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

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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$_{\text{max}}$ of the highest PSMA expressing metastasis (PSMA$_{\text{max}}$), SUV$_{\text{max}}$ of the lowest PSMA expressing metastasis (PSMA$_{\text{min}}$), and average SUV$_{\text{max}}$ of all metastases (PSMA$_{\text{average}}$) amongst other PET parameters were measured for each patient. A log-rank cutoff-finder was used to determine low (lowPSMA$_{\text{average}}$) and high (highPSMA$_{\text{average}}$) average PSMA expression as well as low (lowPSMA$_{\text{min}}$) and high (highPSMA$_{\text{min}}$) minimal PSMA expression.

**Results:** PSMA$_{\text{average}}$ was a significant prognosticator of overall survival in contrast to PSMA$_{\text{max}}$ (HR: 0.959; p = 0.047 vs. HR: 0.992; p = 0.231). Optimal log rank cut-offs were: PSMA$_{\text{average}}$ = 14.3; PSMA$_{\text{min}}$ = 10.2. Patients with low average PSMA expression (lowPSMA$_{\text{average}}$) had significantly shorter survival compared to those with high average expression (highPSMA$_{\text{average}}$) (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$_{\text{min}}$) had shorter survival compared to those without a low PSMA expressing metastasis (highPSMA$_{\text{min}}$) (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$_{\text{average}}$, but with lowPSMA$_{\text{min}}$, had an intermediate overall survival (11.4 months; longer compared to lowPSMA$_{\text{average}}$, 5.3 months, p = 0.002; but shorter compared to highPSMA$_{\text{min}}$, 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$_{\text{max}}$ 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$_{\text{max}}$ 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 |
| SUVmax, SUV mean, SUV peak | 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**

| Parameter | Median [IQR] |
|-----------|--------------|
| Hemoglobin (g/dl) | 147.0 [193.0] |
| Alanine transaminase (U/l) | 31.6 [227.0] |
| Aspartate aminotransferase (U/l) | 32.5 [24.0] |
| Lactate dehydrogenase (U/l) | 16.0 [11.0] |
| Alkaline phosphatase (U/dl) | 10.4 [2.4] |
| Prostate-specific antigen (ng/ml) | 284.0 [805.0] |

**Metastases**

| Metastases | N [%] |
|------------|-------|
| Bone | 78 [92%] |
| Lymph node | 68 [80%] |
| Liver | 26 [31%] |
| Lung | 20 [24%] |
| Brain | 1 [1%] |

**Previous therapies**

| Therapy | N [%] |
|---------|-------|
| 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 on 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 SUVmax) were segmented and SUVmax, SUVmean, SUVpeak were reported for each metastasis. Metastases were delineated using relative thresholding (50% of local SUVmax). On a per patient level, the mean of all SUVmax measurements (PSMAaverage), the maximum SUVmax measurement (PSMAmax), the lowest SUVmax measurement (PSMAmin) and the standard deviation of the SUVmax measurements (PSMAstd) were noted.

PSMA measurements were analyzed as continuous parameter and in binarized form. When PSMAaverage was binarized using optimized log rank thresholds, the group with low PSMAaverage was denoted lowPSMAaverage (highPSMAaverage threshold: 14.3). When PSMAmin was binarized, patients with low PSMA expressing metastases were denoted lowPSMAmin (without low PSMA expressing metastases: highPSMAmin threshold: 10.2). The volumetric fraction of low PSMA expressing tumor was determined by dividing the volume of metastases with low PSMA expression (SUVmax ≤ 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 SUVmax tests: optimal log rank cut-off for PSMAmax, PSMAmin, PSMAaverage, PSMAstd. Other SUV parameters (SUVmean, SUVpeak) were only analyzed to further corroborate SUVmax 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 PSMAmax measurements was 44.6 SUV (range 7.1–181.6), whereas the median of PSMAaverage measurements was 18.9 (range 4.6–129.8). The median intensity was 31.6 (range 4.7–159.7) for the highest SUVpeak. A detailed report on SUV parameters is provided by Table 2.

**Baseline PET-parameters and overall survival**

Regarding SUVmax, neither the highest (PSMAmax: HR: 0.992; p = 0.231; 95% CI: 0.979–1.005), nor the lowest (PSMAmin: HR: 0.890; p = 0.118; 95% CI: 0.768–
value per patient were significant prognosticators of overall survival, but the average value was (PSMAaverage: HR = 0.959; p = 0.047; 95% CI: 0.921–0.999). The same was true for SUVmean (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 SUVmax, SUVmean and SUVpeak) are given by Table 3. Figure 1 depicts the overall survival stratified according to the quartiles of PSMAaverage and PSMAmin.

There were no relevant correlations between PSMAmin and PSMAaverage (R² = 0.30, p < 0.001; Figure 2) or PSMAmin and PSMAstd (R² = 0.18, p < 0.001), but PSMAstd and PSMAaverage were significantly correlated (R² = 0.78, p < 0.001; Figure 3).

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

| Measurement selected per patient | 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]                             | 29.3 [4.6–128.9]                           | 6.3 [2.6–29.4]                            |
| SUVpeak                          | 12.1 [3.7–46.5]                             | 31.6 [4.7–159.7]                           | 5.7 [1.8–22.4]                            |

Abbreviation: Squared brackets = range.

**Table 3. Baseline PSMA PET parameters and overall survival**

| Measurement selected per patient | HR | 95% CI | P     |
|----------------------------------|----|--------|-------|
| SUVmax                           | 0.959 | 0.921-0.999 | 0.047 |
| Highest (PSMAmax)                | 0.992 | 0.979-1.005 | 0.231 |
| Lowest (PSMAmin)                 | 0.890 | 0.768-1.030 | 0.118 |
| Std (PSAstd)                     | 0.936 | 0.877-0.999 | 0.048 |
| SUVmax/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 | 0.563 |
| Std                              | 0.932 | 0.822-1.057 | 0.274 |
| SUVmean                          | 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 | 0.802-0.996 | 0.042 |
| SUVpeak                          | 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**

| highPSMAaverage | lowPSMAaverage | Sum |
|-----------------|----------------|-----|
| lowPSMAmin      | 49             | 20  | 69  |
| highPSMAmin     | 16             | 0   | 16  |
| Sum             | 65             | 20  | 85  |

Low PSMA expression and overall survival

In a first approach, a liver specific SUV threshold was used (1.5 × SUVmean liver) in analogy to Hofman et al. In our cohort, zero patients had a SUVmax 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, PSMAaverage and PSMAmin were binarized to determine low and high PSMA expression. The optimized log rank threshold for PSMAaverage to stratify according to overall survival was 14.3 SUV (log rank: p < 0.001; estimated median: 15.1 vs. 5.3 months; high PSMAaverage: HR = 0.268, 95%CI = 0.140–0.512, p < 0.001) and 10.2 for PSMAmin (log rank: p = 0.003; estimated median: 21.3 vs. 7.9 months; high PSMAmin: 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 highPSMAmin had longer survival compared to patients classified as both lowPSMAmin and highPSMAaverage (estimated median: 21.3 vs. 11.4 months; p = 0.02; HR = 0.3; 95% CI = 0.186–0.710; p = 0.003). Multivariate Regression (including binarized PSMAmin and lowPSMAaverage and adjusted for presence of liver metastases) confirmed both highPSMAmin and highPSMAaverage to be significant positive prognosticators of overall survival (highPSMAaverage: HR = 0.473; p = 0.044; 95%CI = 0.229–0.980 | highPSMAmin: 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 (>9.7% vs. <9.7%; p = 0.023; 6.4 vs. 11.4 months; only lowPSMAmin patients).

**Influence of liver metastases and Cabazitaxel therapy**

There were no significant differences between patients with and without liver metastases regarding PSMAmin (9.1 vs. 8.9; p = 0.418) or PSMAaverage (18.3 vs. 20.1, p = 0.264; Figure 3). The same was true for a positive/negative history of Cabazitaxel therapy.
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 |
|----------------------------------|---------------|--------|---------------|---------------|
| SUVmax                           | Average       | 14.3   | <0.001        | 20            | 20             |
|                                  | Highest       | n.a.   | n.s.          | 15.1          | 7.8–23.4       |
|                                  | Lowest        | 10.2   | 0.003         | 69            | 7.9            |
|                                  | Std           | 3.0    | <0.001        | 9             | 3.2            |
|                                  | Average       | 6.2    | 0.003         | 42            | 7.2            |
|                                  | Highest       | n.a.   | n.s.          | 21.3          | 9.0–17.4       |
|                                  | Lowest        | n.a.   | n.s.          | 13.2          | 13.2–29.3      |
|                                  | Std           | n.a.   | n.s.          | 16            | 21.3           |
| SUVmean / SUVmean/liver          | Average       | 9.3    | <0.001        | 17            | 6.4            |
|                                  | Highest       | n.a.   | n.s.          | 15.1          | 10.1–20.1      |
|                                  | Lowest        | 7.1    | 0.004         | 65            | 8.6            |
|                                  | Std           | 1.9    | <0.001        | 9             | 3.2            |
|                                  | Average       | 9.7    | <0.001        | 19            | 6.4            |
|                                  | Highest       | n.a.   | n.s.          | 25.5          | 15.1–36.0      |
|                                  | Lowest        | 6.6    | 0.004         | 65            | 8.6            |
|                                  | Std           | n.a.   | n.s.          | 25.5          | 15.1–36.0      |

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.
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 neuron 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 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, highPSMA\textsubscript{min} patients had a significantly longer overall survival. Taken together with PSMA\textsubscript{average}, patients were stratified based on PSMA-PET measurements into those with low (lowPSMA\textsubscript{average}), intermediate (highPSMA\textsubscript{average} and lowPSMA\textsubscript{min}) or high (highPSMA\textsubscript{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 prognostic parameter for overall survival. Future studies have to elucidate, if the occurrence of low PSMA expressing metastases (i.e. lowPSMA\textsubscript{min}) is sequentially followed by the tendency to PSMA decrease in all metastases (i.e. lowPSMA\textsubscript{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 not considered in the analysis. The $SUV_{\text{mean}}$ 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 $SUV_{\text{max}}$). Therefore, $SUV_{\text{mean}}$ results are not directly comparable with other studies. Because of that, only $SUV_{\text{max}}$ based PET parameters (PSMA\textsubscript{max}, PSMA\textsubscript{average}, PSMA\textsubscript{min}, PSMA\textsubscript{std}) were used for hypotheses generation.

Conclusion

In this retrospective analysis, PSMA\textsubscript{average} was a significant prognosticator for overall survival of mCRPC patients treated with Lu-PSMA therapy, whereas PSMA\textsubscript{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.

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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
et 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.

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