

*cancers*



## Article

---

# Upregulation of PSMA Expression by Enzalutamide in Patients with Advanced mCRPC

---

Florian Rosar, Robert Neher, Caroline Burgard, Johannes Linxweiler, Mathias Schreckenberger, Manuela A. Hoffmann, Mark Bartholomä, Fadi Khreish and Samer Ezziddin

## Special Issue

Radionuclide Therapy and Hybrid Imaging in Oncology

Edited by

Dr. Amir Sabet and Prof. Dr. Frank Grünwald



<https://doi.org/10.3390/cancers14071696>

## Article

# Upregulation of PSMA Expression by Enzalutamide in Patients with Advanced mCRPC

Florian Rosar <sup>1</sup> , Robert Neher <sup>1</sup>, Caroline Burgard <sup>1</sup> , Johannes Linxweiler <sup>2</sup>, Mathias Schreckenberger <sup>3</sup>, Manuela A. Hoffmann <sup>3</sup> , Mark Bartholomä <sup>1</sup> , Fadi Khreish <sup>1</sup>  and Samer Ezziddin <sup>1,\*</sup> 

<sup>1</sup> Department of Nuclear Medicine, Saarland University, 66421 Homburg, Germany; florian.rosar@uks.eu (F.R.); s8ronehe@stud.uni-saarland.de (R.N.); caroline.burgard@uks.eu (C.B.); mark.bartholomae@uks.eu (M.B.); fadi.khreish@uks.eu (F.K.)

<sup>2</sup> Department of Urology, Saarland University, 66421 Homburg, Germany; johannes.linxweiler@uks.eu

<sup>3</sup> Department of Nuclear Medicine, Johannes Gutenberg-University, 55131 Mainz, Germany; mathias.schreckenberger@unimedizin-mainz.de (M.S.); manuela1hoffmann@bmv.g.bund.de (M.A.H.)

\* Correspondence: samer.ezziddin@uks.eu; Tel.: +49-6841-16-22201; Fax: +49-6841-16-24692

**Simple Summary:** The prostate-specific membrane antigen (PSMA) which shows overexpression on the cell surface of prostate cancer cells, provides a specific target for molecular imaging and radioligand therapy. In this study, we investigated PSMA upregulation by enzalutamide, an established androgen axis drug, in a cohort ( $n = 30$ ) of patients with advanced, metastatic, castration-resistant prostate cancer (mCRPC). Our results show that short-term enzalutamide medication significantly increases PSMA expression in patients with mCRPC. Therefore, enzalutamide may provide a potential enhancer medication for PSMA-targeted radioligand therapy.



**Citation:** Rosar, F.; Neher, R.; Burgard, C.; Linxweiler, J.; Schreckenberger, M.; Hoffmann, M.A.; Bartholomä, M.; Khreish, F.; Ezziddin, S. Upregulation of PSMA Expression by Enzalutamide in Patients with Advanced mCRPC. *Cancers* **2022**, *14*, 1696. <https://doi.org/10.3390/cancers14071696>

Academic Editor: Janina Baranowska-Kortylewicz

Received: 4 February 2022

Accepted: 24 March 2022

Published: 26 March 2022

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

**Abstract:** In this study, we investigated upregulation of prostate-specific membrane antigen (PSMA) by enzalutamide in a cohort ( $n = 30$ ) of patients with advanced metastatic castration-resistant prostate cancer (mCRPC). Patients were examined by [ $^{68}\text{Ga}$ ]Ga-PSMA-11 PET/CT pre- and post-enzalutamide medication (mean  $13 \pm 7$  days). Imaging results were compared based on quantification of whole-body PSMA tumor burden: total lesion PSMA (TLP) and normalized TLP values to liver (TLP-LR) and to parotid gland (TLP-PR). In addition, lesion-based analyses were performed. The median (mean) increases in TLP, TLP-LR and TLP-PR after enzalutamide medication were 10.1% (20.2%), 29.5% (34.8%) and 27.6% (24.4%), respectively. These increases were statistically significant ( $p = 0.002$ ,  $p < 0.001$ , and  $p < 0.001$ ), while prostate-specific antigen (PSA) serum values did not change significantly ( $p = 0.483$ ). The increase was independent of prior patient exposure to enzalutamide.  $\text{SUV}_{\text{max}}$  increased substantially ( $>10\%$ ) in 49.6% of target lesions. The relative change was significantly higher in the subgroup of lesions with  $\text{SUV}_{\text{max}} < 10$  ( $p < 0.001$ ). In conclusion, short-term enzalutamide medication significantly increases PSMA expression in patients with mCRPC, irrespective of prior enzalutamide exposure. The relative PSMA upregulation effect seems to be more pronounced in lesions with only moderate baseline PSMA expression. Enzalutamide may provide a potential enhancer medication for PSMA-targeted radioligand therapy.

**Keywords:** prostate cancer; mCRPC; PSMA; enzalutamide; upregulation

## 1. Introduction

Prostate cancer is one of the most frequent malignancies in men worldwide [1]. While most patients with metastatic prostate cancers initially respond well to androgen-deprivation therapy (ADT), virtually all patients ultimately develop metastatic castration-resistant prostate cancer (mCRPC) during disease course [2,3]. In this mCRPC setting, various treatments have proven to prolong survival, including the following: chemotherapy with docetaxel [4] or cabazitaxel [5]; therapy with new androgen axis drugs (NAAD),

such as abiraterone [6] or enzalutamide [7]; therapy with PARP-inhibitors, such as olaparib [8]; and bone-seeking therapy with  $^{223}\text{Ra}$  [9]. These therapies have different therapeutic mechanisms. For example, enzalutamide targets multiple steps in the signaling pathway of androgen receptor (AR) and inhibits the binding of androgen to AR, the nuclear translocation and DNA binding of androgen–AR complex and the recruitment of coactivators [7,10]. Besides these established therapies, radioligand therapy (RLT), targeting the prostate-specific membrane antigen (PSMA) using PSMA ligands labeled with a beta emitter (e.g.,  $^{177}\text{Lu}$ Lu-PSMA-617), has shown promising survival data in patients with mCRPC in various retrospective studies [11–13], in prospective phase II trials [14,15] and in a recently published phase III trial [16]. PSMA is a type-II transmembrane glycoprotein, which shows overexpression on the cell surface of prostate carcinoma cells [17] providing new ways of targeted molecular imaging (PSMA PET/CT) and radioligand therapy (PSMA-RLT) [18,19]. In principle, intense PSMA expression forms the basis for a potential response to PSMA-RLT. The expression of PSMA, however, varies from patient to patient and even between different metastases, which may impact the outcome of PSMA-RLT [20]. It is known from preclinical research that PSMA expression in prostate cancer cells is inversely modulated by the AR-signaling cascade, eventually involving the FOLH1 gene, which encodes PSMA [21,22]. Some preclinical and clinical studies have shown that PSMA expression can be increased by enzalutamide [23–29], which we also recently communicated in a small cohort of advanced mCRPC patients [30]. However, more data is needed to support these findings. In this subsequent study, we investigated PSMA upregulation by enzalutamide in a larger cohort of advanced mCRPC patients.

## 2. Methods

### 2.1. Patients and Ethics

Data of thirty patients with mCRPC in preparation for PSMA-RLT were included in this monocenter study and were analyzed retrospectively. All patients received a baseline  $^{68}\text{Ga}$ PSMA-11 PET/CT, followed by oral enzalutamide medication and a second  $^{68}\text{Ga}$ PSMA-11 PET/CT. The mean duration of enzalutamide medication was  $13 \pm 7$  days, starting  $7 \pm 11$  days after baseline PET/CT and continuing until the second PET/CT. The mean time between both PET/CT scans was  $20 \pm 12$  days. Twenty-eight patients received 160 mg and two patients 80 mg due to mild side effects of previous enzalutamide treatment. The mean age of the patients was  $69 \pm 9$  years. All patients were heavily pretreated and had a high tumor burden with bone metastases (27/30), lymph node metastases (22/30) and visceral metastases (6/30). Detailed information about the patients is presented in Table 1.

$^{68}\text{Ga}$ PSMA-11 PET/CT was performed on a compassionate use basis under the German Pharmaceutical Act §13 (2b). Patients gave written consent after being thoroughly informed about the risks and potential side effects of this examination and enzalutamide medication. Additionally, patients consented to publication of any resulting data in accordance with the Declaration of Helsinki. The retrospective analysis was approved by the local Institutional Review Board (ethics committee permission number 140/17, approved on 13 July 2017).

### 2.2. Image Acquisition

For PET imaging, a mean activity of  $127 \pm 18$  MBq  $^{68}\text{Ga}$ PSMA-11 was applied, followed by a 500 mL infusion of NaCl 0.9%. In accordance with standard procedures for prostate cancer imaging [31], the time between injection and PET acquisition was  $64 \pm 9$  min. All PET/CT scans were performed using a Biograph mCT 40 PET/CT scanner (Siemens Medical Solutions, Knoxville, TN, USA)  $^{18}\text{F}$ -accredited by European Association of Nuclear Medicine (EANM) Research Ltd. With respect to  $^{68}\text{Ga}$ , inhouse phantom measurements were also routinely performed to allow valid PET quantification. The PET acquisition was conducted from vertex to proximal femur with a 3 min acquisition time per bed position (extended field of view: 21.4 cm). CT data was acquired in low-dose

technique using an X-ray tube voltage of 120 keV and a modulation of the tube current by applying ‘CARE Dose4D’ with a maximal tube current time product of 30 mAs. The PET data sets were reconstructed using an iterative 3D OSEM (ordered subset expectation maximization) algorithm (3 iterations, 24 subsets) with Gaussian filtering and slice thickness of 5 mm. Random correction, decay correction, scatter attenuation and attenuation correction were applied.

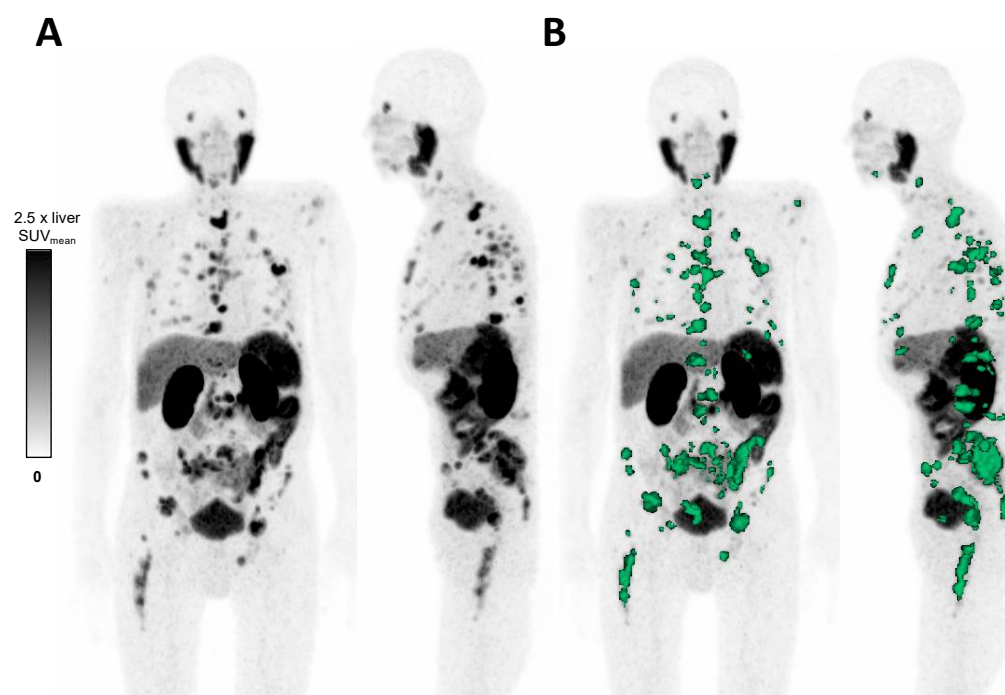
**Table 1.** Characteristics of the patient cohort.

| Characteristics              | Mean $\pm$ SD/n (%) |
|------------------------------|---------------------|
| Age (years)                  | 69 $\pm$ 9          |
| Enzalutamide medication      |                     |
| Dose                         |                     |
| 160 mg/day                   | 28 (93.3%)          |
| 80 mg/day                    | 2 (6.7%)            |
| Duration (days)              | 13 $\pm$ 7          |
| Prior treatments             |                     |
| Prostatectomy                | 11 (36.7%)          |
| Radiation                    | 7 (23.3%)           |
| ADT                          | 30 (100%)           |
| NAAD                         | 26 (86.7%)          |
| Enzalutamide                 | 18 (60.0%)          |
| Abiraterone                  | 21 (70.0%)          |
| Enzalutamide and Abiraterone | 13 (43.4%)          |
| Chemotherapy                 | 26 (86.7%)          |
| Docetaxel                    | 26 (86.7%)          |
| Cabazitaxel                  | 14 (46.7%)          |
| Docetaxel and Cabazitaxel    | 14 (46.7%)          |
| $^{223}\text{Ra}$ therapy    | 2 (6.7%)            |
| Sites of metastases          |                     |
| Bone                         | 27 (90.0%)          |
| Lymph node                   | 22 (73.4%)          |
| Visceral                     | 6 (20.0%)           |

ADT—androgen-deprivation therapy; NAAD—new androgen axis drugs; SD—standard deviation.

### 2.3. Quantification of PSMA Expression

To evaluate PSMA expression, the whole-body total lesion PSMA (TLP), defined as the summed products of  $\text{SUV}_{\text{mean}} \times \text{PSMA-based tumor volume}$ , was quantified in [ $^{68}\text{Ga}$ ]Ga-PSMA-11 PET/CT for each patient pre- and post-enzalutamide medication (patient-based analysis). Quantitative analyses were performed using Syngo.via (Enterprise VB 60, Siemens, Erlangen, Germany) by applying a semi-automatic tumor segmentation algorithm (Figure 1). Following Ferdinandus et al., a standard uptake value (SUV) threshold of 3.0 was set to delineate tumor lesions [32]. Considering the physiological organ uptake in healthy liver tissue (in general  $\text{SUV} > 3.0$ ), a SUV threshold of  $1.5 \times \text{SUV}_{\text{mean}}$  of the liver was applied for intrahepatic metastases [33]. Physiological uptake in organs, e.g., kidneys or salivary glands, were manually excluded. In addition, we normalized TLP values to healthy PSMA-positive tissue as liver and parotid gland: TLP-to-liver ratio (TLP-LR) and TLP-to-parotid gland ratio (TLP-PR). TLP-LR and TLP-PR were calculated as the quotient of TLP and  $\text{SUV}_{\text{mean}}$  of the respective organ. To evaluate the effect of enzalutamide on metastases with different PSMA expression (lesion-based analysis), an independent reader with long experience in molecular imaging (F.K.) determined up to ten bone metastases per patient in baseline [ $^{68}\text{Ga}$ ]Ga-PSMA-11 PET/CT (up to five with moderate and up to five with high PSMA expression). All metastases had to be larger than 1 cm in diameter to allow valid PET quantification.  $\text{SUV}_{\text{max}}$  of the identified lesions were measured in baseline and at follow up [ $^{68}\text{Ga}$ ]Ga-PSMA-11 PET/CT.



**Figure 1.** (A) MIP (maximum intensity projection) of [ $^{68}\text{Ga}$ ]Ga-PSMA-11 PET in an advanced mCRPC patient. (B) Semi-automatic tumor segmentation by Syngo.via (Enterprise VB 60, Siemens, Erlangen, Germany). SUV windowing was set from 0 to 2.5 times  $\text{SUV}_{\text{mean}}$  of the liver. The delineated tumor volume is shown in green.

For statistical analysis Wilcoxon matched pairs signed rank test and Mann–Whitney test were applied using Prism 8 (GraphPad Software, San Diego, CA, USA) to determine significant differences. A  $p$  value  $< 0.05$  was regarded as statistically significant.

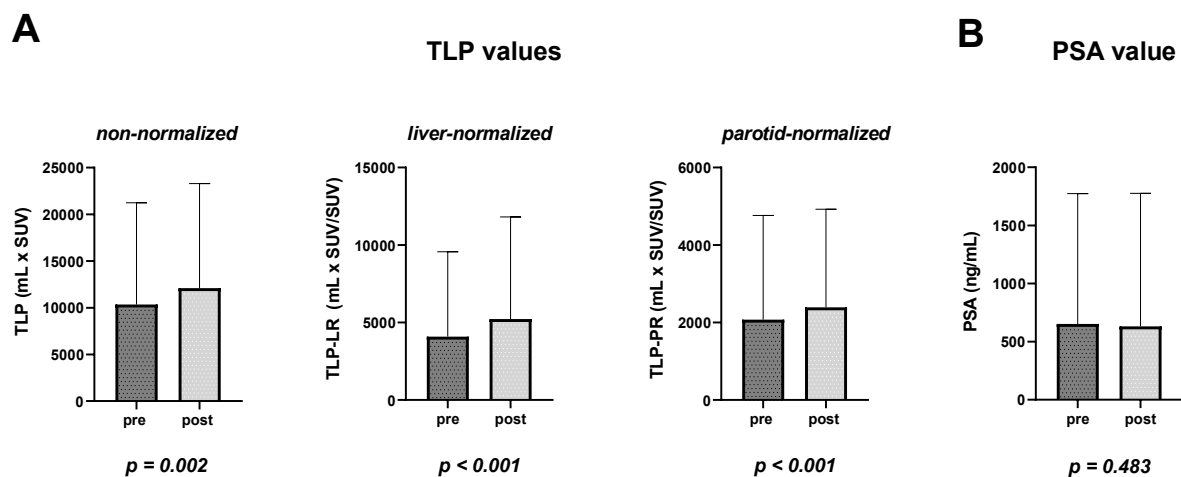
### 3. Results

In the patient-based analysis, a significantly higher TLP ( $10,920 \pm 10,910 \text{ mL} \times \text{SUV}$  vs.  $12,608 \pm 11,418 \text{ mL} \times \text{SUV}$ , matched pairs signed rank test  $p = 0.002$ ) was measured after enzalutamide medication (mean  $13 \pm 7$  days) in the follow-up PET/CT examination compared to baseline PET/CT. Consistently, TLP-LR ( $4096 \pm 5464 \text{ mL} \times \text{SUV}/\text{SUV}$  vs.  $5228 \pm 6583 \text{ mL} \times \text{SUV}/\text{SUV}$ ,  $p < 0.001$ ) and TLP-PR ( $2072 \pm 2690 \text{ mL} \times \text{SUV}/\text{SUV}$  vs.  $2391 \pm 2530 \text{ mL} \times \text{SUV}/\text{SUV}$ ,  $p < 0.001$ ) values showed a significant increase, whereas prostate-specific antigen (PSA) serum levels ( $652 \pm 1122 \text{ ng/mL}$  vs.  $631 \pm 1145 \text{ ng/mL}$ ,  $p = 0.483$ ) did not change significantly (Figure 2). Additionally, uptake in reference organs (liver and parotid gland) and in the kidney did not change significantly ( $p = 0.155$ ,  $p = 0.309$ ,  $p = 0.465$ ). The overall tumor burden varied from patient to patient; the individual TLP, TLP-LR and TLP-PR values pre- and post-enzalutamide medication are presented in Figure 3. In concordance with TLP quantification, increased uptake could also be identified visually on [ $^{68}\text{Ga}$ ]Ga-PSMA-11 PET/CT images. Figure 4 shows a representative example of increased tracer uptake in a [ $^{68}\text{Ga}$ ]Ga-PSMA-11 PET/CT of an mCRPC patient after 18 days of enzalutamide medication, despite a decrease in the serum PSA level.

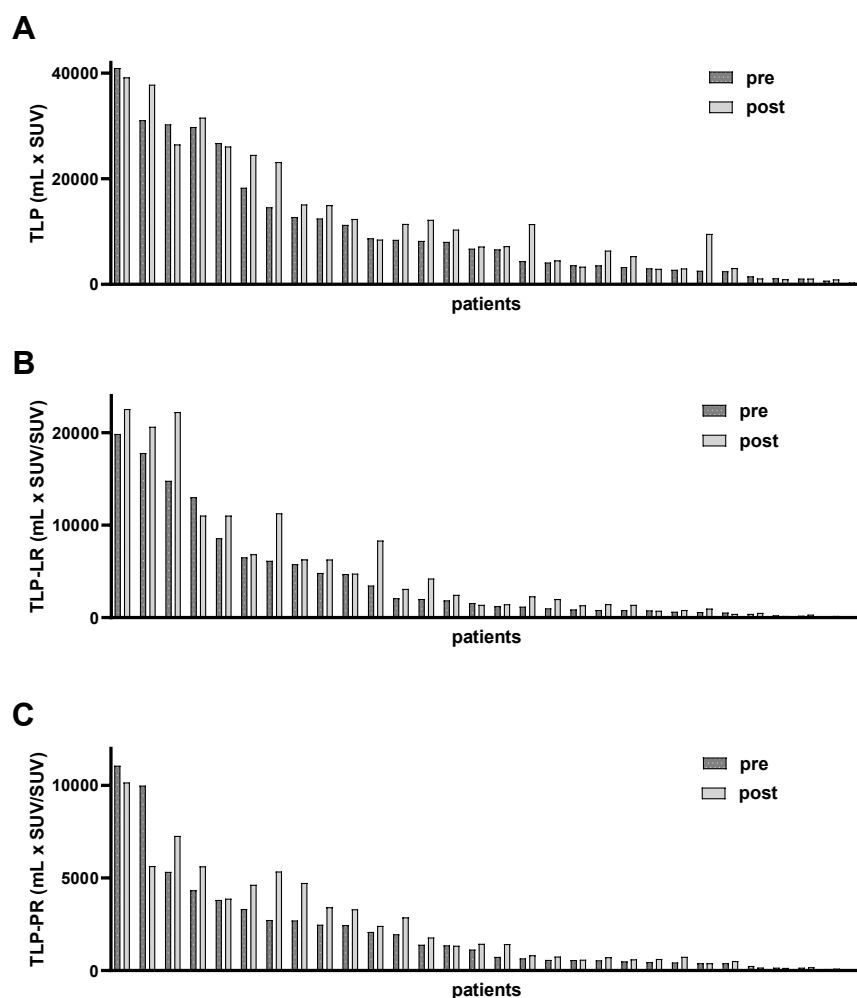
The overall median (mean  $\pm$  standard deviation) increased in non-normalized TLP, TLP-LR and TLP-PR after enzalutamide medication was 10.1% ( $20.2 \pm 36.1\%$ ), 29.5% ( $34.8 \pm 43.1\%$ ) and 27.6% ( $24.4 \pm 33.4\%$ ), respectively.

In total, 15/30 (50.0%), 21/30 (70.0%) and 21/30 (70.0%) patients showed a substantial increase in TLP, TLP-LR and TLP-PR of more than 10%, respectively. The individual relative changes in TLP, TLP-LR and TLP-PR levels for all patients are shown in Figure 5. Of note, the increment in the subgroup of patients previously treated with enzalutamide was comparable to that in patients without prior enzalutamide therapy for all three parameters—

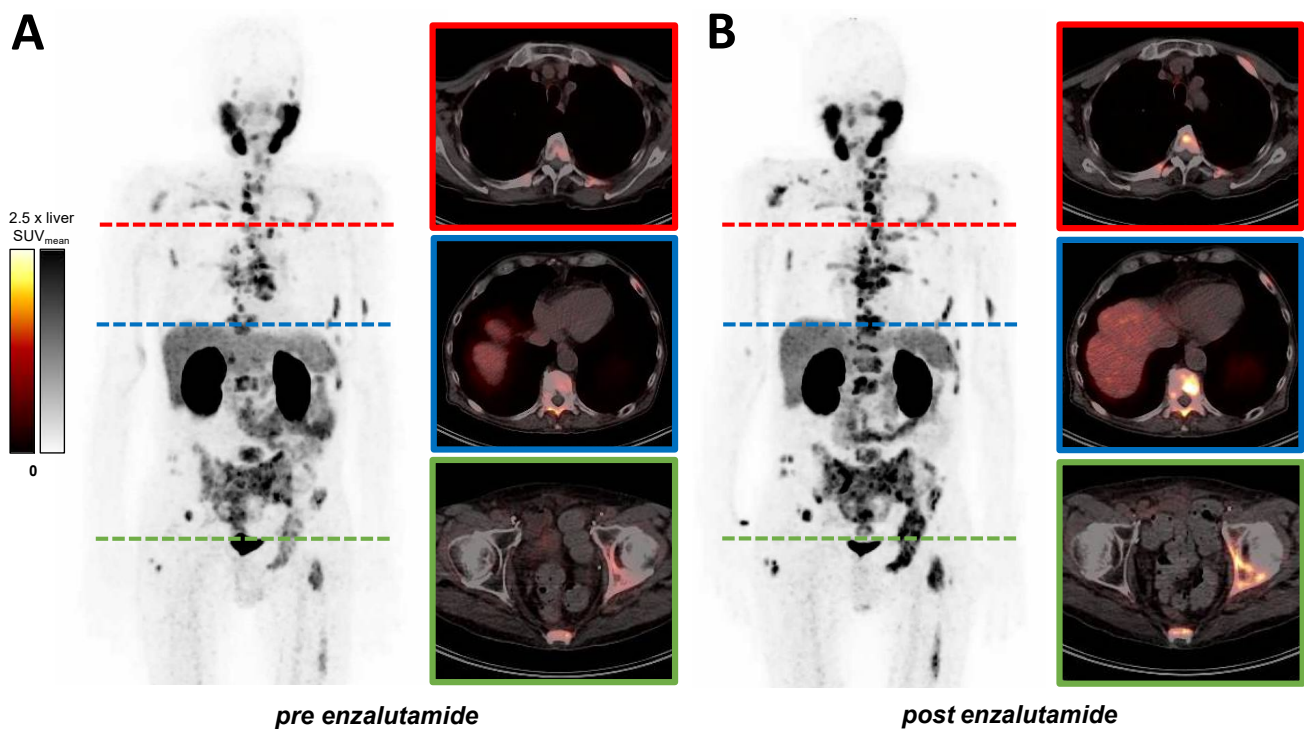
TLP, TLP-LR and TLP-PR ( $p = 0.20, 0.98$  and  $0.60$ ). In contrast, a substantial decrease in TLP, TLP-LR and TLP-PR was observed in 4/30 (13.3%), 5/30 (16.7%) and 3/30 (10.0%) patients.



**Figure 2.** (A) Non-normalized total lesion PSMA (TLP), liver-normalized TLP (TLP-LR), parotid-normalized TLP (TLP-PR); (B) prostate-specific antigen (PSA) serum levels pre- and post-enzalutamide medication.

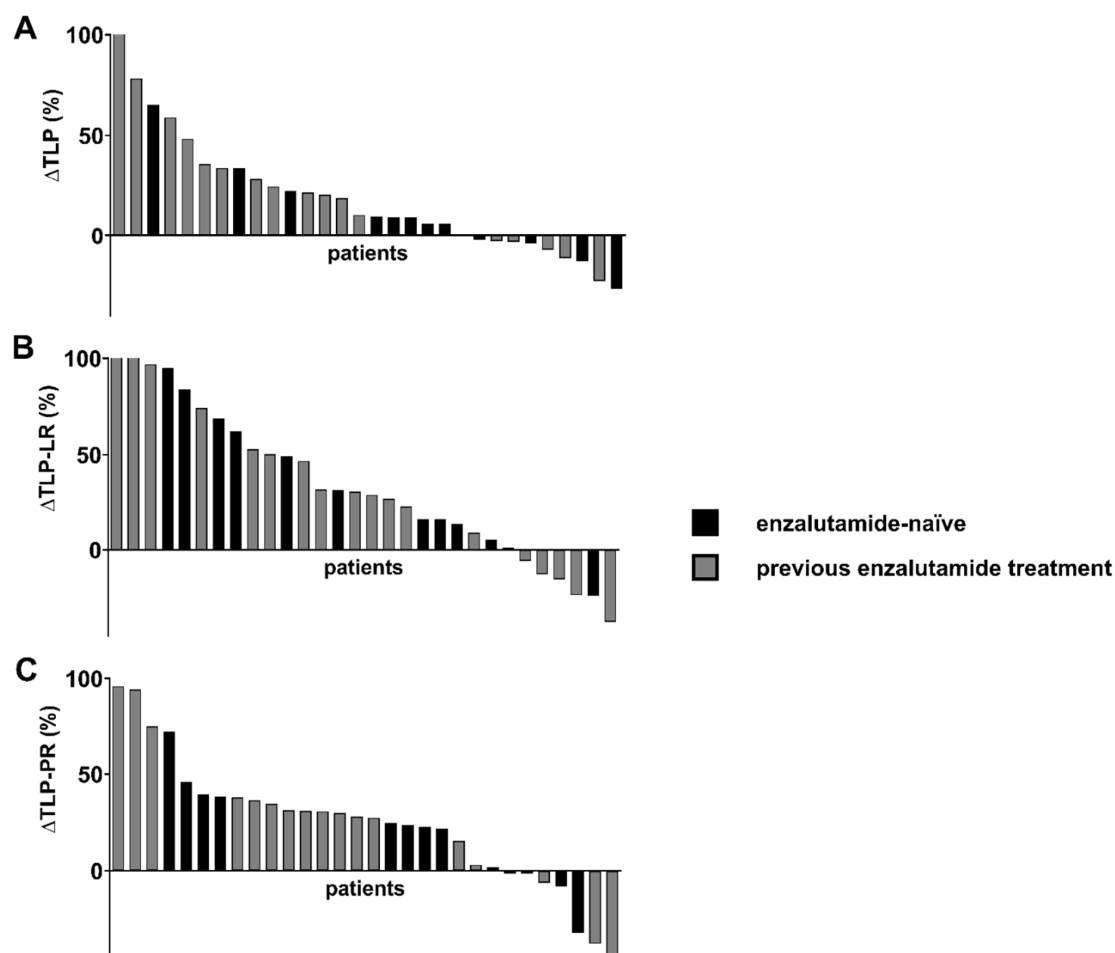


**Figure 3.** Waterfall plots of individual (A) non-normalized total lesion PSMA (TLP), (B) liver-normalized TLP (TLP-LR) and (C) parotid-normalized TLP (TLP-PR) values pre- and post-enzalutamide medication.

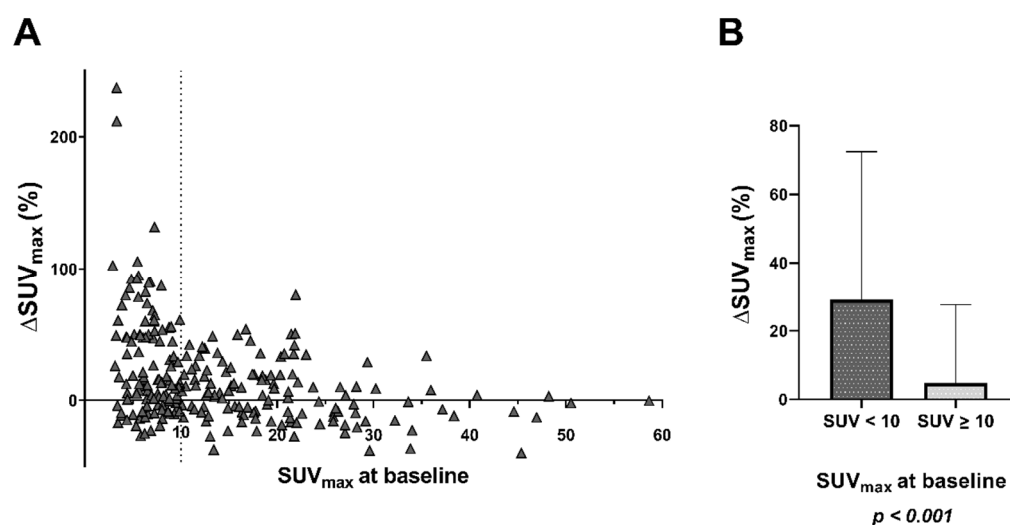


**Figure 4.** Example of a 68-year-old patient with predominantly bone metastases. Increased uptake can be seen in presented MIP (maximum intensity projection) and exemplary transversal slices of [ $^{68}\text{Ga}$ ][Ga-PSMA-11 PET/CT (A) baseline (TLP 3611 mL  $\times$  SUV, TLP-LR 840 mL  $\times$  SUV/SUV, TLP-PR 455 mL  $\times$  SUV/SUV, PSA 408 ng/mL) and (B) after 18 days of enzalutamide medication (TLP 6440 mL  $\times$  SUV (+78.3%), TLP-LR 1464 mL  $\times$  SUV/SUV (+74.3%), TLP-PR 688 mL  $\times$  SUV/SUV (+38.0%), PSA 189 ng/mL (−53.7%)). SUV windowing was set from 0 to 2.5 times  $\text{SUV}_{\text{mean}}$  of the liver.

In lesion-based analysis, 242 bone lesions of 27 patients with bone metastases (maximum of 10 per patient) were determined as target lesions and analyzed: 115 with an  $\text{SUV}_{\text{max}} < 10$  and 127 with an  $\text{SUV}_{\text{max}} \geq 10$ . The mean  $\text{SUV}_{\text{max}}$  of all lesions was  $14.4 \pm 10.8$  at baseline and  $15.5 \pm 10.2$  after enzalutamide medication. This difference was statistically significant ( $p < 0.001$ ). Lesion-related change in  $\text{SUV}_{\text{max}}$  was highly heterogeneous intra- and inter-individually. In total, 120/242 (49.6%) metastases showed a substantial increase of  $>10\%$ . Figure 6 presents the relative changes of  $\text{SUV}_{\text{max}}$  for each lesion, as well as categorized in subgroups with  $\text{SUV}_{\text{max}} < 10$  and  $\text{SUV}_{\text{max}} \geq 10$ , respectively. The relative change was significantly higher in the subgroup with  $\text{SUV} < 10$  ( $30.4 \pm 42.6\%$  vs.  $4.8 \pm 22.8\%$ ,  $p < 0.001$ ). However, in both groups, there were also a quite number of lesions with decreasing uptake.



**Figure 5.** Waterfall plots of individual relative changes in (A) non-normalized total lesion PSMA (TLP), (B) liver-normalized TLP (TLP-LR) and (C) parotid-normalized TLP (TLP-PR) after medication with enzalutamide. Black—enzalutamide-naïve patients. Grey—patients with previous enzalutamide treatment. Values over 100% were cropped for simplification.



**Figure 6.** Relative change of  $\text{SUV}_{\text{max}}$  in 242 bone metastases (maximum of 10 per patient) after enzalutamide medication (A) for each lesion individually, (B) categorized in subgroups with initial  $\text{SUV}_{\text{max}} < 10$  and  $\text{SUV}_{\text{max}} \geq 10$ , respectively.

#### 4. Discussion

In this study, we examined the PSMA upregulation by antiandrogen therapy with enzalutamide. After short-term medication with enzalutamide, whole-body total lesion PSMA (TLP) increased significantly, while PSA serum values did not change significantly. This constellation indicates that PSMA expression was significantly increased by enzalutamide medication and the measured increase in PSMA molecules was not caused by disease progression. The increase in TLP was corroborated by normalized TLP values to healthy PSMA-positive tissue as liver and parotid gland (TLP-LR, TLP-PR). Interestingly, PSMA upregulation was highly heterogeneous intra- and inter-individually, with the clinically meaningful observation that lesions with moderate baseline PSMA expression show higher potential upregulation than lesions with high baseline PSMA expression. Some patients showed a substantial decrease in PSMA expression in target lesion or total tumor burden, which might be attributed to therapeutic response to enzalutamide.

To the best of our knowledge, this is the first study in a larger patient cohort ( $n = 30$ ) showing that antiandrogen therapy with enzalutamide amplifies PSMA expression in mCRPC. The presented data are consistent with our preliminary experience in  $n = 10$  patients [30] and other clinical [23,24] and preclinical studies [25–29] on enzalutamide. Emmett et al. observed in  $n = 7$  mCRPC patients (6 receiving enzalutamide and 1 abiraterone) that PSMA expression increased significantly after a short time and subsequently plateaued [23]. A variability in PSMA expression after enzalutamide medication was also noted by Aggarwal et al., with an increase in almost half of analyzed target lesions in  $n = 8$  patients with prostate cancer (4 castration-resistant and 4 castration-sensitive patients) [24]. In a preclinical setting, Murga et al. and Kranzbühler et al. observed an increased PSMA intensity by enzalutamide in prostate cancer cell lines as LNCaP and C4-2 [25,26]. In analogy, Evans et al. and Lückerrath et al. demonstrated increased PSMA expression in murine prostate cancer xenograft models after treatment with enzalutamide [27,28]. Recently, Staniszevska et al. were able to confirm these results in 22Rv1, C4-2 and LNCaP cells as well as in xenograft bearing mice and communicated this effect also in  $n = 1$  mCRPC patient receiving enzalutamide [29]. Another similar clinical case was reported by Wondergem et al., who noticed increased PSMA uptake in lymph node metastases after initiation of enzalutamide treatment, whereas lymph node volume and PSA decreased over time [34]. Besides enzalutamide, PSMA upregulation was also described for LHRH analogues and other antiandrogen drugs, as abiraterone or dutasteride [25,26,28,35–39]. In prostate cancer cells, abiraterone and dutasteride showed analogous preclinical PSMA upregulation effects to enzalutamide [25,26].

This effect on PSMA expression should be considered when interpreting PSMA imaging after initiation or modification of hormonal treatment to avoid false interpretation [23]. For example, when evaluating response by molecular imaging, the phenomenon of upregulation should be taken into account when biochemical response is stable or regressive, but uptake increases in PSMA PET/CT.

More importantly, this effect on PSMA expression potentially has also important implications on PSMA-RLT. Our results strongly suggest that enzalutamide can be used to upregulate PSMA expression and thus amplify the antitumor mechanism of PSMA-RLT. Interestingly, in our study the effect of upregulation in the subgroup of patients who were previously treated with enzalutamide was comparable to that in enzalutamide-naïve patients. This is in line with our preliminary experience [30] and offers the possibility to use enzalutamide in combination with PSMA-RLT independent of prior enzalutamide treatment. For this reason, enzalutamide may provide a potential enhancing medication for PSMA-RLT, which has also been suggested by other groups [23,25,26]. With regard to the observation that lesions with only moderate baseline PSMA expression (such as  $SUV_{max} < 10$ ) show higher potential upregulation than lesions with high baseline PSMA expression, the approach of applying enzalutamide for aiming at induction of higher PSMA expression may particularly be motivating in patients with low-uptake patterns of mCRPC. Additionally, patients with inadequate response to PSMA-RLT might benefit from co-

medication with enzalutamide. Moreover, the combination of both therapeutics may also provide a synergistic antitumor effect regardless of any PSMA upregulation phenomenon, especially in enzalutamide-naïve patients. Furthermore, it should be examined whether enzalutamide can be used to recruit patients for PSMA-RLT who were previously ineligible due to insufficient PSMA expression [29]. These approaches should be evaluated in larger studies, ideally in prospective clinical trials.

## 5. Limitations

The study has several limitations. Most importantly, the data are based on a retrospective monocenter study with a limited number of patients, which precluded further analyses to systematically determine the required time and dose of enzalutamide. In particular, the varying duration of enzalutamide medication and single follow-up PET/CT setting limits further deductions. In addition, lesion-based analysis was only performed for bone metastases as the majority of patients had dominant osseous tumor burden. Analyses and comparisons of other sites of metastases would also be of interest and should be performed in a larger cohort.

## 6. Conclusions

Our results show that short-term enzalutamide medication significantly increases tumoral PSMA expression in patients with metastatic castration-resistant prostate cancer, irrespective of prior exposure to the drug. The relative PSMA upregulation effect seems to be more pronounced in lesions with only moderate baseline expression of the target. Enzalutamide may offer potential augmentation of PSMA-RLT by enhancing the antitumor mechanism, certainly deserving evaluation as synergistic RLT co-medication in a prospective setting.

**Author Contributions:** Conceptualization, F.R., F.K. and S.E.; methodology, F.R., R.N. and S.E.; formal analysis, F.R., R.N., C.B., M.A.H. and F.K.; investigation, F.R., C.B., M.B., F.K. and S.E.; data curation, R.N. and F.K.; writing—original draft preparation, F.R., R.N., C.B. and F.K.; writing—review and editing, M.B., J.L., M.S. and S.E.; visualization, F.R., R.N., C.B. and M.A.H.; supervision, J.L., M.S., M.B. and S.E. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of Ärztekammer des Saarlandes/Saarbrücken (decision 140/17, approved on 13 July 2017).

**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

**Data Availability Statement:** The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Bray, F.; Ferlay, J.; Soerjomataram, I.; Siegel, R.L.; Torre, L.A.; Jemal, A. Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J. Clin.* **2018**, *68*, 394–424. [[CrossRef](#)] [[PubMed](#)]
2. Kirby, M.; Hirst, C.; Crawford, E.D. Characterising the Castration-Resistant Prostate Cancer Population: A Systematic Review: The Epidemiology of CRPC. *Int. J. Clin. Pract.* **2011**, *65*, 1180–1192. [[CrossRef](#)] [[PubMed](#)]
3. Watson, P.A.; Arora, V.K.; Sawyers, C.L. Emerging Mechanisms of Resistance to Androgen Receptor Inhibitors in Prostate Cancer. *Nat. Rev. Cancer* **2015**, *15*, 701–711. [[CrossRef](#)] [[PubMed](#)]
4. Tannock, I.F.; Horti, J.; Oudard, S.; James, N.D.; Rosenthal, M.A. Docetaxel plus Prednisone or Mitoxantrone plus Prednisone for Advanced Prostate Cancer. *N. Engl. J. Med.* **2004**, *351*, 1502–1512. [[CrossRef](#)]
5. De Bono, J.S.; Oudard, S.; Ozguroglu, M.; Hansen, S.; Machiels, J.-P.; Kocak, I.; Gravis, G.; Bodrogi, I.; Mackenzie, M.J.; Shen, L.; et al. Prednisone plus Cabazitaxel or Mitoxantrone for Metastatic Castration-Resistant Prostate Cancer Progressing after Docetaxel Treatment: A Randomised Open-Label Trial. *Lancet* **2010**, *376*, 1147–1154. [[CrossRef](#)]
6. De Bono, J.S.; Logothetis, C.J.; Molina, A.; Fizazi, K.; North, S.; Chu, L.; Chi, K.N.; Jones, R.J.; Goodman, O.B.; Saad, F.; et al. Abiraterone and Increased Survival in Metastatic Prostate Cancer. *N. Engl. J. Med.* **2011**, *364*, 1995–2005. [[CrossRef](#)]

7. Scher, H.I.; Fizazi, K.; Saad, F.; Taplin, M.-E.; Sternberg, C.N.; Miller, K.; de Wit, R.; Mulders, P.; Chi, K.N.; Shore, N.D.; et al. Increased Survival with Enzalutamide in Prostate Cancer after Chemotherapy. *N. Engl. J. Med.* **2012**, *367*, 1187–1197. [\[CrossRef\]](#)
8. De Bono, J.; Mateo, J.; Fizazi, K.; Saad, F.; Shore, N.; Sandhu, S.; Chi, K.N.; Sartor, O.; Agarwal, N.; Olmos, D.; et al. Olaparib for Metastatic Castration-Resistant Prostate Cancer. *N. Engl. J. Med.* **2020**, *382*, 2091–2102. [\[CrossRef\]](#)
9. Parker, C.; Nilsson, S.; Heinrich, D.; Helle, S.I.; O’Sullivan, J.M.; Fosså, S.D.; Chodacki, A.; Wiechno, P.; Logue, J.; Seke, M.; et al. Alpha Emitter Radium-223 and Survival in Metastatic Prostate Cancer. *N. Engl. J. Med.* **2013**, *369*, 213–223. [\[CrossRef\]](#)
10. Schalken, J.; Fitzpatrick, J.M. Enzalutamide: Targeting the Androgen Signalling Pathway in Metastatic Castration-Resistant Prostate Cancer. *BJU Int.* **2016**, *117*, 215–225. [\[CrossRef\]](#)
11. Rahbar, K.; Ahmadzadehfard, H.; Kratochwil, C.; Haberkorn, U.; Schäfers, M.; Essler, M.; Baum, R.P.; Kulkarni, H.R.; Schmidt, M.; Drzezga, A.; et al. German Multicenter Study Investigating <sup>177</sup>Lu-PSMA-617 Radioligand Therapy in Advanced Prostate Cancer Patients. *J. Nucl. Med.* **2017**, *58*, 85–90. [\[CrossRef\]](#)
12. Barber, T.W.; Singh, A.; Kulkarni, H.R.; Niepsch, K.; Billah, B.; Baum, R.P. Clinical Outcomes of <sup>177</sup>Lu-PSMA Radioligand Therapy in Earlier and Later Phases of Metastatic Castration-Resistant Prostate Cancer Grouped by Previous Taxane Chemotherapy. *J. Nucl. Med.* **2019**, *60*, 955–962. [\[CrossRef\]](#)
13. Khreish, F.; Ghazal, Z.; Marlowe, R.J.; Rosar, F.; Sabet, A.; Maus, S.; Linxweiler, J.; Bartholomä, M.; Ezziddin, S. <sup>177</sup>Lu-PSMA-617 Radioligand Therapy of Metastatic Castration-Resistant Prostate Cancer: Initial 254-Patient Results from a Prospective Registry (REALITY Study). *Eur. J. Nucl. Med. Mol. Imaging* **2022**, *49*, 1075–1085. [\[CrossRef\]](#)
14. Hofman, M.S.; Violet, J.; Hicks, R.J.; Ferdinandus, J.; Thang, S.P.; Akhurst, T.; Iravani, A.; Kong, G.; Ravi Kumar, A.; Murphy, D.G.; et al. [<sup>177</sup>Lu]-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*, 825–833. [\[CrossRef\]](#)
15. Hofman, M.S.; Emmett, L.; Sandhu, S.; Iravani, A.; Joshua, A.M.; Goh, J.C.; Pattison, D.A.; Tan, T.H.; Kirkwood, I.D.; Ng, S.; et al. [<sup>177</sup>Lu]Lu-PSMA-617 versus Cabazitaxel in Patients with Metastatic Castration-Resistant Prostate Cancer (TheraP): A Randomised, Open-Label, Phase 2 Trial. *Lancet* **2021**, *397*, 797–804. [\[CrossRef\]](#)
16. Sartor, O.; de Bono, J.; Chi, K.N.; Fizazi, K.; Herrmann, K.; Rahbar, K.; Tagawa, S.T.; Nordquist, L.T.; Vaishampayan, N.; El-Haddad, G.; et al. Lutetium-177-PSMA-617 for Metastatic Castration-Resistant Prostate Cancer. *N. Engl. J. Med.* **2021**, *385*, 1091–1103. [\[CrossRef\]](#)
17. Ghosh, A.; Heston, W.D.W. Tumor Target Prostate Specific Membrane Antigen (PSMA) and Its Regulation in Prostate Cancer. *J. Cell. Biochem.* **2004**, *91*, 528–539. [\[CrossRef\]](#)
18. Schwarzenboeck, S.M.; Rauscher, I.; Bluemel, C.; Fendler, W.P.; Rowe, S.P.; Pomper, M.G.; Asfhar-Oromieh, A.; Herrmann, K.; Eiber, M. PSMA Ligands for PET Imaging of Prostate Cancer. *J. Nucl. Med.* **2017**, *58*, 1545–1552. [\[CrossRef\]](#)
19. Fendler, W.P.; Rahbar, K.; Herrmann, K.; Kratochwil, C.; Eiber, M. <sup>177</sup>Lu-PSMA Radioligand Therapy for Prostate Cancer. *J. Nucl. Med.* **2017**, *58*, 1196–1200. [\[CrossRef\]](#)
20. Seifert, R.; Seitzer, K.; Herrmann, K.; Kessel, K.; Schäfers, M.; Kleesiek, J.; Weckesser, M.; Boegemann, M.; Rahbar, K. Analysis of PSMA Expression and Outcome in Patients with Advanced Prostate Cancer Receiving <sup>177</sup>Lu-PSMA-617 Radioligand Therapy. *Theranostics* **2020**, *10*, 7812–7820. [\[CrossRef\]](#)
21. Vaz, S.; Hadaschik, B.; Gabriel, M.; Herrmann, K.; Eiber, M.; Costa, D. Influence of Androgen Deprivation Therapy on PSMA Expression and PSMA-Ligand PET Imaging of Prostate Cancer Patients. *Eur. J. Nucl. Med. Mol. Imaging* **2020**, *47*, 9–15. [\[CrossRef\]](#)
22. Bakht, M.K.; Oh, S.W.; Youn, H.; Cheon, G.J.; Kwak, C.; Kang, K.W. Influence of Androgen Deprivation Therapy on the Uptake of PSMA-Targeted Agents: Emerging Opportunities and Challenges. *Nucl. Med. Mol. Imaging* **2017**, *51*, 202–211. [\[CrossRef\]](#)
23. Emmett, L.; Yin, C.; Crumbaker, M.; Hrubby, G.; Kneebone, A.; Epstein, R.; Nguyen, Q.; Hickey, A.; Ihsheish, N.; O’Neill, G.; et al. Rapid Modulation of PSMA Expression by Androgen Deprivation: Serial <sup>68</sup>Ga-PSMA-11 PET in Men with Hormone-Sensitive and Castrate-Resistant Prostate Cancer Commencing Androgen Blockade. *J. Nucl. Med.* **2019**, *60*, 950–954. [\[CrossRef\]](#)
24. Aggarwal, R.; Wei, X.; Kim, W.; Small, E.J.; Ryan, C.J.; Carroll, P.; Cooperberg, M.; Evans, M.J.; Hope, T. Heterogeneous Flare in Prostate-Specific Membrane Antigen Positron Emission Tomography Tracer Uptake with Initiation of Androgen Pathway Blockade in Metastatic Prostate Cancer. *Eur. Urol. Oncol.* **2018**, *1*, 78–82. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Murga, J.D.; Moorji, S.M.; Han, A.Q.; Magargal, W.W.; DiPippo, V.A.; Olson, W.C. Synergistic Co-Targeting of Prostate-Specific Membrane Antigen and Androgen Receptor in Prostate Cancer: Synergy Targets: PSMA and Androgen Receptor. *Prostate* **2015**, *75*, 242–254. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Kranzbühler, B.; Salemi, S.; Umbricht, C.A.; Müller, C.; Burger, I.A.; Sulser, T.; Eberli, D. Pharmacological Upregulation of Prostate-Specific Membrane Antigen (PSMA) Expression in Prostate Cancer Cells. *Prostate* **2018**, *78*, 758–765. [\[CrossRef\]](#) [\[PubMed\]](#)
27. Evans, M.J.; Smith-Jones, P.M.; Wongvipat, J.; Navarro, V.; Kim, S.; Bander, N.H.; Larson, S.M.; Sawyers, C.L. Noninvasive Measurement of Androgen Receptor Signaling with a Positron-Emitting Radiopharmaceutical That Targets Prostate-Specific Membrane Antigen. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 9578–9582. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Lückerrath, K.; Wei, L.; Fendler, W.P.; Evans-Axelsson, S.; Stuparu, A.D.; Slavik, R.; Mona, C.E.; Calais, J.; Rettig, M.; Reiter, R.E.; et al. Preclinical Evaluation of PSMA Expression in Response to Androgen Receptor Blockade for Theranostics in Prostate Cancer. *EJNMMI Res.* **2018**, *8*, 96. [\[CrossRef\]](#)
29. Staniszevska, M.; Fragoso Costa, P.; Eiber, M.; Klose, J.M.; Wosniack, J.; Reis, H.; Szarvas, T.; Hadaschik, B.; Lückerrath, K.; Herrmann, K.; et al. Enzalutamide Enhances PSMA Expression of PSMA-Low Prostate Cancer. *Int. J. Mol. Sci.* **2021**, *22*, 7431. [\[CrossRef\]](#)

30. Rosar, F.; Dewes, S.; Ries, M.; Schaefer, A.; Khreish, F.; Maus, S.; Bohnenberger, H.; Linxweiler, J.; Bartholomä, M.; Ohlmann, C.; et al. New Insights in the Paradigm of Upregulation of Tumoral PSMA Expression by Androgen Receptor Blockade: Enzalutamide Induces PSMA Upregulation in Castration-Resistant Prostate Cancer Even in Patients Having Previously Progressed on Enzalutamide. *Eur. J. Nucl. Med. Mol. Imaging* **2020**, *47*, 687–694. [\[CrossRef\]](#)
31. Fendler, W.P.; Eiber, M.; Beheshti, M.; Bomanji, J.; Ceci, F.; Cho, S.; Giesel, F.; Haberkorn, U.; Hope, T.A.; Kopka, K.; et al. <sup>68</sup>Ga-PSMA PET/CT: Joint EANM and SNMMI Procedure Guideline for Prostate Cancer Imaging: Version 1.0. *Eur. J. Nucl. Med. Mol. Imaging* **2017**, *44*, 1014–1024. [\[CrossRef\]](#)
32. Ferdinandus, J.; Violet, J.; Sandhu, S.; Hicks, R.J.; Ravi Kumar, A.S.; Iravani, A.; Kong, G.; Akhurst, T.; Thang, S.P.; Murphy, D.G.; et al. Prognostic Biomarkers in Men with Metastatic Castration-Resistant Prostate Cancer Receiving [<sup>177</sup>Lu]-PSMA-617. *Eur. J. Nucl. Med. Mol. Imaging* **2020**, *47*, 2322–2327. [\[CrossRef\]](#)
33. Rosar, F.; Wenner, F.; Khreish, F.; Dewes, S.; Wagenpfeil, G.; Hoffmann, M.A.; Schreckenberger, M.; Bartholomä, M.; Ezziddin, S. Early Molecular Imaging Response Assessment Based on Determination of Total Viable Tumor Burden in [<sup>68</sup>Ga]Ga-PSMA-11 PET/CT Independently Predicts Overall Survival in [<sup>177</sup>Lu]Lu-PSMA-617 Radioligand Therapy. *Eur. J. Nucl. Med. Mol. Imaging* **2022**, *49*, 1584–1594. [\[CrossRef\]](#)
34. Wondergem, M.; van der Zant, F.; Broos, W.; Knol, R. Increased PSMA Expression in Castration-Resistant Prostate Cancer Metastases 3 Months After Initiation of Enzalutamide Indicated by <sup>18</sup>F-DCFPyL PET/CT. *Clin. Nucl. Med.* **2019**, *44*, 582–584. [\[CrossRef\]](#)
35. Leitsmann, C.; Thelen, P.; Schmid, M.; Meller, J.; Sahlmann, C.-O.; Meller, B.; Trojan, L.; Strauss, A. Enhancing PSMA-Uptake with Androgen Deprivation Therapy—A New Way to Detect Prostate Cancer Metastases? *Int. Braz. J. Urol. Off. J. Braz. Soc. Urol.* **2019**, *45*, 459–467. [\[CrossRef\]](#)
36. Wright, G.L.; Grob, B.M.; Haley, C.; Grossman, K.; Newhall, K.; Petrylak, D.; Troyer, J.; Konchuba, A.; Schellhammer, P.F.; Moriarty, R. Upregulation of Prostate-Specific Membrane Antigen after Androgen-Deprivation Therapy. *Urology* **1996**, *48*, 326–334. [\[CrossRef\]](#)
37. Meller, B.; Bremmer, F.; Sahlmann, C.O.; Hijazi, S.; Bouter, C.; Trojan, L.; Meller, J.; Thelen, P. Alterations in Androgen Deprivation Enhanced Prostate-Specific Membrane Antigen (PSMA) Expression in Prostate Cancer Cells as a Target for Diagnostics and Therapy. *EJNMMI Res.* **2015**, *5*, 66. [\[CrossRef\]](#)
38. Hope, T.A.; Truillet, C.; Ehman, E.C.; Afshar-Oromieh, A.; Aggarwal, R.; Ryan, C.J.; Carroll, P.R.; Small, E.J.; Evans, M.J. <sup>68</sup>Ga-PSMA-11 PET Imaging of Response to Androgen Receptor Inhibition: First Human Experience. *J. Nucl. Med.* **2017**, *58*, 81–84. [\[CrossRef\]](#)
39. Mathy, C.S.; Mayr, T.; Kürpig, S.; Meisenheimer, M.; Dolscheid-Pommerich, R.C.; Stoffel-Wagner, B.; Kristiansen, G.; Essler, M.; Muders, M.H.; Bundschuh, R.A. Antihormone Treatment Differentially Regulates PSA Secretion, PSMA Expression and <sup>68</sup>Ga-PSMA Uptake in LNCaP Cells. *J. Cancer Res. Clin. Oncol.* **2021**, *147*, 1733–1743. [\[CrossRef\]](#)