Journal of Nuclear Medicine, published on May 1, 2020 as doi:10.2967/jnumed.120.242263

^{177}Lu-EB-PSMA radioligand therapy with escalating doses in patients with metastatic castration-resistant prostate cancer

Jie Zang[1,2*], Qingxing Liu[1,2*], Huimin Sui[1,2], Rongxi Wang[1,2], Orit Jacobson[3], Xinrong Fan[4#], Zhaohui Zhu[1,2#], Xiaoyuan Chen[3#]

[1]Department of Nuclear Medicine, Peking Union Medical College (PUMC) Hospital, Chinese Academy of Medical Science and Peking Union Medical College, Beijing 100730, China

[2]Beijing Key Laboratory of Molecular Targeted Diagnosis and Therapy in Nuclear Medicine, Beijing 100730, China

[3]Laboratory of Molecular Imaging and Nanomedicine (LOMIN), National Institute of Biomedical Imaging and Bioengineering (NIBIB), National Institutes of Health (NIH), Bethesda, Maryland, 20892, USA

[4]Department of Urology, Peking Union Medical College (PUMC) Hospital, Chinese Academy of Medical Science and Peking Union Medical College, Beijing 100730, China

* These authors contributed equally to the article.

First author: Jie Zang, 1 Shuaifuyuan, Dongcheng District, Beijing, China. Telephone: +8615901495106. Email: 15901495106@163.com.

Co-first author: Qingxing Liu, 1 Shuaifuyuan, Dongcheng District, Beijing, China. Telephone: +8617810258849. Email: smu_lqx@163.com.

Corresponding contact: Xinrong Fan, 1 Shuaifuyuan, Dongcheng District, Beijing, China. Telephone: +8613691000350. Email: pumcfxr@126.com; Zhaohui Zhu, 1 Shuaifuyuan, Dongcheng

District, Beijing, China. Telephone: +8613611093752. Email: 13611093752@163.com; Xiaoyuan Chen, 35A Convent Dr, Rm GD937, Bethesda, MD 20892-3957. Email: shawn.chen@nih.gov

Running title: ^{177}Lu-EB-PSMA therapy in mCRPC

ABSTRACT

Purpose: This study is designed to assess the safety and therapeutic response to ^{177}Lu-EB-PSMA treatment with escalating doses in patients with metastatic castration-resistant prostate cancer (mCRPC).

Methods: With institutional review board approval and informed consent, patients were randomly divided into three groups: Group A (n = 10) were treated with 1.18 ± 0.09 GBq/dose of ^{177}Lu-EB-PSMA. Group B (n = 10) were treated with 2.12 ± 0.19 GBq/dose of ^{177}Lu-EB-PSMA. Group C (n = 8) were treated with 3.52 ± 0.58 GBq/dose of ^{177}Lu-EB-PSMA. Eligible patients received up to three cycles of ^{177}Lu-EB-PSMA therapy, at eight-week intervals.

Results: Due to disease progression or bone marrow suppression, 4 out of 10, 5 out of 10, and 5 out of 10 patients completed three cycles therapy as planned in Groups A, B, and C, respectively. The prostate-specific antigen (PSA) response was correlated with treatment dose, with PSA disease control rates in Group B (70%) and C (75%) being higher than that in Group A (10%) (P = 0.007), but no correlation between Group B and Group C was found. ^{68}Ga-PSMA PET/CT showed response in all the treatment groups, however, there was no significant difference between the three groups. Hematologic toxicity study found that platelets in Group B and Group C decreased more than those in Group A, and that Grade 4 thrombocytopenia occurred in 2 (25.0%) patients in Group C. No serious nephritic or hepatic side effects were observed.

Conclusion: This study demonstrates that 2.12 GBq/dose of ^{177}Lu-EB-PSMA seems to be safe and adequate in tumor treatment. Further investigations with increased number of patients are warranted.

Key words: radioligand therapy, ^{177}Lu, Evans blue, prostate-specific membrane antigen (PSMA), metastatic castration-resistant prostate cancer (mCRPC).

INTRODUCTION

Prostate cancer is one of the most common types of cancer worldwide, and the second most frequent cause of cancer deaths for adult men (*1*). Most patients with prostate cancer die of metastatic castration-resistant prostate cancer (mCRPC) (*2, 3*). Radioligand therapy targeting prostate specific membrane antigen (PSMA) which is overexpressed in most prostate cancer and even further increased in metastatic and castration-resistant carcinomas has been demonstrated as an effective and safe therapy in men with mCRPC (*4-15*). The European Association of Nuclear Medicine (EANM) procedure guidelines for radionuclide therapy with ^{177}Lu-labeled PSMA ligands was issued in 2019 (*16*). So far, the two most common radiotherapeutic agents are ^{177}Lu-PSMA-617 and ^{177}Lu-PSMA I&T. They are small molecules, which are cleared relatively quickly from the circulation, so the radiotherapy requires high therapy activity and frequent administrations.

To increase tumor uptake and improve radiotherapeutic efficacy, Evans blue modified PSMA-617 (EB-PSMA-617) that binds to both serum albumin and PSMA was synthesized and conjugated to DOTA chelator and labeled with ^{177}Lu. Preclinical studies showed that EB-PSMA-617 had significantly higher accumulation in PSMA positive tumors and highly effective radiotherapeutic efficacy (*17*). Then, we performed the first-in-human study of ^{177}Lu-EB-PSMA-617 to evaluate its safety, dosimetry and therapeutic response, and found that ^{177}Lu-EB-PSMA-617 had 2.15-5.68-fold higher tumor accumulation than ^{177}Lu-PSMA-617, and all the patients were well tolerated at a low dose (*18*).

This translational study is designed to assess the safety and therapeutic response to ^{177}Lu-EB-PSMA-617 treatment with escalating doses and multiple administrations in patients with mCRPC, to provide guidance about optimal doses in ^{177}Lu-EB-PSMA-617 treatment.

MATERIALS AND METHODS

Patients

This study was registered at clinicaltrials.gov (NCT03780075) and approved by the Institutional Review Board of Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, and all subjects signed written informed consent. A total of 28 patients with histologically confirmed prostate cancer were recruited between April 2018 and June 2019 in this prospective study. The inclusion criteria were progressive mCRPC, increasing blood PSA levels, PSMA expression of distant metastases as determined by ^{68}Ga-PSMA-617 PET/CT within one week before treatment. Patients were not eligible if they had clinically significant impaired bone marrow, liver, or kidney function with a hemoglobin level of less than 9.0 g/dL, a white blood cell count of less than 2.5×10^9/L, a platelet count of less than 75×10^9/L, a serum creatinine (CRE) level of more than 150μmol/L, a total bilirubin level of more than 60μmol/L and a serum albumin level of more than 3.0 g/dL. Patients with Eastern Cooperative Oncology Group (ECOG) performance status score more than 2 were also excluded from the study.

Patients stratified to receive ^{177}Lu-EB-PSMA were randomly divided into three groups. Group A (10 patients, 70 ± 9 years old) were treated with 1.18 ± 0.09 GBq/dose (31.79 ± 2.44 mCi) of ^{177}Lu-EB-PSMA. Group B (10 patients, 70 ± 6 years old) were treated with 2.12± 0.19 GBq/dose (57.39 ± 5.29 mCi) of ^{177}Lu-EB-PSMA. Group C (8 patients, 68 ± 4 years old) were treated with 3.52 ± 0.58 GBq/dose (95.12 ± 15.56 mCi) of ^{177}Lu-EB-PSMA.

Treatment Regimen and Follow-up

Preparation of EB-PSMA-617 and ^{177}Lu labeling were performed as described previously (*18*). Patients received intravenous hydration (2000 mL of 0.9% NaCl) starting at 30 min before ^{177}Lu administration. The radiopharmaceutical diluted in 100 mL of normal saline was co-administered slowly in an intravenous infusion for over 15 – 25 min. To minimize dry mouth syndrome, patients received ice packs over the parotid and submandibular glands at 30 min before administration of the radiopharmaceutical. Eligible patients in each group received up to three cycles of ^{177}Lu-EB-PSMA therapy, at eight weekly intervals.

Blood tests including hematologic status, liver function, and renal function were performed before and every two weeks after each cycle of treatment for a period of 8 weeks. The serum PSA response was documented monthly until 8 weeks after the last treatment cycle. Adverse events were categorized using the Common Toxicity Criteria for Adverse Events 5.0.

Response Assessment

PSA Response. According to the recommendations of the Prostate Cancer Working Group 3 (PCWG3) (*19*), biochemical response was classified as the following: partial response (PR) if PSA decrease ≥50%, progressive disease (PD) if PSA increase ≥ 25% and stable disease (SD) if PSA increase <25% or PSA decrease <50%.

^{68}Ga-PSMA PET/CT Response. All patients underwent ^{68}Ga-PSMA-617 whole-body PET/CT acquisitions 8 weeks after each cycle of treatment. The molecular response was classified according to adapted PERSIST 1.0 criteria (*20*). Complete response (CR) was complete resolution of ^{68}Ga-PSMA-617 uptake in the target lesions. Partial response (PR) was defined as >30% decrease in the SUV_{max} uptake of the target lesions from the baseline scan, and > 30% increase in the SUV_{max} value of the target lesions from the baseline scan was taken as progressive disease (PD). Neither CR, PR nor PD was considered stable disease (SD) that was <30% decrease or <30% increase of the target lesion. Changes of SUV (ΔSUV) between pre- and post-therapeutic PET were calculated.

Data Analysis and Statistics

Calculations were performed using SPSS (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY). P-values <0.05 was accepted as statistically significant. All quantitative data were expressed as the mean ± SD, and the differences among groups were

compared with one-way ANOVA analysis or Student's t-tests. The chi-square test (χ^2) was used to compare treatment response rate.

RESULTS

Patients

Because of disease progression, bone marrow suppression or death, all ten patients in Group A received cycle one of ^{177}Lu-EB-PSMA, and 9 (90%) and 4 (40%) patients received cycles two and three, respectively. All ten patients in Group B received cycle one of ^{177}Lu-EB-PSMA, and 9 (90%) and 5 (50%) patients received cycles two and three, respectively. In addition, all eight patients in Group C received cycle one of ^{177}Lu-EB-PSMA, and 8 (100%) and 5 (63%) patients received cycles two and three, respectively. The patients' basic characteristics and flow chart are listed in supplementary Table S1 and Figure 1, respectively.

Safety Evaluation

Administration of ^{177}Lu-EB-PSMA-617 was well tolerated, with no immediate adverse effects recorded during injection and no treatment-related deaths. One death occurred in Group A due to disease progression and one death occurred in Group B due to pulmonary embolism. No death was observed in Group C at least 2 months after the last cycle of treatment.

In Group A, Grade 1 xerostomia was observed in 3 (30.0%) patients, and Grade 1 nausea was observed in 2 (20.0%) patients. In Group B, 5 (50%) patients experienced Grade 1 xerostomia, and three (30%) patients experienced Grade 1 nausea. In Group C, Grade 1-2 xerostomia occurred in 4 (50.0%) patients, and Grade 1 nausea occurred in 2 (25.0%) patients.

Hematological parameters within 8 weeks after the first cycle of treatment were collected for all patients in each group. The mean counts of white blood cell, hemoglobin and platelet over 8 weeks are shown in Figure.2A. White blood cell showed their nadir at week 6-8 and platelet at week 6 in all groups. No differences of hemoglobin were observed before and after the first cycle of treatment in all groups. For patients who completed 3 cycles, the white blood cell, hemoglobin and platelet counts over the complete 24 weeks period are shown in Figure 2B. Platelet dropped the most, followed by white blood cell. A slight decrease in hemoglobin count was observed. In addition, the decrease of platelet in Group B and Group C was higher than that in Group A. However, the mean counts of platelet were still in the reference range. Details are shown in supplementary Table S2.

All patients who had received at least one cycle of ^{177}Lu-EB-PSMA were included in safety analyses. In Group A, Grade 3 anemia occurred in 4 (40.0%) patients (two patients had Grade 2 anemia at baseline). In Group B, Grade 3 anemia, leucopenia and thrombocytopenia occurred in 2 (20.0%), 1 (10.0%) and 1(10.0%) patients, respectively. In Group C, Grade 3-4 anemia, leucopenia and thrombocytopenia occurred in 3 (37.5%),

1 (12.5%) and 3 (37.5%) patients, respectively (one patient with thalassaemia showed both Grade 4 anemia and thrombocytopenia). Most patients with Grade 3-4 hematologic side effects had prior chemotherapy and diffuse bone metastasis. Details are shown in supplementary Table S3.

No nephrotoxicity or hepatotoxicity (grade 3 or 4) occurred within 2 months period of observation after the last cycle of therapy of all the patients. For the patients who completed 3 cycles, the mean CRE, alanine aminotransferase (ALT) and aspartate transferase (AST) over 24 weeks are shown in Figure 2C.

Treatment Efficacy

Improvement in Clinical Symptoms. Except for two patients who died, ECOG scores of the other patients remained stable during therapy. In Group A, 3 patients (30%) had partial remission of pain, 4 (40%) reported no changes and 3 (30%) had worsening bone pain. Three patients (30%) in Group B had partial remission of pain, 5 (50%) reported no changes and 2 (20%) had worsening bone pain. In Group C, 1 patient (13%) had complete resolution of pain, 1 (13%) reported partial remission, 6 (85%) showed no changes.

PSA Response. After the first treatment cycle, decline in the PSA level was observed in 3 (30.0%), 7 (70.0%) and 5 (62.5%) patients in the three dose groups. A decline in the PSA level of greater than 50% (PR) occurred in 0 (0%), 4 (40.0%), and 2 (25.0%) of patients in Groups A, B and C, respectively. Compared with the baseline PSA value, 2 (22.2%), 6 (66.6%) and 6 (75.0%) patients in the three dose groups showed a PSA decline

2 months after the second cycle, with 2 (22.2%), 5 (55.5%) and 5 (62.5%) showing a PSA decline >50%. At two months after the third treatment cycle, 1 (25.0%), 4 (80.0%) and 4 (80.0%) patients showed a PSA decline, with 1 (25.0%), 2 (40.0%) and 4 (80.0%) showing a PSA decline >50% in three groups, respectively. The waterfall plots in Figure 3 represent the PSA response at 8 weeks after each cycle of treatment.

In total, PSA response before and 8 weeks after the last cycle of treatment in all enrolled patients who had received at least one cycle of therapy were analyzed and we found that 1 (10.0%) patient had PR and 9 (90%) patients had PD response in Group A. In addition, 4 (40.0%), 3 (30%) and 3 (30%) patients showed PR, SD, and PD in Group B, and 5 (62.5%), 1 (12.5%) and 2 (25.0%) patients showed PR, SD, and PD in Group C. The disease control rates (PR+SD) in Groups B and C were higher than that in Group A (Group A *vs.* Group B *vs.* Group C: 10% *vs.* 70% *vs.*75%, $P = 0.007$; Group A *vs.* Group B: 10% *vs.* 70%, $P = 0.02$; Group A *vs.* Group C: 10% *vs.* 75%, $P = 0.012$; Group B *vs.* Group C: 70% *vs.* 75%, $P = 1.00$, chi-square test). Table 1 shows an overview of the response data after each cycle of therapy for three groups according to PSA-level measurements in further detail.

There is a significant correlation between patient response to the first cycle and third cycle compared to baseline PSA ($P = 0.027$). A total of 14 patients accepted three cycles of treatment in all Groups. Among them, 11 patients showed PSA decline after the first cycle, and 9 (81.8%) also showed PSA decline 2 months after the third cycle when

compared to their baseline PSA value. Three patients that did not show any PSA decline after the first cycle also showed no response after the third cycle.

^{68}Ga-PSMA PET/CT response. After the first treatment cycle, according to the adapted PERSIST 1.0 criteria, 6 (60%), 3 (30%) and 1 (10%) patients in Group A showed PR, SD and PD, respectively; 5 (50%), 4 (40%) and 1 (10%) patients had PR, SD and PD in Group B, respectively. The responses of PR, SD, and PD were demonstrated for 4 (50.0%), 2 (25.0%), and 2 (25%) patients in Group C, respectively. When the bone metastasis with comparable baseline SUV_{max} from 10.0–40.0 were selected from the three groups for comparison, there was no significant difference as demonstrated by ΔSUV from ^{68}Ga-PSMA PET/CT before and after the treatment (-36.00 ± 23.35%, n = 36 *vs.* -38.63 ± 42.57%, n = 27 *vs.* -33.83 ± 39.63%, n = 19, P = 0.895).

An objective radiologic response at 8 weeks after the second cycle of treatment was demonstrated for 4 of 9 patients (Group A), 5 of 9 patients (Group B), and 5 of 8 patients (Group C). The response of PR was demonstrated for 3 (33.3%), 3 (33.3%), and 3 (37.5%) patients in three groups, respectively. After the third cycle of therapy, 2 of 4, 5 of 5 and 4 of 5 patients repeated ^{68}Ga-PSMA PET/CT in three groups, respectively. In the end, 1 (25.0%), 3 (60.0%) and 2 (40%) patients showed PR in three groups. There was no major difference in ^{68}Ga-PSMA PET/CT response after each cycle of treatment among the three groups. Details are shown in Table 2. The representative ^{68}Ga-PSMA PET/CT images in three groups after each cycle of therapy was shown in Figure 4.

DISCUSSION

The present study showed that PSA response and hematological toxicity were related with treatment activity. Patients in Group B and Group C had better PSA response than those in Group A. Platelets in Group B and C also decreased more than in Group A, however, in most patients, the absolute counts were still in the reference range.

Recent studies showed that^{177}Lu-PSMA-617 and ^{177}Lu-PSMA I&T radionuclide therapy is a safe and effective approach in the treatment of mCRPC patients. A PSA decrease ≥50% was seen in 25-40% patients from the first cycle of ^{177}Lu-PSMA-617 with the average doses of 5.9-6.1 GBq (*21-23*). This study showed that a decline in the PSA level of greater than 50%after the first cycle occurred in 0%, 40 %, and 25.0% of patients in three groups, respectively. In a recent phase II trial of ^{177}Lu-PSMA-617 radioligand therapy, 57% patients showed a PSA decline more than 50% with all patients in analysis (*24*). In our study, a decline in the PSA level of greater than 50% (PR) occurred in 10%, 40.0%, and 62.5% of patients in the Groups A, B, and C, respectively. The PSA response rate in Groups B and C seems to be similar to that of 5.9-8.7 GBq of ^{177}Lu-PSMA-617 therapy. In addition, the disease control rates (PR+SD) in Groups B (70%) and C (75%) were higher than that in Group A (10%), but there was no significant difference between Group B and Group C, probably due to the limited number of patients.

Yordanova *et al.* found that 43% patients showed PR with ^{68}Ga-PSMA PET/CT response according to adapted PERSIST criteria after 1 cycle of ^{177}Lu-PSMA-617 treatment (*23*). In

our study, 6 (60%), 5 (50%) and 4 (40%) patients showed PR in three groups after the first cycle treatment, respectively. However, there was no difference in response rate among the three groups, again likely due to the limited number of patients. Moreover, when we chose comparable bone metastases with baseline SUV_{max} from 10.0–40.0 from three groups, there was still no significant differences as demonstrated by ΔSUV. The imaging-based response did not correlate well with the PSA response in some patients. This is in accordance with the findings of a study showing that the therapy effects on SUV from ^{68}Ga-PSMA-PET/CT are mostly independent of PSA response (*25*). A preclinical study found that PSMA uptake in the tumor was directly associated with the number of tumor cells and that decreased PSMA uptake after therapy was not due to treatment-induced changes but rather reliably reflects the number of living tumor cells (*26*). In addition, the changes in ^{68}Ga-PSMA PET/CT reflect the changes in PSMA receptor expression, and do not represent changes in the tumor as a whole, because some tumors also express other receptors, such as somatostatin receptors. These findings might also explain the discordance between ^{68}Ga-PSMA PET/CT response and PSA changes in our study.

Hematological toxicity is the most commonly reported adverse side effect related to ^{177}Lu-PSMA therapy, especially for the patients with a heavy burden of bone metastases and borderline marrow function. Due to albumin binding, ^{177}Lu-EB-PSMA-617 also had significantly higher effective dose in red bone marrow than ^{177}Lu-PSMA-617, as also in kidneys and liver. Based on dose limit of 2 Gy to red marrow and the dosimetry result in

our previous study, patients can be injected with as much as 34 GBq of ^{177}Lu-EB-PSMA. In the current study, no serious nephrotoxicity or hepatotoxicity was observed. After the first cycle of treatment, only Grade 3 anemia occurred in 1 patient who had thalassaemia, but was not diagnosed before the treatment in Group C, this rate is in accordance with a German multicenter study that showed 10% Grade 3–4 anemia in patients with ^{177}Lu-PSMA-617 therapy (*7*). A recent phase II trial reported that the most common toxic effects possibly were grade 3neutropenia in 7%, grade 3 anemia in 23%, and grade 3 or 4 thrombocytopenia in 27% patients (*24*). In our study, Grade 3 anemia occurred in 40.0% in Group A, and Grade 3 anemia occurred in 20.0%, leucopenia in 10.0% and thrombocytopenia 10.0% in the Group B. In Group C, Grade 3-4 anemia, leucopenia and thrombocytopenia occurred in 37.5%, 12.5% and 37.5% patients. As the dose escalated, platelets in Groups B and C decreased more than in Group A, but the absolute counts were still in the reference range in most patients. To weigh the advantages and disadvantages, patients in the Group B had better treatment response and acceptable hematological side effects, and ^{177}Lu-EB-PSMA treatment should be carefully preformed with 2.12-GBq doses and monitored more closely, especially for patients with diffuse red marrow infiltration.

^{177}Lu-EB-PSMA allowed the radioactivity to remain in the target, prolonged tumor retention and maximized the therapeutic effect with lower doses. Relatively low dose (2.12 GBq) of ^{177}Lu-EB-PSMA can achieve the similar therapeutic effect as 5.9-8.7 GBq of

^{177}Lu-PSMA-617, improve utilization of ^{177}Lu, while making the delivery to vulnerable normal organs within the acceptable level. At the same time, lower doses of ^{177}Lu can lower the cost and reduce radiation exposure to medical workers and the public environment. This new compound has great potential to be used in patients with mCRPCs.

There were several limitations in our study. Firstly, the most prominent of these is the small number of patients in each group. Secondly, our study lacked long-term observations of side effects and survival analysis, this part will be implemented in our future studies. Thirdly, we did not set ^{177}Lu-PSMA-617 therapy as the control group, so the data in our study could only be compared with the literature. Further studies with more patients subjected to ^{177}Lu-EB-PSMA therapy with ^{177}Lu-PSMA-617 therapy as the control group are warranted, but patients with diffuse bone marrow involvement should be vetted carefully.

CONCLUTION

This study demonstrates that PSA response and hematological toxicity were related with treatment activity. Grade 4 thrombocytopenia observed in 3.52 GBq/dose Group might imply that the potential for further dose escalations is limited, and 2.12 GBq/dose of ^{177}Lu-EB-PSMA with relatively high efficacy and acceptable side effects seems to be the optimal dose from the trade-off.

ACKNOWLEDGMENTS

This work was supported by National Natural Science Foundation of China projects (81871392), Chinese Academy of Medical Science Major Collaborative Innovation Project (2019-I2M-1-001), and Intramural Research Program of the National Institute of Biomedical Imaging and Bioengineering of the National Institutes of Health.

DISCLOSURE

The authors declare no conflict of interest.

KEY POINTS:

Question: Is ^{177}Lu-EB-PSMA therapy with escalating doses and multiple administrations safe and effective in patients with mCRPC and what is the optimal dose?

Pertinent findings: In this clinical study, a total of 28 patients with mCRPC were randomly divided into three dose groups for ^{177}Lu-EB-PSMA treatment. The prostate-specific antigen (PSA) response was correlated with treatment dose, with PSA disease control rates in the 2.12 GBq group (70%) and 3.52 GBq group (75%) being higher than that in the 1.18 GBq group (10%) (P = 0.007).

Implications for patient care: 2.12 GBq/dose of ^{177}Lu-EB-PSMA seems to be the best choice in balancing safety with adequacy in tumor treatment.

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. *CA Cancer J Clin.* 2017,67:7-30.

2. Kirby M, Hirst C, Crawford ED. Characterising the castration-resistant prostate cancer population: a systematic review. *Int J Clin Pract.* 2011;65:1180-1192.

3. Hotte SJ, Saad F. Current management of castrate-resistant prostate cancer. *Curr Oncol.* 2010;17 (Suppl 2):S72-79.

4. Yadav MP, Ballal S, Tripathi M, *et al.* (177)Lu-DKFZ-PSMA-617 therapy in metastatic castration resistant prostate cancer: safety, efficacy, and quality of life assessment. *Eur J Nucl Med Mol Imaging.* 2017;44:81-91.

5. Rahbar K, Schmidt M, Heinzel A, *et al.* Response and tolerability of a single dose of 177Lu-PSMA-617 in patients with metastatic castration-resistant prostate cancer: amulticenter retrospective analysis. *J Nucl Med.* 2016;57:1334-1338.

6. Rahbar K, Bogeman M, Yordanova A, *et al.* Delayed response after repeated (177)Lu-PSMA-617 radioligand therapy in patients with metastatic castration resistant prostate cancer. *Eur J Nucl Med Mol Imaging.* 2018;45:243-246.

7. Rahbar K, Ahmadzadehfar H, Kratochwil C, *et al.* German multicenter study investigating 177Lu-PSMA-617 radioligand therapy in advanced prostate cancer patients. *J Nucl Med.* 2017;58:85-90.

8. Kulkarni HR, Singh A, Schuchardt C, *et al.* PSMA-based radioligand therapy for metastatic castration-resistant prostate cancer: The Bad Berkaexperience since 2013. *J Nucl Med.* 2016;57(Suppl 3):97s-104s.

9. Ahmadzadehfar H, Wegen S, Yordanova A, *et al.* Overall survival and response pattern of castration-resistant metastatic prostate cancer to multiple cycles of radioligand therapy using [(177)Lu]Lu-PSMA-617. *Eur J Nucl Med Mol Imaging.* 2017;44:1448-1454.

10. Baum RP, Kulkarni HR, Schuchardt C, *et al.* 177Lu-Labeled prostate-specific membrane antigen radioligand therapy of metastatic castration-resistant prostate cancer: safety and efficacy. *J Nucl Med*. 2016;57:1006-1013.

11. Heck MM, Tauber R, Schwaiger S, *et al.* Treatment outcome, toxicity, and predictive factors for radioligand therapy with (177)Lu-PSMA-I&T in metastatic castration-resistant prostate cancer. *Eur Urol*. 2019;75:920-926.

12. Ahmadzadehfar H, Rahbar K, Kurpig S, *et al.* Early side effects and first results of radioligand therapy with (177)Lu-DKFZ-617 PSMA of castrate-resistant metastatic prostate cancer: a two-centre study. *EJNMMI Res*. 2015;5:114.

13. Kratochwil C, Giesel FL, Stefanova M, *et al.* PSMA-targeted radionuclide therapy of metastatic castration-resistant prostate cancer with 177Lu-labeled PSMA-617. *J Nucl Med*. 2016;57:1170-1176.

14. Rahbar K, Bode A, Weckesser M, *et al.* Radioligand therapy with 177Lu-PSMA-617 as anovel therapeutic option in patients with metastatic castration resistant prostate cancer. *Clin Nucl Med*. 2016;41:522-528.

15. Ghosh A, Heston WD. Tumor target prostate specific membrane antigen (PSMA) and its regulation in prostate cancer. *J Cell Biochem.* 2004;91:528-539.

16. Kratochwil C, Fendler WP, Eiber M, *et al.* EANM procedure guidelines for radionuclide therapy with (177)Lu-labelled PSMA-ligands ((177)Lu-PSMA-RLT). *Eur J Nucl Med Mol Imaging*. 2019;46:2536-2544.

17. Wang Z, Tian R, Niu G,*et al.* Single low-dose injection of evans blue modified PSMA-617 radioligand therapy eliminates prostate-specific membrane antigen positive tumors. *Bioconjug Chem*. 2018;29:3213-3221.

18. Zang J, Fan X, Wang H, *et al.* First-in-human study of (177)Lu-EB-PSMA-617 in patients with metastatic castration-resistant prostate cancer. *Eur J Nucl Med Mol Imaging.* 2019;46:148-158.

19. Scher HI, Morris MJ, Stadler WM, *et al.* Trial design and objectives for castration-resistant prostate cancer: updated recommendations from the prostate cancer clinical trials working group 3. *J Clin Oncol*. 2016;34:1402-1418.

20. Wahl RL, Jacene H, Kasamon Y, Lodge MA. From RECIST to PERCIST: evolving considerations for PET response criteria in solid tumors. *J Nucl Med*. 2009;50 Suppl 1:122S-150S.

21. Rathke H, Giesel FL, Flechsig P, *et al.* Repeated (177)Lu-labeled PSMA-617 radioligand therapy using treatment activities of up to 9.3 GBq. *J Nucl Med*. 2018;59:459-465.

22. Brauer A, Grubert LS, Roll W, *et al.* (177)Lu-PSMA-617 radioligand therapy and outcome in patients with metastasized castration-resistant prostate cancer. *Eur J Nucl Med Mol Imaging*. 2017;44:1663-1670.

23. Yordanova A, Linden P, Hauser S, *et al.* Outcome and safety of rechallenge [(177)Lu]Lu-PSMA-617 in patients with metastatic prostate cancer. *Eur J Nucl Med Mol Imaging*. 2019;46:1073-1080.

24. Hofman MS, Violet J, Hicks RJ, *et al.* [(177)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.

25. Taeger P, Hammes J, Hohberg M, *et al.* Discrete evaluation of multi-cycle Lu-177-PSMA-617-therapy effects on bone versus lymph node metastases in patients with metastasized castration-resistant prostate cancer (mCRPC). *J Nucl Med*. 2018;59(supplement 1):523.

26. Hillier SM, Kern AM, Maresca KP, *et al.* 123I-MIP-1072, a small-molecule inhibitor of prostate-specific membrane antigen, is effective at monitoring tumor response to taxane therapy. *J Nucl Med*.2011;52:1089-1093.

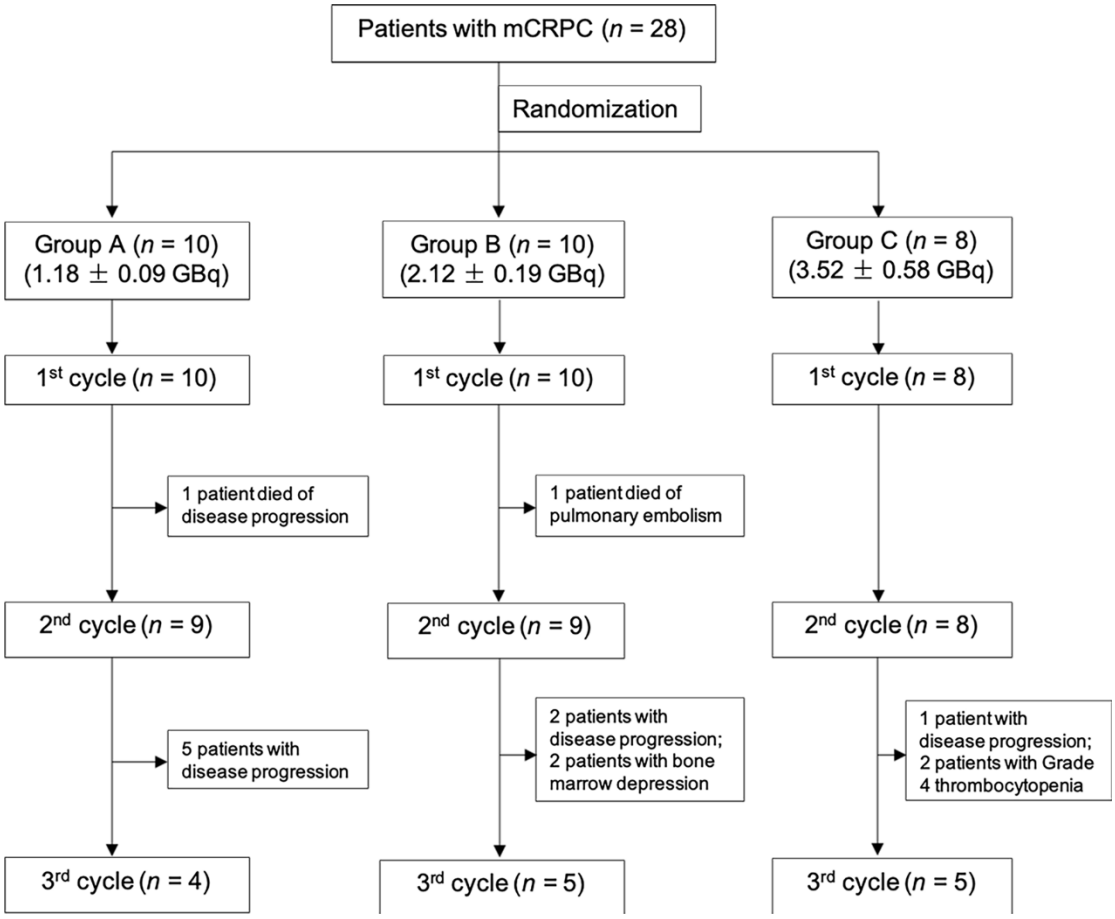

FIGURE 1. Patients flow chart of the three randomized groups.

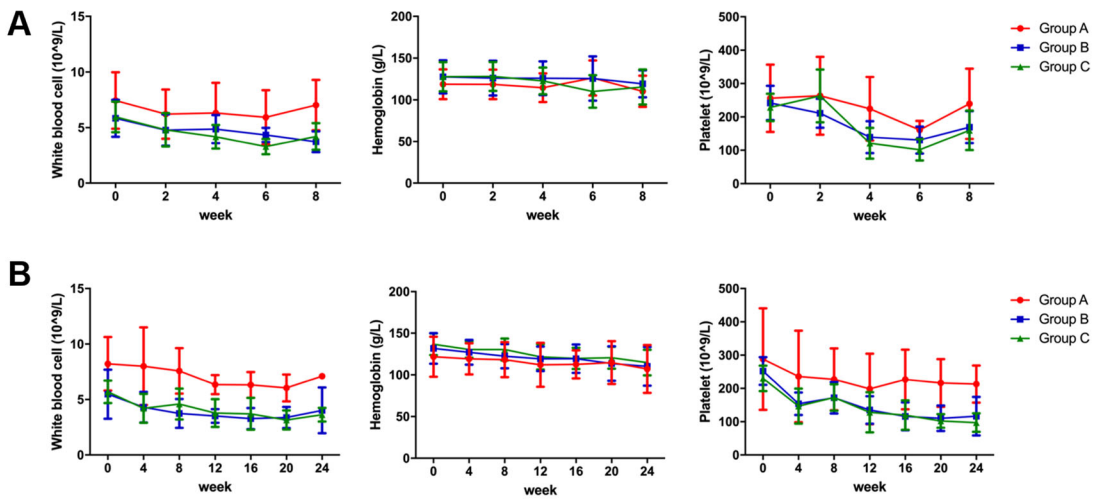

FIGURE 2. Hematologic toxicity,nephrotoxicity and hepatotoxicity. A showed the change of white blood cell, hemoglobin and platelet over 8 weeksafter the first cycleof all the patients, and B showed the change of hematological parametersover 24 weeks for patients accepted three cyclesof ^{177}Lu-EB-PSMAtherapy for the three groups. C showed the mean CRE, ALT, and AST over 24 weeks for patients accepted three cycles of therapy.

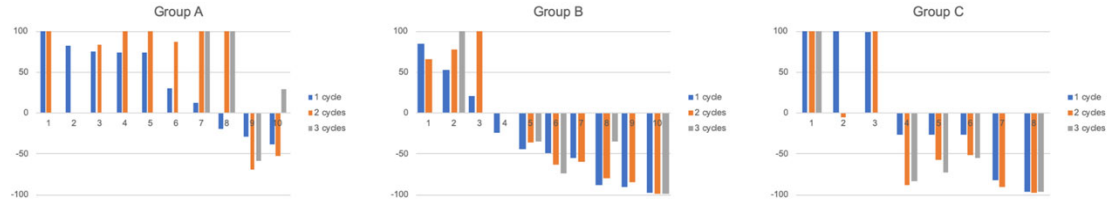

FIGURE 3. Waterfall graphs of PSA responses as compared to baseline levels after each cycle of treatment for the three groups.PSA increase 100% was cropped due to simplification.

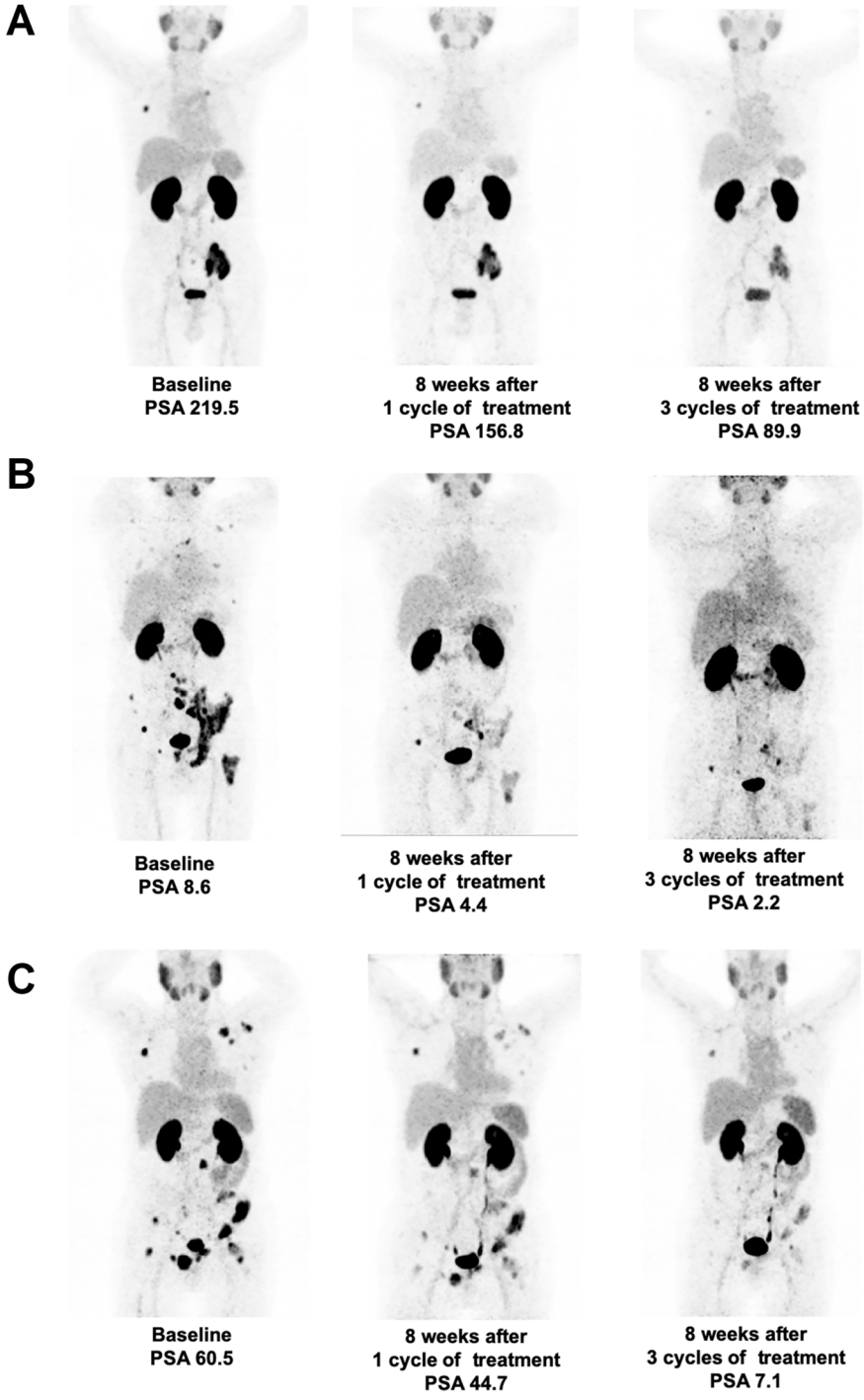

FIGURE 4. The representative patients for ^{68}Ga-PSMA PET/CT and PSA response evaluation in Group A (A), Group B (B) and Group C (C).

TABLE1. PSA response after each cycle of therapy for three groups.

PSA response	Group A				Group B				Group C			
	1st	2nd	3rd	all*	1st	2nd	3rd	all*	1st	2nd	3rd	all*
PR	0/10 (0.0%)	2/9 (22.2%)	1/4 (25.0%)	1/10 (10.0%)	4/10 (40.0%)	5/9 (55.5%)	2/5 (40.0%)	4/10 (40.0%)	2/8 (25.0%)	5/8 (62.5%)	4/5 (80.0%)	5/8 (62.5%)
SD	4/10 (40.0%)	0/9 (0.0%)	0/4 (0.0%)	0/10 (0.0%)	4/10 (40.0%)	1/9 (11.1%)	2/5 (40.0%)	3/10 (30.0%)	3/8 (37.5%)	1/8 (12.5%)	0/5 (0.0%)	1/8 (12.5%)
PD	6/10 (60.0%)	7/9 (77.7%)	3/4 (75.0%)	9/10 (90.0%)	2/10 (20.0%)	3/9 (33.3%)	1/5 (20.0%)	3/10 (30.0%)	3/8 (37.5%)	2/8 (25.0%)	1/5 (20.0%)	2/8 (25.0%)

all*: PSA response before and 8 weeks after the last cycle of treatment in all enrolled patients who had received at least one cycle of therapy were analyzed.

TABLE2. ^{68}Ga-PSMA PET/CT response after each cycle of therapy for three groups

^{68}Ga-PSMA PET/CT response	Group A			Group B			Group C		
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
PR	6/10 (60.0%)	3/9 (33.3%)	1/4 (25.0%)	5/10 (50.0%0	3/9 (33.3%)	3/5 (60.0%)	4/8 (50.0%)	3/8 (37.5%)	2/5 (40.0%)
SD	3/10 (30.0%)	0/9 (0.0%)	0/4 (0.0%)	4/10 (40.0%0	2/9 (22.2%)	2/5 (40.0%)	2/8 (25.0%)	1/8 (12.5%)	2/5 (40.0%)
PD	1/10 (10.0%)	1/9 (11.1%)	1/4 (25.0%)	1/10 (10.0%)	0/9 (0.0%)	0/5 (0.0%)	2/8 (25.0%)	1/8 (12.5%)	0/5 (0.0%)
Not performed	0/10 (0.0%)	5/9 (55.5%)	2/4 (50.0%)	0/10 (0.0%)	4/9 (44.4%)	0/5 (0.0%)	0/8 (0.0%)	3/8 (37.5%)	1/5 (20.0%)

TABLE S1. Baseline Characteristics of Patients

Characteristics	Value in dose group		
	Group A	**Group B**	**Group C**
Number	10	10	8
Age(y)			
Mean	69.7	69.6	67.8
Range	56-84	62-81	60-72
ECOG			
0	2 (20.0%)	3（30.0%）	4（50.0%）
1	7 (70.0%)	7（70.0%）	3（37.5%）
2	1 (10.0%)	0（0.0%）	1（12.5%）
Gleason score			
Mean	8.2	8.0	8.4
Range	7-10	6-10	8-10
Initial PSA level (ng/mL)			
Median	217.7	220.1	36.2
Range	10.5-2287.0	2.1-1501.2	2.4-215.6
Localization of metastases			
Lymph node	2	4	3
Bone	10	10	7
Liver	0	1	0
Lung	1	1	1
Previous therapy			
Prostatectomy	1 (10.0%)	2 (20.0%)	4 (50.0%)
External-beamradiation therapy	6 (60.0%)	4 (40.0%)	3 (37.5%)
Chemotherapy	7 (70.0%)	9 (90.0%)	7 (87.5%)
223Ra	0 (0.0%)	0 (0.0%)	0 (0.0%)
LHRH analogs	9 (90.0%)	11(100.0%)	7 (87.5%)
Bicalutamide	10 (100.0%)	11(100.0%)	8 (100.0%)
Abiraterone	9 (90.0%)	9 (90.0%)	7 (87.5%)
Enzalutamide	4 (40.0%)	3 (30.0%)	4 (50.0%)
Average dose (GBq)	1.18 ± 0.09	2.12± 0.19	3.52 ± 0.58

TABLE S2. Changes of hematological parameters between baseline and 8 weeks after each cycle of treatment for all three groups.

Group	Δ White blood cell			Δ Hemoglobin			Δ Platelet		
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
Group A	-9.6±18.0%	-13.5±24.2%	-11.8±22.5%	-7.4±8.1%	-14.5±25.8%	-17.3± 18.0%	-26.8±23.0%	-22.4±37.4%	-4.3±52.7%
Group B	-33.9±17.9%	-34.0±26.1%	-21.46±50.4%	-7.4±8.1%	-15.2±26.6%	-18.9±8.6%	-34.8±8.5%	-60.5±13.9%	-56.8±17.5%
Group C	-26.8±23.0%	-44.9±23.5%	-31.8±23.1%	-8.1±9.3%	-18.6±10.7%	-14.6±8.7%	-34.8±8.5%	-59.0±21.9%	-56.1±11.1%
P value	0.030	0.044	0.709	0.749	0.925	0.849	0.026	0.009	0.043

TABLE S3. Hematological toxicity according to CTCAE v.5.0.

Side effects		White blood cell				Hemoglobin				Platelet			
group	CTCAE-grade	baseline	1st	2nd	3rd	baseline	1st	2nd	3rd	baseline	1st	2nd	3rd
Group A	G-0	9	8	8	3	7	3	3	1	10	10	7	4
	G-1	1	2	1	1	1	4	2	2	0	0	2	0
	G-2	0	0	0	0	2	3	0	0	0	0	0	0
	G-3	0	0	0	0	0	0	4	1	0	0	0	0
	G-4	0	0	0	0	0	0	0	0	0	0	0	0
Group B	G-0	9	6	2	1	8	6	3	2	10	7	4	1
	G-1	1	2	4	2	1	3	2	1	0	2	3	3
	G-2	0	2	2	1	1	1	2	2	0	1	1	1
	G-3	0	0	1	1	0	0	2	0	0	0	1	0
	G-4	0	0	0	0	0	0	0	0	0	0	0	0
Group C	G-0	8	1	1	0	5	4	3	2	8	5	2	2
	G-1	0	5	2	3	2	3	2	2	0	2	2	3
	G-2	0	2	4	2	1	0	0	1	0	1	1	0
	G-3	0	0	1	0	0	1	2	0	0	0	1	0
	G-4	0	0	0	0	0	0	1	0	0	0	2	0