An ^{89}Zr -labeled PSMA tracer for PET/CT imaging of prostate cancer patients

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ABSTRACT

The short half-life of existing prostate-specific membrane antigen (PSMA) tracers limits their time for internalization into tumor cells after injection, which is an essential prerequisite for robust detection of tumor lesions with low PSMA expression on PET/CT scans. Due to its longer half-life, the ^{89}Zr -labeled ligand ^{89}Zr -PSMA-Df allows acquisition of PET scans up to 6 days after injection, thereby overcoming the above limitation. We investigated whether ^{89}Zr -PSMA-Df allowed more sensitive detection of weak PSMA-positive prostate cancer lesions.

Methods: We selected 14 prostate cancer patients with biochemical recurrence who exhibited no PSMA-positive lesions on a PET scan acquired with existing PSMA tracers (^{68}Ga -PSMA-11, ^{18}F -JK-PSMA-7). Within 5 weeks after the negative scan, we performed a second PSMA-PET scan using ^{89}Zr -PSMA-Df (117 ± 16 MBq, PET acquisition within 6 days of injection).

Results: ^{89}Zr -PSMA-Df detected 15 PSMA-positive lesions in 8/14 patients, who had a PET-negative reading of their initial PET scans with existing tracers. In these 8 patients, the new scans revealed localized recurrence of disease (3/8), metastases in lymph nodes (3/8), or lesions at distant sites (2/8). Based on these results, patients received lesion-targeted radiotherapies (5/8), androgen deprivation therapies (2/8), or no therapy (1/8). The plausibility of 14/15 lesions was supported by histology, clinical follow-up after radiotherapy or subsequent imaging. Furthermore, comparison of the 15 ^{89}Zr -PSMA-Df-positive lesions with their correlates on the original PET scan revealed that established tracers exhibited mild accumulation in 7/15 lesions but contrast-to-noise ratios (CNR) were too low for robust detection of these lesions (CNR 2.4 ± 3.7 for established tracers vs. 10.2 ± 8.5 for ^{89}Zr -PSMA-Df, $p=0.0014$). The SUV_{max} of the 15 ^{89}Zr -PSMA-Df-positive lesions (11.5 ± 5.8) was significantly higher than the SUV_{max} on the original PET scans (4.7 ± 2.8 , $p=0.0001$). Kidneys were the most exposed organ with doses of 3.3 ± 0.7 mGy/MBq. The effective dose was 0.15 ± 0.04 mSv/MBq.

Conclusion: In patients with weak PSMA expression, a longer period of time might be needed for ligand internalization than that offered by existing PSMA tracers to make lesions visible on PET/CT scans. Hence, ^{89}Zr -PSMA-Df might be of significant benefit to patients in whom the search for weak PSMA-positive lesions is challenging. Radiation exposure should be weighed against the potential benefit of metastasis-directed therapy or salvage radiotherapy, which we initiated in 36% (5/14) of our patients based on their ^{89}Zr -PSMA-Df PET scans.

INTRODUCTION

In a substantial number of prostate cancer patients, prostate-specific antigen (PSA) serum levels rise after surgery or radiotherapy (biochemical recurrence, BCR). Early localization of recurrent tumor lesions is critical for selecting the accurate salvage therapy to improve the survival of these patients. Prostate-specific membrane antigen (PSMA)-PET/CT imaging is widely used for localizing prostate cancer after BCR, and an extensive series of clinical studies has established the increased detection rate of PSMA tracers relative to alternative PET tracers or imaging techniques (1,2). Nevertheless, PSMA PET scans fail to localize tumor lesions in approximately 20% of the patients with BCR (3). In a series of prostatectomies, PSMA staining intensity was reported as absent or weak for 23% and 19% of patients with malignant tissue and Gleason scores of 3+4 or 4+3, respectively (4). Furthermore, negative PSMA-PET scans are significantly associated with weak PSMA levels based on immunohistochemistry (5). This might explain why PSMA PET scans reveal negative results in a substantial number of prostate cancer patients, even at high PSA levels. Patients with detectable but weak PSMA expression in the recurrent tumor lesions pose a challenge for existing PSMA-ligands.

One limitation of existing PSMA tracers is the short half-life of their radioactive labels (^{18}F : 1.8 hrs, ^{68}Ga : 1.1 hrs), which means that PET images must be acquired within 3 hours of injection. Experimental data suggest, however, that internalization of PSMA ligands gradually increases over 24 hours (6). Ligand internalization is an important prerequisite for tracer accumulation in recurrent tumor lesions. Moreover, mildly increased tumor-to-background ratios could be observed with existing tracers when PET/CT scans were acquired later (3 hrs vs. 1 hr) after tracer injection (7,8). Hence, if PET/CT images could be acquired much later, such as days after injection, even prostate cancer lesions with weak PSMA expression might become detectable on PSMA-PET scans.

Given that existing PSMA tracers cannot overcome this limitation due to their short half-lives, we explored the value of a new ^{89}Zr -labeled PSMA tracer (^{89}Zr -PSMA-Df) in prostate patients with BCR. Unlike existing PSMA tracers, the long half-life of the ^{89}Zr label (77 hours) allows image acquisition several days after tracer injection. Furthermore, our *ex-vivo* data on LNCaP tumor xenograft-bearing mice revealed that ^{89}Zr -PSMA-Df exhibited an increased tumor-to-background ratio compared with the widely used PSMA tracers ^{68}Ga -PSMA-11 and ^{18}F -JK-PSMA-7 due to a prolonged period for ligand internalization (6). Moreover, many existing PSMA tracers are excreted through the kidney, and ^{89}Zr -PSMA-Df might improve the detection of tumor lesions in lymph nodes near the ureter after renal clearance. Here, we present the first-in-human application of ^{89}Zr -PSMA-Df for PET imaging in 14 prostate cancer patients after BCR. Using ^{89}Zr -PSMA-Df, we aimed to identify tumor lesions for

metastasis-directed therapy (MTD) or salvage-radiotherapy (S-RT) in these 14 patients, who had negative PET scans using existing PSMA tracers (^{68}Ga -PSMA-11, ^{18}F -JK-PSMA-7). We also compared ^{89}Zr -PSMA-Df-positive lesions with their correlates in the initial, negative PET scans and examined the clinical plausibility of the ^{89}Zr -PSMA-Df-positive lesions.

MATERIALS AND METHODS

Patient characteristics

Patients with biochemically relapsed prostate cancer underwent PET/CT imaging with the widely used tracers ^{68}Ga -PSMA-11 or ^{18}F -JK-PSMA-7 as part of clinical routine diagnostics. The first PET scan was read as entirely PSMA-negative (13/14) or exhibited PSMA-positive findings exclusively in or near the urinary tract, which were interpreted as residual activity in the urine (1/14). When the irradiation of an empirical field within the prostate fossa was no longer an option, since either salvage radiotherapy had already been performed after prostatectomy (11/14), or salvage radiotherapy after prostatectomy had been refused by the patient before imaging (2/14), or radiotherapy had already been performed as the first-line therapy (1/14), we offered a second PET scan with ^{89}Zr -PSMA-Df. Between December 2019 and July 2020, 14 patients were interested in a second PET scan because these patients were in good condition and expressed a clear preference for a MTD over an ADT. The 14 patients were selected from an overall group of 633 patients, who received PSMA PET/CT within the 8 months of recruitment.

In these 14 patients (average age of 62.1 ± 8.6 years), we performed a second PET scan using ^{89}Zr -PSMA-Df within 5 weeks of the first scan. Most patients (11/14) had undergone two therapy lines for prostate cancer (i.e., prostatectomy followed by salvage radiotherapy with an empirical field). Two patients had undergone only initial prostatectomy, and one patient had received radiotherapy alone. Table 1 provides more details on patient characteristics. Since the option of MDT or S-RT depend on the exact localization of the tumor and no other PSMA tracers or imaging options with comparable sensitivity are available, we determined that the benefit of the PET imaging outweighed the radiation exposure of an additional PET/CT scan using the ^{89}Zr -labeled ligand. The Institutional Review Board (IRB) approved this study and the use of the data for a retrospective analysis (21-1167). All subjects signed a written informed consent to PET imaging and the use of their data for a retrospective analysis. All procedures were performed in compliance with the regulations of the responsible local authorities (District Administration of Cologne, Germany).

Tracer preparation

^{89}Zr -PSMA-Df was produced following applicable good manufacturing practice (6). The precursor for the ^{89}Zr -based PSMA-vector, EuK-2Nal-AMCHA-N-sucDf-Fe (E: glutamic acid, u: urea, K: lysine, 2Nal: 2-naphthyl-alanine, AMCHA: traxamic acid) (ABX, Radeberg, Germany), was formed by the pharmacophore EuK coupled to a naphthyl linker, and the chelator agent N-sucDf-Fe. The N-sucDf-Fe moiety functionalized the molecule for labeling with ^{89}Zr . It proved to be a suitable chelator for ^{89}Zr . Labeling of the precursor Fe-N-PSMA-Df with ^{89}Zr required a multistep procedure due to the presence of Fe(III), which was removed by transchelation to EDTA (100:1) at 35 °C for 30 min, forming [Fe(III)EDTA]. The purification of the Fe(III)-free compound from by-products such as EDTA and [Fe(III)EDTA] was performed using a Sep-Pak[™] C₁₈ plus light cartridge (130 mg sorbent per cartridge, 55-105 µm particle size) (Waters Corporation, Massachusetts, USA) and PD MidiTrap[™] G-10 column (>700 Mr, 5.3 ml bed package of Sephadex[™] G-10) (GE Healthcare, Illinois, USA). After elution of PSMA-Df, the radiolabeling procedure was performed by adjusting the pH of a solution of ^{89}Zr in 1 M oxalic acid to 6.8–7.2 with 1 M sodium carbonate, 0.5 M HEPES (pH 6.8) and 0.25 M sodium acetate (5 mg/1 ml gentisic acid, 50 µl). Unbound ^{89}Zr was then efficiently removed by solid phase extraction using a Sep-Pak[™] C₁₈ plus light cartridge.

Specification of ^{89}Zr -PSMA-Df and quality control included assessment of radiochemical purity ($\geq 97\%$), pH-value (5.0-8.0), endotoxin content (≤ 11.7 IE/ml), testing for sterility, and chemical purity (HEPES ≤ 40 µg/ml, ethanol $\leq 10\%$). During this study, none of the 7 syntheses failed to reach these specifications.

Imaging and reading

All PET/CT images were acquired from mid-thigh to the tip of the skull on a Biograph mCT 128 Flow PET/CT scanner (Siemens Healthineers, Erlangen, Germany) and reconstructed using an ultra-high-definition algorithm. For existing PSMA tracers, PET scans were acquired 1 hour (^{68}Ga -PSMA-11, 153 ± 30 MBq, 4 patients) or 2 hours (of ^{18}F -JK-PSMA-7, 286 ± 26 MBq, 10 patients) after injection. For ^{89}Zr -PSMA-Df (117 ± 16 MBq), we acquired PET/CT scans 2 and 3 days after tracer injection (10 patients). For logistical reasons, we slightly deviated from this protocol for 4 patients (images acquired on days 1 and 2, or on days 1 and 3, or on days 2 and 6, or on days 1, 2, and 6, respectively). Acquisition times were adapted to the PSMA ligand with a flow motion bed speed of 1.5 mm/sec for ^{68}Ga -PSMA-11 and ^{18}F -JK-PSMA-7, of 0.9 mm/sec for the first ^{89}Zr -PSMA-Df PET scan, and of 0.6 mm/sec for the subsequent ^{89}Zr -

PSMA-Df PET scans. CT scans (slice thickness of 5.0 mm, pitch 1.2) were acquired using a low-dose technique with kV and mA modulation adapted to the patient's size.

Because we applied lower activities of ^{89}Zr -PSMA-Df than of ^{68}Ga -PSMA-11 or ^{18}F -JK-PSMA-7, we measured the signal-to-noise ratio (SNR) and the contrast-to-noise ratio (CNR) to describe the image quality obtained with ^{89}Zr -PSMA-Df on the Biograph mCT 128 Flow PET/CT scanner (9). The signal-to-noise ratio (SNR) was calculated as the ratio of SUV_{mean} to standard deviation in a VOI with 3 cm diameter in the liver. The contrast-to-noise ratio (CNR) was calculated as the ratio of the difference of the SUV_{mean} in the lesion and the SUV_{mean} in the background to standard deviation in the background. The SUV_{mean} in a lesion was obtained by delineating a VOI at 41% of the SUV_{max} of the same lesion. The SUV_{mean} of the background and its standard deviation were measured in a 3 cm VOI in the local background around the lesion.

For cross-calibration of the ^{89}Zr label, we filled an ^{89}Zr -phantom with 6.283 l of a specific activity of ^{89}Zr (43.287 MBq/l). The activity of ^{89}Zr , measured by the PET/CT scanner, was 35.597 MBq/l, resulting in a dose cross-calibration factor of 1.216. To avoid overestimating the SUV in ^{89}Zr -PSMA-Df-positive tumor lesions with the UHD algorithm (10), we did not multiply ^{89}Zr -PSMA-Df SUV-values by the cross-calibration factor for ^{89}Zr . The cross-calibration factors for ^{68}Ga and ^{18}F were 1.01 and 1.02, respectively.

PET/CT scans were interpreted according to published criteria for standardization of PSMA-PET/CT interpretation (11,12) by a team of two specialists in nuclear medicine and one radiologist. Any disagreement was resolved in consensus. The same team interpreted PET/CT scans acquired with ^{89}Zr -PSMA-Df and existing PSMA tracers (^{68}Ga -PSMA-11, ^{18}F -JK-PSMA-7). Statistical analyses were performed with Microsoft Excel, comparing the SUV values in the areas with a suspicious PSMA accumulation, the signal-to-noise ratios, and the contrast-to-noise ratios.

Dosimetric measurement

Estimation of the kidney dose was determined on the basis of 3 patients who received PET/CT scans with an interval between 2 scans of at least 2 days. The following assumptions were made for the estimation: Between time 0 (injection) and the first measuring point the time-activity-curve has a constant progression. All measuring points were integrated numerically using trapezoidal approximation. From the last measuring point to infinity, a mono-exponential function was fitted and integrated. As the effective half-life could not be accurately determined from two measurement points in 2 of the 3 patients, the physical half-life of ^{89}Zr was used instead.

RESULTS

PSMA-positive lesions with ^{89}Zr -PSMA-Df PET

We acquired ^{89}Zr -PSMA-Df PET/CT scans of 14 patients with BCR, who had been examined with ^{68}Ga -PSMA-11 (n=4) or ^{18}F -JK-PSMA-7 (n=10) less than 5 weeks previously without any PSMA-positive tumor lesions being revealed (Table 1). In 8/14 patients (57%), ^{89}Zr -PSMA-Df identified at least one PSMA-positive lesion (15 additional lesions in total). We detected these lesions in the prostate or prostate fossa of 3 patients, in lymph nodes of 3 patients, and at distant lesions for 2 patients (bone marrow, lung). In addition, we interpreted PSMA-positive lesions according to PSMA-RADS version 1.0 and the miTNM classification (Table 1). To avoid false-positive interpretations due to low signal-to-noise ratios, we applied the following additional criteria: i) We interpreted a lesion as PSMA-positive, if it could be detected on 2 independent scans (14/15 lesions). ii) We interpreted a PSMA-positive lesion as a PSMA-positive lymph node or as a suspicious lung lesion, if we detected a radiological correlate on the parallel low-dose CT scan.

To examine which aspects might have contributed to the detection of these 15 additional lesions, we compared lesions identified by ^{89}Zr -PSMA-Df with the corresponding areas on the initial PET scans (^{68}Ga -PSMA-11, ^{18}F -JK-PSMA-7), using the mediastinal blood pool as a reference. This comparison revealed that 7/15 lesions also exhibited a mild tracer accumulation on the PET scans acquired with ^{68}Ga -PSMA-11 or ^{18}F -JK-PSMA-7 (average ratio of the tumor area to the mediastinal blood pool for both tracers: 3.1 ± 2.4 ; ^{68}Ga -PSMA-11: 2.6 ± 1.8 ; ^{18}F -JK-PSMA-7: 3.5 ± 2.9), but that this signal was not strong enough to allow robust detection of those lesions.

In contrast, PET/CT scans acquired with ^{89}Zr -PSMA-Df exhibited a significantly higher ratio of the 15 lesions to the mediastinal blood pool (14.2 ± 7.7 , $p < 0.0001$, paired t-test), and this difference remained significant when comparing the signal of ^{89}Zr -PSMA-Df against ^{68}Ga -PSMA-11 ($p = 0.0072$, 7 lesions, Fig. 1, 2) and ^{18}F -JK-PSMA-7 ($p = 0.026$, 8 lesions, Fig. 3) separately (Tables 2, 3). When we used the SUV_{max} as an alternative measure, these differences were similarly significant ($p = 0.0002$), and the corresponding numbers were 4.7 ± 2.0 (^{68}Ga -PSMA-11), and 4.7 ± 3.5 (^{18}F -JK-PSMA-7), and 11.1 ± 5.8 (^{89}Zr -PSMA-Df).

Furthermore, like many existing PSMA tracers, ^{68}Ga -PSMA-11 and ^{18}F -JK-PSMA-7 are excreted through the kidney, which interferes with the detection of lesions near the ureter due to residual activity in the urine. The ^{89}Zr -PSMA-Df PET scans were uncompromised by residual activity in the urinary tract, since they were acquired after the tracer was fully cleared from the bloodstream. This might have facilitated the detection of tumor lesions near the ureter (Supplemental Fig. 1).

Image quality

Signal-to-noise ratios (SNR) of ^{89}Zr -PSMA-Df were 2.1 ± 0.5 and 2.1 ± 0.4 in the first and second PET scans, respectively. These ratios were significantly lower than those of established tracers (SNR of ^{68}Ga -PSMA-11: 3.7 ± 0.9 , $p=0.0034$; SNR of ^{18}F -JK-PSMA-7: 7.7 ± 1.3 , $p<0.0001$, paired t-test) (Table 4). However, ^{89}Zr -PSMA-Df PET/CT exhibited significantly higher contrast-to-noise ratios (CNR) in PSMA-positive lesions (10.2 ± 8.5 and 11.0 ± 10.1 in scans 1 and 2), compared with ^{68}Ga -PSMA-11 (4.5 ± 4.6 , $p=0.0016$), ^{18}F -JK-PSMA-7 (0.7 ± 1.0 , $p=0.036$), or ^{68}Ga - and ^{18}F -PSMA tracers in combination (2.4 ± 3.7 , $p=0.0014$, paired t-test) (Table 5, Supplemental Table 1). This suggests that the detection of weak PSMA-avid lesions was facilitated by significantly higher CNR of ^{89}Zr -PSMA-Df.

Timing between tracer injection and ^{89}Zr -PSMA-Df PET scans

Finally, we investigated whether the time between injection and image acquisition had a substantial impact on the sensitivity of ^{89}Zr -PSMA-Df. For all tumor patients, we acquired PET/CT scans with ^{89}Zr -PSMA-Df on 2 or more days after tracer injection (2 whole body scans for 13 patients, 3 whole body scans for 1 patient). Most lesions (14/15) were visible on all consecutive scans. Only one lesion became visible on day 6 only (SUV_{max} 8.1) and could not be detected previously (day 1: SUV_{max} 3.2; day 2: SUV_{max} 2.5). Signal-to-noise ratio (SNR) did not differ significantly between first (2.1 ± 0.5) and second ^{89}Zr -PSMA-Df PET scans (2.1 ± 0.4) ($p=0.79$, paired t-test). Contrast-to-noise ratios (CNR) of the 15 PSMA-positive lesions did not differ significantly between scans 1 and 2 either (10.2 ± 8.5 vs. 11.0 ± 10.1 , $p=0.69$, paired t-test). Similar results were obtained when using the SUV_{max} instead (11.5 ± 5.8 vs. 9.9 ± 5.1 , $p=0.27$) (Tables 2, 5). This suggests that the exact time of acquisition has no more than a minor impact on detection of PSMA lesions, as long as PET/CT images are acquired at least 2 days after tracer injection when ligand internalization has reached a steady state.

Verification and therapeutic consequences

We verified ^{89}Zr -PSMA-Df-positive lesions in 5/8 patients by histology (1 patient) and clinical follow-up (4 patients, drop in PSA levels after metastasis-directed radiotherapy). Based on the results of the ^{89}Zr -PSMA-Df PET/CT scan, these 5 patients received MDT or S-RT (3 patients: prostate fossa, 1 patient: PSMA-positive lymph-nodes, 1 patient: solitary bone marrow metastasis). Another 2 patients with ^{89}Zr -PSMA-Df-positive PET scans received androgen deprivation therapy since they were not eligible for MDT. One patient with a PSMA-positive coin lesion in the lung exhibited stable disease in a follow-up

CT after 7 months and PSA levels remained stable for 11 months, so that the urologists pursued watchful waiting for this patient. Further data on clinical follow-up are presented in Table 1.

Dosimetric measurement

The ^{89}Zr -PSMA-Df PET scans exhibited the highest tracer activity in the kidneys, the organ with the highest radiation exposure. We calculated the kidney dose to be 3.3 ± 0.73 mGy/MBq. The overall effective dose (ICRP 60) was 0.15 ± 0.04 mSv/MBq.

Adverse events

All patients tolerated the tracer injection and the PET/CT examination well. We asked each patient whether they had experienced any adverse side effects when the PET-results were communicated with the patient in-person and again when the therapeutic consequences were discussed on the phone. None of the patients reported nausea, diarrhea, dizziness or any other adverse events or side effects during these conversations.

DISCUSSION

Our study revealed that ^{89}Zr -PSMA-Df has the ability to localize lesions with weak PSMA expression in patients with biochemical recurrence (BCR) when the preceding ^{68}Ga -PSMA-11 or ^{18}F -JK-PSMA-7 PET scans have been read as PSMA-negative. In our small group, localization was successful in more than half the prostate cancer patients (8/14). This observation is in marked contrast to the results obtained with other PSMA tracers we have examined recently. For example, when comparing ^{68}Ga -PSMA-11 with ^{18}F -DCFPyL, we obtained an ^{18}F -DCFPyL-positive PET scan after a prior ^{68}Ga -PSMA-11-negative scan in only 1/25 patients (3,13). Similarly, ^{18}F -JK-PSMA-7 (1/10 patients) and ^{18}F -PSMA-1007 (0/7 patients) rarely revealed a positive scan after a previous negative PET scan (14,15). Hence, our study demonstrates that ^{89}Zr -PSMA-Df has the ability to identify tumor lesions with detectable but low PSMA expression.

In this study, ^{89}Zr -PSMA PET was offered with the goal of initiating MDT and delaying the start of ADT with the risk of later castration resistance. MDT is described as promising therapeutic approach in men with hormone-sensitive oligometastatic prostate cancer with up to 3 metastases in international guidelines, but its efficacy depends on the exact and sensitive localization of all tumor lesions (16,17). In a phase 2 randomized study, MDT of the PSMA-positive lesions improved the progression-free survival and decreased the risk of new lesions in the PSMA PET at 6 months (18). Based on the ^{89}Zr -PSMA-Df PET

scans, an MDT or S-RT could be initiated in 5/14 patients. However, this clinical benefit required an ^{89}Zr -PSMA-Df PET/CT scan, resulting in a radiation exposure of 3.3 ± 0.73 mGy/MBq for the kidneys and an overall effective dose of 0.15 ± 0.04 mSv/MBq. Our dosimetry estimates suggest that the effective dose of ^{89}Zr -PSMA-Df is lower than that of an ^{89}Zr -labeled antibody in metastatic castration-resistant prostate cancer patients (0.44 mSv/MBq), but the renal dose of ^{89}Zr -PSMA-Df is higher than that of an ^{89}Zr -labeled antibody (0.73 mSv/MBq) (19). Dosimetry estimates in larger patient cohorts will be required to establish ^{89}Zr -PSMA-Df in routine clinical diagnostics.

Multiple orthogonal observations suggest that the prolonged acquisition time after tracer injection led to increased accumulation of ^{89}Zr -PSMA-Df in prostate cancer lesions. First, comparison of matched PET scans revealed that ^{68}Ga -PSMA-11 and ^{18}F -JK-PSMA-7 also accumulated in the lesions identified by ^{89}Zr -PSMA-Df, suggesting that the three ligands consistently bound to the surface of these tumor cells. However, prolonged time was required for sufficient ligand internalization, so that these lesions could only be identified on ^{89}Zr -PSMA-Df PET/CT scans. Second, experimental studies suggest that internalization of PSMA ligands increases over time, thereby gradually enhancing the signal-to-background ratio (6). Third, recent clinical studies have indicated that the performance of existing tracers can be marginally improved by acquiring PET images with ^{68}Ga -PSMA-11 or ^{18}F -JK-PSMA-7 at later time points (7,8). Fourth, many tracers are excreted through the kidney, and acquisition at later time points facilitates detection of lesions near the ureter due to low residual activity in the urine. As such, ^{89}Zr -PSMA-Df might be particularly suitable for detecting tumor lesions with low PSMA expression.

Our study further suggests that images with ^{89}Zr -PSMA-Df can be acquired anytime 48-72 hours after tracer injection. The exact acquisition time point within this period has only a marginal impact on tracer sensitivity (half-life of ^{89}Zr -PSMA-Df is 77 hours). But, our logistics for tracer injection and PET scans on different days were designed for a few patients with a rare constellation of inclusion criteria. Very late time points (up to 6 days after tracer injection) might occasionally identify additional lesions, but should be combined with earlier image acquisition time points, since PET/CT scans after 6 days exhibit a decrease in the signal-to-noise ratio. In future, the technology of a digital PET/CT will allow lower activities to be applied than those used here with ^{89}Zr -PSMA-Df (20).

Hence, our data provide a solid rationale to further evaluate the performance of ^{89}Zr -PSMA-Df in prospective clinical trials and overcome potential limitations of our first-in-human study. Since our study was not designed as a prospective clinical trial, readers were not blinded regarding the PSMA-PET tracers. Furthermore, the sensitivity of ^{89}Zr -PSMA-Df cannot be compared in an unbiased manner, since ^{89}Zr -PSMA-Df PET scans were performed only in patients with a negative, prior PET scan using ^{68}Ga -

PSMA-11 or ^{18}F -JK-PSMA-7. It might have increased the sensitivity of the ^{89}Zr -PSMA-Df PET that readers could revisit their initial interpretation of the ^{68}Ga -PSMA-11 PET or the ^{18}F -JK-PSMA-7 PET scans based on the ^{89}Zr -PSMA-Df PET scan in ambiguous cases.

CONCLUSION

^{89}Zr -PSMA-Df allows more time for ligand internalization and renal clearance before image acquisition, whereas existing PSMA tracers require image acquisition within a few hours of injection. Our findings suggest that ^{89}Zr -PSMA-Df might be used in men with BCR after a PET/CT scan with established PSMA tracers was read as negative. In particular, acquiring ^{89}Zr -PSMA-Df PET/CT scans 2 or 3 days after tracer injection might be beneficial in patients with detectable but low PSMA expression in the recurrent tumor lesions, in which the search for PSMA-positive lesions has proven challenging.

DISCLOSURE

B.N. and A.D. have applied for a patent on ^{18}F -JK-PSMA-7. No other potential conflicts of interest relevant to this article exist.

KEY POINTS:

QUESTIONS: Does ^{89}Zr -PSMA-Df exhibit a higher detection rate for subtle tumor lesions compared with existing PSMA tracers?

PERTINENT FINDINGS: ^{89}Zr -PSMA-Df detected PSMA-positive lesions in 8/14 prostate cancer patients with a negative PET scan acquired previously with existing PSMA-PET tracers. The majority of the PSMA-positive patients had oligometastatic status or a local relapse.

IMPLICATIONS FOR PATIENT CARE: Based on the ^{89}Zr -PSMA-Df PET scan, metastasis-directed radiotherapy was initiated in 5/8 patients. ^{89}Zr -PSMA-Df may therefore offer a benefit to patients with weak PSMA positivity, in whom the localization of recurrent tumor lesions has proved challenging using existing PSMA tracers. Our data suggest that ^{89}Zr -PSMA-Df might be used in combination with established PSMA tracers, but larger clinical cohorts will be required to characterize and confirm the clinical benefits of ^{89}Zr -PSMA-Df.

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Table 1

Patient No.	Indication	Gleason score, TNM	PSA value, dt PSA, iPSA	PSMA tracer used in PET-1	Number of lesions interpreted as PSMA-positive with ⁸⁹ Zr-PSMA-Df in PET-2	Therapeutic consequence	Verification
1	BCR after RT	n.a. n.a.	3.2 > 12 mon. 4.21	¹⁸ F-JK-PSMA-7	Prostate lobe right PSMA-RADS 4 Prostate lobe left PSMA-RADS 4 miT2mN0M0	Biopsy, S-RT at a PSA level of 4.2	Histologically confirmed on both sides. Decrease in PSA level to 1.5 after S-RT
2	BCR after RPE and S-RT	4+3 pT2 cN0	0.88 > 12 mon. 4.53	⁶⁸ Ga-PSMA-11	Coin lesion in the lung, dignity unclear PSMA-RADS 3C miT0N0M1c	Watchful waiting with low-dose CT of the lung	Stable in the CT of the lung after 7 mon., PSA stable after 11 mon., suggestive of vascular malformation
3	BCR after RPE and S-RT	3+4 pT3a pN0	7.2 4 mon. 12	¹⁸ F-JK-PSMA-7	3 l.n. retroperitoneal PSMA-RADS 3A l.n. supraclav. left PSMA-RADS 3A miT0N1bM0	ADT after rapid increase of PSA to 14.6	Increase in PSA level before ADT
4	BCR after RT and S-RPE	4+5 Tx pN1	0.46 3 mon. n.a.	¹⁸ F-JK-PSMA-7	none PSMA-RADS 0 miT0N0M0	Watchful waiting with PSMA PET/CT	PSMA-neg. bone metastases detected after 9 mon.
5	BCR after RPE. ADT until 12 mon. before	5+5 pT4 pN0	0.31 n.a. 444	¹⁸ F-JK-PSMA-7	none PSMA-RADS 0 miT0N0M0	Start with ADT at a PSA level of 1.74. S-RT of the prostate fossa	Decrease in PSA level to 0.03 after S-RT and ADT
6	BCR after RPE and S-RT	4+4 pT2a pN0	0.72 7 mon 13.15	⁶⁸ Ga-PSMA-11	l.n. iliac right PSMA-RADS 3A Os ilium left PSMA-RADS 3B miT0N1aM1b	RT of the solitary bone metastasis at a PSA level of > 1, RT of the l.n. iliac right 9 mon. later	Decrease in PSA level to 0.71 after RT; ¹⁸ F-JK-PSMA PET/CT after 9 mon.: progress of the l.n. iliac right
7	BCR after RPE and S-RT	3+4 pT3b pN0	0.62 3 mon. 7.1	¹⁸ F-JK-PSMA-7	none PSMA-RADS 0 miT0N0M0	Watchful waiting with PSMA PET/CT	PET/CT after 6 mon. also PSMA-neg. at a PSA level of 1.3
8	BCR after RPE and S-RT	3+4 pT2 pN1	4.4 5 mon. 24	⁶⁸ Ga-PSMA-11	2 l.n. retroperitoneal PSMA-RADS 3A l.n. supraclav. left PSMA-RADS 3A miT0N1bM0	All 3 PSMA-pos. l.n. treated with S-RT; start of temporary ADT	Decrease in PSA level to 0.17 ng/ml
9	BCR after RPE and S-RT	4+3 pT3 pN0	1.1 > 12 mon. 14	¹⁸ F-JK-PSMA-7	none PSMA-RADS 0 miT0N0M0	Watchful waiting	MRI also without finding
10	BCR after RPE. S-RT refused before PET	4+4 pT3a pN0	0.54 10 mon. 7.7	¹⁸ F-JK-PSMA-7	Prostate fossa right PSMA-RADS 4 miT2uN0M0	S-RT	Decrease in PSA level to 0.3 after S-RT
11	BCR after RPE and S-RT. ADT until 2 y before	4+5 pT3b pN0 R1	0.81 n.a. 184	¹⁸ F-JK-PSMA-7	none PSMA-RADS 0 miT0N0M0	Start with ADT at a PSA level of 1.71	MRI also without finding
12	BCR after RPE and S-RT	3+4 n.a.	1.78 4 mon. 6.2	¹⁸ F-JK-PSMA-7	none PSMA-RADS 0 miT0N0M0	Start with ADT for 1 y	MRI also without finding
13	BCR after RPE and S-RT	4+3 pT3b pN0	2.01 1 mon. 8.5	⁶⁸ Ga-PSMA-11	l.n. iliac right PSMA-RADS 3A miT0N1aM0	Start with ADT at a PSA level of 3.8	MRI with enlarged l.n. iliac right
14	BCR after RPE. S-RT not recommended before PET	3+4 pT2c pN0	0.7 7 mon. n.a.	¹⁸ F-JK-PSMA-7	Prostate fossa left PSMA-RADS 4 miT2uN0M0	S-RT	Decrease in PSA level to 0.09 after S-RT (6 weeks after the end of S-RT)

Table 1. Data from 14 patients whose PET-1 scans were interpreted as PSMA-negative. Eight of the 14 patients showed PSMA-positive lesions with ^{89}Zr -PSMA-Df. In 6 patients, the PSMA-negative interpretation from PET-1 was confirmed by the ^{89}Zr -PSMA-Df PET-2. The location of each PSMA-positive lesion detected with ^{89}Zr -PSMA-Df is described for each patient.

Abbreviations: BCR, biochemical recurrence; dtPSA, doubling time of PSA; iPSA, initial PSA; l.n., lymph node; mon., months; n.a., not available; RPE, radical prostatectomy; RT, radiotherapy; S-RT, salvage radiotherapy; SUV, standardized uptake value

Table 2

Patient No.	Lesions interpreted as PSMA-positive with ^{89}Zr -PSMA-Df in PET-2					Corresponding area with ^{18}F -JK-PSMA-7 in PET-1	Corresponding area with ^{68}Ga -PSMA-11 in PET-1
	PET finding	SUV _{max} d 1	SUV _{max} d 2	SUV _{max} d 3	SUV _{max} d 6	SUV _{max} (2 h)	SUV _{max} (1 h)
1	Prostate lobe right Prostate lobe left		13.3 9.5	13.1 7.1		5.4 4.6	
2	Lung left (dignity unclear)		5.7		6.7		4.1
3	l.n. retroperitoneal l.n. retroperitoneal l.n. retroperitoneal l.n. supraclav. left		3.1 17.0 14.2 12.1	6.1 11.0 8.9 6.9		1.4 2.4 1.7 2.2	
6	l.n. iliac right Os ilium left		7.8 14.3	13.2 15.8			4.3 8.2
8	l.n. retroperitoneal l.n. retroperitoneal l.n. supraclav. left	11.5 12.2 3.2	9.6 8.3 2.5		15.6 7.2 8.1		3.1 6.0 2.0
10	Prostate fossa right		14.7	22.8	11.1	10.9	
13	l.n. iliac right		7.6	4.5			5.5
14	Prostate fossa left		25.5	12.8		8.7	

Table 2. Data from 8 patients whose PET-2 scans were interpreted as PSMA-positive with ^{89}Zr -PSMA-Df. When one or several PSMA-positive areas were detected by ^{89}Zr -PSMA-Df PET, the SUV_{max} within the corresponding area of the ^{18}F -JK-PSMA-7 or the ^{68}Ga -PSMA-11 PET was measured. The SUV_{max} of ^{89}Zr -PSMA-Df PET-2 (first measurement) was significantly higher than the SUV_{max} of the PET-1 scan with ^{68}Ga -PSMA-11 or ^{18}F -JK-PSMA-7 in the corresponding areas ($p=0.0001$, paired t-test).

Abbreviations: d, day; h, hour; l.n., lymph node; SUV, standardized uptake value

Table 3

Patient No.	Lesions interpreted as PSMA-positive with ^{89}Zr -PSMA-Df in PET-2					Corresponding area with ^{18}F -JK-PSMA-7 in PET-1	Corresponding area with ^{68}Ga -PSMA-11 in PET-1
	PET finding	Ratio $\text{SUV}_{\text{max}} : \text{MBP}_{\text{mean}}$ d 1	Ratio $\text{SUV}_{\text{max}} : \text{MBP}_{\text{mean}}$ d 2	Ratio $\text{SUV}_{\text{max}} : \text{MBP}_{\text{mean}}$ d 3	Ratio $\text{SUV}_{\text{max}} : \text{MBP}_{\text{mean}}$ d 6	Ratio $\text{SUV}_{\text{max}} : \text{MBP}_{\text{mean}}$ (2 h)	Ratio $\text{SUV}_{\text{max}} : \text{MBP}_{\text{mean}}$ (1 h)
1	Prostate lobe right Prostate lobe left		13.5 9.7	10.1 5.5		4.6 4.0	
2	Lung left (dignity unclear)		7.6		13.3		3.3
3	l.n. retroperitoneal l.n. retroperitoneal l.n. retroperitoneal l.n. supraclav. left		4.8 26.2 21.9 18.6	11.3 20.4 16.5 12.7		0.8 1.3 0.9 1.2	
6	l.n. iliac right Os ilium left		10.5 19.4	19.9 23.9			0.9 1.7
8	l.n. retroperitoneal l.n. retroperitoneal l.n. supraclav. left	8.5 9.1 2.4	9.9 8.6 2.5		21.7 10.0 11.3		1.8 3.5 1.2
10	Prostate fossa right		11.9	17.8	n.a.	6.7	
13	l.n. iliac right		19.6	11.5			6.1
14	Prostate fossa left		28.1	12.0		8.4	

Table 3. Data from 8 patients whose PET-2 scans were interpreted as PSMA-positive with ^{89}Zr -PSMA-Df. When one or several PSMA-positive areas were detected by ^{89}Zr -PSMA-Df PET, the SUV_{max} within the corresponding area of the ^{18}F -JK-PSMA-7 or the ^{68}Ga -PSMA-11 PET was measured. The SUV_{max} within the suspicious area was divided by the SUV_{mean} in the mediastinal blood pool. The ratio SUV_{max} (lesion) : SUV_{mean} (MBP) was a measure of the contrast between the suspicious area and the background. The ratio of the ^{89}Zr -PSMA-Df PET-2 (first measurement) was significantly higher than the ratio of the PET-1 scan with ^{68}Ga -PSMA-11 or ^{18}F -JK-PSMA-7 in the corresponding areas ($p < 0.0001$, paired t-test). In patient ID 10 the PET scan on day 6 was confined to the pelvis and the ratio between lesion and the MBP was not calculated.

Abbreviations: d, day; h, hour; l.n., lymph node; MBP, mediastinal blood pool; n.a., not available; SUV, standardized uptake value

Table 4

Patient No.	⁸⁹ Zr-PSMA-Df PET/CT					¹⁸ F-JK-PSMA-7 PET/CT	⁶⁸ Ga-PSMA-11 PET/CT
	Parameter (liver)	d 1	d 2	d 3	d 6	2 h	1 h
1	SUVmean ± SD SNR		2.3±1.2 1.9	2.1±1.0 2.0		13.1±2.4 5.5	
2	SUVmean ± SD SNR		2.7±1.3 2.1		2.1±1.4 1.5		6.0±1.4 4.5
3	SUVmean ± SD SNR		2.4±1.8 1.4	2.1±1.2 1.8		16.3±2.0 8.1	
4	SUVmean ± SD SNR	2.9±1.0 2.8		1.9±0.8 2.4		8.5±1.1 8.1	
5	SUVmean ± SD SNR		2.6±1.2 2.3	2.6±1.2 2.2		13.4±1.8 7.6	
6	SUVmean ± SD SNR		2.2±1.4 1.6	2.0±0.9 2.2			5.0±1.7 3.0
7	SUVmean ± SD SNR	2.5±1.1 2.3	2.3±1.0 2.3			10.6±1.7 6.4	
8	SUVmean ± SD SNR	3.0±1.1 2.6	2.7±0.9 3.0		2.3±1.4 1.6		4.5±1.0 4.5
9	SUVmean ± SD SNR		2.5±1.2 2.1	2.5±1.2 2.1		10.7±1.4 7.8	
10	SUVmean ± SD SNR		2.7±1.3 2.2	1.9±0.9 2.2		11.9±2.4 5.0	
11	SUVmean ± SD SNR		2.8±1.2 2.4	2.3±0.9 2.6		14.7±1.7 8.5	
12	SUVmean ± SD SNR		2.7±1.3 2.1	2.5±1.0 2.5		14.1±2.2 6.4	
13	SUVmean ± SD SNR		1.5±1.3 1.1	1.5±0.9 1.7			3.5±1.3 2.8
14	SUVmean ± SD SNR		2.9±1.0 2.9	2.4±1.3 1.9		14.6±2.9 5.1	

Table 4. Image quality obtained with ⁸⁹Zr-PSMA-Df, ¹⁸F-JK-PSMA-7, and ⁶⁸Ga-PSMA-11 on the Biograph mCT 128 Flow PET/CT scanner. The signal-to-noise ratio (SNR) was calculated as the ratio of SUV_{mean} to standard deviation in a VOI with 3 cm diameter in the liver. Image quality was significantly higher with ¹⁸F-JK-PSMA-7 (p<0.0001, paired t-test) and ⁶⁸Ga-PSMA-11 (p=0.0034) than with ⁸⁹Zr-PSMA-Df.

Abbreviations: d, day; h, hour; n.a., not available; SD, standard deviation; SNR, signal-to-noise ratio; SUV, standardized uptake value

Table 5

Patient No.	Contrast-to-noise ratio in the lesions interpreted as PSMA-positive with ^{89}Zr -PSMA-Df in PET-2					Corresponding area with ^{18}F -JK-PSMA-7 in PET-1	Corresponding area with ^{68}Ga -PSMA-11 in PET-1
	PET finding	CNR d 1	CNR d 2	CNR d 3	CNR d 6	CNR (2 h)	CNR (1 h)
1	Prostate lobe right Prostate lobe left		3.8 3.5	3.0 1.1		0.9 0.6	
2	Lung left (dignity unclear)		20.3		23.7		12.1
3	l.n. retroperitoneal l.n. retroperitoneal l.n. retroperitoneal l.n. supraclav. left		1.1 8.3 8.6 18.8	3.1 6.6 4.7 11.9		0.1 1.1 -0.2 2.9	
6	l.n. iliac right Os ilium left		11.5 17.7	20.6 37.9			3.0 9.4
8	l.n. retroperitoneal l.n. retroperitoneal l.n. supraclav. left	4.6 3.9 3.0	4.9 6.5 3.1		12.2 5.5 13.0		0.03 1.7 0.7
10	Prostate fossa right		3.5	13.2	8.4	-0.2	
13	l.n. iliac right		12.9	8.0			4.3
14	Prostate fossa left		30.8	16.7		-0.3	

Table 5. Contrast-to-noise ratio (CNR) of 15 lesions in 8 patients whose ^{89}Zr -PSMA-Df PET scans (PET-2) were interpreted as PSMA-positive. When one or several PSMA-positive areas were detected by ^{89}Zr -PSMA-Df PET, the SUV_{mean} within the corresponding area of the ^{18}F -JK-PSMA-7 or the ^{68}Ga -PSMA-11 PET was measured. The contrast-to-noise ratio (CNR) was calculated as the ratio of the difference of the SUV_{mean} in the lesion and the SUV_{mean} in the background to standard deviation in the background. The SUV_{mean} of the background and its standard deviation were measured in a 3 cm VOI in the local background around the lesion. The CNR of ^{89}Zr -PSMA-Df PET-2 (first measurement) was significantly higher than the CNR of the PET-1 scan with ^{68}Ga -PSMA-11 ($p=0.0016$, paired t-test) or ^{18}F -JK-PSMA-7 ($p=0.036$).

Abbreviations: CNR, contrast-to-noise ratio; d, day; h, hour; l.n., lymph node; SUV, standardized uptake value; VOI, volume of interest

Figure 1

FIGURE 1. ^{68}Ga -PSMA-11 PET with MIP (A), PET/low-dose CT fusion image (C) and ^{89}Zr -PSMA-Df PET 3 days p.i. with MIP (B), PET/low-dose CT fusion image (D) of patient No. 6 with BCR after prostatectomy and salvage radiotherapy. The images are highly suggestive of a PSMA-positive osteomedullary metastasis in the os ilium left, clearly visible with ^{89}Zr -PSMA-Df (red arrows in B, D). The PSMA overexpression in the os ilium left with ^{68}Ga -PSMA-11 was faint (yellow arrow in C). The small lesion is retrospectively visible in the MRI scan (blue arrow in E) without any correlate in the low-dose CT. After the PSA level further increased above 1 ng/ml, the bone metastasis was irradiated and PSA levels dropped to 0.7 ng/ml. Nine months later, an ^{18}F -JK-PSMA-7 PET/CT revealed further progression of the tumor in the right iliac lymph node with weak ^{89}Zr -PSMA-Df expression (green arrow in B), and the lymph node was locally irradiated.

Abbreviations: d, day; Gd, Gadolinium; p.i., post injection

Figure 2

FIGURE 2. ^{68}Ga -PSMA-11 PET with MIP (A), PET/low-dose CT fusion images (C) and ^{89}Zr -PSMA-Df PET 6 days p.i. with MIP (B), PET/low-dose CT fusion images (D) of patient No. 8 with BCR after prostatectomy and salvage radiotherapy. The images are highly suggestive of PSMA-positive lymph node metastasis supraclavicular left and retroperitoneal left, visible with ^{89}Zr -PSMA-Df (red arrows in B, D). The lymph nodes were PSMA-negative with ^{68}Ga -PSMA-11 (yellow arrow in C). The patient underwent S-RT and temporary ADT. The PSA level dropped to 0.17 ng/ml.

Abbreviations: ADT, androgen deprivation therapy; d, day; p.i., post injection; S-RT, salvage radiotherapy

Figure 3

FIGURE 3. ^{18}F -JK-PSMA-7 PET with MIP (A), PET/low-dose CT fusion images (D) and ^{89}Zr -PSMA-Df PET 2 and 3 days p.i. with MIP (B, C), PET/low-dose CT fusion images (E) of patient No. 3 with BCR after prostatectomy and salvage radiotherapy. The images are highly suggestive of PSMA-positive lymph node metastasis supraclavicular left and retroperitoneal, visible with ^{89}Zr -PSMA-Df (red arrows in B, C, E). The lymph node supraclavicular left was PSMA-negative with ^{18}F -JK-PSMA-7 and the lymph node retroperitoneal was faint PSMA-positive with ^{18}F -JK-PSMA-7 (yellow arrow in A). Due to a rapid increase in the PSA level, the patient underwent ADT.

Abbreviations: ADT, androgen deprivation therapy; d, day; p.i., post injection

GRAPHICAL ABSTRACT

Supplemental Material

Table S1

Patient No.	Contrast-to-noise ratio (CNR) in the lesions interpreted as PSMA-positive with ⁸⁹ Zr-PSMA-Df in PET-2					Corresponding area with ¹⁸ F-JK-PSMA-7 in PET-1	Corresponding area with ⁶⁸ Ga-PSMA-11 in PET-1
	PET finding	d 1	d 2	d 3	d 6	2 h	1 h
1	Prostate lobe right, CNR		3.78	2.98		0.94	
	SUV _{mean} lesion-SUV _{mean} bckg):SD bckg		(7.25-1.13):1.62	(7.51-2.03):1.84		(3.30-2.24):1.13	
	Prostate lobe left		3.48	1.14		0.59	
	SUV _{mean} lesion-SUV _{mean} bckg):SD bckg		(6.77-1.13):1.62	(4.12-2.03):1.84		(2.91-2.24):1.13	
2	Lung left (dignity unclear), CNR		20.33		23.74		12.13
	SUV _{mean} lesion-SUV _{mean} bckg):SD bckg		(3.81-0.15):0.18		(4.62-0.11):0.19		(2.42-0.48):0.16
3	I.n. retroperitoneal		1.12	3.09		0.09	
	SUV _{mean} lesion-SUV _{mean} bckg):SD bckg		(2.14-0.85):1.15	(4.06-0.66):1.10		(1.16-1.10):0.68	
	I.n. retroperitoneal		8.31	6.56		1.01	
	SUV _{mean} lesion-SUV _{mean} bckg):SD bckg		(10.41-0.85):1.15	(7.88-0.66):1.10		(1.79-1.10):0.68	
	I.n. retroperitoneal		8.63	4.71		-0.16	
	SUV _{mean} lesion-SUV _{mean} bckg):SD bckg		(10.78-0.85):1.15	(5.84-0.66):1.10		(0.99-1.10):0.68	
6	I.n. supraclav. Left		18.78	11.91		2.85	
	SUV _{mean} lesion-SUV _{mean} bckg):SD bckg		(7.01-0.25):0.36	(5.47-0.35):0.43		(1.51-0.57):0.33	
	I.n. iliac right		11.51	20.58			3.02
	SUV _{mean} lesion-SUV _{mean} bckg):SD bckg		(4.64-0.38):0.37	(10.72-0.43):0.5			(2.64-0.80):0.61
8	Os ilium left		17.67	37.90			9.41
	SUV _{mean} lesion-SUV _{mean} bckg):SD bckg		(9.88-0.34):0.54	(11.61-0.24):0.3			(4.87-0.54):0.46
	I.n. retroperitoneal	4.55	4.92		12.15		0.03
	SUV _{mean} lesion-SUV _{mean} bckg):SD bckg	(6.13-1.35):1.05	(6.87-1.26):1.14		(9.52-0.71):0.75		(1.75-1.71):1.16
	I.n. retroperitoneal	3.89	6.46		5.51		1.69
	SUV _{mean} lesion-SUV _{mean} bckg):SD bckg	(5.43-1.35):1.05	(8.63-1.26):1.14		(4.84-0.71):0.75		(3.67-1.71):1.16
	I.n. supraclav. Left	2.98	3.08		12.98		0.65
	SUV _{mean} lesion-SUV _{mean} bckg):SD bckg	(2.49-0.79):0.57	(1.71-0.54):0.38		(6.43-0.46):0.46		(1.78-1.41):0.57

10	Prostate fossa right SUV _{mean} lesion-SUV _{mean} bckg):SD bckg		3.46 (10.50-1.91):2.48	13.22 (15.25-1.1):1.07	8.35 (7.11-0.51):0.79	-0.19 (7.43- 11.56):21.4	
13	I.n. iliac right SUV _{mean} lesion-SUV _{mean} bckg):SD bckg		12.91 (5.89-0.21):0.44	8.03 (3.33-0.20):0.39			4.25 (3.46-0.78):0.63
14	Prostate fossa left SUV _{mean} lesion-SUV _{mean} bckg):SD bckg		30.83 (17.31-0.66):0.54	16.67 (11.35-0.68):0.64		-0.26 (6.53- 11.5):18.96	

Table S1. Contrast-to-noise ratio of 15 lesions in 8 patients whose ⁸⁹Zr-PSMA-Df PET scans (PET-2) were interpreted as PSMA-positive. When one or several PSMA-positive areas were detected by ⁸⁹Zr-PSMA-Df PET, the SUV_{mean} within the corresponding area of the ¹⁸F-JK-PSMA-7 or the ⁶⁸Ga-PSMA-11 PET was measured. The contrast-to-noise ratio (CNR) was calculated as the ratio of the difference of the SUV_{mean} in the lesion and the SUV_{mean} in the background to standard deviation in the background. The SUV_{mean} of the background and its standard deviation were measured in a 3 cm VOI in the local background around the lesion. The CNR of ⁸⁹Zr-PSMA-Df PET-2 (first measurement) was significantly higher than the CNR of the PET-1 scan with ⁶⁸Ga-PSMA-11 (p=0.0016, paired t-test) or ¹⁸F-JK-PSMA-7 (p=0.036).

Abbreviations: CNR, contrast-to-noise ratio; bckg, background; d, day; h, hour; I.n., lymph node; SUV, standardized uptake value; VOI, volume of interest

Figure S1

FIGURE S1. ^{18}F -JK-PSMA-7 PET with MIP (A), attenuation corrected PET scan (C), PET/low-dose CT fusion image (D) and ^{89}Zr -PSMA-Df PET 2 days p.i. with MIP (B), attenuation corrected PET scan (E), PET/low-dose CT fusion image (F) of patient No. 10 with BCR after prostatectomy. Standard field salvage radiotherapy had been refused by the patient before the ^{89}Zr -PSMA-Df PET scan. The PSMA-positive relapse in the right prostate fossa is clearly visible with ^{89}Zr -PSMA-Df without the overlay of the residual activity in the urine (red arrows in B, E, F). Residual activity of ^{89}Zr -PSMA-Df is seen in the rectum. The ^{18}F -JK-PSMA-7 PET was misinterpreted as activity in the ureter, but the relapse is retrospectively also PSMA-positive with ^{18}F -JK-PSMA-7 (yellow arrow in C, D). Based on the findings of the ^{89}Zr -PSMA-Df PET scan, the patient underwent salvage-radiotherapy, and the PSA level dropped from 0.54 ng/ml to 0.3 ng/ml.

Abbreviations: d, day; MIP, maximum intensity projection; p.i.; post injection