater agreement concerning PSMA-ligand uptake was 0.88 (95% CI, 0.740 to 1.000; 94% agreement), i.e. “almost perfect agreement”.

Cohen`s kappa for inter-rater agreement was 0.66 (95% CI, 0.440 to 0.870; 84% agreement), i.e. “substantial agreement”.

Serum Levels of Neuroendocrine Markers are Not Associated with Early Progression

In the total study population, 19 (38%) of 50 patients showed early progression (PSA progression (*n*=16) and/or new metastases (*n*=10)). In the subgroup, 13 (45%) of 29 patients showed early progression (PSA progression (*n*=11) and/or new metastases (*n*=6)). Intense PSMA-ligand tumor uptake protected from early progression (OR 0.2029 (95% CI, 0.05604 to 0.6721), *P*=0.0111 for entire group), whereas neuroendocrine markers did not predict early progression (**Supplemental Table 3**). Median PFS (**Figure 3**) was 92 days in patients with low PSMA-ligand uptake, and not yet reached in patients with intense uptake (HR 0.3464; 95% CI, 0.1354 to 0.8863; *P*=0.0196 for entire group).

Immunohistochemical Analysis Reveals Heterogeneous Neuroendocrine Marker Expression

Neuroendocrine marker expression demonstrated marked intralesional and interpatient heterogeneity. Two of three primary tumor specimens at the time of prostate cancer diagnosis and one of two metastases at the time of mCRPC stained positively for NSE (5% to 15% of tumor cells) (**Supplemental Table 4, Figure 2**). Marker expression was not associated with treatment response (Fisher`s exact test, *P*=1.0).

DISCUSSION

Pre-treatment neuroendocrine marker levels were elevated in the majority of patients prior to their first PSMA-targeted RLT, even after exclusion of potential confounding factors. However, marker levels had no value for prediction of response or short-term progression-free survival.

Rathke et al. have observed that CgA showed no significance for outcome prediction, but had some predictive value for disease progression (*3*). The present project evaluated a more comprehensive panel of three neuroendocrine markers. Consistent with both the marked interindividual variability of these markers and their absent significance for response prediction or prognosis, neuroendocrine marker expression in histological specimens also demonstrated intralesional and interpatient heterogeneity, and was not associated with treatment response.

Importantly, although the role of biomarkers for early prediction of response to PSMA-ligand RLT is evolving, studies have delivered inconsistent results so far. Ferdinandus and colleagues found that the number of platelets, but not LDH levels, was an independent factor for response prediction (*11*). Other studies identified serum level of LDH before RLT as independent predictor of response (*3*). In this study, intense PSMA-ligand uptake in metastases emerged as the single predictor of treatment response. Others also found strong predictive value of intense tumor uptake on posttherapeutic ^{177}Lu-PSMA-617 scintigraphy (*3*), but – unlike our study - reported an additional value of serum markers.

There is a more consistent notion that PSMA imaging biomarkers are useful for response prediction. From PSMA PET, e.g., prior work showed that uptake of the parotids correlated with absorbed dose, and whole-body tumor dose was associated with PSA response at 12 wk (*12*). We found no association between neuroendocrine biomarkers and low tumor uptake of PSMA ligand, rejecting the presumption that serum-linked evidence of NED leads to clinically detectable, relevant loss of PSMA expression. Indeed, a recent biopsy study indicated that half of the patients with no histologic PSMA expression in the castration-resistant metastatic phase were already negative at initial, castration-sensitive diagnosis (*13*). Of note, lowest PSMA expression was observed in liver metastases, but none of these biopsies demonstrated overt NED (*13*).

Some limitations should be acknowledged. First, the single-center and retrospective design of our work may be associated with inherent limitations. Second, although we applied particular care to account for potential influencing factors, neuroendocrine markers may be elevated for various reasons. However, individual elevations were consistent across different markers, and in line with published evidence (*6*). Third, we did not evaluate the significance of baseline PSMA PET, because scans were acquired at different institutions with different radiotracers, scanners and protocols, confounding analyses, and tumor uptake of PSMA-617 and other compounds, e.g. PSMA-11, is different. Finally, prostatic adenocarcinoma with neuroendocrine differentiation must not be confused with small cell carcinoma of the prostate, a high-grade, morphologically and histologically distinct malignant neoplasm.

CONCLUSION

Neuroendocrine secretory profiles are abnormal in the majority of advanced mCRPC patients, and highly heterogeneous. Elevated neuroendocrine markers do not predict treatment failure in PSMA-targeted RLT, and patients should not be excluded from RLT based on serum-linked evidence of NED. By contrast, high ligand uptake was confirmed to be crucial for achieving tumor-response.

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None.

DISCLOSURE

No potential conflict of interest relevant to this article was reported.

KEY POINTS

QUESTION: Do circulating neuroendocrine biomarkers demonstrate predictive or early prognostic significance in patients commencing RLT with ^{177}Lu-PSMA-617?

PERTINENT FINDINGS: In a cohort study evaluating outcomes in 50 men with mCRPC, abnormal neuroendocrine biomarker profiles indicating therapy-induced neuroendocrine-like trans-differentiation were found in the majority of patients. However, biomarker levels did not predict treatment failure or early progression.

IMPLICATIONS FOR PATIENT CARE: Neuroendocrine markers do not predict outcome of PSMA-targeted RLT, and RLT should not be withheld on the ground of serum-linked evidence of NED.

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Figure legends

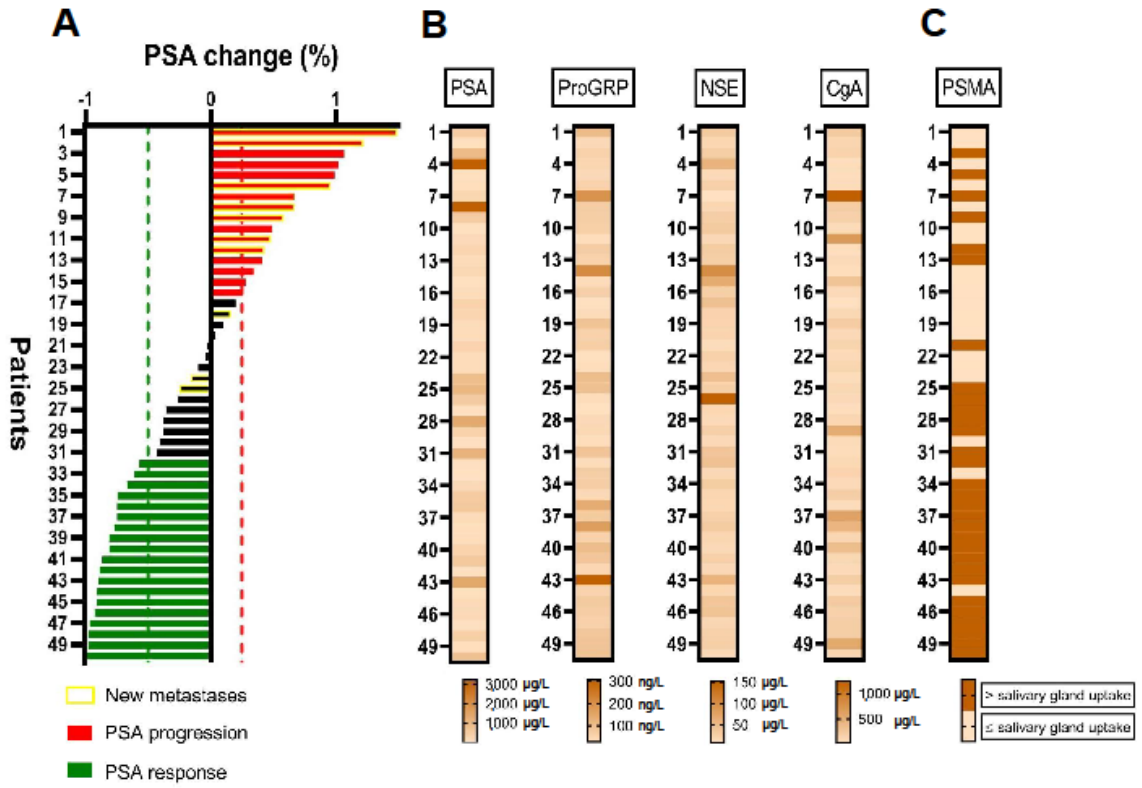


Figure 1 *Treatment response to ^{177}Lu-PSMA-617 radioligand therapy and neuroendocrine markers in the total study population (n=50).* Graphical representation (waterfall plot) of PSA change after 2 cycles of ^{177}Lu-PSMA-617 RLT presented in order of increasing PSA response ranging from +149% to -100% (**A**). *Green line indicates PSA response, red line indicates PSA progression.* Graphical representation of the degree of interindividual heterogeneity (**B**) in PSA levels and neuroendocrine marker levels prior to therapy, depicted as heat map ranging from low serum levels (*light beige*) to high serum levels (*dark brown*). High marker levels are observed in both responders and non-responders, and are randomly distributed. PSMA ligand uptake in metastases (**C**) depicted as categorical heat map. High PSMA ligand uptake is significantly associated with treatment response (*P*=0.0030).

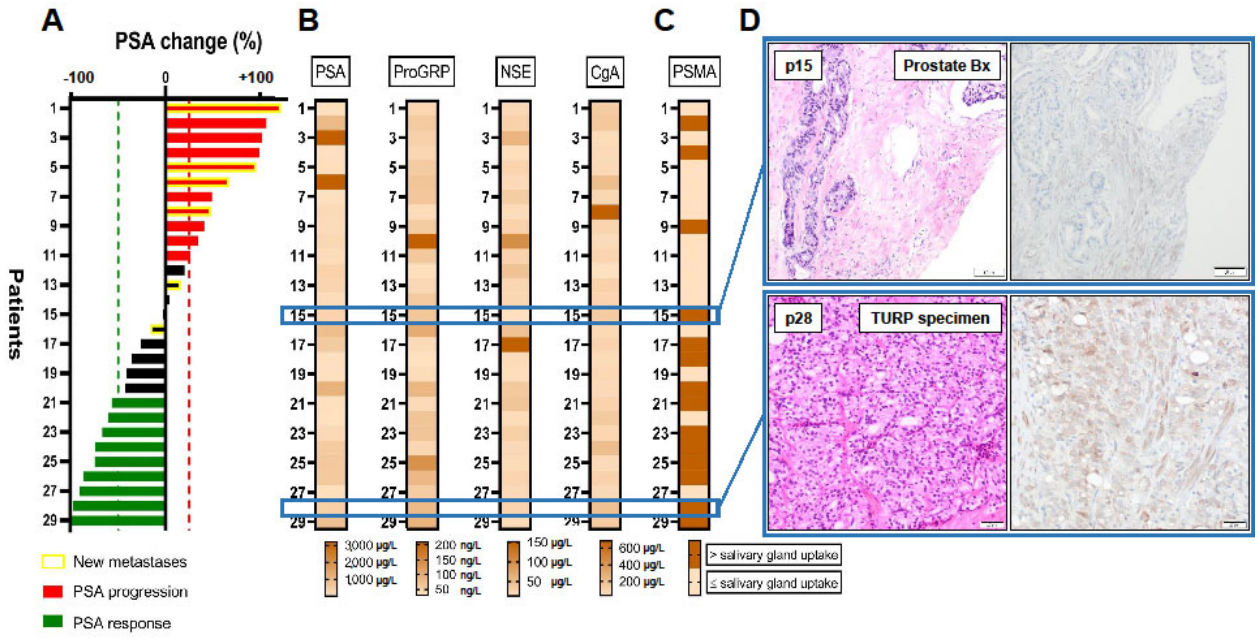
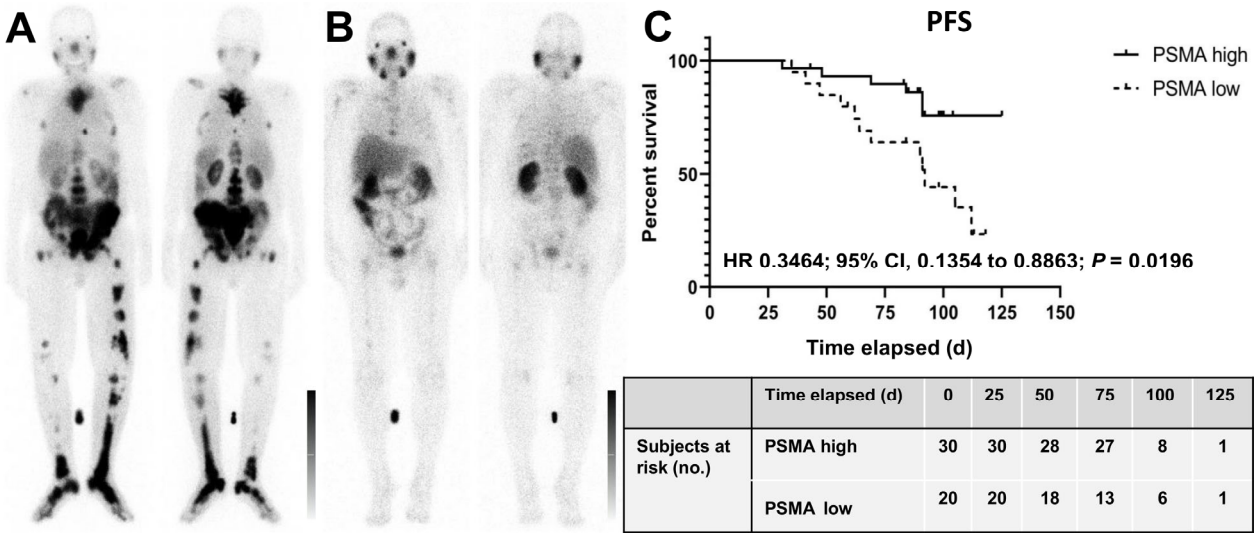


Figure 2 *Treatment response to ^{177}Lu-PSMA-617 radioligand therapy and neuroendocrine markers in the subgroup without factors potentially influencing marker levels (n=29).* Graphical representation of PSA change after 2 cycles of ^{177}Lu-PSMA-617 RLT presented in order of increasing PSA response ranging from +122% to -100% (**A**). *Green line indicates PSA response, red line indicates PSA progression.* Interindividual heterogeneity (**B**) in PSA levels and neuroendocrine marker levels prior to therapy. High marker levels are observed in both responders and non-responders. PSMA ligand uptake in metastases (**C**) depicted as categorical heat map. High PSMA ligand uptake is significantly associated with PSA response (*P*=0.0439). Examples of immunohistochemistry (**D**): Prostate biopsy of a non-responder (*upper row*) to ^{177}Lu-PSMA-617 at time of prostate cancer diagnosis containing prostate adenocarcinoma cancer cells (Hematoxylin & eosin stain (HE), *left panel*) with no detectable NSE expression (IHC, *right panel*); two of three serum markers were elevated at time of PSMA-targeted therapy (ProGRP, 164 ng/l; CgA 136 µg/l), consistent with therapy-

induced neuroendocrine differentiation. Transurethral resection of prostate (TURP) specimen of a responder (*lower row*) to ^{177}Lu-PSMA-617 at time of prostate cancer diagnosis containing prostate adenocarcinoma cancer cells (HE, *left panel*) with moderate cytoplasmatic NSE expression (IHC, *right panel*); all three serum markers were elevated at time of PSMA-targeted therapy (ProGRP, 81 ng/l; NSE, 25 µg/l; CgA 196 µg/l), consistent with neuroendocrine differentiation and pre-existing NSE protein expression in primary tumor.



	Time elapsed (d)	0	25	50	75	100	125
Subjects at risk (no.)	PSMA high	30	30	28	27	8	1
	PSMA low	20	20	18	13	6	1

Figure 3 *High PSMA-ligand expression and risk of early progression in patients receiving ^{177}Lu-PSMA-617 radioligand therapy (n=50).* Intense (**A**) and low (**B**) PSMA-ligand tumor uptake on posttherapeutic ^{177}Lu-PSMA-617 whole-body scans. Patients with intense tumor uptake had reduced risk of early progression (median PFS not yet reached vs 92 days in patients with low PSMA-ligand uptake; *P*=0.0196) (**C**).

SUPPLEMENTAL DATA

Supplemental Materials and Methods

GMP-compliant Preparation of the PSMA-targeting Ligand ^{177}Lu-PSMA-617

Lutetium-177 was purchased from itg (Isotope Technologies Garching GmbH, Germany) as GMP-certified ^{177}Lu-$LuCl_3$ in 0.04M HCl-solution (EndolucinBeta^{TM}, 40 GBq/mL) in no carrier added (n.c.a.) quality. The precursor PSMA-617 was obtained from Endocyte/ABX (USA/Germany) in GMP quality. The radiosynthesis was performed on a Gaia/Luna GMP automated radiosynthesizer (Elysia-raytest GmbH, Germany) using a sterile, single-use cassette and reagent kit (ABX, Germany). Per patient dose, 100-125 µg PSMA-617 precursor was dissolved in 800 µl buffer solution (gentisic acid/sodium ascorbate/HCl). Between 7.0-9.0 GBq ^{177}Lu-$LuCl_3$ per patient was provided in the sterile, rubber sealed delivery vial (10 ml), which served as reaction vessel in the automated process. The ^{177}Lu-labelling step was conducted at 95 °C for 30 min. The product solution was transferred into a product vial via a sterile filter and diluted by 10-15 mL 0.9% NaCl. Patient doses were calculated and dispensed into 50 mL syringes with addition of 0.9% NaCl by a self-designed automated dispensing system. The radiosynthesizer and the dispensing system were both housed in a laminar air flow class-A glovebox under controlled conditions.

RadioHPLC as primary quality control was performed on a Merck HPLC system equipped with two L-7100 pumps, a L-7200 autosampler, a L-7400 UV/Vis detector, a D-7000 interface d-line and a GABI radiodetector (Elysia-raytest, Germany), and a Gemini C18, 5 µm, 100 Å column (250 x 4.6 mm) (Phenomenex, Germany). As eluent

phosphate buffer (pH 2) and methanol was used in a gradient system at a flow of 0.6 ml/min. Production batches were further tested for pH, sterility, endotoxins and radionuclide purity (gamma spectroscopy). ^{177}Lu-PSMA-617 was always of flawless quality with radiochemical purity of ≥95% and a peptide content of 14.3-15.6 µg/GBq.

Ex vivo analysis of prostate cancer tissue

Three primary tumor samples and two specimens of metastases were available, and evaluated for marker protein expression. Immunohistochemistry (IHC) was performed using mouse NSE antibody (1:1600 dilution; M0873; monoclonal [clone BBS/NC/VI-H14]; DAKO; Agilent Technologies: Santa Clara, California, United States) and mouse CgA antibody (1:100 dilution; NCL-L-CHROM-430; monoclonal [clone 5H7]; Leica Biosystems, Newcastle Upon Tyne, United Kingdom), using the Benchmark Ultra System (Ventana Systems, Tucson, AZ, USA). Marker expression for each sample was graded by a pathologist, and the overall percentage of marker positivity across the entire stained tumor sample was determined.

Supplemental Table 1 Characteristics of study population (*n* = 50).

Parameter	Total study population	Subgroup without influencing factors	Subgroup with influencing factors	*P* Value
Number (n)	50 (100%)	29 (58%)	21 (42%)	-
Age (years)	71.9 ±7.9 (52-86)	70.2 ± 8.4 (52 to 84)	74.4 ± 6.5 (63 to 86)	0.08
Gleason grade (median (range))	8 (6 to 10)	8 (6 to 10)	8 (7 to 9)	0.60
Blood-based parameters*				
Pre-treatment PSA (µg/l)	423.8 ± 674.7 (3.8 to 3205)	498.4 ± 809.8 (3.8 to 3205)	320.8 ± 422.1 (5.3 to 1394)	0.67
ProGRP (ng/ml)	75 ± 71 (29 to 305)	65 ± 37 (29 to 210)	89 ± 64 (30 to 305)	0.08
NSE (µg/l)	31 ± 24 (9 to 152)	33 ± 29 (13 to 152)	28 ± 14 (9 to 67)	0.89
CgA (µg/l)	191 ± 212 (33 to 1241)	119 ± 118 (33 to 687)	290 ± 271 (46 to 1241)	**0.0001**
Hemoglobin (g/dL)	11.5 ± 1.6 (7.3 to 13.9)	11.7 ± 1.7 (8.6 to 13.9)	11.3 ± 1.6 (7.3 to 13.8)	0.29
Erythrocyte count (10^6/µL)	3.9 ± 0.6 (2.6 to 4.8)	4.0 ± 0.6 (2.7 to 4.8)	3.8 ± 0.5 (2.6 to 4.7)	0.18
Leukocyte count (10^3/µL)	6.9 ± 2.1 (3.2 to 13.6)	6.7 ± 2.1 (3.5 to 13.6)	7.2 ± 2.0 (3.2 to 11.6)	0.11
Platelets (10^3/µL)	238 ± 82 (107 to 480)	243 ± 74 (131 to 378)	231 ± 93 (107 to 480)	0.37
Alkaline phosphatase (U/L)	230 ± 201 (43 to 950)	235 ± 210 (43 to 950)	224 ± 192 (44 to 636)	0.71
Lactate dehydrogenase (µmol/L)	383 ± 283 (83 to 1694)	378 ± 323 (83 to 1694)	383 ± 220 (195 to 954)	0.25
Site of metastases (no. of patients)				
Bone	45 (90%)	27 (93%)	18 (86%)	0.64
Lymph nodes	40 (80%)	23 (79%)	17 (81%)	1.0
Liver	14 (28%)	9 (31%)	5 (24%)	0.75
Lung	5 (10%)	4 (14%)	1 (5%)	0.38
Previous therapy (no. of patients)				
Androgen-deprivation therapy	50 (100%)	29 (100%)	21 (100%)	1.0
Arbiraterone acetate	35 (70%)	21 (72%)	14 (67%)	0.76
Enzalutamide	32 (64%)	16 (55%)	16 (76%)	0.15
Chemotherapy				
Docetaxel (1st line)	44 (88%)	26 (90%)	18 (86%)	0.69
Cabazitaxel (2nd line)	13 (26%)	9 (31%)	4 (19%)	0.52
Carboplatin (2nd/3rd line)	3 (6%)	2 (7%)	1 (5%)	1.0
External radiation therapy	41 (82%)	24 (83%)	17 (81%)	1.0

Supplemental Table 2 Univariate predictors of treatment response after 2 cycles of ^{177}Lu-PSMA-617

Parameter	Total study population (*n* = 50)			Subgroup without non-oncologic marker influencing factors (*n* = 29)		
	OR	95% CI	*P* value	OR	95% CI	*P* value
Blood-based parameters						
Hemoglobin	1.415	0.9682 to 2.191	0.0904	1.277	0.774 to 2.271	0.3574
Erythrocyte count	1.432	0.5034 to 4.423	0.5097	1.069	0.2515 to 4.988	0.9279
Thrombocyte count	1.004	0.9972 to 1.012	0.2449	1.003	0.925 to 1.015	0.5420
Leucocyte count	1.054	0.7915 to 1.405	0.7146	1.106	0.7485 to 1.636	0.5916
Lactate dehydrogenase (LDH)	0.9989	0.9959 to 1.001	0.4077	0.9966	0.9886 to 1.001	0.2484
Alkaline phosphatase (ALP)	0.9990	0.9956 to 1.002	0.5229	0.9995	0.9948 to 1.003	0.7934
Alanine transaminase (ALT)	0.9649	0.9035 to 1.001	0.1444	0.9245	0.7910 to 1.008	0.2611
Aspartate transaminase (AST)	0.9886	0.9666 to 1.006	0.2322	0.9474	0.8502 to 0.9962	0.1706
Gamma-glutamyltransferase (GGT)	0.9961	0.9842 to 1.000	0.2896	0.9891	0.9593 to 1.000	0.3635
Prostate-specific antigen (PSA)	0.9993	0.9977 to 1.000	0.2687	0.9995	0.99676 to 1.001	0.4872
Progastrin-releasing peptide (ProGRP)	1.010	0.9983 to 1.026	0.1269	1.009	0.9871 to 1.034	0.4125
Neuron-specific enolase (NSE)	1.004	0.7034 to 1.359	0.9794	0.9637	0.8763 to 1.008	0.3092
Chromogranin-A (CgA)	1.001	0.9979 to 1.004	0.6010	1.000	0.9905 to 1.007	0.9972
Imaging parameters						
Lymph node metastases	0.5385	0.1285 to 2.238	0.3860	0.3529	0.05138 to 2.353	0.2702
Osseous metastases	0.1250	0.006099 to 0.9356	0.0735	0.4211	0.01533 to 11.52	0.5578
Hepatic metastases	0.3636	0.07269 to 1.411	0.1690	0.6190	0.07652 to 3.589	0.6105
Intense PSMA-ligand uptake	11.77	2.743 to 82.81	**0.0030**	6.500	1.194 to 52.38	**0.0439**

CI – confidence interval; OR – Odds ratio

Supplemental Table 3 Univariate predictors of early progression after 2 cycles of ^{177}Lu-PSMA-617

Parameter	Total study population (*n*=50)			Subgroup without non-oncologic NE marker influencing factors (*n*=29)		
	OR	95% CI	P value	OR	95% CI	P value
Blood-based parameters						
Hemoglobin	0.7111	0.4755 to 1.024	0.0765	0.8595	0.5342 to 1.354	0.5145
Erythrocyte count	0.7864	0.2735 to 2.248	0.6490	1.379	0.3557 to 5.832	0.6441
Thrombocyte count	0.9946	0.9860 to 1.002	0.1823	0.9887	0.9753 to 0.9997	0.0633
Leucocyte count	1.080	0.8127 to 1.445	0.5919	0.9647	0.6536 to 1.385	0.8433
Lactate dehydrogenase (LDH)	1.002	0.9995 to 1.004	0.1594	1.002	0.9991 to 1.006	0.2624
Alkaline phosphatase (ALP)	1.001	0.9984 to 1.004	0.3892	1.001	0.9972 to 1.005	0.6508
Alanine transaminase (ALT)	1.011	0.9844 to 1.041	0.4063	1.014	0.9754 to 1.068	0.4862
Aspartate transaminase (AST)	1.010	0.9942 to 1.027	0.2170	1.024	0.9988 to 1.062	0.1062
Gamma-glutamyltransferase (GGT)	0.999	0.9979 to 1.001	0.8871	0.9997	0.9970 to 1.001	0.7294
Prostate-specific antigen (PSA)	1.001	0.998 to 1.002	0.1678	1.001	0.9997 to 1.002	0.2697
Progastrin-releasing peptide (ProGRP)	1.001	0.9888 to 1.013	0.8601	1.004	0.9832 to 1.028	0.6884
Neuron-specific enolase (NSE)	1.007	0.9816 to 1.035	0.5772	1.002	0.9733 to 1.031	0.8860
Chromogranin-A (CgA)	1.001	0.9977 to 1.003	0.6768	1.003	0.9966 to 1.016	0.4111
Clinical parameters						
Lymph node metastases	0.5385	0.1285 to 2.238	0.3860	0.7692	0.1190 to 4.949	0.7751
Osseous metastases	_	_	0.1424*	_	_	0.4877*
Hepatic metastases	1.269	0.3487 to 4.493	0.7110	0.4500	0.07621 to 2.252	0.3437
Intense PSMA-ligand uptake	0.2029	0.05604 to 0.6721	**0.0111**	0.1364	0.02206 to 0.6610	**0.0192**

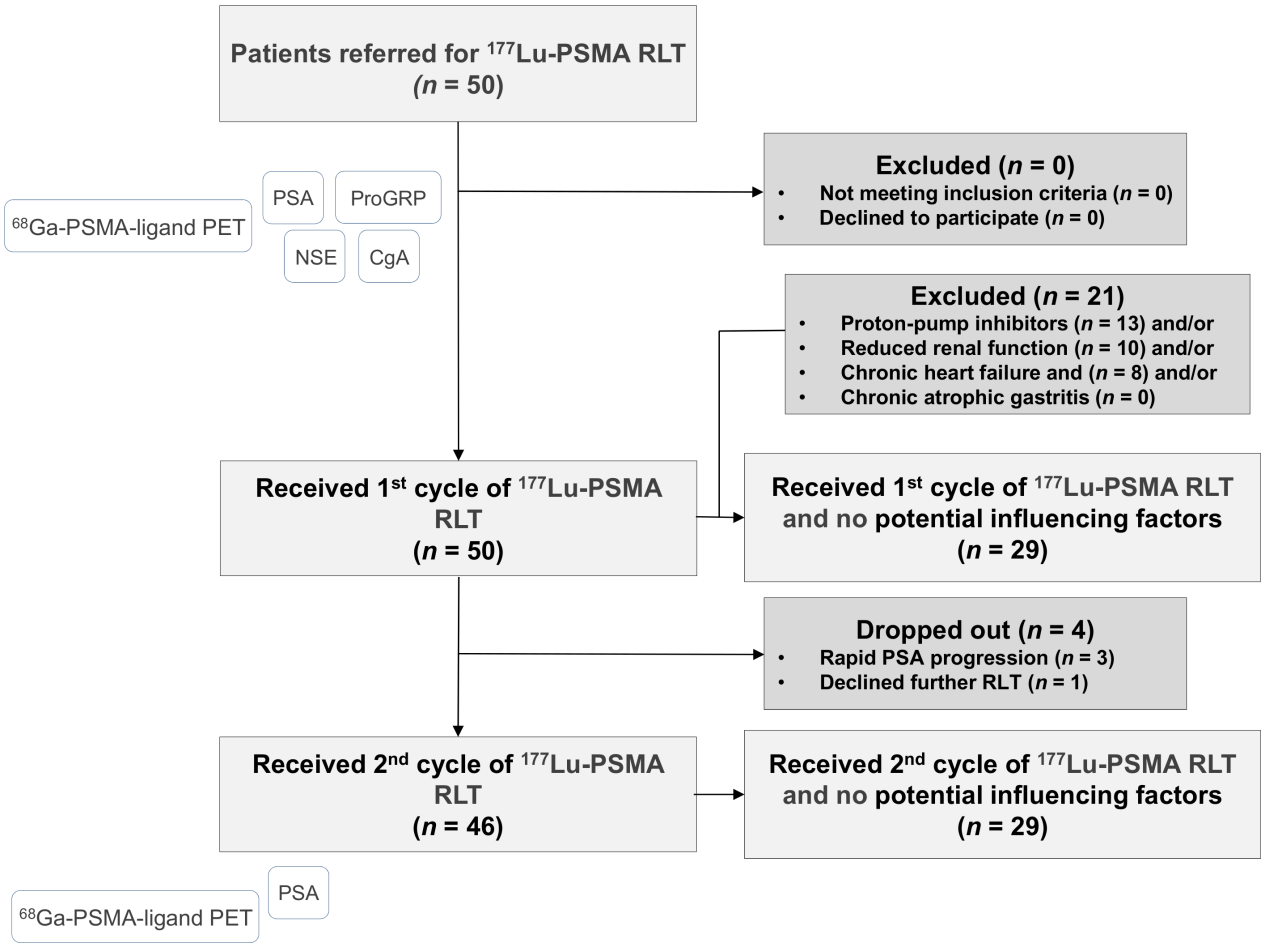
**P values were calculated using Fisher`s exact test because a logistic regression could not be fitted for patients with osseous metastases (all patients who progressed had bone metastases). CI – confidence interval; OR – Odds ratio*

Supplemental Table 4 Neuroendocrine marker expression, neuroendocrine serum marker levels, and outcome in patients with available histological specimens (*n* = 5)

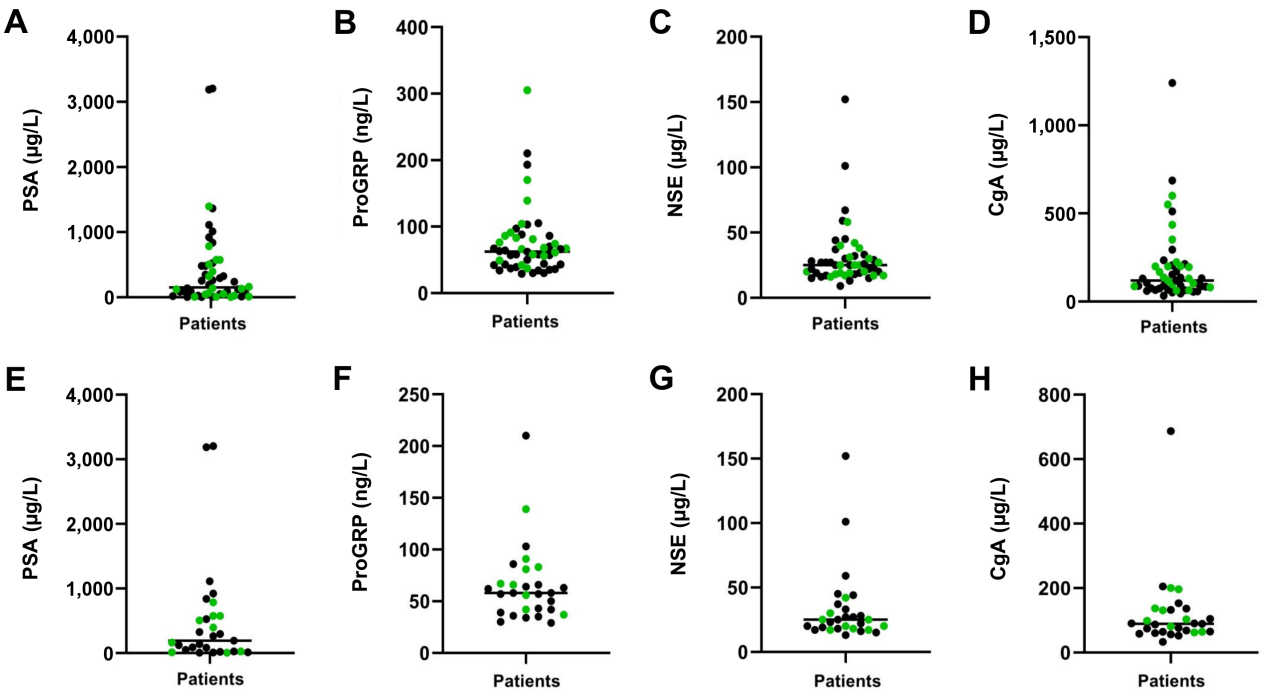
Specimen	Hormone-responsiveness	Serum markers			Immunohistochemistry		PSMA-ligand uptake	Outcome after 2 cycles of ^{177}L PSMA	
		ProGRP (ng/l)	NSE (µg/l)	CgA (µg/l)	NSE	CgA		PSA response	Early progres
Prostate biopsy	castration-sensitive	64	13	136	-	-	intense	n	n
Prostatectomy	castration-sensitive	35	16	74	+	-	low	n	n
TURP specimen	castration-sensitive	81	25	196	+ to ++	-	intense	y	n
Peritoneal metastasis	castration-resistant	68	40	168	+	-	intense	y	n
Bone metastasis	castration-resistant	170	31	435	-	-	intense	y	n

CgA - chromogranin-A; NSE - neuron-specific enolase; ProGRP - progastrin-releasing peptide; PSA – prostate-specific antigen; PSMA – prostate-specific membrane antigen; TURP - transurethral resection of *the* prostate

Supplemental Figures



Supplemental Figure 1 *Flowchart of study cohort included in the analysis (n = 50).* 50 consecutive patients with mCRPC commencing ^{177}Lu-PSMA-617 radioligand therapy were included in the analysis, and had undergone pretreatment ^{68}Ga-PSMA ligand PET, assessment of PSA levels and screening for neuroendocrine differentiation. Outcome was evaluated after 2 cycles of ^{177}Lu-PSMA-617, based on PSA change and restaging PET. Analyses were performed for both the total study population and a subgroup of patients without non-oncologic factors potentially influencing serum levels of neuroendocrine markers (*n* = 29).



Supplemental Figure 2 *Pre-treatment serum levels of secreted neuroendocrine markers in mCRPC patients commencing ^{177}Lu-PSMA-617 radioligand therapy.* Serum PSA for comparison (**A**). Markers were elevated in the majority of patients, and showed marked interindividual variability (ProGRP, range 29-305 ng/l (**B**); NSE, range 9-152 µg/l (**C**); and CgA, range 33-1241 µg/l (**D**)). *Upper limit of normal is 40 ng/l for ProGRP, 16 µg/l for NSE, and 76 µg/l for CgA.* In the subgroup of patients without potential non-oncologic influencing factors (*n* = 29), PSA and markers demonstrated a similar distribution with high interindividual variability (**E-H**). *Responders are marked in green.*