IVYSPRING INTERNATIONAL PUBLISHER

Theranostics

2020; 10(17): 7812-7820. doi: 10.7150/thno.47251

Research Paper

Analysis of PSMA expression and outcome in patients with advanced Prostate Cancer receiving ^{177}Lu-PSMA-617 Radioligand Therapy

Robert Seifert[1,2,3,4], Konstantin Seitzer[1,4,5], Ken Herrmann[2,3,4], Katharina Kessel[1], Michael Schäfers[1,4], Jens Kleesiek[3,6], Matthias Weckesser[1,4], Martin Boegemann[4,5] and Kambiz Rahbar[1,4]✉

1. Department of Nuclear Medicine, University Hospital Münster, Münster, Germany.
2. Department of Nuclear Medicine, University Hospital Essen, Essen, Germany.
3. German Cancer Consortium (DKTK).
4. West German Cancer Center.
5. Department of Urology, University Hospital Münster, Münster, Germany.
6. Division of Radiology, German Cancer Research Center, Heidelberg, Germany.

✉ Corresponding author: Kambiz Rahbar, MD, Department of Nuclear Medicine; University Hospital Muenster, Albert-Schweitzer-Campus 1, D-48149 Münster, Germany. Tel.: +49-251-8347362; Fax: +49-251-8347363; E-mail: rahbar@uni-muenster.de.

Received: 2020.04.21; Accepted: 2020.05.22; Published: 2020.06.19

Abstract

Rationale: PSMA-PET-CT enables measuring molecular expression of prostate-specific membrane antigen (PSMA) *in vivo*, which is the target molecule of ^{177}Lu-PSMA-617 (Lu-PSMA) therapy. However, the correlation of PSMA expression and overall survival (OS) in patients treated with Lu-PSMA therapy is currently unclear; especially with regard to coexistence of high and low PSMA expressing metastases. To this end, this retrospective single arm study elucidates the correlation of PSMA expression and overall survival in patients treated with Lu-PSMA therapy. Additionally, PET based criteria to define low PSMA expression were explored.

Methods: Eighty-five patients referred to Lu-PSMA therapy were included in the analysis. Pretherapeutic ^{68}Ga-PSMA-PET-CT scans were available for all patients. SUV_{max} of the highest PSMA expressing metastasis ($PSMA_{max}$), SUV_{max} of the lowest PSMA expressing metastasis ($PSMA_{min}$), and average SUV_{max} of all metastases ($PSMA_{average}$) amongst other PET parameters were measured for each patient. A log-rank cutoff-finder was used to determine low ($lowPSMA_{average}$) and high ($highPSMA_{average}$) average PSMA expression as well as low ($lowPSMA_{min}$) and high ($highPSMA_{min}$) minimal PSMA expression.

Results: $PSMA_{average}$ was a significant prognosticator of overall survival in contrast to $PSMA_{max}$ (HR: 0.959; p = 0.047 vs. HR: 0.992; p = 0.231). Optimal log rank cut-offs were: $PSMA_{average}$ = 14.3; $PSMA_{min}$ = 10.2. Patients with low average PSMA expression ($lowPSMA_{average}$) had significantly shorter survival compared to those with high average expression ($highPSMA_{average}$) (5.3 vs. 15.1 months; p < 0.001; HR: 3.738, 95%CI = 1.953–7.154; p < 0.001). Patients with low PSMA expressing metastases ($lowPSMA_{min}$) had shorter survival compared to those without a low PSMA expressing metastasis ($highPSMA_{min}$) (p = 0.003; 7.9 months vs. 21.3; HR: 4.303, 95%CI = 1.521–12.178; p = 0.006). Patients that were classified as $highPSMA_{average}$ but with $lowPSMA_{min}$ had an intermediate overall survival (11.4 months; longer compared to $lowPSMA_{average}$, 5.3 months, p = 0.002; but shorter compared to $highPSMA_{min}$, 21.3 months, p = 0.02).

Conclusion: Low average PSMA expression is a negative prognosticator of overall survival. Absence of low PSMA expressing metastases is associated with best overall survival and the maximum PSMA expression seems not suited to prognosticate overall survival. Low PSMA expression might therefore be a negative prognosticator for the outcome of patients treated with Lu-PSMA therapy. Future studies are warranted to elucidate the degree of low PSMA expression tolerable for Lu-PSMA therapy.

Key words: PSMA radioligand therapy; PSMA PET; prostate cancer; prognosticator

Introduction

There are only limited therapeutic options for patients with metastatic castration-resistant prostate cancer (mCRPC) [1]. However, the treatment of mCRPC patients with ^{177}Lu-PSMA-617 (Lu-PSMA) achieves biochemical response (> 50% decline of prostate-specific antigen blood levels) in 45-64% of patients [2–4]. Yet, the identification of mCRPC patients who will benefit from Lu-PSMA therapy is still an unmet clinical issue [5,6].

Prostate-specific membrane antigen (PSMA) targeted positron emission tomography computed tomography (PET-CT) can visualize the target molecule of Lu-PSMA therapy *in vivo*, which is also referred to as theranostics [6–9]. The molecular expression of PSMA should be directly linked to Lu-PSMA efficacy. Therefore, procedure guidelines of the European Association of Nuclear Medicine (EANM) for Lu-PSMA therapy demand a PSMA-PET-CT acquisition to evaluate therapy eligibility [10]. However, there are contradictory reports on the implications of PSMA targeted imaging: It has been reported that PSMA-PET is not suited to predict response to Lu-PSMA therapy [11]. On the other hand, high tumor uptake in post Lu-PSMA therapy scintigraphies is a prognosticator of survival [12]. It remains currently unclear to what extent PSMA expression measured by PSMA-PET-CT can predict response and prognosticate overall survival and thus, ultimately assess eligibility for Lu-PSMA therapy. Moreover, there is no reasonable definition of low PSMA expression.

The first prospective Phase II trial by Hofman et al. has addressed the issue of eligibility assessment pragmatically by requesting an arbitrarily defined minimum PSMA-PET uptake of metastases to undergo Lu-PSMA therapy [3,13]. The minimum PSMA uptake of any metastasis was defined as 1.5 times the mean liver uptake [3]. Only patients whose SUV_{max} exceeded this minimum activity at any metastatic site were eligible for Lu-PSMA therapy. It seems plausible that PSMA-PET uptake should be linked to therapeutic efficacy of Lu-PSMA therapy. However, it appears difficult to translate the trial inclusion criteria to the clinical routine, as it was not part of the study evaluation. By applying a SUV_{max} based criterion, Lu-PSMA therapy might be withheld from patients due to their low PSMA expression that still might have benefited from therapy. Therefore, the aim of the present study was to investigate the relevance of PSMA-PET parameters for the overall survival of patients treated by Lu-PSMA therapy. Additionally, multiple PSMA-PET parameters were employed to distinguish between patients with low and high PSMA expression. Finally, survival time, presence of liver metastases and history of second line chemotherapy were compared between patients with low and high PSMA expression.

Methods

Patients

All patients who were referred for ^{177}Lu-PSMA-617 therapy at the Department of Nuclear Medicine in Muenster between December 2014 and October 2018 were considered in this retrospective analysis. Inclusion criterion was the presence of a ^{68}Ga-PSMA-11 PSMA-PET-CT examination prior to administration of the first therapy cycle showing any uptake of the tracer in the target lesions. The decision for Lu-PSMA-617 therapy was made by the institutional interdisciplinary tumor board on a case by case basis. Prerequisites for ^{177}Lu-PSMA-617 therapy were: castration-resistance (mCRPC), sustained androgen deprivation therapy, if no contraindication was present at least one line of taxane chemotherapy, PSMA-positive metastases, sufficient hematological reserve, and sufficient kidney as well as liver function [10]. Pretherapeutic PSMA-PETs were assessed visually for presence of PSMA positive metastases; no quantitative PSMA-PET related inclusion criteria were applied. Foci that were not caused by physiological uptake and showed higher activity than the surrounding tissue were assessed as sufficient for Lu-PSMA therapy. A detailed patient characteristic is given by *Table 1.*

PSMA-PET imaging procedure

^{68}Ga-PSMA-11 was produced according to manufactures recommendations (precursor delivered by ABX GmbH, Radeberg, Germany). A Siemens Biograph mCT (Siemens Healthineers, Knoxville, TN, United States) was used for image acquisition. PET-CT acquisitions were started 60 minutes after tracer injection. Low-dose or diagnostic CT were acquired immediately prior to PET acquisition for anatomical orientation and attenuation correction. PET reconstruction was done using the standard software as provided by the manufacturer and an iterative time-of-flight algorithm without PSF correction. The median interval between PET acquisition and therapy start was 32 (IQR: 22) days.

PSMA therapy preparation and administration

^{177}Lu-PSMA-617 was prepared as described elsewhere (Lutetium: ITG Isotopes Technology, Garching, Germany; precursor: ABX advanced biochemical compounds, Radeberg, Germany) [14]. Lu-PSMA was administered every 8 weeks until severe adverse

reactions, altered therapy regime, progression, or death occurred.

Table 1. Patient characteristics

Patient characteristics	N [%]	Median [IQR]; survival: Median [CI]
Number of patients	85 [100%]	
Age (years)		73.1 [11.4]
Estimated overall survival time (months)		11.4 [8.0–14.7]
>50% PSA decline from baseline	39 [46%]; n = 80, follow up not present for 5 patients.	
PSMA therapy		
Number of cycles		3.0 [4]
Cumulated activity (GBq)		19.3 [24.8]
Baseline blood parameters		
Alkaline phosphatase (U/l)		147.0 [193.0]
Lactate dehydrogenase (U/l)		316.5 [227.0]
Aspartate aminotransferase (U/l)		32.5 [24.0]
Alanine transaminase (U/l)		16.0 [11.0]
Hemoglobin (g/dl)		10.4 [2.4]
Prostate-specific antigen (ng/ml)		284.0 [805.0]
Metastases		
Bone	78 [92%]	
Lymph node	68 [80%]	
Liver	26 [31%]	
Lung	20 [24%]	
Brain	1 [1%]	
Previous therapies		
Docetaxel	68 [80%]	
Cabazitaxel	20 [24%]	
Abiraterone	72 [85%]	
Enzalutamide	72 [85%]	

Blood parameters were not available for all patients; Abbreviations: Std = standard deviation; CI = confidence interval.

PSMA-PET image analysis

The analysis of PSMA-PET images was done semi-automatically using the research prototype software MI Whole Body Analysis Suite (MIWBAS, v1.0, Siemens Medical Solutions USA, Inc., Knoxville, TN), which has been described in detail before [15]. Briefly, all PSMA avid foci were automatically pre-selected based by a pre-defined threshold; foci with a PET volume smaller than 0.5 ml (segmented by 50% of local SUVmax as threshold) were discarded. Missed pathological foci were manually added, if necessary. PSMA avid foci that were caused by physiological tracer accumulation were semi automatically removed from the analysis.

All PSMA avid metastases (regardless of SUV_{max}) were segmented and SUV_{max}, SUV_{mean}, SUV_{peak} were reported for each metastasis. Metastases were delineated using relative thresholding (50% of local SUV_{max}). On a per patient level, the mean of all SUVmax measurements ($PSMA_{average}$), the maximum SUVmax measurement ($PSMA_{max}$), the lowest SUV_{max} measurement ($PSMA_{min}$) and the standard deviation of the SUV_{max} measurements ($PSMA_{std}$) were noted.

PSMA measurements were analyzed as continuous parameter and in binarized form. When $PSMA_{average}$ was binarized using optimized log rank thresholds, the group with low $PSMA_{average}$ was denoted low$PSMA_{average}$ (high$PSMA_{average}$; threshold: 14.3). When $PSMA_{min}$ was binarized, patients with low PSMA expressing metastases were denoted low$PSMA_{min}$ (without low PSMA expressing metastases: high$PSMA_{min}$; threshold: 10.2). The volumetric fraction of low PSMA expressing tumor was determined by dividing the volume of metastases with low PSMA expression ($SUV_{max} \leq 10.2$) by the whole-body tumor volume.

Statistical analysis

SPSS 25 (IBM, NY, USA) was used for log rank tests, Pearson correlation, Mann-Whitney-U test, Fisher's exact test and uni- as well as multivariate Cox-regression. R and the maxstat package were used for finding the optimal log rank cut-off of continuous variables [16,17]. P values < 0.05 were regarded as statistically significant. To correct for log-rank test alpha error accumulation, significance was assumed when $p < 0.0125$ (Bonferroni correction for 4 SUV_{max} tests: optimal log rank cut-off for $PSMA_{max}$, $PSMA_{min}$, $PSMA_{average}$, $PSMA_{std}$. Other SUV parameters (SUV_{mean}, SUV_{peak}) were only analyzed to further corroborate SUV_{max} findings and therefore not regarded for Bonferroni correction. Values are presented together with the interquartile range (IQR).

Results

PSMA therapy and patient characteristics

A detailed patient characteristic is given by *Table 1*. Median therapy interval (including therapy pauses) was 8.2 (IQR: 3.3) weeks, median therapeutic activity was 6.2 (IQR: 1.2) GBq. The median cumulated dose was 23.7 (IQR: 25.7) GBq. Eighty percent of all patients had received taxane based chemotherapy (Docetaxel or Cabacitaxel), whereas 100% patients had received androgen deprivation therapy and 97.7% had received next generation androgen receptor targeted therapy (Enzalutamide or Abiraterone).

Descriptive statistics of baseline PET parameter measurements

The median of $PSMA_{max}$ measurements was 44.6 SUV (range 7.1–181.6), whereas the median of $PSMA_{average}$ measurements was 18.9 (range 4.6–129.8). The median intensity was 31.6 (range 4.7 –159.7) for the highest SUV_{peak}. A detailed report on SUV parameters is provided by *Table 2*.

Baseline PET-parameters and overall survival

Regarding $SUV_{max,}$ neither the highest ($PSMA_{max}$: HR: 0.992; p = 0.231; 95% CI: 0.979–1.005), nor the lowest ($PSMA_{min}$: HR: 0.890; p = 0.118; 95% CI: 0.768–

1.030) value per patient were significant prognosticators of overall survival, but the average value was ($PSMA_{average}$: HR: 0.959; p = 0.047; 95% CI: 0.921–0.999). The same was true for SUV_{mean} (highest: HR= 0.989; p = 0.241; 95% CI= 0.970–1.008; average: HR = 0.941; p = 0.045; 95% CI= 0.887–0.999; lowest: HR = 0.799; p = 0.052; 95% CI = 0.638–1.002). Details (including the standard deviation of SUV_{max}, SUV_{mean} and SUV_{peak}) are given by *Table 3*. *Figure 1* depicts the overall survival stratified according to the quartiles of $PSMA_{average}$ and $PSMA_{min}$.

There were no relevant correlations between $PSMA_{min}$ and $PSMA_{average}$ (R^2 = 0.30, p < 0.001; *Figure 2*) or $PSMA_{min}$ and $PSMA_{std}$ (R^2 = 0.18, p < 0.001), but $PSMA_{std}$ and $PSMA_{average}$ were significantly correlated (R^2 = 0.78, p < 0.001; *Figure 3*).

Table 2. SUV parameters of the presented patient cohort (n = 85)

PET parameter	Median of the *average* value of all patients	Median of the *highest* value of all patients	Median of the *lowest* value of all patients
SUVmax	18.9 [5.9–73.4]	44.6 [7.1–181.6]	8.9 [2.7–40.9]
SUVmean	13.0 [4.0–51.4]	29.5 [4.6–129.8]	6.3 [2.6–29.4]
SUVpeak	12.1 [3.7–46.5]	31.6 [4.7–159.7]	5.7 [1.8–22.6]

Abbreviation: Squared brackets = range.

Table 3. Baseline PSMA PET parameters and overall survival

	Measurement selected per patient	HR	95%CI	P
SUV_{max}	Average ($PSMA_{average}$)	0.959	0.921–0.999	**0.047**
	Highest ($PSMA_{max}$)	0.992	0.979–1.005	0.231
	Lowest ($PSMA_{min}$)	0.890	0.768–1.030	0.118
	Std ($PSMA_{std}$)	0.936	0.877–0.999	**0.048**
SUV_{max} / $SUV_{mean\ liver}$	Average	0.963	0.895–1.036	0.313
	Highest	0.996	0.975–1.017	0.701
	Lowest	0.904	0.728–1.123	0.363
	Std	0.932	0.822–1.057	0.274
SUV_{mean}	Average	0.941	0.887–0.999	**0.045**
	Highest	0.989	0.970–1.008	0.241
	Lowest	0.799	0.638–1.002	0.052
	Std	0.904	0.820–0.996	**0.042**
SUV_{peak}	Average	0.941	0.882–1.004	0.064
	Highest	0.989	0.972–1.007	0.227
	Lowest	0.736	0.533–1.016	0.062
	Std	0.918	0.844–0.999	**0.048**

Abbreviations: HR = Hazard ratio; CI = confidence interval; Std = standard deviation.

Table 4. Overlap of PET stratification

	$highPSMA_{average}$	$lowPSMA_{average}$	Sum
$lowPSMA_{min}$	49	20	*69*
$highPSMA_{min}$	16	0	*16*
Sum	*65*	*20*	*85*

Low/$highPSMA_{min}$ = patients with or without metastases that had a SUV_{max} above or below 10.2; high/$lowPSMA_{average}$ = patients with an average SUV_{max} of all metastases above or below 14.3. SUV threshold values resemble optimized log rank cut-offs.

Low PSMA expression and overall survival

In a first approach, a liver specific SUV threshold was used (1.5 × SUV*mean* liver) in analogy to Hofman et al. In our cohort, zero patients had a SUV_{max} below the liver specific threshold. Patients (n=8) with at least one tumor lesion below the liver specific SUV threshold did not have a significantly shorter overall survival time (log rank: p = 0.335; 7.5 vs.13.2 months; HR: 1.588; p = 0.340; 95% CI: 0.615–4.101).

In a second approach, $PSMA_{average}$ and $PSMA_{min}$ were binarized to determine low and high PSMA expression. The optimized log rank threshold for $PSMA_{average}$ to stratify according to overall survival was 14.3 SUV (log rank: p < 0.001; estimated median: 15.1 vs. 5.3 months; high $PSMA_{average}$: HR = 0.268, 95%CI = 0.140–0.512, p<0.001) and 10.2 for $PSMA_{min}$ (log rank: p = 0.003; estimated median: 21.3 vs. 7.9 months; high $PSMA_{min}$: HR = 0.232, 95%CI = 0.082–0.658; p = 0.006). Taken together, both classifiers stratified patients into high, intermediate or low overall survival. *Table 4* presents the intersection of these two classifiers; *Figure 2* depicts the overall survival according to them. Patients that were classified $highPSMA_{min}$ had longer survival compared to patients classified as both $lowPSMA_{min}$ and $highPSMA_{average}$ (estimated median: 21.3 vs. 11.4 months; p = 0.02; HR = 0.3; 95%CI = 0.102–0.877; p = 0.028); patients classified as both $lowPSMA_{min}$ and $highPSMA_{average}$ had longer survival compared to $lowPSMA_{average}$ (estimated median: 11.4 vs. 5.3 months; p = 0.002; HR = 0.364; 95%CI = 0.186–0.710; p = 0.003). Multivariate Regression (including binarized $PSMA_{min}$ and $lowPSMA_{average}$ and adjusted for presence of liver metastases) confirmed both $highPSMA_{min}$ and $highPSMA_{average}$ to be significant positive prognosticators of overall survival ($highPSMA_{average}$: HR = 0.473; p = 0.044; 95%CI = 0.229–0.980 | $highPSMA_{min}$: HR = 0.300; p = 0.028; 95%CI = 0.102–0.878 | liver metastases absence: HR = 0.476; p = 0.033; 95%CI = 0.240–0.943). Optimized threshold values (both absolute and relative to liver activity) for other SUV parameters are shown by *Table 5*. The median volumetric fraction of low PSMA expressing tumor volume was 3.6% (IQR: 13.8) for the proposed optimized log rank threshold (10.2 SUV). The volumetric fraction of low PSMA expressing tumor volume could significantly stratify the overall survival time (>9.7% vs. =<9.7%; p = 0.023; 6.4 vs. 11.4 months; only $lowPSMA_{min}$ patients).

Influence of liver metastases and Cabazitaxel therapy

There were no significant differences between patients with and without liver metastases regarding $PSMA_{min}$ (9.1vs.8.9; p = 0.418) or $PSMA_{average}$ (18.3 vs. 20.1, p = 0.264; *Figure 3*). The same was true for a positive/negative history of Cabazitaxel therapy

($PSMA_{min}$ 8.9 vs. 9.1; p = 0.615; $PSMA_{average}$ 15.9 vs. 20.1, p = 0.138; *Figure 3*). There were no statistically significant associations between the presence of liver metastases and $PSMA_{min}$ status (p = 1.000) or $PSMA_{average}$ status (p = 0.164). Likewise, there were no statistically significant associations between the history of Cabazitaxel therapy and $PSMA_{min}$ status (p = 0.338) or $PSMA_{average}$ status (p = 0.547). In accordance with a previous study of our group, presence of liver metastases (HR = 2.775; p = 0.001; 95%CI = 1.481–5.198) as well as history of second line chemotherapy (HR = 3.047; p = 0.002; 95%CI = 1.523–6.093) were significant negative prognosticators of overall survival [18].

Discussion

The implication of low PSMA expression for the overall survival of patients treated with Lu-PSMA therapy was investigated in the present study. To this end, the correlation of overall survival and various PSMA-PET parameters was elucidated. Additionally, different PET uptake criteria have been employed to group patients into low or high PSMA expression. The highest pathological PSMA expression of a given patient under Lu-PSMA therapy ($PSMA_{max}$) could not prognosticate overall survival. Low average PSMA expression of all metastases ($PSMA_{average}$) was associated with shorter overall survival. The absence of low PSMA expressing metastases prior to Lu-PSMA therapy was associated with best overall survival. The volumetric fraction of low PSMA expressing metastases was a negative prognosticator of overall survival.

Table 5. Binarized baseline PSMA PET parameters and overall survival

	Measurement selected per patient	Ideal cut-off	P	Below cut-off			Above cut-off		
				n	Median Survival	95%CI	n	Median Survival	95%CI
SUV_{max}	*Average (=$PSMA_{average}$)*	14.3	**<0.001**	20	5.3	2.3–8.8	65	15.1	7.8–23.4
	Highest (=$PSMA_{max}$)	n.a.	n.s.						
	Lowest (=$PSMA_{min}$)	10.2	**0.003**	69	7.9	4.6–11.2	16	21.3	n.a.
	Std (=$PSMA_{std}$)	3.0	**<0.001**	9	3.2	0.0–6.9	76	13.2	9.0–17.4
SUV_{max} / $SUV_{mean\ liver}$	*Average*	6.2	**0.003**	42	7.2	5.8–8.6	43	21.3	13.2–29.3
	Highest	n.a.	n.s.						
	Lowest	n.a.	n.s.						
	Std	n.a.	n.s.						
SUV_{mean}	*Average*	9.3	**<0.001**	17	6.4	1.7–11.1	68	15.1	10.1–20.1
	Highest	n.a.	n.s.						
	Lowest	7.1	**0.004**	65	8.6	4.3–12.9	20	25.5	15.1–36.0
	Std	1.9	**<0.001**	9	3.2	0.0–6.9	76	13.2	9.1–17.4
SUV_{peak}	*Average*	9.7	**<0.001**	19	6.4	3.7–9.1	66	13.2	8.4–18.1
	Highest	n.a.	n.s.						
	Lowest	6.6	**0.004**	65	8.6	4.3–12.9	20	25.5	15.1–36.0
	Std	n.a.	n.s.						

Abbreviations: CI = confidence interval; Std = standard deviation; n.a. = not available; n.s. = not statistically significant. Bonferroni adjustment: p < 0.0125 is regarded statistically significant. Ideal cut-off was found by log rank cut-off finder.

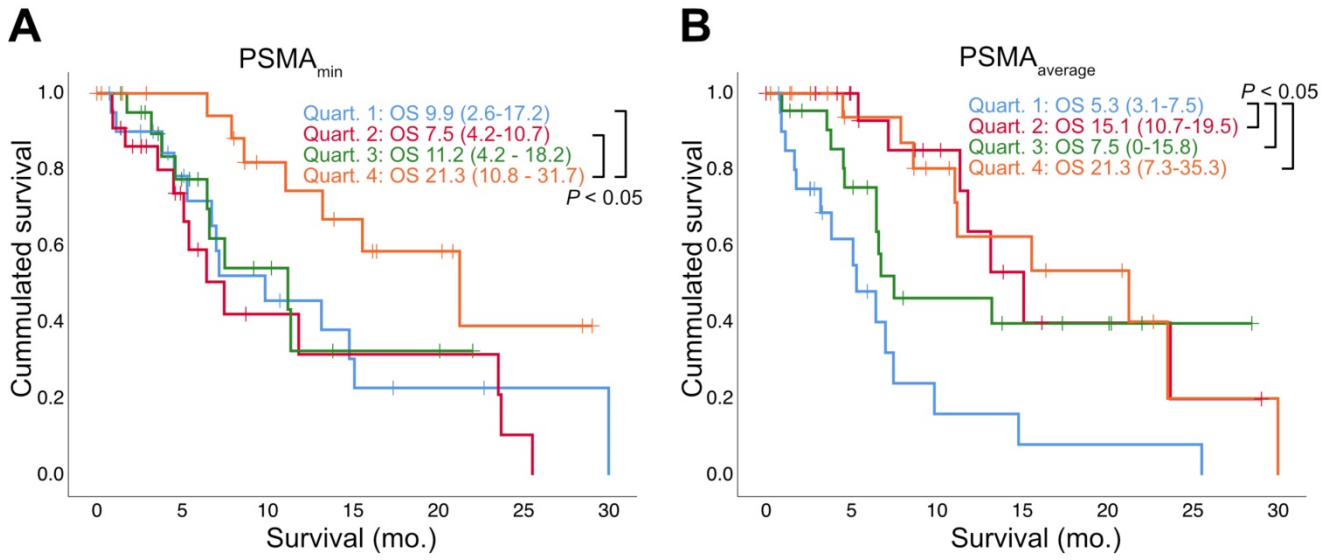

Figure 1. Survival stratified by quartiles of PSMA-PET parameters. Estimated median overall survival (OS) in months (mo.) is shown together with the 95% Confidence Interval (in parentheses). Log rank test was used to compare OS between quartiles.

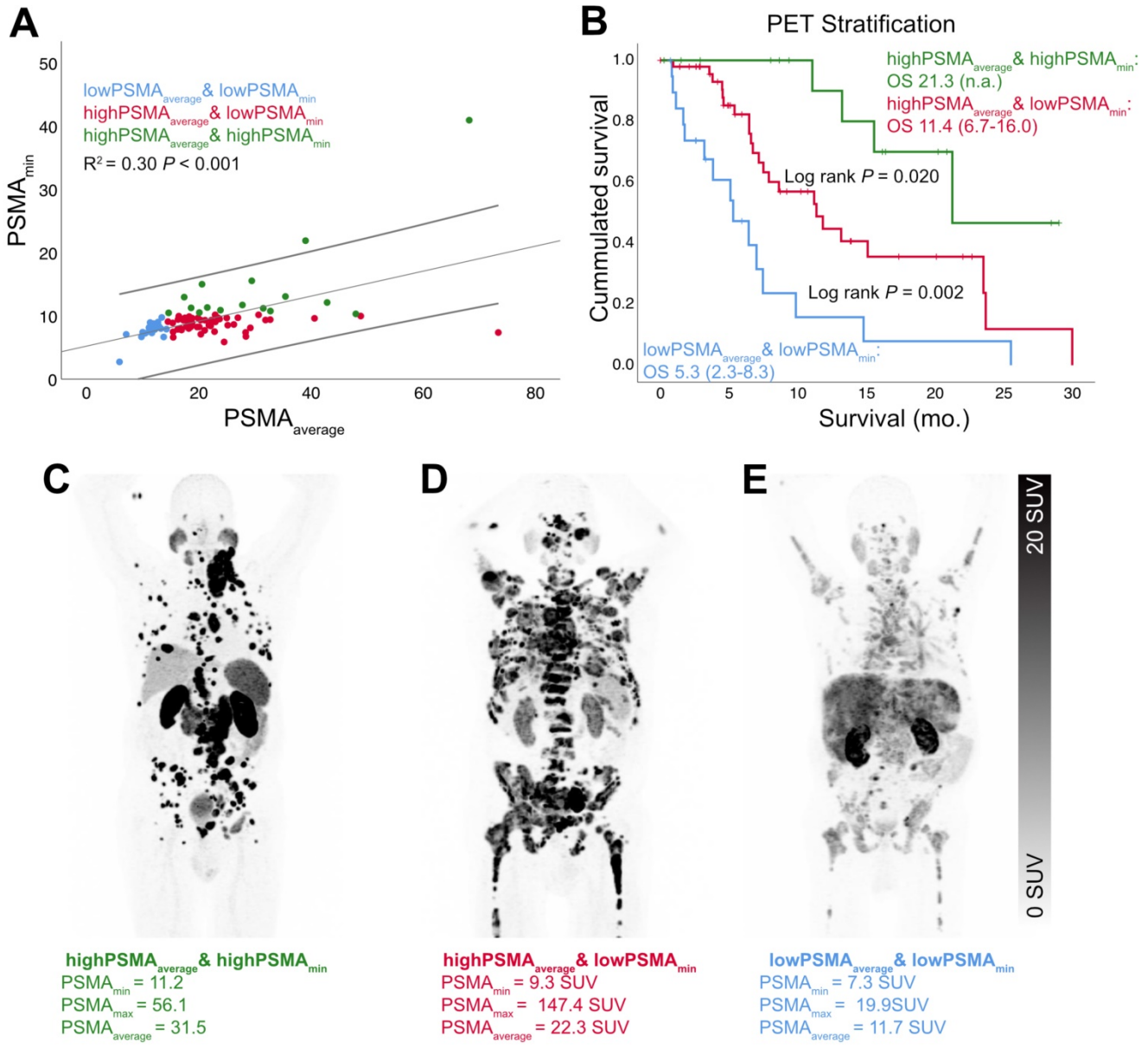

Figure 2. Survival stratified by high, intermediate and low PSMA expression. There was no relevant correlation between $PSMA_{min}$ and $PSMA_{average}$ (A, linear regression and 95% CI interval). Therefore, the combination of both PET parameters enabled an optimized stratification according to overall survival (B). Exemplary patients of the high (C), intermediate (D) and low (E) overall survival group were shown additionally.

To date, there is no reasonable definition of low or high PSMA expression. However, patients in whom the PSMA expression was assessed low were not considered for Lu-PSMA therapy in the Australian Phase II trial of Hofman et al. [3,19]. Yet, the benefit of the employed PSMA expression-based inclusion criteria could not be evaluated in the very same trial. Interestingly, some patients from the present cohort that received Lu-PSMA therapy had a lower maximum PSMA expression (SUV_{max} 7.1) compared to the Hofman et al. cohort (SUV_{max} 22.1) [3]. Therefore, the present study retrospectively applied the PSMA-PET criterion of Hofman et al., to evaluate, if patients would have been judged eligible for Lu-PSMA therapy [3]. In the present cohort, the maximum PSMA expression of each patient was above the liver specific threshold of Hofman et al. (1.5 × SUV_{mean} of liver) and therefore all would have met the inclusion criterion of Hofman et al. [3]. Eight patients had at least one metastasis with a SUV_{max} below this liver specific threshold. Still, there was no significant stratification of patients according to overall survival in the present cohort. Finally, in the present patient cohort, $PSMA_{max}$ was not a significant prognosticator of overall survival, which is in line with a recent publication of Ferdinandus et al. [20]. Therefore, the maximum pathological PSMA expression seems unsuited to predict the therapy response of patients who show a minimum SUV_{max} of 7.1 at any metastatic site.

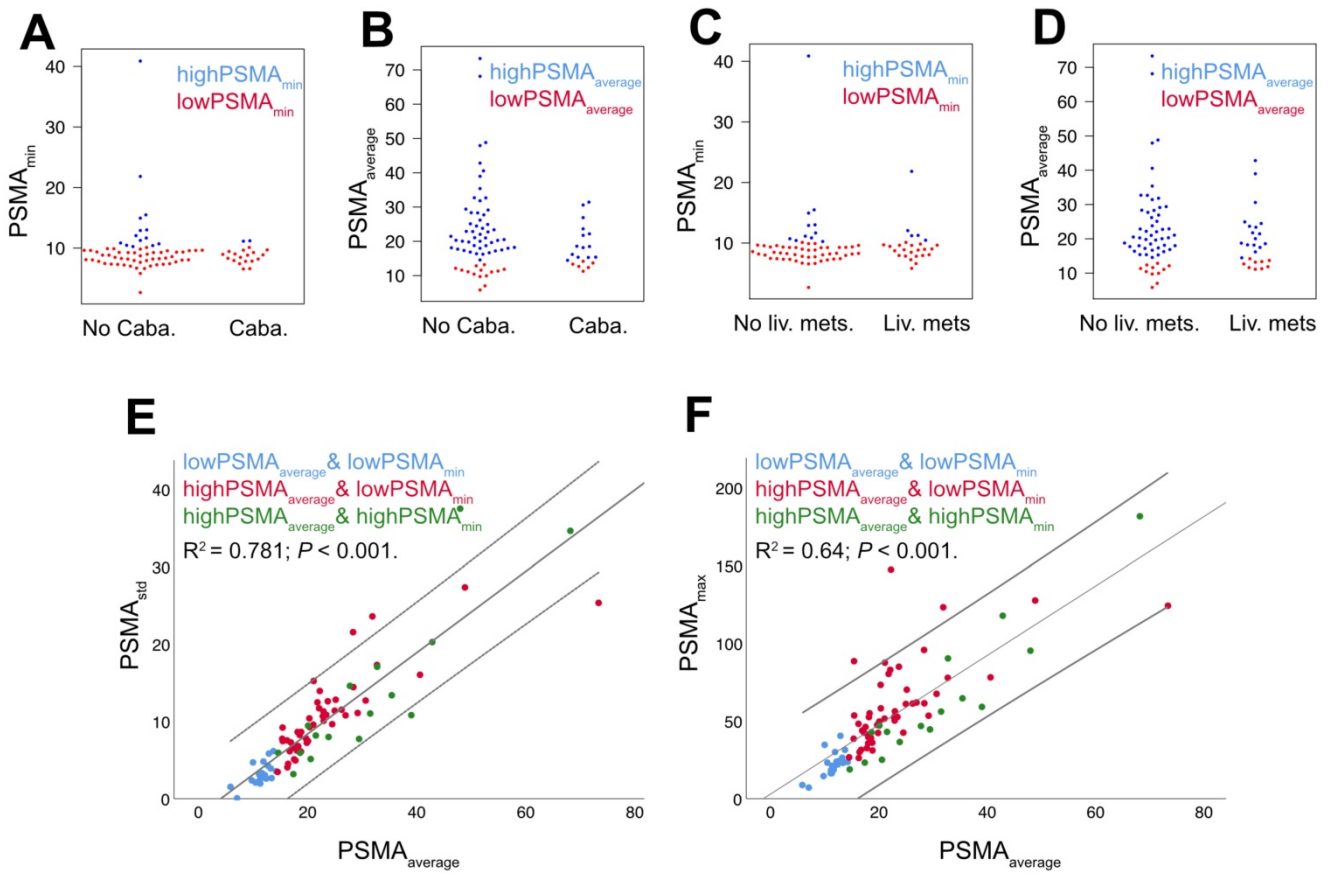

Figure 3. Relations of baseline PSMA-PET parameters. PSMA-PET parameters were grouped by history of Cabazitaxel chemotherapy (= Caba; A+B) or presence of liver metastases (= Liv. mets; C+D); there were no statistically significant differences. There was a high correlation of $PSMA_{std}$ and $PSMA_{average}$ and a moderate correlation of $PSMA_{max}$ and $PSMA_{average}$ (E+F, linear regression and 95% CI interval).

Patients with low average PSMA expression had short overall survival compared to those with high average PSMA expression and/or no low PSMA expressing metastases. Yet it remains unclear, if these patients still benefited from Lu-PSMA therapy. There is only limited evidence that the overall survival would have been worse, if Lu-PSMA therapy would not have been administered. The survival of patients with low PSMA expression that received Lu-PSMA therapy was longer in the current cohort (6.4 months) compared to patients excluded from the Lu-PSMA Phase II trial of Hofman et al. (2.5 months) [19]. Other studies have employed the survival of historic control cohorts for comparison [14]. But these comparisons might be heavily influenced by the individual metastatic spread and tumor differentiation, especially given the small patient numbers. Therefore, no rationale for PSMA-PET based exclusion criteria for Lu-PSMA can be provided by the given study. However, it seems unfavorable to use the maximum PSMA expression as decision criterion which did not correlate with overall survival.

Other prognosticators of Lu-PSMA therapy outcome have been identified in previous studies [18,21]. Amongst others, the presence of liver metastases and history of second line Cabazitaxel chemotherapy were negative prognosticators of overall survival [18,22]. Interestingly, there were no statistically significant associations between those predictors and the $PSMA_{average}$ or $PSMA_{min}$ status.

Due to genetic and non-genetic variations, cancer cells both in primary tumors and metastases become heterogeneous during the course of the disease [23,24]. In prostate cancer, neuroendocrine differentiation of cancer cells may occur in advanced stages and especially after lasting androgen deprivation therapy [25–29]. Prostate cancer cell markers like prostate-specific antigen are lost during dedifferentiation, whereas neuroendocrine markers like neurone specific enolase are gained [26,30]. Neuroendocrine differentiation is generally associated with poor overall survival [31]. Interestingly, Rathke et al. could show that the neuroendocrine marker chromogranin A is a moderately negative predictor for response in patients treated by Lu-PSMA therapy [12]. In the present study, the average PSMA expression was a positive prognosticator for overall survival. Interestingly, average PSMA expression and variation of PSMA uptake (measured as $PSMA_{std}$) between metastases

were highly correlated. Both findings might be partly explained by dedifferentiation of prostate cancer cells in metastases. This might be contradictory, as one could assume that decreased average PSMA expression is associated with more heterogeneity between metastases (i.e. side by side presence of metastases with low and with high PSMA expression). However, it remains unclear if the shorter overall survival of patients with low PSMA expressing metastases is due to dedifferentiated and more aggressive tumor phenotypes, or due to reduced efficacy of Lu-PSMA therapy.

PSMA PET can visualize PSMA expression in the living patient and Lu-PSMA therapy targets PSMA expressing cells. Therefore, it has been hypothesized that PSMA PET can predict the achieved radiation dose, which is deposited in the tumor, and thereby indirectly predict the treatment response. The work of Violet et al. had shown that the mean whole body PSMA PET uptake indeed correlated with the absorbed doses of Lu-PSMA therapy [32]. Moreover, patients that obtained doses <10 Gy had unfavorable PSA response rates [32]. However, no survival data was studied by Violet et al. [32]. In contrast, the present manuscript could show that mean whole body PSMA expression is a significant predictor of survival.

The overall survival of patients that present strong PSMA expressing metastases, but likewise have less PSMA expressing metastases is currently unclear. Interestingly, high$PSMA_{min}$ patients had a significantly longer overall survival. Taken together with $PSMA_{average}$, patients were stratified based on PSMA-PET measurements into those with low (low$PSMA_{average}$), intermediate (high$PSMA_{average}$ and low$PSMA_{min}$) or high (high$PSMA_{min}$) PSMA expression. Thereby, patients could be stratified in those with good, intermediate and poor overall survival. This is depicted by *Figure 2*. Moreover, the volumetric fraction of low PSMA expressing metastases was a negative prognosticator for overall survival. Future studies have to elucidate, if the occurrence of low PSMA expressing metastases (i.e. low$PSMA_{min}$) is sequentially followed by the tendency to PSMA decrease in all metastases (i.e. low$PSMA_{average}$) in the process of the disease. Moreover, it might be warranted to elucidate, if this sequence is caused by occurrence and spared of dedifferentiated tumor cell phenotypes.

The present study has limitations. It was conducted retrospectively and is therefore prone to selection biases. The patient collective might not be comparable to the Phase II trial of Hofman et al. or other retrospective analyses [13]. However, retrospective studies are mandatory for the planning of prospective trials, especially for inclusion criteria definition. In the present analysis, no FDG PET-CT was employed for patient selection. Therefore, PSMA negative metastases that show strong FDG uptake might have evaded the analysis. Additionally, small metastases (<0.5 ml) were no considered in the analysis. The SUV_{mean} heavily depends on the segmentation method. In contrast to previous approaches, we did not use a fixed SUV threshold (e.g. SUV > 3) but relative thresholding (50% of local SUV_{max}). Therefore, SUV_{mean} results are not directly comparable with other studies. Because of that, only SUV_{max} based PET parameters ($PSMA_{max}$, $PSMA_{average}$, $PSMA_{min}$, $PSMA_{std}$) were used for hypotheses generation.

Conclusion

In this retrospective analysis, $PSMA_{average}$ was a significant prognosticator for overall survival of mCRPC patients treated with Lu-PSMA therapy, whereas $PSMA_{max}$ was not. Patients without low PSMA expressing metastases had the best overall survival. Future studies are warranted to elucidate the degree of low PSMA expression tolerable for Lu-PSMA therapy.

Abbreviations

PET: Positron Emission Tomography; PSMA: Prostate Specific Membrane Antigen.

Acknowledgements

We acknowledge support from the Open Access Publication Fund of the University of Muenster.

Compliance with Ethical Standards

Disclosure of potential conflicts of interest

KR has received consultant fees from Bayer and lectureship fees from Janssen Cilag, Amgen, AAA and SIRTEX. KR is a clinical consultant for ABX. The University of Muenster has received consulting fees from ABX Advanced Biochemical Compounds, Radeberg, Germany for KR. MB has received consultant and lectureship fees from Bayer, Janssen Cilag, Astellas, ABX, Sanofi, Eisai, EUSApharm, Pfizer, BMS, MSD, AstraZeneca, Merck, Amgen, Novartis, Exelixis and Roche. The authors declare that they have no conflict of interest regarding this study.

Research involving human participants and/or animals

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

ethical standards. Data analysis was done retrospectively and was approved by the local ethics committee (No. 2016-585-f-S, Ethikkommission der Ärztekammer Westfalen-Lippe und der Westfälischen Wilhelms-Universität Münster).

Informed consent

Lu-PSMA therapy was done on a case by case basis in the clinical routine and only after recommendation by the local tumor board. Informed consent for the therapy was present for all patients.

References

1. Sartor O, de Bono JS. Metastatic Prostate Cancer. N Engl J Med. 2018; 378: 645–57.
2. Rahbar K, Bögeman M, Yordanova A, et al. Delayed response after repeated 177Lu-PSMA-617 radioligand therapy in patients with metastatic castration resistant prostate cancer. Eur J Nucl Med Mol Imaging. 2018; 45: 243–6.
3. Violet J, Sandhu S, Iravani A, et al. Long term follow-up and outcomes of re-treatment in an expanded 50 patient single-center phase II prospective trial of Lutetium-177 (177Lu) PSMA-617 theranostics in metastatic castrate-resistant prostate cancer. J Nucl Med. 2019; 10.2967/jnumed.119.236414.
4. Rahbar K, Ahmadzadehfar H, Kratochwil C, et al. German Multicenter Study Investigating 177 Lu-PSMA-617 Radioligand Therapy in Advanced Prostate Cancer Patients. J Nucl Med. 2017; 58: 85–90.
5. Donin NM, Reiter RE. Why Targeting PSMA Is a Game Changer in the Management of Prostate Cancer. J Nucl Med. 2018; 59: 177–82.
6. Virgolini I, Decristoforo C, Haug A, Fanti S, Uprimny C. Current status of theranostics in prostate cancer. Eur J Nucl Med Mol Imaging. 2018; 45: 471–95.
7. Zamboglou C, Schiller F, Fechter T, et al. 68 Ga-HBED-CC-PSMA PET/CT Versus Histopathology In Primary Localized Prostate Cancer: A Voxel-Wise Comparison. Theranostics. 2016; 6: 1619–28.
8. Zamboglou C, Drendel V, Jilg CA, et al. Comparison of 68 Ga-HBED-CC PSMA-PET/CT and multiparametric MRI for gross tumour volume detection in patients with primary prostate cancer based on slice by slice comparison with histopathology. Theranostics. 2017; 7: 228–37.
9. Zamboglou C, Carles M, Fechter T, et al. Radiomic features from PSMA PET for non-invasive intraprostatic tumor discrimination and characterization in patients with intermediate- and high-risk prostate cancer - a comparison study with histology reference. Theranostics. 2019; 9: 2595–605.
10. Kratochwil C, Fendler WP, Eiber M, et al. EANM procedure guidelines for radionuclide therapy with 177Lu-labelled PSMA-ligands (177Lu-PSMA-RLT). Eur J Nucl Med Mol Imaging. 2019; 46: 2536–44.
11. Ferdinandus J, Eppard E, Gaertner FC, et al. Predictors of Response to Radioligand Therapy of Metastatic Castrate-Resistant Prostate Cancer with 177 Lu-PSMA-617. J Nucl Med. 2017; 58: 312–9.
12. Rathke H, Holland-Letz T, Mier W, et al. Response Prediction of 177 Lu-PSMA-617 Radioligand Therapy Using Prostate-Specific Antigen, Chromogranin A, and Lactate Dehydrogenase. J Nucl Med. 2020; 61: 689–95.
13. Hofman MS, Violet J, Hicks RJ, et al. [177Lu]-PSMA-617 radionuclide treatment in patients with metastatic castration-resistant prostate cancer (LuPSMA trial): a single-centre, single-arm, phase 2 study. Lancet Oncol. 2018; 19: 825–33.
14. Rahbar K, Bode A, Weckesser M, et al. Radioligand Therapy With 177Lu-PSMA-617 as A Novel Therapeutic Option in Patients With Metastatic Castration Resistant Prostate Cancer. Clin Nucl Med. 2016; 41: 522–8.
15. Seifert R, Herrmann K, Kleesiek J, et al. Semi-automatically quantified tumor volume using Ga-68-PSMA-11-PET as biomarker for survival in patients with advanced prostate cancer. J Nucl Med. 2020; 10.2967/jnumed.120.242057.
16. R Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. 2018.
17. Hothorn T, Lausen B. On the exact distribution of maximally selected rank statistics. Comput Stat Data Anal. 2003; 43: 121–37.
18. Kessel K, Seifert R, Schäfers M, et al. Second line chemotherapy and visceral metastases are associated with poor survival in patients with mCRPC receiving 177 Lu-PSMA-617. Theranostics. 2019; 9: 4841–8.
19. Thang SP, Violet J, Sandhu S, et al. Poor Outcomes for Patients with Metastatic Castration-resistant Prostate Cancer with Low Prostate-specific Membrane Antigen (PSMA) Expression Deemed Ineligible for 177Lu-labelled PSMA Radioligand Therapy. Eur Urol Oncol. 2019; 2: 670–6.
20. Ferdinandus J, Violet J, Sandhu S, et al. Prognostic biomarkers in men with metastatic castration-resistant prostate cancer receiving [177Lu]-PSMA-617. Eur J Nucl Med Mol Imaging. 2020; 6–11.
21. Ahmadzadehfar H, Schlolaut S, Fimmers R, et al. Predictors of overall survival in metastatic castration-resistant prostate cancer patients receiving [177Lu]Lu-PSMA-617 radioligand therapy. Oncotarget. 2017; 8: 103108–16.
22. von Eyben FE, Kulkarni HR, Baum RP. Metastatic extent predicts survival as patients with metastatic castration-resistant prostate cancer are treated with 177 Lu-PSMA radioligand therapy. Theranostics. 2020; 10: 4900–2.
23. Marusyk A, Almendro V, Polyak K. Intra-tumour heterogeneity: a looking glass for cancer? Nat Rev Cancer. 2012; 12: 323–34.
24. Gerlinger M, Rowan AJ, Horswell S, et al. Intratumor Heterogeneity and Branched Evolution Revealed by Multiregion Sequencing. N Engl J Med. 2012; 366: 883–92.
25. Pouessel D, Gallet B, Bibeau F, et al. Liver metastases in prostate carcinoma: Clinical characteristics and outcome. BJU Int. 2007; 99: 807–11.
26. Yuan TC, Veeramani S, Lin MF. Neuroendocrine-like prostate cancer cells: Neuroendocrine transdifferentiation of prostate adenocarcinoma cells. Endocr Relat Cancer. 2007; 14: 531–47.
27. Molenaar JPF, Baten A, Blokx WAM, Hoogendam A. Development of Carcinoid Tumour in Hormonally Treated Adenocarcinoma of the Prostate. Eur Urol. 2009; 56: 874–7.
28. Wang HT, Yao YH, Li BG, Tang Y, Chang JW, Zhang J. Neuroendocrine Prostate Cancer (NEPC) Progressing from conventional prostatic adenocarcinoma: Factors associated with time to development of nepc and survival from NEPC Diagnosis-A systematic review and pooled analysis. J Clin Oncol. 2014; 32: 3383–90.
29. Conteduca V, Oromendia C, Eng KW, et al. Clinical features of neuroendocrine prostate cancer. Eur J Cancer. 2019; 121: 7–18.
30. Bakht MK, Derecichei I, Li Y, et al. Neuroendocrine differentiation of prostate cancer leads to PSMA suppression. Endocr Relat Cancer. 2019; 26: 131–46.
31. Aggarwal R, Huang J, Alumkal JJ, et al. Clinical and genomic characterization of treatment-emergent small-cell neuroendocrine prostate cancer: A multi-institutional prospective study. J Clin Oncol. 2018; 36: 2492–503.
32. Violet J, Jackson P, Ferdinandus J, et al. Dosimetry of 177Lu-PSMA-617 in metastatic castration-resistant prostate cancer: Correlations between pretherapeutic imaging and whole-body tumor dosimetry with treatment outcomes. J Nucl Med. 2019; 60: 517–23.