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Concise title: PREDICTORS OF OVERALL AND DISEASE FREE SURVIVAL IN METASTATIC CASTRATION-RESISTANT PROSTATE CANCER PATIENTS RECEIVING ^{225}Ac-PSMA-617 RADIOLIGAND THERAPY

Short title: Predictors of survival in ^{225}Ac-PSMA therapy

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ABSTRACT

Background: Metastatic prostate carcinoma over-expresses the prostate specific membrane antigen (PSMA) making this antigen a suitable target for radioligand therapy of the disease. Here we report on our experience in a series of 73 castrate resistant prostate carcinoma patients treated with ^{225}Ac-PSMA-617, identifying variables predictive for overall and progression free survival following ^{225}Ac-PSMA-617 treatment. **Methods:** ^{225}Ac-PSMA-617 was administered to patients with mCRPC who had exhausted available therapy options for their disease. Full blood count, glomerular filtration rate and liver function test were obtained at baseline and on follow-up for evaluation of toxicity. ^{68}Ga-PSMA PET/CT was obtained at baseline, prior to every treatment cycle and on follow-up for patients' selection for treatment, to determine the activity of treatment agent to be adminsitered and for response assessment. Serial (prostate specific antigen) PSA was obatianed for PSA response assessment. **Results:** Seventy-three men (mean age=69 years, range:45-85) with mCRPC were treated with 210 cycles of ^{225}Ac-PSMA-617. In 70% of patients, a PSA decline of ≥50% was obtained while 83% of patients had any PSA decline. In 29% of patients, all lesions on ^{68}Ga-PSMA-PET resolved in response to treatment. During follow-up, 23 patients experienced disease progression while 13 patients died from their disease. The estimated median progression free survival (PFS) and overall survival (OS) were 15.2 months (95% CI: 13.1 – 17.4) and 18 months (95% CI: 16.2 – 19.9) respectively. On univariate analyses factors such as baseline PSA, any PSA decline, PSA decline ≥50%, prior chemotherapy, prior radiation therapy, baseline hemoglobin level were associated with longer PFS and OS (all $p<0.05$). On multivariate analyses, only prior Lu-PSMA therapy and a PSA decline ≥50% remained significant in their association with a longer PFS while only PSA decline ≥50% remained significantly associated with OS. Xerostomia was seen in 85% of patients, none was severe enough to warrant discontinuing treatment. Anaemia was seen in 27 patients, no patients with grade IV bone marrow toxicity was seen. Renal failure grade III-IV was seen in five patients with baseline renal impairement. **Conclusion:** In this study, a PSA decline of ≥ 50% proved significantly associated with OS and PFS in multivariate analysis following treatment with ^{225}Ac-PSMA-617. Furthermore, previous ^{177}Lu-PSMA treatment was negatively associated with PFS in both uni- and multivariate analysis.

Keywords: prostate carcinoma, Actinium-225, PSMA, radioligand therapy, PSA response

INTRODUCTION

Prostate specific membrane antigen (PSMA) has been shown to be significantly over-expressed in prostate carcinoma as compared to normal and benign hypertrophic prostate tissue (*1,2*). Furthermore, its overexpression in prostate carcinoma has been shown to directly relate to the tumor's Gleason score and disease stage. Of interest, PSMA bears two distinct enzyme activities, it can either hydrolyze N-Acetyl-L-Aspartyl-L-Glutamate (NAAG) to aspartate and glutamate (NAALDase activity) or release glutamic acid from folate polyglutamate resulting in the release of folic acid (*3*). In recent years, modified forms of NAALDase inhibitors mimicking the NAAG substrate that are internalized intracellularly following binding to PSMA have been designed and used as PSMA targeting agents for prostate carcinoma imaging as well as treatment (*1*). In terms of treatment, both ^{177}Lu- labelled PSMA-617 and PSMA-I§T have been applied in compassionate use programs in metastatic castrate resistant prostate carcinoma patients that had run out of approved treatment options. However, only 45% of thus treated patients show a siginificant decline in PSA levels ($\geq$ 50%) whereas approximately 30% of patients do not respond at all (*4,5*). Radium-223 dichloride is an approved alpha-emitting radionuclide with survival benefit in patients with predominatly bone metastases of prostate cancer (6). As opposed to beta-emitting isotopes such as ^{177}Lu that predominantly induce single stranded breaks, alpha-emitting isotopes have significantly higher linear energy transfer values and thus a greater potential for inducing double strand breaks, DNA cluster breaks and thus also for cell kill. Their short range (< 0.1 mm) may also prove beneficial for reducing toxicity to radiosensitive normal tissues that are invaded by prostate cancer cells, e.g. normal bone marrow (*3*). Accordingly, the development of radiolabeled PSMA targeting inhibitors has been recently shifted torwards alpha-emitting PSMA targeting inhibitors. Of the limited alpha-emitting radionuclides that are suitable for clinical application, ^{225}Actinium (Ac) ($T_{1/2}$=9.9d) and its short-lived daughter radionuclide ^{213}Bismuth(Bi) ($T_{1/2}$= 46 min) have been most extensively studied (*3,7,8*). Becauses of it's decay scheme, with 6 daughter products (^{221}Fr, ^{217}At, ^{213}Bi, ^{213}Po,^{209}Pb and ^{209}Tl) with several alpha and beta-decays, ^{225}Ac appears promising for clinical applications and preliminary results obtained using PSMA-617 labelled with ^{225}Ac in patients suffering from advanced prostate carcinoma have shown its therapeutic potential (*9,10*). In a series of 40 advanced prostate carcinoma patients by Kratochwil et al., ^{225}Ac-PSMA-617 resulted in a prostate specific antigen (PSA) decline of more than 50% in 63% of patients with a median duration of tumor control of 9 months (versus a median duration of 10 months for first-line arbiraterone treatment) (*9*).

Here we report on our experience in a series of 73 castrate resistant prostate carcinoma patients treated with ^{225}Ac-PSMA-617, identifying variables predictive for overall and progression free survival following ^{225}Ac-PSMA-617 treatment.

PATIENTS AND METHODS

In the this retrospective study, we report our experience on the use of ^{225}Ac-PSMA-617 in the treatment of consecutive patients with histologically proven metastatic castration resistant prostate carcinoma that relapsed after initial therapy with radical prostatectomy, bilateral orchidectomy, external beam radiotherapy and/or prostate brachytherapy and subsequent androgen deprivation therapy with gonadotropin-releasing hormone analogs. Inclusion criteria for treating patients with ^{225}Ac-PSMA-617 included a life expectancy of at least 6 months, widespread disease precluding treatment with radiotherapy with a curative intent, completed chemotherapy or patients' refusal of chemotherapy and lack of access to second generation anti-androgen therapy (arbiraterone and enzalutamide). Exclusion criteria were impaired bone marrow function (hemoglobin concentration < 6 g/dl, platelet counts < 25 x 10^9/L or white blood cell count < 3.0 x 10^9), compromised renal function defined as glomerular filtration rate of < 30ml/min/1.73 m2 body surface area or impaierd liver function defined as albumin < 25g/l, uptake of ^{68}Ga-PSMA-11 below twice the physiologic uptake in the normal liver on PET/CT imaging. The decision to treat patients with ^{225}Ac-PSMA-617 was made by the local interdisciplinary tumor board. All patients were well informed of the fact that ^{225}Ac-PSMA-617 is an experimental treatment agent not approved either in South-Africa or elsewhere in the world and of the possible adverse events related to this treatment especially dry mouth, bone marrow suppression, renal impairment and the possibility of any other side effects that may be unknown at this time. We obtained a section 21 approval from the South African Health Products Regulatory Authority for compasionate use of ^{225}Ac-PSMA-617 and all patients treated gave a written informed consent.

Patient Preparation

All patients underwent ^{68}Ga-PSMA-11 PET/CT imaging as part of their initial assessment to determine their suitability for ^{225}Ac-PSMA-617 treatment. Patients were considered suitable for treatment if all metastatic lesions

identified demonstrated an uptake greater than twice the normal physiological liver uptake. Synthesis of ^{68}Ga-PSMA-11 and PET/CT imaging were performed as previoulsy reported.

Preparation and Administration of ^{225}Ac-PSMA-617.

The PSMA-617 precursor (ABX advanced biochemical compounds, Radeberg, Germany) was labeled with ^{225}Ac (JRC, Karlsruhe, Germany) in-house and administered to patients as previously reported (*10*). The initial administration was 8 MBq. Administered activity was de-escalated in subsequent cycles to 7,6 or 4 MBq based on response to earlier administered treatment as previously described (10). Treatment was repeated every 8 weeks.

Safety

All patients were observed for a minimum of four hours after ^{225}Ac-PSMA-617 adminsitration to detect any immediate side effects. Within two weeks before the first cycle of treatment, all patients had determination of their hemoglobin level, leucocyte count, platelet count, glomerular filtration rate (GFR), and liver function tests for baseline assessment. Except when clinical situations warrants more frequent follow-up, these blood tests were repeated two weeks prior to subsequent cycles of treatment (i.e. every eight weeks). After completion of treatment cycles, these blood tests were repeated every 12 weeks until disease progression or death. Patients who developed toxicity were followed-up until resolution or death. In addition to blood tests, patients reported any observed side effects during treatment or on follow-up. During patients' visit for every cycle of treatment and for follow-up evaluations, each patient was asked about side effects known to occur with PSMA-based radioligand therapy including dry mouth, dry eyes, dysgeusia, weight loss, anorexia, fatigue, constipation, and dyspepsia. Toxicity was defined according to the common terminology criteria for adverse events version 5.0 (CTCAE v5.0).

Evaluation of Response to Treatment

Treatment response was assessed using serial measurements of PSA. PSA was obtained at baseline and subsequently every 4 weeks to determine PSA response to therapy. ^{68}Ga-PSMA PET/CT imaging was repeated every

8 weeks (before each subsequent cycle of treatment was administered) to guide the adminsitered activity adminsitered in subsequent treatment cycles as we have previously described (10). PSA response and progression were determined using the Prostate Cancer Working Group 3 criteria defined as a PSA decline $\geq$ 50% as being a biochemical significant response (*11*). Additionally, any change in PSA values was documented and analyzed. Follow-up ^{68}Ga-PSMA-11 PET/CT imaging was used to define resolution of initially identified metastatic lesions on baseline scan.

Statistical Analysis

Statistical analysis was performed using the commercially available software package SPSS version 25.0. The Kolmogorov-Smirnov test was used to check the normality of data distribution. Quantitative variables were compared using paired student's t test and ANOVA or Mann-Whitney test and Kruskall test if their distribution was not normal. The Chi-square test and Fisher's exat test were used to determine differences in proportions when appropriate.

Progression free survival was calculated from the date of the first ^{225}Ac-PSMA-617 treatment. Persistent or recurrent disease at a presenting primary site was scored as local disease failure. Progression free survival took into account all disease events, including local, regional and distant failures. Patients were censored at the time of last follow-up or death. Overall survival was defined as the time from the date of the first ^{225}Ac-PSMA-617 treatment until death or until last follow-up (right censored data). Overall survival took into account all deaths.

For univariate and regression analysis we dichotomized values according to the median values of the study cohort in the case of continuous variables. We also dichotomized the following clinical covariates : disease stage (IVA versus IVB), previous radiotherapy, previous chemotherapy, the presence of visceral metastases, PSA reponse $\geq$ 50%, any decline in PSA and undetectable PSA. Local recurrence free as well as overall survival times were estimated by the Kaplan-Meier method and log rank testing to examine the predictive value of dichotomized values and other clinical risk factors for local disease control and overall survival. Multivariate analysis was performed using Cox-regression analysis, including in sequential order of statistical significance those variables that were found to be significant in univariate analyis followed by the interactive terms.

RESULTS

Patient Characteristics

Seventy-three castratation resistant metastasized prostate carcinoma patients were included in the study. Median age of the patient population under study was 69 yrs (range: 45-85 yrs). Thirty-seven patients presented with an Eastern Cooperative Oncology Group (ECOG) score 0, 23 pts with an ECOG score 1, 11 pts with and ECOG score 2 and 2 pts with an ECOG score 3. Seven patients presented with isolated lymph node involvement (stage IVA disease) whereas the remaining 66 patients all had bone metastases (stage IVB disease) and 6 out of these also presented with visceral metastases (3 patients with liver metastases, 2 with lung metastases and one patient with both liver and brain metastases). Among the 66 patients with bone metastases, 28 patients had widespread diffuse skeletal metastases in the typical superscan pattern on ^{68}Ga-PSMA-11 PET/CT including two patients with associated visceral metastases (supplemenatry table 1).

Twenty-seven patients had received previous chemotherapy, 37 had received previous radiation therapy, one patient had received previous arbiraterone and enzelutamide treatment and 10 patients had recevied previous ^{177}Lu-PSMA-617 therapy.

A total number of 210 cycles were administered; 17 pts received one cycle, 18 patients received 2 cycles, 14 pts received 3 cycles, 11 pts received 4 cycles, 9 pts received 5 cycles, 3 pts received 6 cycles and one patient received 8 cycles. Patient follow-up ranged from 2 to 22 months (median=9). Median number of cycles administered was 3 (range: 1 to 8 cycles). Additional patient information is shown in table 1.

Following ^{225}Ac-PSMA-617 treatment, fifty-one patients (70%) presented with a PSA decline $\geq$ 50% and 60 patients (83%) with any decline in PSA (see Fig 1). Following ^{225}Ac-PSMA-617 treatment, PSMA-PET imaging became negative in 21 patients i.e. avidity similar to background bloodpool activity (Supplementary table 2).

Safety

Administration of ^{225}Ac-PSMA-617 was well tolerated. No patient showed any feature of immediate toxicity to its adminstration. No patient dropped out of the study on the basis of toxicity or any other reason. The commenest

toxicity seen was grade I-II dry mouth observed in 85% of patients. No patient with grade III dry mouth was seen and no patient discontinued treatment due to this side effect. Four patients each had dry eye and dysgeusia in addition to dry mouth. No patient with grade IV bone marrow toxicity was seen. Anemia was the most common manifestation of hematotoxicity seen in 37% of patients (22 patients with grade I-II and 5 patients with grade III anemia). Any grade of renal failure was seen in 32% of patients (grade I-II=15, grade III=3, and grade IV=2). The details of toxicity seen in the treated patients is shown in table 2.

Side effects were prevalent among patients with superscan including xerostomia (25/28), hematologic toxicity in 57% of patients half of whom had grade III/IV toxicity, renal toxicity (7/28), weight loss (10/28) and fatigue (14/28).

Overall Survival

At the time of data analysis, 13 patients had died and all deaths seemed to be directly related to the underlying prostate adenocarcinoma. Estimated median OS for all study participants was 18 months (95% CI: 16.2 – 19.9). We found no significant difference in the estimated median OS between the group treated for mCRPC with chemotherapy, second-line hormonal therapy or ^{177}Lu-PSMA-617 and those who were not, 7 versus 9.5 months respectively, p=0.333. Similarly, there was no significant difference in estimated median OS median patients who had superscan versus those who did not, 8.5 versus 9.0 respectivley, p=0.330 (supplementary table 3). On univariate analysis, the following factors were significantly associated with a favourable OS; baseline PSA (p=0.035), any PSA decline (p<0.001), PSA decline $\geq$ 50% (p<0.001), Hb level (p=0.035), previous chemotherapy (p=0.049), and previous radiotherapy (p=0.013). When included in the multivariate model including age and Gleason score as covariates, out of the aforementionned variables, only PSA decline $\geq$ 50% retained its statistical significance (p=0.025) (see table 3 and Fig 2). Estimated OS for patients with a PSA decline $\geq$ 50% was 20.1 months versus 10.5 months for a PSA decline < 50%.

Progression Free Survival

During follow-up, 23 patients progressed. The estimated PFS for the entire study participants was 15.2 months (95% CI: 13.1 – 17.4). We found no significant difference in the median estimated PFS between patients who had superscan pattern versus those who did not. However, patients prior treatment for mCRPC had a negative impact on PFS (supplememntary table 3). On univariate analysis, the following variables proved significantly related to PFS: baseline PSA (p=0.020), PSA decline $\geq$ 50% (p<0.001), any decline in PSA (p<0.001), undetectable PSA (p=0.021), disease stage (p= 0.048), Alkaline phosphatase levels (p= 0.040), Hb (p=0.041), negative PSMA PET scan (p=0.001), previous radiation therapy (p= 0.002), and previous ^{177}Lu-PSMA therapy (p<0.001) (see table 3 and Figs 3A and 3B). When included in the multivariate model including age and Gleason score as covariates, out of the aforementionned variables, previous treatment with ^{177}Lu-PSMA (p=0.05) and percentage PSA decline $\geq$ 50% retained their statistical significance (p<0.001). Estimated PFS for patients with a PSA decline $\geq$ 50% was 17.9 months versus 6.6 months for a PSA decline < 50%. Estimated PFS was much shorter in patients who received ^{177}Lu-PSMA therapy for their disease prior to Ac-PSMA-617 therapy (5.1 months, CI: 3.8 – 6.5) compared with patients who did not (16.5 months, CI: 14.3 – 18.7).

DISCUSSION

In this study we report on the response rate and progression-free as well as overall survival following ^{225}Ac-PSMA-617 therapy in a series of 73 mCRPC patients.

According to the recommendation of the Prostate Cancer Clinical Trials Working Group 2 (*11*), response to therapy in mCRPC patients should be assessed by PSA response and is commonly defined as a $\geq$ 50% decline in PSA levels. In our series, 70% of patients presented a PSA decline $\geq$ 50%, which is significantly higher when compared to the response rates reported in literature for ^{177}Lu-PSMA ranging from 34 to 59% (*4,5*). This finding might partly be explained by the heterogeneous patient cohorts included in the various studies and in particular by the number of treatments exploited before therapy with ^{177}Lu- or ^{225}Ac-PSMA617 which makes a direct comparison difficult. However, the high response rates observed in our study, and also in previous studies reported by Kratochwil et al. (9),

are probably also due to the significantly higher linear energy transfer values and thus the anticipated greater potential for inducing double strand breaks and cell kill of alpha-emitting agents when compared to beta-emitting agents. In our series, a PSA decline of $\geq$ 50% proved the only significant factor associated with a favourable OS in multivariate analysis with a median estimated overall survival in patients with a PSA decline of $\geq$ 50% of 20.1 months versus 10.5 months for patients presenting a decline in PSA levels < 50%. This finding agrees with the outcome from the only published phase II on PSMA-based radioligand therapy where Hofman et al. also reported a PSA decline ≥50% to have significant survival benefit (12). To date, to the best of our knowledge, only four studies have reported on the factors governing overall and disease free survival of mCRPC patients following ^{177}Lu-PSMA therapy in multivariate analysis, respectively studies by Bräuer et al., Heck et al. and two studies by Ahmadzadefahr et al. (*13-16*). These studies included 59, 100, 52 and 100 mCRPC patients treated with ^{177}Lu-PSMA. In none of these studies, PSA levels of $\geq$ 50% after the first treatment cycle were associated with a significantly longer OS in multivariate analysis. This is not at all surprising given the overall lower biochemical response to ^{177}Lu-PSMA treatment with a lower percentage of patients presenting with a decline in PSA levels $\geq$ 50% resulting in unbalanced groups for univariate analysis and thus a lower statistical power. In this regard, the study by Ahmadzadefahr et al. including 100 patients, is of interest, as in their series, dichotomising patients according a lower threshold for percentage PSA response of respectively $\geq$ 14 %, yielding two more balanced groups in number for comparison, did prove associated with a significantly longer median OS (70 weeks (approximately 16 months) ; 95% CI: 39.5-100.5) when compared to patients with a decline of PSA < 50% (49 weeks (approximately 11 months) ; CI: 30.2-67.8 weeks) in both uni- and multivariate analysis (*16*). As opposed to some of these studies, the level of serum Hb and the presence of visceral metastases proved not significantly related to overall survival in multivariate analysis in our study (*16*). In cancer patients, a low level of Hb is common and has been well documented to impair quality of life and reduce locoregional disease control conferred by radiation therapy. The reported prognostic importance of low Hb in radiation oncology has been assumed to relate to a reduction of molecular oxygen levels, thereby attenuating radiation-induced damage and ultimate cell death (*17,18*). A number of studies show that the oxygen enhancement ratio (the ratio of dose administered under hypoxia to aerated conditions needed to achieve the same biological effect) is 1.0 when LET is greater than 165 kev/μm,varies from 2.0 to 1.3 when LET is between 61 and 110 keV/μm and increases to 2.4 when LET reduces to 26 keV/μm (*19*). Cell death induced by alpha radiation has also been demonstrated to be independent of cellular oxygenation (*20*). Accordingly, the significantly higher LET value of ^{225}Ac, (100 keV/μm) versus that of ^{177}Lu, (0.7 keV/μm), results in

cell kill of ^{225}Ac being significantly less dependent on oxygen and Hb levels when compared to ^{177}Lu which may in part explain the lack of predictive power of Hb values in our series in multiovariate analysis. With regard to visceral visceral metastases, our series included only a limited number of patients with visceral metastases which may have impacted statistical power.

Results obtained in large cohorts of mCRPC patients using firstline chemotherapy and arbiraterone acetate have previously shown baseline PSA to be predictive for overall survival in uni- and mulivariate analysis. Using data from a phase III trial of 1050 patients (Cancer and Leukemia Group B CALGB-90401), an updated prognostic model for predicting overall survival following first-line chemotherapy prostate castration resistant prostate cancer was defined by Halabi et al. Nine parameters proved significantly associated with OS, including PSA pretreatment values (*21*). Likewise, in a recent observational study reporting on data collected from 481 chemotherapy-naïve patients treated with arbiraterone acetate plus corticoids of choice, recruited in three European countries, baseline PSA values proved one of the six factors associated with overall surival in multivariate analysis (*22*). In our series, both baseline PSA levels and alkaline phospatase levels proved significantly related to OS in univariate analysis but not in multivariate analysis, suggesting that for ^{225}Ac-PSMA-617 treatment it is not the number of lesions present but their intrinsic sensitivity to radiation that governs response to therapy and thus overall survival.

We additionally assessed the prognostic value of various variables on PFS and found previous treatment with ^{177}Lu-PSMA to be significantly associated in uni- and multivariate analysis with PFS, suggesting previous ^{177}Lu-PSMA treatment may have induced an increased resistance to radiation. Various studies have demonstrated that radioresistance of cancer is due to the existence of intrinsic cancer stem cells (CSCs), termed prostaspheres in prostate carcinoma, which represent a small radioresistant cell subpopulation (*23*). When compared to adherent prostate cancer cells (non-stem cells), cells in prostaspheres were shown to exhibit higher expression of DNA repair proteins following exposure to ionizing radiation, which efficiently repair radiation induced DNA injury confering a survival benefit (*24*). Also, non-stem cell prostate cancer cells were shown to be able to differentiate into CSCs via a process termed epithelial-mesenchymal transition (EMT) (*23*). Similar to CSCs, prostate cancer cells that have obtained an EMT phenotype have proven more resistant to radiation therapy (*25*). Finally, in a study by Wang et al., the proportion of prostate cancer stem-cell like cells in a human prostate cancer cell culture was shown to increase significantly

following exposure to radiation suggesting that radiation may eliminate the radiosensitive adult cancer cells in the culture e.g. by inducing apoptosis, resulting in the enrichment of radioresistant CSCs (*26*). Future studies investigating the underlying pathways that drive radioresistance in prostaspheres and EMT-transformed prostate cancer cells may lead to the development of treatment approaches that block the generation of radioresistance thereby potentially improving outcome following ^{177}Lu-PSMA617/I&T and ^{225}Ac-PSMA-617 treatment.

^{225}Ac-PSMA-617 treatment was associated with tolerable side effects. The majority of our patients experienced dry mouth commonly seen after the first cycle of treatment. This was not severe enough to warrant treatment discontinuation in any patients. This is contratry to the report by Kratochwil et al. in whom four of 40 patients discontinued treatment due to intolerable xerostomia (*9*). We routinely titrate administered activity of the therapy agent against residual disease as seen on ^{68}Ga-PSMA PET/CT done prior to every cycle of treatment. All patients are initially treated with 8 MBq for their first cycle of treatment and the activity of the treatment agent adminsitered is reduced in subsequent treatment cycles according to the residual disease seen. This de-escalation of treatment is, perhaps, responsible for the lesser severity of dry mouth seen in our series. Dry mouth occurred with other gastrointestinal side effects including dysgeusia, contipation and weight loss. Hematoxicity seen in our cohort was transient and no patient had grade IV hematotoxicity. We reported grade I-II anaemia in 22 patients, leucopenia in seven patients and thrombocytopenia in 6 patients. While these bone marrow toxicities are transient, their incident is relatively high as ^{225}Ac is expected to spare the functional bone marrow due to its short range. We believe that the incidence of marrow toxicity seen in this study is related to the preponderance of skeletal metastases seen in 66 patients including 28 patients with superscan pattern on ^{68}Ga-PSMA-11 PET/CT. Eight cases of Grade III hematoxicities were seen in 5 patients (grade III anaemia=5, grade III leucopenia=2, and grade III thrombocytiopenia=1) all of whom had impaired bone marrow function at baseline assessment resulting from toxicity from their previous treatments. Grade III-IV renal failure was seen in five patients all of whom had renal failure (grade II) prior to ^{225}Ac-PSMA-617 therapy.

The main limitation of our study is its single center set-up and its retrospective design. In line with the ongoing phase-three randomized VISION study, which will compares ^{177}Lu-PSMA-617 plus best standard of care to best standard of care alone investigations using OS as primary endpoint, prospective-designed studies assessing the

potential benefit of ^{225}Ac-PSMA-617 on PFS and OS are mandatory. Compared with some previously reported patients population treated with PSMA-based radioligand therapy, our cohort appear less heavily pre-treated. In our study, these heavily pre-treated patients had a significantly shorter duration to biochemical progression compared with patients in whom ^{225}Ac-PSMA-617 was introduced earlier in their treatment history. On the whole, being heavily pre-treated did not impact on the OS in our cohort.

CONCLUSION

In this study, a PSA decline of ≥ 50% proved significantly associated with OS and PFS in multivariate analysis following treatment with ^{225}Ac-PSMA-617. Furthermore, previous ^{177}Lu-PSMA treatment was negatively associated with PFS in both uni- and multivariate analysis.

KEY POINTS

QUESTION: What are the factors predictive of overall and disease free survivals in patients with metastatic castration-resistant prostate carcinoma treated with Ac-PSMA-617 therapy?

PERTINENT FINDING: In a cohort of 73 patients with castration-resistant prostate carcinoma treated with ^{225}Ac-PSMA-617, there was PSA response in 83% of patients with 70% recording a PSA decline ≥ 50%. Estimated median overall and progression free survivals were 18 and 15.2 months respectively. A PSA decline of ≥ 50% was the strongest predictor of OS and PFS.

IMPLICATIONS: ^{225}Ac-PSMA-617 is a viable treatment option for patients with castration-rseistant prostate carcinoma who have failed convdentional therapy. Response is durable and side effects are tolerable.

Disclosures: FLG is a stock holder of Endocyte, Inc. West Lafayette, IN, USA. The other authors declare no conflict of interests. Ethical approval: All subjects signed a written informed consent. All procedures performed in this retrospective evaluation were in accordance with the ethical standard of our institions and 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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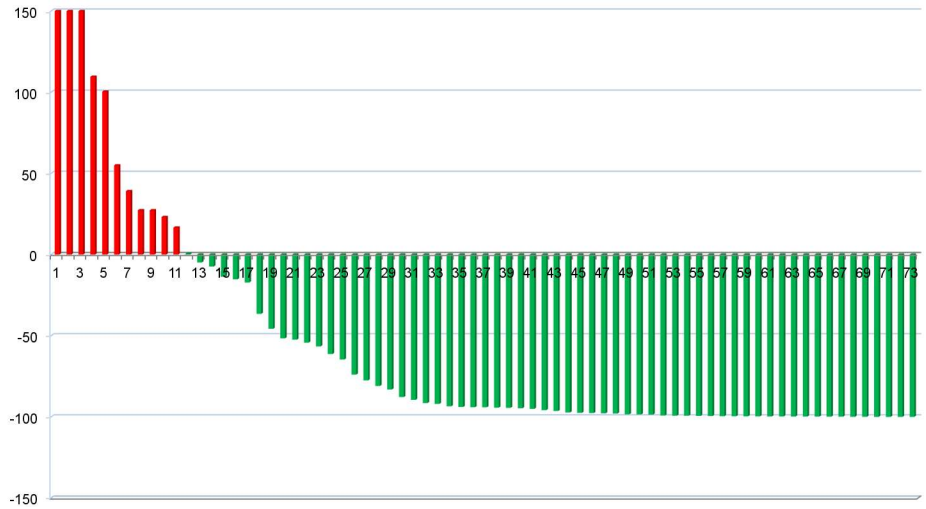

Figure 1. Waterfall plot demonstrating the percentage change in PSA values following treatment with ^{225}Ac-PSMA-617 in the patients studied (X-axis: number of patients, Y-axis: percentage change)

Figure 2. Kaplan Meier plot of overall survival (OS) in months versus % PSA decline of > 50% (red curve) and % PSA decline < 50% (blue curve)

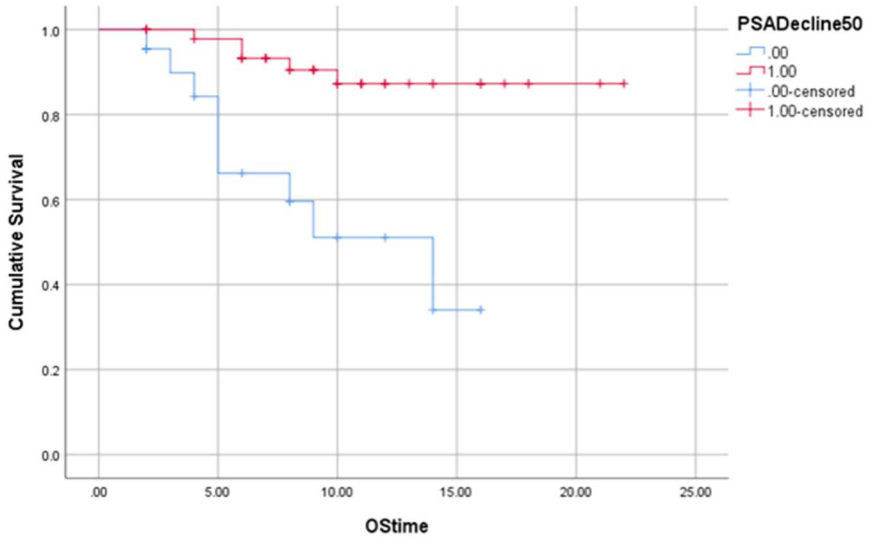

Figure 3A. Kaplan Meier plot of progression free survival (PFS) in months versus % PSA decline > 50% (red curve) and % PSA decline < 50% (blue curve)

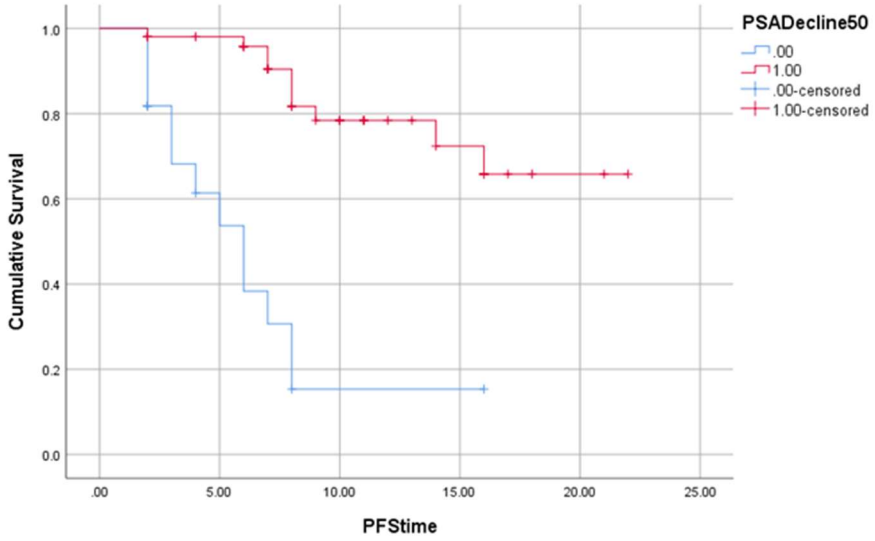

Figure 3B. Kaplan Meier plot of progression free survival (PFS) in months versus previous pre-treatment with 177Lu-PSMA-617 (red curve) or not (blue curve)

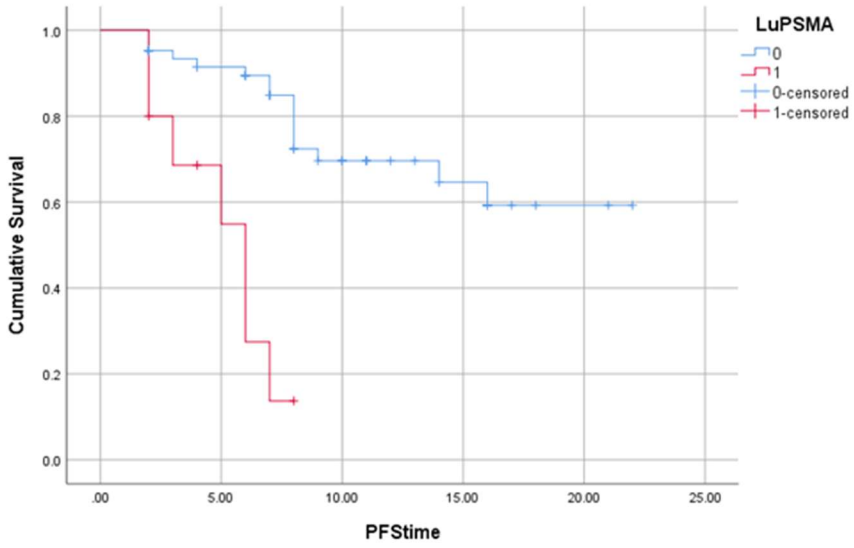

Table 1: Patient Characteristics

Characteristic	This report
Patients included (n)	73
Median age (years)	69
Age ≥ 75 years (%)	29
ECOG score =0/1 (%)	82
ECOG score ≥ 2 (%)	18
Median PSA (ng/mL)	57.2
Median alkaline phosphatase (IU/L)	154
Alkaline phosphatase > 220IU/L (%)	27
Median hemoglobin level (g/dL)	11.7
Hemoglobin ≤ 10 g/dL (%)	30
Bone metastases (%)	90
Superscan (%)	38
Visceral metastases	
Lung (%)	3
Liver (%)	5
Brain (%)	1
Local therapy to the prostate	
Prostatectomy (%)	33
Radiation therapy (%)	14
No local therapy (%)	53
Therapy for castrate-resistant disease	
Chemotherapy (%)	37
Abiraterone (%)	1
Enzalutamide (%)	1
^{177}Lu-PSMA-617 (%)	14
Estimated median OS (months)	18

ECOG: Eastern Cooperative Oncology Group; OS: overall survival.

Table 2: Toxicity profile of 73 patients treated with 225Ac-PSMA-617

	Grade I-II	Grade III	Grade IV
Dry mouth	62 (85%)	0	0
Dry eyes	4 (5%)	0	0
Anorexia	23 (32%)	0	0
Nausea	15 (21%)	0	0
Vomiting	4 (5%)	0	0
Constipation	19 (26%)	0	0
Fatigue	37 (51%)	0	0
Weight loss	28 (38%)	0	0
Dyspepsia	3 (4%)	0	0
Dysgeusia	4 (5%)	0	0
Anemia	22 (30%)	5 (7%)	0
Leucopenia	7 (10%)	2 (3%)	0
Thrombocytopenia	6 (8%)	1 (1%)	0
Hypoalbuminemia	14 (19%)	0	0
Renal failure	18 (25%)	3 (4%)	2 (3%)
Dysuria	13 (18%)	0	0

Table 3: Univariate analysis of the relationship between the studied variables and progression free (PFS) and overall survival (OS)

	OS, p(Log-rank)	PFS, p(Log-rank)
Age	P=0.994	P=0.394
Gleason score	P=0.163	P=0.120
ECOG score	P=0.152	P=0.171
Baseline PSA (ng/mL)	**P=0.035**	**P=0.020**
PSA decline ≥ 50%	**P<0.001**	**P<0.001**
Any PSA decline	**P<0.001**	**P<0.001**
PSA undetectable	P=0.119	**P=0.021**
Stage IVA/IVB	P=0.576	**P=0.048**
Visceral metastases	P=0.806	P=0.147
Number of treatment cycles	P=0.409	P=0.166
Alkaline phosphatase level	P=0.318	**P=0.040**
Hemoglobin level	**P=0.035**	**P=0.041**
Platelets count	P=0.478	p=0.698
WBC count	P=0.358	P=0.442
Negative ^{68}Ga-PSMA PET post treatment	P=0.175	**P=0.001**
Previous chemotherapy	**P=0.049**	P=0.169
Previous radiotherapy	**P=0.013**	**P=0.002**
Previous ^{177}Lu-PSMA therapy	P=0.146	**P<0.001**

PSA: prostate specific antigen; ECOG: Eastern Cooperative Oncology group; WBC: white blood cell

Supplementary Table 1: Characteristics of patients with superscan

Variables	Mean ± SD	Range
Age (years)	67.32 ± 8.10	48 – 80
Gleason score	8.00 (7.00 – 9.00)*	6 – 10
Baseline PSA	144.24 (18.53 – 1620.40)*	1.10 – 4495.00
Baseline ALP	330.00 (177.75 – 451.75)*	84.00 – 728.00
Baseline Hb	10.15 ± 2.30	6.10 – 16.00
Baseline Platelet	264.00 (179.00 – 358.00)*	29.00 – 762.00
	Frequency (n = 28)	**Percent**
ECOG		
0	13	46.4
1	10	35.7
2	5	17.9
Heavily pretreated		
Yes	16	57.1
No	12	42.9
Site of metastasis**		
Bone	28	100.0
Liver	1	3.6
Lungs	1	3.6
Nodes	16	57.1

***: Median (Inter-quartile range); **: Multiples sites; PSA: Prostate Specific Antigen; ALP: Alkaline phosphatase; Hb: Hemoglobin; ECOG: Eastern Cooperative Oncology Group**

Supplementary table 2: Characteristics of patients who achieved negative ^{68}Ga-PSMA-11 scan post ^{225}Ac-PSMA-617 therapy

Variables	Mean ± SD	Range
Age (years)	69.48 ± 9.98	45 – 84
Gleason score	8.00 (7.00 – 9.00)*	6 – 10
Baseline PSA	15.33 (5.05 – 354.60)*	1.20 – 4495.00
Baseline ALP	131.00 (72.00 – 258.50)*	62.00 – 549.00
Baseline Hb	12.03 ± 2.06	7.40 – 15.20
Baseline Platelet	256.00 (209.00 – 377.50)*	127.00 – 470.00
	Frequency (n = 21)	**Percent**
ECOG		
0	12	57.1
1	5	23.8
2	4	19.0
Heavily pretreated		
Yes	4	19.0
No	17	81.0
Site of metastasis**		
Bone	16	76.2
Lungs	1	4.8
Nodes	13	61.9

***: Median (Inter-quartile range); **: Multiples sites; PSA: Prostate Specific Antigen; ALP: Alkaline phosphatase; Hb: Hemoglobin; ECOG: Eastern Cooperative Oncology Group**

Supplementary table 3: Sub-group analyses

		OS			PFS	
Variable	**Range**	**Median (IQR)**	***p* value**	**Range**	**Median (IQR)**	***p* value**
Superscan						
Yes	2.0 – 18.0	8.5 (4.0 – 11.8)	0.330	2.0 – 18.0	6.5 (2.3 – 10.0)	0.293
No	2.0 – 22.0	9.0 (6.0 – 14.0)		2.0 – 22.0	8.0 (4.5 – 11.5)	
Heavily pretreated						
Yes	2.0 – 22.0	7.0 (4.5 – 13.0)	0.333	2.0 – 22.0	6.0 (2.0 – 8.0)	**0.010***
No	2.0 – 21.0	9.5 (6.0 – 13.8)		2.0 – 21.0	8.5 (6.0 – 13.8)	
Negative PSMA PET						
Yes	4.0 – 22.0	13.0 (8.5 – 16.0)	**<0.001***	4.0 – 22.0	13.0 (8.0 – 16.0)	**<0.001***
No	2.0 – 21.0	8.0 (4.0 – 10.8)		2.0 – 21.0	6.0 (2.0 – 8.0)	

NB: Mann-Whitney U test used; *: *p* value < 0.05