Journal of Nuclear Medicine, published on October 10, 2019 as doi:10.2967/jnumed.119.234559

Patients resistant against PSMA-targeting alpha-radiation therapy often harbor mutations in DNA-repair associated genes

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Running title: PSMA-TAT and mutated DNA-repair genes

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ABSTRACT

Prostate-specific membrane antigen (PSMA) targeting alpha-radiation therapy (TAT) is an emerging treatment modality for metastatic castration-resistant prostate cancer. There is a subgroup of patients with poor response despite sufficient expression of PSMA in their tumors. The aim of this work was to characterize PSMA-TAT non-responding lesions by targeted next-generation sequencing (tNGS). **Methods:** Out of 60 patients treated with ^{225}Ac-PSMA-617, we identified 10 patients that presented with a poor response despite sufficient tumor-uptake in PSMA-PET/CT. We were able to perform CT-guided biopsies with histologic validation of the non-responding lesions in seven of these non-responding patients. Specimens were analyzed by tNGS interrogating 37 DNA damage-repair associated genes. **Results:** In the seven tumor samples analyzed, we found a total of 15 whole-gene deletions, deleterious or presumably deleterious mutations affecting *TP53* (n=3); *CHEK2* (n=2), *ATM* (n=2); *BRCA1, BRCA2, PALB2, MSH2, MSH6, NBN, FANCB* and *PMS1* (n=1 each). The average number of deleterious or presumably deleterious mutations was 2.2 (range, 0-6) per patient. In addition, several variants of unknown significance in *ATM*, *BRCA1*, *MSH2*, *SLX4*, *ERCC*- and various *FANC*-genes were detected. **Conclusion:** Patients with resistance to PSMA-TAT despite PSMA-positivity frequently harbor mutations in DNA-damage-repair and checkpoint genes. While the causal role of these alterations in the patient outcome remains to be determined, our findings encourage future studies combining PSMA-TAT and DNA-damage-repair targeting agents such as Poly(ADP-ribose)-Polymerase inhibitors.

KEYWORDS: Ac-225, next-generation-sequencing, tumor genome, PSMA-617, 225Ac

INTRODUCTION

Prostate-specific membrane antigen (PSMA) targeting radio-ligand therapy is an emerging and promising approach to treat metastatic castration-resistant prostate cancer. A phase-2 trial found a serum prostate-specific antigen (PSA) response rate for the beta-particle emitting radiopharmaceutical ^{177}Lu-PSMA-617 of 57% (*1*). High anti-tumor activity was also reported for alpha-particle emitting ^{225}Ac-PSMA-617; a recent study, however relying on different inclusion criteria, achieved PSA-responses in 63% (*2*). Other drugs that have been evaluated in phase-2/3 trials for therapy of prostate cancer during the last decade only achieved PSA response rates of 54% (enzalutamid), 39% (cabazitaxel), 29% (abiraterone) and 10% ($^{223}RaCl_2$) (*3*).

Alpha-radiation is characterized by a high linear energy transfer causing 2,000-7,000 ion pairs per µm in water, i.e. one ionization event every 2 nm. As the diameter of the DNA double-helix is about 2 nm, the transverse of a single alpha particle is enough to induce, often blunt-ended, double-stranded DNA breaks (*4*). Beta-radiation results in fewer than 20 ion pairs per µm and the transverse of a single beta-particle only causes single-stranded DNA breaks, while higher absorbed doses are needed to achieve double-strand breaks, then often with cohesive ends (*4*). Taking into account that it is more challenging for a cell to repair a blunt-ended double-strand break than a sticky-ended or only single-stranded DNA-break, it seems reasonable that fewer patients should be resistant against PSMA-targeted alpha-radiation therapy (TAT) (*5*). However, the reported subgroup of 37% patients with poor response or early resistance against ^{225}Ac-PSMA-617 is still surprisingly large (*2*).

PSMA-PET/CT is routinely used as a stratification tool to select patients with PSMA-positive tumor phenotypes toward PSMA-TAT. However, only a moderate correlation of pre-therapy standard uptake values (SUV), tumor absorbed dose and treatment response was observed (*6,7*). Obviously, in addition to quantitative tumor-targeting, accessory factors are influencing response to PSMA-TAT. Preclinical and early clinical studies reported that particular DNA-damage repair associated gene-mutations (DRMs) can either increase or decrease the radio-sensitivity of prostate cancers (*8-14*) and thus might represent one of these cofactors.

In parallel to the development of PSMA-TAT, the poly ADP ribose polymerase (PARP)-inhibitor olaparib has been evaluated for metastatic castration-resistant prostate

cancer and received FDA “breakthrough designation” for patients with a germline or somatic mutation of *ATM* or *BRCA1/2*. Anti-tumor activity was also observed in patients positive for other DRMs (*15,16*). Therefore, genetically characterization of our patients was warranted by clinical indication.

Given the important role of DNA-damage-repair gene alterations in the response to DNA-damaging agents including PSMA-TAT and the clinical relevance for the use of PARP-inhibitors, the aim of this pilot study was to evaluate the frequency of these defects in patients insufficiently responding to ^{225}Ac-PSMA-617 therapy.

MATERIALS AND METHODS

Patient Characteristics

The patient cohort has been selected from a total of 60 patients treated with ^{225}Ac-PSMA-617, 40 of them have already been described previously (*2*). In a first step patients with increasing PSA levels and progression of radiologically evaluable lesions upon treatment were identified. Retrospective re-evaluation of PSMA-PET in comparison to a second imaging modality identified ten patients where the poor response could be related to a faint uptake of PSMA-ligands in at least some viable tumor lesions (Fig-1), resulting in a collective with homogenously PSMA-positive lesions. Of ten patients with a poor response despite sufficient PSMA-expression, i.e. SUV >10 (equals approx. 2-fold liver and 1.2-fold salivary gland uptake), seven patients with suitable lesions for biopsy (lymph-node or soft-tissue metastases or large osteolytic bone lesion) agreed into tissue sampling, three patients (after being informed about the challenging location of their lesions) refused biopsy. A flow-chart (Fig-1) demonstrates patient selection. The clinical indication to perform these biopsies was to select patients either suitable for treatment with Olaparib (if DRM-positive) or platin-containing chemotherapy (in case of de-differentiation or neuroendocrine trans-differentiation). Detailed patient characteristics are summarized in (Table 1).

All patients provided written informed consent. The retrospective evaluation of data acquired with clinical indication during PSMA-therapy (vote S-321-2012) and genetic analysis (votes S-085/2012 and S-051/2017) were approved by the ethic committee of the University Heidelberg and carried out in accordance with the updated Declaration of Helsinki.

PSMA-PET/CT, Treatment Emission Scans and Imaging Guided Biopsies

All PSMA-PET/CT were performed with a clinical routine protocol on a Biograph mCT Flow scanner (Siemens, Erlangen, Germany) using ^{68}Ga-PSMA-11.

Post-therapy ^{225}Ac-PSMA-617 scans were acquired using the 440-keV γ-coemission of ^{213}Bi (26% emission probability), the 218-keV γ-coemission of ^{221}Fr (12%), and the bremsstrahlung of ^{209}Pb with a scan speed of 10 cm/min on a 2.54-cm-crystal (1-in) γ-camera (Hawkeye; GE Healthcare) equipped with a high-energy collimator.

CT-guided biopsy of one of the most PSMA-avid tumor sites (liver, 2x retrop LN, os illium, skin, axillary LN) was performed using PSMA-PET/CT for localization (Fig 2). Each biopsy was performed according to interventional standard procedure, adequately adapted to patient needs. Mean 5 (range 2-10) core-cut specimens (17G, length 15-22mm) were taken and fixed in buffered formalin. Conventional histopathology validated that viable tumor tissue was successfully sampled in all cases.

Histopathology and PSMA Immunostainings

Following hematoxylin/eosin-staining, representative sections were stained for PSMA by immunohistochemistry. Sections were deparaffinized in xylene and rehydrated in a graded ethanol series. Antigen retrieval was performed with a steam cooker using retrieval buffer (Target Retrieval Solution, Dako, Denmark). A mouse monoclonal antibody against PSMA (clone 3E6, Dako) was used at a 1:100 dilution and incubated overnight at 4°C. Immunodetection was performed using the Histostain-Plus detection kit (Invitrogen, Carlsbad, USA) according to manufacturer's recommendations. Stained sections were scanned using a Nanozoomer 2.0-HT Scansystem (Hamamatsu Photonics, Japan).

Targeted Next-Generation-Sequencing (tNGS)

Two separate targeted next-generation-sequencing panels, the Oncomine BRCA Research Assay (Thermo Fisher Scientific, Waltham, MA, USA) and a proprietary panel (HDRv1) that was developed at our institution; were used for mutation detection in the following 37 genes involved in DNA damage, checkpoint signaling or DNA repair: *BRCA1, BRCA2, as well as ATM, ATR, BARD1, BRIP1, CHEK1, CHEK2, FAM175A, FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, FANCM, ERCC2, ERCC4, ERCC5, MLH1, MSH2, MSH6, PALB2, PMS1, PMS2, RAD50, RAD51C, RAD51D, RECQL4, MRE11A, NBN, SLX4, TP53, XRCC2.*

Library preparation and semiconductor sequencing was performed as described earlier (*17,18*). In short, for both panels amplicon libraries were prepared using two primer pools with 5/10 ng of DNA and the Ion AmpliSeq Library Kit v2.0 (Thermo Fisher Scientific), respectively. Subsequent to PCR amplification, primer end sequences were partially digested using FuPa reagent, and ligated to barcoded sequencing adapters (Ion

Xpress Barcode Adapters, Life Technologies). The final libraries were purified using AMPure XP magnetic beads (Beckman Coulter, Krefeld, Germany) and quantified using the Ion Library Quantitation Kit (Thermo Fisher Scientific) on a StepOne system (Thermo Fisher Scientific). The individual libraries were diluted to a final concentration of 50 pM, combined and processed to library amplification and enrichment on the Ion Chef system together with the Ion 520 & Ion 530 Kit-Chef (both Thermo Fisher Scientific). The processed libraries were then loaded on an Ion 530 Chip, generating a mean coverage of 1,000-3,000 fold per amplicon.

Data analysis was performed using the Ion Torrent Suite Software (version 5.8.0) as described previously (*19*). After base calling, the reads were aligned against the human genome (hg19) using the TMAP algorithm within the Torrent Suite. Variant calling was performed with the variant caller plugin (version 5.8.7-1) applying a frequency > 5% and a minimum coverage of 200 reads. Variant annotation was performed using Annovar (*20*). Annotations included information about nucleotide and amino acid changes of RefSeq annotated genes, COSMIC and dbSNP entries as well as detection of possible splice site mutations. For data interpretation and verification, the aligned reads were visualized using the IGV browser (Broad Institute, Cambridge, MA, USA) (*21*).

RESULTS

Patient Cohort - Potentially DNA-damaging Pretreatments

The patient collective analyzed here presents a selection (n = 7 out of initially 60) of advanced stage patients. All patients were previously treated with standard androgen deprivation therapy (five patients GnRH axis and non-steroidal anti-androgen; one patient anti-androgen and one patient GnRH axis only). 7/7 patients received abiraterone and 5/7 patients also enzalutamide. All patients had previously received docetaxel and 4 of them additionally cabazitaxel. Olaparib was never considered before PSMA-TAT. None of these standard treatment modalities are directly interfering with DNA integrity or repair.

In addition, all patients had a history of external beam radiotherapy: 4/7 on the prostatic bed, 5/7 on the pelvic lymphatic drainage and 5/7 focusing on symptomatic bone lesions. Previous systemic beta-emitter-based radio-ligand therapy was administered in 4/7 patients, 2 of them with non-response directly from the start, 2 had acquired resistance after 3 and 7 months of ^{177}Lu-PSMA617. These previous therapies are inherently pro-mutagenic and could affect tumor mutational burden and they may also induce some selection bias toward radio-resistant tumors even in advance of PSMA-TAT. Such heavily pretreated patients are inherently bearing a high risk for inter-lesion heterogeneity, which was addressed by selective, imaging-guided biopsies dedicated to PSMA-positive but PSMA-TAT resistant lesions (Fig.-3).

Tumor-phenotype Evaluation per PSMA-PET/CT Before and After Therapy

The mean SUV_{max} of five progressive index lesions was 21.0 +/- 10.1 (median 17.8) before therapy and 23.3 +/- 8.5 (median 24.5) after ^{225}Ac-PSMA617 therapy. Thus, the tumor uptake of PSMA-ligands rather increased under PSMA-targeting therapy, indicating that non-response to the radiopharmaceutical was caused by radio-resistance and not by insufficient expression of the target receptor. In two patients new lesions occurred under PSMA-TAT, also these newly occurring lesions were still sufficiently PSMA-positive (SUV_{max} 17 and 37). In patients that presented with mixed-response, i.e. simultaneously remission in some and progression of other lesions, there was no relevant difference in the mean SUVs of responding versus non-responding lesions;

there was even a patient in whom the non-responding lesions showed a trend toward higher SUVs than the responding ones (Fig.-4).

Next-Generation-Sequencing

The 7 patients not responding to PSMA-TAT were further analyzed for possible genetic alterations in genes related to the DNA damage-repair system (table-2). Most abundantly alterations causing a loss of function were found in *TP53* (3/7 patients), *ATM* (2/7), and *CHEK2* (2/7), with one patient having a *TP53 and CHEK2* co-mutation. While both, deleterious mutations as well as whole gene deletions, were seen for *TP53* and *ATM*, both patients with a *CHEK2* alteration harbored a deleterious mutation. Further loss of function mutations or whole gene deletion were detected once in the analyzed sample set for *BRCA1*, *PMS1*, *PALB2* and *FANCB*, *NBN*, *MSH2*, *MSH6* and *BRCA2*, respectively.

In total we found at least one genetic alteration negatively affecting the DNA damage-repair system in 6/7 patients. In one tumor (Patient 3) low-level amplifications of *ATR*, *BRIP1* and *SLX4* were detected additionally.

Treatment Activity versus Number of Mutations

Correlating the genetic alterations with the parameters of the PSMA-TAT therapy, we observed that the two patients who had received the highest ^{225}Ac treatment dose were harboring the lowest number of gene deletions (20 MBq / deletion of *PALB2* and 22 MBq / no gene deletion and only a variant of unknown significance within *MSH2*). In contrast, the patient with the lowest exposure to alpha-radiation presented with two deletions (12 MBq / *ATM* and *TP53* deletion). Multiple gene deletions were found in patients that had received an intermediate activity of ^{225}Ac (14-18 MBq / 3-4 genes deleted (table-2)). Thus, we found no indication for an association that the number of (DNA damage-repair) gene deletions after PSMA-TAT are correlating to the dose of alpha-therapy itself.

DISCUSSION

It is well accepted that PSMA-targeting therapies, regardless whether based on ^{177}Lu or ^{225}Ac and regardless whether based on small-molecules or antibody-drug-conjugates are non-efficient in tumors with poor expression of the target receptor PSMA. However, there are also non-responding lesions despite sufficient PSMA expression. The underlying resistance-mechanisms of these tumors have not yet been identified.

In this work, tumor lesions progressing under ^{225}Ac-PSMA-617 despite intense expression of the target receptor PSMA were genetically characterized by tNGS of 37 DNA damage-repair associated genes to evaluate whether these patients may be candidates for treatment with PARP-inhibitors. We found at least one, often even multiple (average 2.2 per patient) functional relevant deleterious mutations in six of seven patients analyzed (86%).

There was no clinical indication to evaluate patients still sufficiently responding to PSMA-TAT. Without a control-group evaluating mutational burden of responding lesions in advance of therapy as the primary limitation of our study design, the interpretation of our results depends on comparison with literature data. In general, the pre-test probability to find such mutations increases with more advanced tumor stages, the number of previous therapies and with the number of analyzed target-genes (*22*). Our cohort is very similar to the patient characteristics evaluated with a 113-gene tNGS panel in the TOPARP-trial (*16*) or with a 25-gene panel focused on DNA-damage-repair in a ^{223}Ra-study (*23*). These studies reported functional relevant mutations in 16/49 (33%) and 10/28 (38%) of their patients, respectively. So in comparison to these previous reports the frequency of 86% DRMs appears to be enriched in our patients.One simple explanation could be that the treatment with a pro-mutagenic radiopharmaceutical is responsible for this finding. However, this idea is not supported by our observation that the number of genetic alterations did not correlate with the cumulative dose of ^{225}Ac-PSMA-617. Nevertheless, previous beta-radiation with ^{90}Y/^{177}Lu-PSMA-617 in four of our patients presents a mentionable difference in comparison to historical controls and also the pro-mutagenic potential of this treatment line still remains to be determined.

Eventually the focus on highly PSMA target-positive cancers, stratified by PSMA-PET/CT already in advance of PSMA-TAT, could introduce some selection bias. Several

groups found high PSMA-expression either measured by immunohistochemistry (*24-26*) or PSMA-PET (*27*) to correlate with traditional negative prognostic factors such as Gleason score, T-stage, d'Amico-criteria and as an independent predictor of prostate cancer recurrence and progression. A similar correlation between Gleason score, tumor stage and prognosis was found for patients with DRMs (*28,29*). One recent work (relying on immunohistochemistry) found higher PSMA-expression in *BRCA2* and *ATM* deficient than in unselected tumor samples (*30*). Thus, a somehow increased overlap between PSMA-PET positive patients and DRM carriers would be reasonable.

Despite all that potential selection biases, the high frequency of DRMs in radio-resistant patients is still a surprise; one might rather expect that patients with a truncated DNA-damage repair pathway should be exceptional sensitive against radio-pharmaceuticals. However due to the complexity of DNA-damage repair, requiring activation of a cascade of several related factors, basic research is more controversial. *ATM* and *CHEK2* serve as sensors of DNA integrity and are known activators of *TP53*, which serves as a signaling checkpoint to discontinue mitosis until DNA-integrity could be restored. Loss of such DNA-damage "recognition and signaling" genes have often been described to promote radio-resistance (*10-12,31,32*). In contrast, the protein product of the *BRCA2* gene plays a key role in the effector downstream of DNA-repair and deficiency at this point of the cascade commonly translates into increased radio-sensitivity (*12,13,33,34*). In the ^{223}Ra-study three out of ten mutation carriers were harboring a *BRCA2* mutation (also the only mutation reported more than once) and belonged to the best responders toward the following ^{223}Ra-therapy (*23*). Also in other epidemiological studies *BRCA2* was found amongst the most common mutations (*16,22*). In contrast, we found 7 deleterious mutations of *ATM*, *CHEK2* or *TP53* but only a single *BRCA2* loss in our PSMA-TAT resistant patients; thus deficient "damage-recognition and signaling" was over-represented in comparison to "damage-repair" related mutations. This pilot study was not designed to explore causal relationships, however based on our interesting observation we encourage to consider DRMs as a control-variable in future PSMA-trials to evaluate their respective value as prognostic biomarkers.

Release of endogenous cancer-specific antigens from radiation induced necrosis can lead to a generalized tumor immune response to these antigens via cross-talk mechanisms, the so called abscopal effect. Thus, combined radio-immuno-therapy was

already considered beneficial in general (*35*). Recent data suggested that a high tumor mutational burden in prostate cancer might further improve its response to immunotherapies (*36,37*). Encouraging clinical results have also been demonstrated for combination of ^{177}Lu-PSMA-617 with radio-sensitizers (*38*). In addition to their specific anti-tumor activity in *ATM* and *BRCA1/2* mutated patients, PARP-inhibitors also act as unspecific radio-sensitizers (*13,14,39*). Thus, regardless whether the observed co-incidence of DRMs and resistance to PSMA-TAT despite PSMA-positivity reflects tumor biology or other selection biases, it seems reasonable to speculate that the combination of PSMA-TAT with immunotherapy or PARP-inhibitors may act complementary.

CONCLUSION

In summary, we found an unexpected high frequency of DRMs in patients insufficiently responding to PSMA-TAT monotherapy. This observation provides a good rationale to evaluate combination therapies of PSMA-TAT with PARP-inhibitors or immunotherapies. Additional studies to explore the value of particular DRMs that may have potential as prognostic biomarkers in advance of PSMA-TAT are required.

DISCLOSURES

U. Haberkorn and C. Kratochwil have a patent application for PSMA-617.
The other authors declare that they have no conflict of interest.

KEY POINTS

QUESTION: Is resistance to PSMA-targeted alpha-therapy associated with mutations in DNA-damage-repair related genes?
PERTINENT FINDINGS: In comparison to literature, the frequency of DNA-damage recognition and signaling-checkpoint genes appears increased in patients with non-response to radio-ligand therapy despite high uptake in PSMA-PET.
IMPLICATIONS FOR PATIENT CARE: Combination therapies of PSMA-targeting alpha-therapy and PARP-inhibitors or immunotherapies might act complementary. Particular mutations could have potential as prognostic biomarkers in advance of PSMA-therapy.

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FIGURE LEGENDS

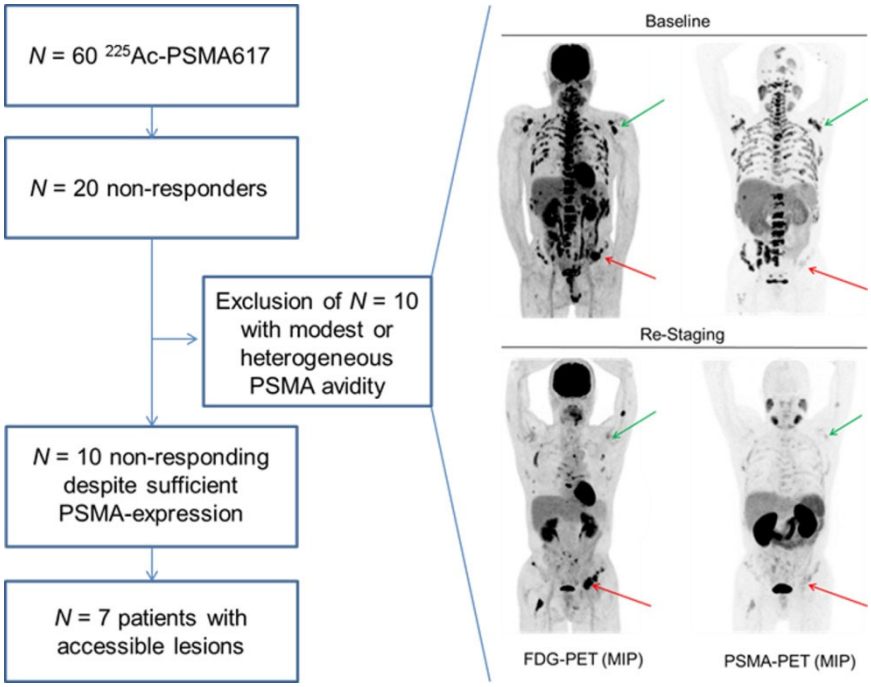

FIGURE 1: Left: Flow-chart of patient selection. Biopsies were only taken from tumor lesions with non-response despite high uptake values in PSMA-PET scans. Right: A patient with several FDG-positive (viable) and visually concordant PSMA-positive lesions (green arrows) at baseline. After 3 cycles of PSMA-targeted therapy residual lesions (FDG-positive) were de-masked to be PSMA-negative (red arrows).

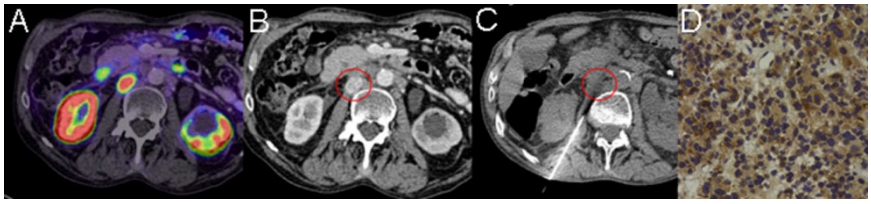

FIGURE 2: In PSMA-PET/CT a residual lymph-node metastasis of SUV 37.7 remained after PSMA-targeted alpha-therapy (A). In contrast-enhanced CT its location was clearly delineated (B). CT-guided biopsy was performed (C) and histopathological validation of the tumor lesion was performed, e.g. per PSMA immunohistochemistry (D).

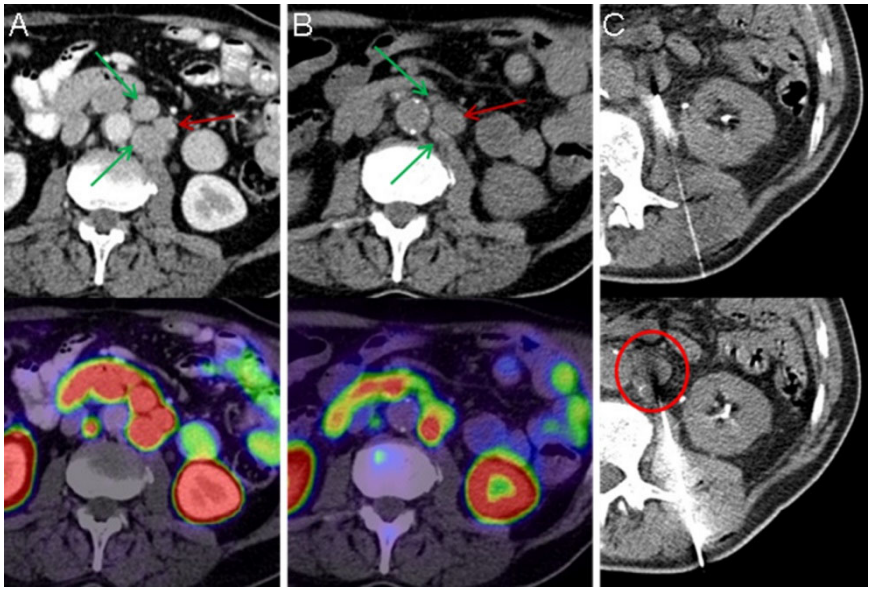

FIGURE 3: Intra-patient tumor-heterogeneity is making tissue-sampling of the most appropriate index-lesion challenging: Baseline PSMA-PET/CT demonstrated a group of 3 lymph-nodes with homogenously, intense uptake of 29.7-35.5 SUV (A, red and green arrows). After ^{225}Ac-PSMA therapy (B) two lesions with SUV 29.7 and 32.0 presented morphological response (green arrows) but the index-lesion with the highest initial uptake (SUV 35.5) presented with increased size (red arrow) and persisting PSMA-uptake (SUV 30.0) and was chosen for imaging-guided biopsy (C).

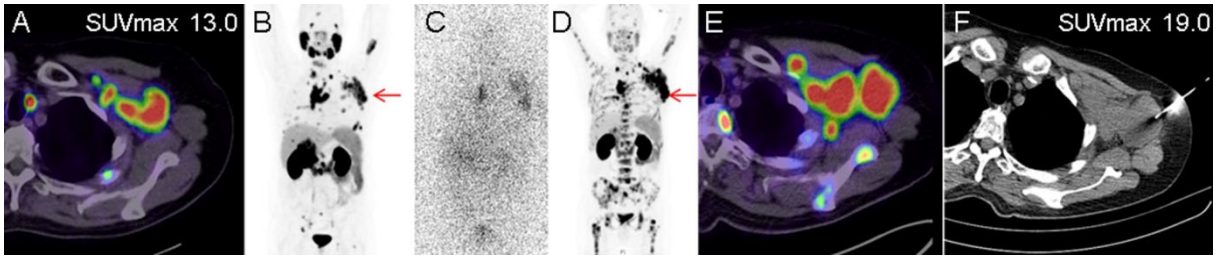

FIGURE 4: Baseline PSMA-PET demonstrated intense uptake of PSMA-ligand in an axillar lymph-node bulk (A, B). Planar emission scan of ^{225}Ac-PSMA validated positive tumor-targeting during therapy (C). Restaging PSMA-PET revealed morphological progression and even increased PSMA-expression of the lesion (D, E). Consecutively, the lesion was chosen for CT-guided biopsy (F).

TABLES

TABLE 1: Patient characteristics

Patient No.	PSA	Location of Metastases	Previous pharmacotherapy	Previous radiotherapy	Previous radioactive drugs
1	227	LN, bone, liver, adrenal	Abi, Doce, Enza	RTxBone	^{177}Lu (27,9 GBq)
2	239	Bone, LN, lung, OTH(skin)	Keto, Estra, Doce, Abi, Trial, Enza	RTxLocal, RTxBone	^{90}Y (7,4 GBq)
3	697	LN, bone, adrenal	Abi, Doce, Trial, Cabazi	RTxPelv, RTxBone	none
4	111	LN, bone	Abi, Enza, Doce	RTxPelv, RTxBone	^{177}Lu (16 GBq)
5	481	liver	Abi, Enza, Doce, Cabazi	RTxLocal, RTxPelv	none
6	759	LN, bone	Abi, Enza, Doce, Cabazi	RTxLocal, RTxPelv, RTxBone	^{177}Lu (44,4 GBq)
7	1658	LN, bone	Abi, Doce, Cabazi, Enstra, Enza	RTxLocal, RTxPelv	none

Prostate-specific antigene (PSA), Lymph-node (LN), Abiraterone (Abi), Docetaxel (Doce), Enzalutamide (Enza), Ketokonazol (Keto), Estramustine (Estra), Cabazitaxel (Cabazi), Radiotherapy (RTx)

TABLE 2: Summery of the observed gene defects and previous exposure to ^{225}Ac-PSMA617

Patient No.	225 Ac dose; kumulativ (fractions)	(Probable) deleterious mutations	Whole-gene deletion	Low-level Amplification	Variants of Unknown Significance
1	12 (6/6)	ATM	TP53-Deletion	-	FAM175A
2	14 (6 /8)	BRCA1, PMS1, 2x TP53	-	-	ATM, BARD1, 3xERCC2, ERCC4, FANCB, FANCG
3	14 (6/8)	CHEK2	FANCB, NBN, ATM	ATR, BRIP1 SLX4	FANCL, RECQL4
4	22 (6/6/6/4)	-	-	-	MSH2
5	20 (8/6/6)	PALB2	-	-	SLX4
6	18 (6/6/6)	TP53, CHEK2	MSH2, MSH6	-	BRCA1
7	18 (8/6/4)	-	BRCA2	-	ERCC2, SLX4, RAD50