

Prostate-Specific Membrane Antigen–Targeting Alpha Emitter via Antibody Delivery for Metastatic Castration-Resistant Prostate Cancer: A Phase I Dose-Escalation Study of ^{225}Ac -J591

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ABSTRACT

PURPOSE Novel therapies are needed to extend survival in metastatic castration-resistant prostate cancer (mCRPC). Prostate-specific membrane antigen (PSMA), a cell surface antigen overexpressed in PC, provides a validated target. This dose-escalation study investigated the safety, efficacy, maximum tolerated dose (MTD), and recommended phase II dose (RP2D) for ^{225}Ac -J591, anti-PSMA monoclonal antibody J591 radiolabeled with the alpha emitter actinium-225.

METHODS Following investigational new drug-enabling preclinical studies, we enrolled patients with progressive mCRPC that was refractory to or who refused standard treatment options (including androgen receptor pathway inhibitor and had received or been deemed ineligible for taxane chemotherapy). No selection for PSMA was performed. Patients received a single dose of ^{225}Ac -J591 at one of seven dose-escalation levels followed by expansion at the highest dose. Primary end point of dose-escalation cohort was determination of dose-limiting toxicity (DLT) and RP2D.

RESULTS Radiochemistry and animal studies were favorable. Thirty-two patients received ^{225}Ac -J591 in an accelerated dose-escalation design (22 in dose escalation, 10 in expansion). One patient (1 of 22; 4.5%) experienced DLT in cohort 6 (80 KBq/kg) but none in cohort 7; MTD was not reached, and RP2D was the highest dose level (93.3 KBq/kg). The majority of high-grade adverse events (AEs) were hematologic with an apparent relationship with administered radioactivity. Non-hematologic AEs were generally of low grade. Prostate-specific antigen (PSA) declines and circulating tumor cell (CTC) control were observed: 46.9% had at least 50% PSA decline at any time (34.4% confirmed PSA response), and protocol-defined CTC count response occurred in 13 of 22 (59.1%).

CONCLUSION To our knowledge, this is the first-in-human phase I dose-escalation trial of a single dose of ^{225}Ac -J591 in 32 patients with pretreated progressive mCRPC demonstrating safety and preliminary efficacy signals. Further investigation is underway.

ACCOMPANYING CONTENT

-  Appendix
-  Data Sharing Statement
-  Protocol

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INTRODUCTION

Despite advances in diagnostic and treatment strategies, prostate cancer is the second most common cause of cancer mortality in men in the United States and worldwide.^{1,2} Despite considerable progress, the median survival of metastatic castration-resistant prostate cancer (mCRPC)

remains <3 years and mCRPC remains incurable.³ Additional innovative treatments are needed to further extend survival and to explore potentially curative options.

Prostate-specific membrane antigen is a cell surface glycoprotein that is commonly overexpressed by PC cells relative to normal prostate cells.^{4,5} The highest expression

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CONTEXT

Key Objective

Is targeting prostate-specific membrane antigen (PSMA) with an antibody radiolabeled with an alpha emitter safe in patients with prostate cancer?

Knowledge Generated

A single dose of ^{225}Ac -J591 was safe with no acute dose-limiting toxicity. Preliminary efficacy as measured by prostate-specific antigen decline and circulating tumor cell count control was demonstrated with or without previous ^{177}Lu -PSMA.

Relevance (M.A. Carducci)

This early phase study of an alpha-emitting PSMA targeted monoclonal antibody is highly encouraging given its novelty and activity in heavily pre-treated metastatic castration-resistant prostate cancer patients, including those with prior lutecium based therapy. Additional early phase studies including combinations will keep this a promising agent for future clinical use.*

*Relevance section written by JCO Associate Editor Michael A. Carducci, MD.

is found with higher grades and in mCRPC.⁴⁻⁶ These characteristics, combined with limited expression on normal tissues, make prostate-specific membrane antigen (PSMA) a logical target for prostate cancer therapy, particularly for high-risk and advanced prostate cancer, as validated by the VISION and TheraP studies.^{7,8}

J591 is a deimmunized monoclonal antibody (mAb) directed at the extracellular domain of human PSMA. J591 was previously evaluated in mCRPC therapy when it was radiolabeled with ^{177}Lu and ^{90}Y , beta particle-emitting radionuclides, and an imaging agent labeled with ^{111}In or ^{89}Zr .⁹⁻¹⁴ In phase I and II mCRPC trials, single-dose and fractionated ^{177}Lu -J591 showed acceptable toxicity, excellent targeting of metastatic sites, and encouraging results for overall survival (OS), prostate-specific antigen (PSA) response, measurable disease response, and circulating tumor cell (CTC) response, leading to a current phase III trial (ClinicalTrials.gov identifier: [NCT04876651](https://clinicaltrials.gov/ct2/show/study?term=NCT04876651)).¹¹⁻¹³

Compared with beta particles (β^- or electrons), alpha particles (α^{2+} or helium nuclei) have severalfold higher linear energy transfer (100 keV/ μm v 0.2 keV/ μm). In addition, the kinetic energy of α particles is very high compared to the energy of β^- particles. For example, ^{225}Ac and its daughters emit four α particles with kinetic energies of 5.8–8.4 MeV. By contrast, the ^{177}Lu atom emits only one electron with an energy of only 0.498 MeV. In addition, α particles have a short range in tissues (<100 μm) compared with the range of high-energy β^- particles (approximately 12 mm), allowing a larger fraction of the total energy to be deposited in the cells, thus increasing the relative biologic effect.¹⁵

The US Food and Drug Administration–approved alpha-emitting radiopharmaceutical radium-223 usually causes only mild adverse events (AEs), primarily myelosuppression

and gastrointestinal.¹⁶ Radium-223 is preferentially deposited in sites of active bone formation, but it is not specifically targeted to prostate cancer and thus is expected to have no effect on soft tissue or visceral metastases. Our hypothesis was that radiolabeling the PSMA-targeting mAb J591 with ^{225}Ac would provide good antitumor activity against a broader range of prostate cancer metastases with acceptable tolerability.

Here, we describe the results of a dual-center phase I clinical trial of ^{225}Ac -J591 to treat progressive, heavily pretreated mCRPC.

METHODS

Radiochemistry and Preclinical Studies

Deimmunized J591 mAb was first conjugated with the bi-functional chelating agent DOTA; DOTA-J591 was prepared under good manufacturing practice conditions as previously described.⁹⁻¹¹ It was then radiolabeled with ^{225}Ac , which was purchased from the Department of Energy, Oak Ridge National Laboratory (Oak Ridge, TN). Initial mouse safety studies were followed by a radioimmunotherapy (RIT) dose-escalation study as given in detail in [Appendix 1](#) (online only).

Patients

Adult patients with documented progressive mCRPC on the basis of Prostate Cancer Working Group 3 criteria were eligible.¹⁷ Patients had previously been treated with at least one androgen receptor pathway inhibitor (ARPI) such as enzalutamide or abiraterone acetate; they also had previously received taxane chemotherapy or refused or been deemed ineligible for taxane chemotherapy. Previous radium-223 and PSMA-targeted lutetium-177 were allowed. All patients had

an Eastern Cooperative Oncology Group performance status of 0–2 and intact organ and marrow function. Full eligibility criteria are available in the protocol ([Appendix 1](#)).

Baseline number and location of lesions were ascertained using three imaging methods: computed tomography (CT) or magnetic resonance imaging (MRI), bone scan, and PSMA positron-emission tomography (PET)/CT (at the Weill Cornell Medicine (WCM) site) using the radiotracer ^{68}Ga -PSMA-11. While PSMA imaging was not required for study entry and patients were not excluded on the basis of PET findings, the baseline ^{68}Ga -PSMA-11 PET/CT scan also served to document PSMA expression at tumor sites for secondary analysis. All patients had to complete a follow-up of at least 12 weeks to be included in the evaluation of response; the planned duration of follow-up was 3 years. Patients had follow-up imaging at 12 weeks (CT/MRI, bone scan, and ^{68}Ga -PSMA-11 PET/CT) and every 12 weeks thereafter (CT/MRI and bone scan).

Trial Design and Treatment

ClinicalTrials.gov identifier: [NCT03276572](#) was an open-label, dual-center phase I dose-escalation trial. Patients received a single dose of ^{225}Ac -J591 at one of seven predetermined dose levels ranging from 13.3 to 93.3 KBq/kg on the basis of predicted organ dosimetry from previous experience with ^{89}Zr -J591, ^{177}Lu -J591, and ^{111}In -J591 as given in detail in [Appendix 1](#). To minimize exposure to subtherapeutic levels of ^{225}Ac -J591, an accelerated dose-escalation design was used in which single-patient cohorts were used, monitoring for 8 weeks for AEs. If the patient did not experience a grade 2 or higher AE that was attributable to treatment, the next patient would be treated at the next dose level, for levels 1–4. On reaching the fifth dose level (or earlier if there was grade >1 toxicity), the study transitioned to a 3 + 3 dose-escalation design. Lower-dose cohorts with two or more patients were backfilled to increase the cohort size to six per dose level.

This trial was approved by the Institutional Review Boards of participating institutions and was conducted according to the Declaration of Helsinki and Good Clinical Practice. All patients gave written informed consent before enrollment and were provided with radiation safety instructions ([Appendix 1](#)).

End Points and Assessments

The primary end point of the dose-escalation study was the percentage of patients who experienced a dose-limiting toxicity (DLT) within 8 weeks of treatment. DLT was defined as grade ≥ 3 nonhematologic AEs attributable to treatment or severe grade 4 hematologic AEs. The maximum tolerated dose (MTD) was defined as the highest dose in cohorts where two or fewer of the six patients experienced DLT. The recommended phase II dose (RP2D) was the highest dose level reached by a cohort in which two or fewer

patients had a DLT and approved by the study steering committee. After determination of RP2D, additional patients were enrolled in an expansion cohort in a Simon two-stage design. If <2 of the first nine evaluable patients (inclusive of six in dose escalation) did not have a >30% PSA decline, enrollment would halt; otherwise, accrual would continue to the target enrollment of 16 patients at RP2D. The new regimen will be declared effective and worthy of further testing if five or more of 16 experience a 30% PSA decline. This two-stage design yields a 0.80 probability of a positive result if the true 30% PSA decline proportion is 40%. It yields a 0.90 probability of a negative result if the true 30% PSA decline proportion is 15%.

Secondary end points included PSA response (confirmed PSA decline $\geq 30\%$ and $\geq 50\%$ from baseline), radiographic response (modified RECIST,¹⁷ progression-free survival [PFS], OS, AE rate, CTC response [[Appendix 1](#)]), and patient-reported outcomes using the Functional Assessment of Cancer Therapy–Prostate and Brief Pain Inventory–Short Form questionnaires.

Patients were closely monitored for AEs throughout the trial, from the initial screening dose of ^{68}Ga -PSMA-11 to the last follow-up visit (assessment at 1, 2, 4, 8, 12, 16, 20, and 24 weeks after ^{225}Ac -J591, then every 4 weeks until progression, then every 6 months for up to 3 years). AEs were evaluated using the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0.

Statistical Analysis

Median biochemical PFS, radiographic PFS, and OS, including survival curves, were estimated using Kaplan–Meier (K–M) methodology. Greenwood's formula was used to calculate 95% CIs for the K–M estimates. Percent change in PSA from baseline is described by mean/median and standard deviation/interquartile range, as appropriate, depending on the distribution of percent change from baseline. Modified RECIST response (ie, complete response [CR], partial response [PR], and CR/PR proportions), CTC count response proportion, and associated 95% CIs were estimated via binomial proportions.

RESULTS

Radiochemistry studies generally demonstrated a purity of >95%, and the immunoreactivity was >80% with stability documented for at least 72 hours. Murine study results are presented in [Appendix 1](#).

Patient Characteristics and Disposition

Between October 2017 and January 2021, 35 patients were enrolled in which 32 were treated with ^{225}Ac -J591 at one of seven radioactivity dose levels; three were not treated: one because of withdrawal before screening and two because of

ineligibility (low platelets and PSA decline during screening). One patient was treated at each of the first four dose levels given the lack of attributable grade >1 AEs. Dose levels 5–7 had six patients each, and the expansion cohort contained an additional 10 patients (Appendix Table A1). The median age was 70 years, and median baseline PSA was 14.9 ng/mL. Most patients were classified as poor prognostic risk by Cancer and Leukemia Group B (CALGB) criteria¹⁸ (Table 1). Seventy-eight percent (25 of 32) of patients had received at least two previous ARPIs, 63% (20 of 32) had previously received chemotherapy, and 46.9% (15 of 32) had previously been treated with ¹⁷⁷Lu-PSMA. Of the 28 treated at the WCM site (ie, those with PSMA PET), all had at least one tumor with PSMA standardized uptake value (SUV)_{max} > liver SUV_{mean}, with 21 of 28 (75%) having the brightest lesion >5 × liver. The median (SUV)_{max} was 40.5 (range, 9.6–138.5). Eleven (39.3%) had lesions seen on PSMA PET, but not CT/bone scan, and two (7.1%) had lesions seen on CT without significant uptake on PSMA PET (lymph node, liver).

Safety, Tolerability, and Patient-Reported Outcomes

Only one patient experienced DLT during dose escalation (dose level 6, 80 KBq/kg, grade 4 anemia and thrombocytopenia); no patients (0 of 6) at the seventh dose level experienced DLT (Table 2). Therefore, the highest dose level, 93.3 KBq/kg, was the RP2D and used in the expansion cohort. MTD was not achieved as only a single patient experienced DLT.

Treatment-emergent AEs are shown in Table 2. The majority of high-grade AEs were hematologic. In addition to the DLT, three patients had grade 3 anemia, two had grade 3 thrombocytopenia, two had grade 4 thrombocytopenia, two had grade 3 neutropenia, and one had grade 4 neutropenia. All were transient. The nonhematologic AEs were generally of grade 1 or 2, with no grade 3 AEs occurring in more than one patient. Neither of the grade 4 nonhematologic AEs were related to treatment. Higher administered radioactivity was associated with higher-grade hematologic AEs (Table 3).

There were no meaningful changes in patient-reported outcomes comparing baseline with 12-week scores (Appendix 1).

Antitumor Activity

Nine (28.1%) patients had a PSA rise as best response, with a PSA decline in the remainder, including 15 (46.9%) with a >50% PSA decline at any time during follow-up (Fig 1A). Confirmed ≥50% PSA (PSA₅₀) response (Appendix 1) was observed in 11 of 32 (34.4%) patients. On multivariable analysis, no variable (including PSMA PET) was significantly associated with PSA₅₀ although receipt of RP2D (odds ratio, 2.89; *P* = .3) tended to be associated with PSA response (Appendix Table A3). Of 11 patients with measurable disease at baseline and any follow-up imaging results, two (18.2%)

TABLE 1. Patient Demographic and Baseline Characteristics

Characteristic	All Patients (N = 32 ^a)
Age, years, median (range)	69.5 (52-89)
PSA, ng/mL, median (range)	149.1 (4.8-7,168)
Race, No. (%)	
White	30 (93.75)
Black	1 (3.125)
Asian/Pacific Islander	1 (3.125)
ECOG performance status, No. (%)	
0	4 (12.5)
1	22 (68.75)
2	6 (18.75)
LDH, U/L, median (range)	231 (140-844)
CALGB prognostic group, ¹⁸ No. (%)	
Low risk	1 (3.1)
Intermediate risk	8 (25)
High risk	23 (72)
Sites of metastases (conventional imaging), No. (%)	
Bone	31 (97)
Lymph node	28 (88)
Liver	5 (16)
Lung	5 (16)
Circulating tumor cells	N = 22
Median (range)	7 (0-500)
0, No. (%)	8 (36)
1-4, No. (%)	3 (14)
≥5, No. (%)	11 (50)
Previous systemic therapy, ^b No. (%)	
≥1 potent AR pathway inhibitor ^c	32 (100)
≥2 potent AR pathway inhibitors ^c	25 (78)
Chemotherapy	20 (63)
¹⁷⁷ Lu-PSMA	15 (47)
Sipuleucel-T	12 (38)
Radium-223	9 (28)

Abbreviations: AR, androgen receptor; CALGB, Cancer and Leukemia Group B; ECOG, Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase; PSA, prostate-specific antigen; PSMA, prostate-specific membrane antigen.

^aOne patient was enrolled in both dose escalation and dose expansion; baseline demographics at the time of each enrollment are included in Table 1.

^bDoes not include surgical or external beam radiation treatments.

^cPotent androgen receptor pathway inhibitors included enzalutamide, abiraterone acetate, apalutamide, and darolutamide.

had PR, seven (63.6%) had stable disease, and two (18.2%) had progression of disease.

CTC response in patients with paired pre-/post-therapy counts, defined per protocol as a drop from ≥5 CTCs (per 7.5 mL of blood) to ≤4 CTCs, or count remaining at ≤4 CTCs at 12 weeks, occurred in 13 of 22 (59.1%; Fig 1B). Twelve of 16 (75%) with initially detectable CTC count declined

TABLE 2. Treatment-Emergent AEs by Grade (N = 32)

AE	All Grade, No. (%)	Grade 1, No. (%)	Grade 2, No. (%)	Grade 3, No. (%)	Grade 4, No. (%)
Fatigue	26 (81)	11 (34)	14 (44)	1 (3)	
Thrombocytopenia	22 (69)	15 (47)	2 (6)	2 (6)	3 (9)
Nausea	22 (69)	18 (56)	4 (12)		
Anorexia	22 (69)	13 (41)	9 (28)		
Pain	14 (44)	9 (28)	5 (16)		
Xerostomia	12 (38)	11 (34)	1 (3)		
Anemia	11 (34)	3 (9)	4 (12)	3 (9)	1 (3)
Neutropenia	10 (31)	4 (12)	3 (9)	2 (6)	1 (3)
Weight loss	9 (28)	8 (25)	1 (3)		
Dysgeusia	6 (19)	6 (19)			
AST elevation	4 (12)	2 (6)	2 (6)		
ALT elevation	4 (12)	2 (6)	1 (3)	1 (3)	
Diarrhea	3 (9)	3 (9)			
Dizziness	7 (22)	5 (16)	2 (6)		
Dyspnea	5 (16)	4 (12)	1 (3)		
Abdominal pain	4 (12)	3 (9)		1 (3)	
Constipation	4 (12)	4 (12)			
Tooth/gum infection	3 (9)	3 (9)			
Hyperkalemia	3 (9)	2 (6)	1 (3)		
Dehydration	2 (6)		2 (6)		
Hypocalcemia	2 (6)		1 (3)	1 (3)	
Hypotension	2 (6)	2 (6)			
Urinary tract infection	2 (6)	1 (3)	1 (3)		
Upper respiratory tract infection	2 (6)	2 (6)			
Fever	1 (3)			1 (3)	
Sepsis	1 (3)			1 (3)	
Hypercalcemia	1 (3)				1 (3)
Pancreatitis	1 (3)		1 (3)		
Deep vein thrombosis	1 (3)		1 (3)		
Lowered ejection fraction	1 (3)			1 (3)	
Renal failure	1 (3)				1 (3)
Pulmonary embolism	1 (3)			1 (3)	
Bilirubin increase	1 (3)			1 (3)	
Muscle soreness	1 (3)			1 (3)	
Cellulitis	1 (3)			1 (3)	
Rectal bleeding	1 (3)	1 (3)			

Abbreviation: AE, adverse event.

(Fig 1B); six (37.5%) became undetectable. Of 13 with baseline unfavorable count, five (41.7%) converted to favorable. Of six with initially undetectable CTC count, five remained undetectable and one (16.7%) increased.

At the time of last follow-up, nearly all patients progressed and/or died (29 of 32). The median PFS was 5.6 months (95% CI, 3.7 to 7.9), and the median OS was 10.7 months (95% CI, 6.5 to 17.2; Fig 2). On multivariable analysis, only

CALGB prognostic grouping was associated with survival (hazard ratio, 3.94; $P = .04$; Appendix Table A3).

DISCUSSION

To our knowledge, this is the first report of a first-in-human prospective trial of a PSMA-targeted alpha emitter. Treatment with a single dose was tolerated by men with pretreated progressive mCRPC, with a majority of patients experiencing

TABLE 3. Treatment-Emergent Hematologic AEs by Dose Level Administered

Cohort	No.	Dose, KBq/kg	Dose, μ Ci/kg	Gr 1-2 Plts	Gr 3-4 Plts	Gr 1-2 ANC	Gr 3-4 ANC	Gr 1-2 Hgb	Gr 3-4 Hgb
1	1	13.3	0.36					1	
2	1	26.7	0.72	1				1	
3	1	40	1.08	1					
4	1	53.3	1.44						1
5	6 ^a	66.7	1.80	3		1			
6	6	80	2.16	5	1	1	1	2	1
7	6	93.3	2.52	3	1	1	1	2	
Exp	10	93.3	2.52	4	3	4	1	1	2

Abbreviations: AE, adverse event; ANC, absolute neutrophil count (neutropenia); Exp, expansion; Gr, grade; Hgb, hemoglobin (anemia); Plts, platelets (thrombocytopenia).

^aBackfilled to provide additional information.

declines in both PSA levels and CTC counts (71.9% and 75%, respectively). This evidence of efficacy was observed without using PSMA PET for patient selection and with nearly half of patients with previous exposure to PSMA-targeted ¹⁷⁷Lu and more than one quarter with previous ²²³Ra. There was no significant difference in efficacy or hematologic toxicity on the basis of previous exposure to ¹⁷⁷Lu-PSMA although this analysis is limited by small sample size.

PSMA targeting in patients dates back to the development of the first series of mAbs against the extracellular domain of PSMA in 1997¹⁹; J591 beta-RIT established the ability to sensitively and specifically target and image disseminated prostate cancer and to generate antitumor responses measured by PSA declines.¹⁰⁻¹³ The preferred approach became a two-dose fractionated regimen, which is now being studied in a phase III registration study (ClinicalTrials.gov identifier: [NCT04876651](#)). As with all beta-emitting radioantibodies, the relatively long circulating time of mAbs plus the bystander effect of targeting a beta emitter to metastatic disease within the bone marrow resulted in dose-limiting myelosuppression.

Targeting of alpha particles is of interest on the basis of their substantially higher linear energy transfer with the ability to cause double-stranded DNA breaks coupled with a much shorter, more precise range offering the possibility that they would cause a less bystander effect particularly in the bone marrow. Early preclinical studies demonstrated significant antitumor activity.²⁰⁻²²

While, to our knowledge, this is the first prospective trial reported, others have shared retrospective data on PSMA-targeted alpha emitters, mostly using the small-molecule PSMA-617 labeled with ²²⁵Ac. A case report of two patients with heavily pretreated mCRPC who received 100 KBq/kg of ²²⁵Ac-PSMA-617 at 8-week intervals demonstrated near CRs by PSA and PSMA PET/CT, and the only toxicity reported was xerostomia.²³ Additional retrospective studies were subsequently published including 14 and 40 patients.^{24,25} Many had PSA declines with only the minority (12.5%) with a PSA

increase as best response, with a subset (10%) stopping treatment because of xerostomia. The initial South African experience with ²²⁵Ac-PSMA-617 included four patients with noncastrate (ie, castration-sensitive) prostate cancer and 13 with mCRPC.²⁶ In a more recent retrospective data set of 73 patients with mCRPC treated with ²²⁵Ac-PSMA-617, 70% had a $\geq 50\%$ PSA decline.²⁷ Eighty-five percent had xerostomia with a low incidence of high-grade AEs although patients with a superscan pattern on PSMA PET had more hematologic and nonhematologic toxicities. Noting that retrospective studies limit the strength of the conclusions drawn, PSMA-alpha radioligand therapy (RLT) has begun prospective clinical trials (ClinicalTrials.gov identifiers: [NCT04597411](#) and [NCT05219500](#), etc).

In addition to benign and malignant prostate cells, PSMA is expressed on the luminal surface of normal salivary and lacrimal glands, proximal renal tubules, and the small intestine. Interestingly, despite sharing specificity for PSMA, patient imaging and biodistribution studies consistently show differential targeting of the mAbs and small-molecule ligands (SMLs) to these PSMA-positive normal tissue sites.²⁸ We hypothesize that normal tissue barriers such as basement membrane and intercellular tight junctions effectively limit mAb penetration to luminal sites of PSMA expression, whereas SMLs are able to readily penetrate these barriers. In contrast to normal tissues, invasive and metastatic cancers have invaded beyond these physiologic barriers.

Salivary and lacrimal gland damage is a function of both the PSMA targeting agent and the isotope. In prospective studies of PSMA-targeted ¹⁷⁷Lu small molecules, xerostomia ranges from 39% to 87%,^{7,8,29-32} whereas in phase I/II studies of ¹⁷⁷Lu-J591 mAb, <2% of patients experienced grade 1 xerostomia.¹¹⁻¹³ There is a further significant increase in such toxicity when ¹⁷⁷Lu is replaced with ²²⁵Ac, reflecting the substantially greater potency of the alpha particles over beta particles with lower salivary gland uptake on post-treatment PET imaging studies.²³⁻²⁷ In the present trial, 12

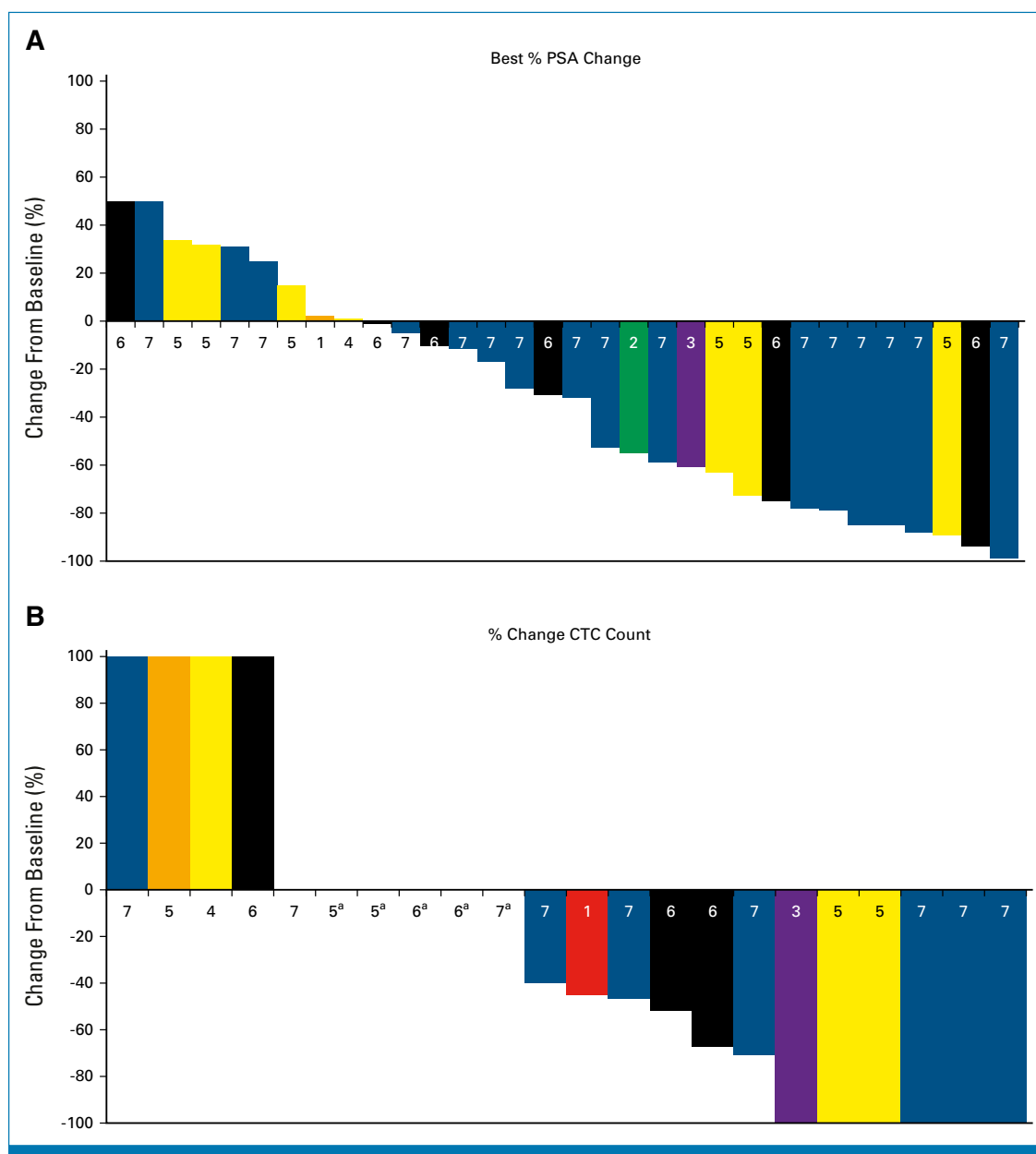


FIG 1. Best PSA and CTC response for each patient. (A) PSA response (N = 32); (B) CTC response (N = 22). *Remained undetectable. Detectable → undetectable. Unfavorable → favorable. CTC, circulating tumor cell; PSA, prostate-specific antigen.

patients experienced xerostomia, 11 of whom had grade 1; 7 of the 12 were in patients previously treated with ^{177}Lu -PSMA. It remains to be seen whether multiple doses of ^{225}Ac -J591 or longer-term follow-up will demonstrate higher frequency or severity of xerostomia, with another early phase I study of PSMA-targeted alpha emitter using an antibody approach (ClinicalTrials.gov identifier: [NCT03724747](https://clinicaltrials.gov/ct2/show/study/NCT03724747)). Longer follow-up is also important to see if there is any significant toxicity in other sites of normal PSMA expression, such as the kidney. One patient on this trial developed renal failure after

disease progression, associated with medication-induced hypotension and intravenous contrast (determined to be unrelated to ^{225}Ac -J591 by nephrology).

In this study, there was no significant association with PSMA PET and outcome. While there are stronger associations with SML PSMA PET and SML ^{177}Lu -PSMA RLT, notably, there are differences in uptake and retention of PSMA mAb and SML.³³ It is unknown if there would have been a tighter association between mAb immunoPET imaging and outcome

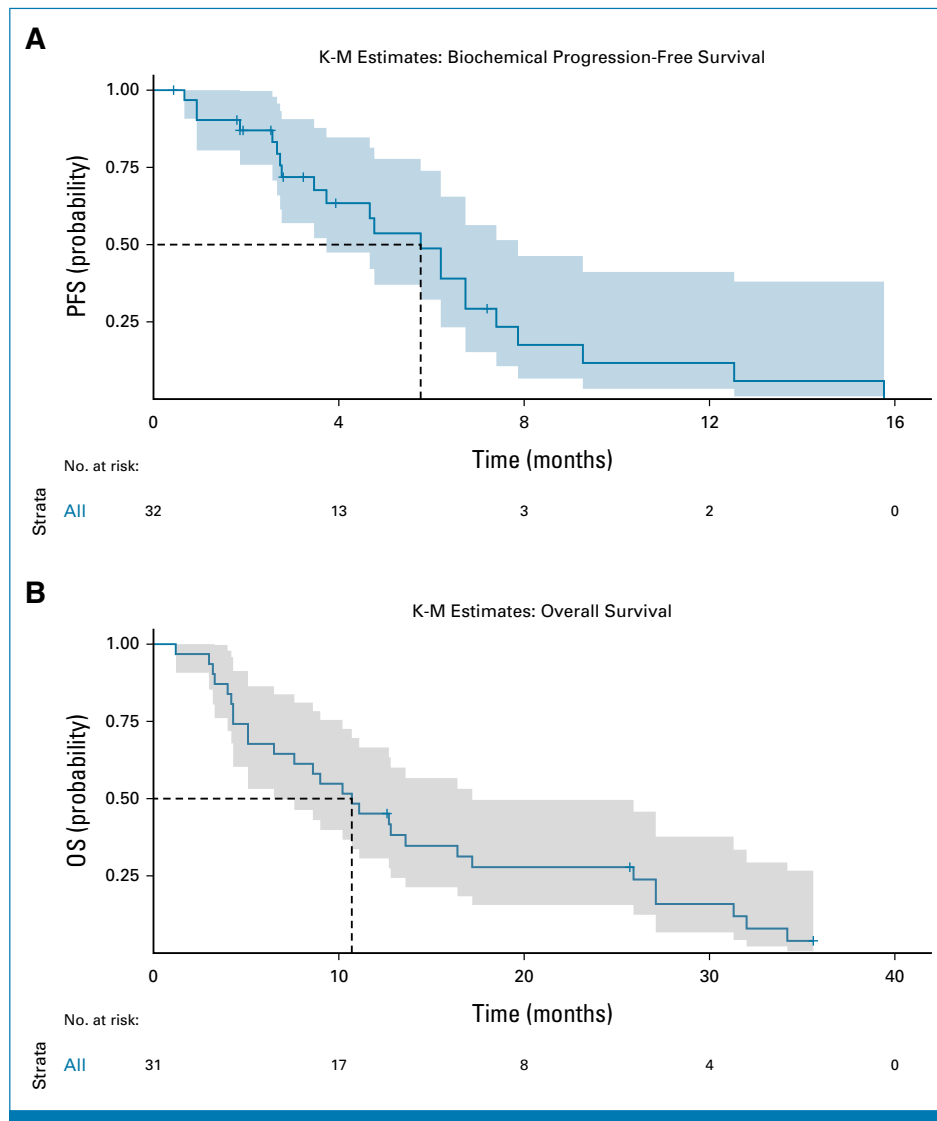


FIG 2. K-M curves for (A) PFS ($n = 32$) and (B) OS ($n = 31$). K-M, Kaplan-Meier; OS, overall survival; PFS, progression-free survival.

with mAb-based RIT. In addition, we hypothesize that mAbs (in particular, those radiolabeled with alpha emitters) may target micrometastatic disease that is not visible with PET.

Liver metastases are associated with poorer prognosis in general, may be associated with worse outcomes with ^{177}Lu -PSMA RLT, and might have lower PSMA expression.^{7,18,33} A post hoc observation (given in detail in [Appendix 1](#)) that two of three patients with liver metastases in this trial had a decrease in measurable tumor volume in the liver after ^{225}Ac -J591 with a fourth patient experiencing stable sub-centimeter liver metastases is consistent with preclinical data that antibodies with longer circulation time have better uptake than small molecules in cells with lower PSMA expression.³⁴ While the longer half-life of Abs provides

increased tumor exposure and improved uptake in tumors, particularly those with lower PSMA expression, the longer half-life likely plays a role in off-target toxicity in the form of bone marrow exposure and myelosuppression. We hypothesize that although the energy level is much higher with alpha emitters, because of their shorter range, myelosuppression may be less significant. The grade 4 thrombocytopenia rate with a single dose of ^{225}Ac -J591 in this study at the RP2D was 9.4% with one (6.3%) platelet transfusion versus 56.3% with grade 4 thrombocytopenia and 41% with transfusions with a single dose of the longer range ^{177}Lu -J591 at the RP2D.¹²

In conclusion, to our knowledge, in this initial first-in-human trial using a single-dose of ^{225}Ac -J591, we found reversible toxicity and good antitumor activity in men with pretreated

progressive mCRPC despite not selecting patients by PSMA PET and with 47% having had previous ^{177}Lu -PSMA. However, since the MTD was not reached and fractionated dosing may improve therapeutic index, a follow-up phase I/II study (ClinicalTrials.gov identifier: [NCT04506567](#)) of multiple and fractionated dosing of ^{225}Ac -J591 is underway to find the highest therapeutic dose that can be given without unacceptable side effects (with or without previous ^{177}Lu -PSMA).

Additional studies are investigating the safety and feasibility of retreatment with ^{225}Ac -J591 (ClinicalTrials.gov identifier: [NCT04576871](#)), whether PSMA-targeted alpha radiation may boost response to anti-PD1 therapy with the AR signaling inhibitor (ClinicalTrials.gov identifier: [NCT04946370](#)), and the safety and efficacy of ^{225}Ac -J591 combined with ^{177}Lu -PSMA I&T (ClinicalTrials.gov identifier: [NCT04886986](#)).

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Prostate-Specific Membrane Antigen–Targeting Alpha Emitter via Antibody Delivery for Metastatic Castration-Resistant Prostate Cancer: A Phase I Dose-Escalation Study of ^{225}Ac -J591

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APPENDIX 1. SUPPLEMENTAL METHODS

Measurement of the Effect—Prostate-Specific Antigen

Biochemical (prostate-specific antigen [PSA]) response: declines of $\geq 30\%$ and $\geq 50\%$ from baseline, confirmed by a second PSA value ≥ 2 weeks later.

Duration of PSA response: time from the first 25% PSA decline until the PSA value is confirmed to increase by 25% above the nadir, provided that the increase is ≥ 2 ng/mL above the nadir.

Biochemical (PSA) progression: $>25\%$ increase above either the baseline level or the nadir, whichever is lowest. PSA must increase by ≥ 2 ng/mL to be considered progression and be confirmed by a second PSA value ≥ 2 weeks later.

Time to PSA progression: interval between treatment and the PSA increase of 25% above nadir, provided that the increase is ≥ 2 ng/mL.

PSA stabilization: any set of values not meeting the criteria for PSA response or PSA progression.

Measurement of the Effect—Radiographic Change in Lesion Size

Measurable disease response will be calculated using RECIST version 1.1 with modifications per Prostate Cancer Working Group 3.

In patients with measurable disease, complete response is defined as complete disappearance of all measurable and evaluable lesions by physical examination or imaging studies and normalization of PSA with no appearance of new lesions for >1 month.

Partial response (PR) is defined as a $\geq 30\%$ reduction in the sum of longest diameters of all measurable lesions and no progression of nontarget lesions or new lesions.

Stable disease is characterized by patients who do not meet the criteria of PR and who are without signs of progressive disease for at least 1 month.

Disease progression is defined as a $>20\%$ increase in the sum of longest diameters of the target lesions or progression of nontarget lesions or the appearance of new lesions.

Bone scan progression (evaluable disease only) requires at least two new lesions seen on a scan subsequent to baseline followed by a repeat scan at least 8 weeks later with at least two new additional lesions.

Measurement of the Effect—Circulating Tumor Cells

We used the CellSearch circulating tumor cell (CTC) enumeration platform, for which it has been established that per 7.5-mL blood sample, a count of ≥ 5 CTCs indicates an unfavorable prognosis and a count of ≤ 4 CTCs indicates a favorable prognosis. Patients who had unfavorable counts at baseline and favorable counts at 12 weeks are considered responders, as are patients who maintained their original favorable count. Patients who maintained their original unfavorable count are considered nonresponders, as were those who transitioned from favorable to unfavorable.

A decrease in CTC counts post-therapy appears to be a favorable marker even if the count remains at least 5, so we analyzed % change in CTC counts; $\geq 50\%$ decline from baseline was also considered response.

In phase III metastatic castration-resistant prostate cancer studies, overall survival (OS) has been associated with conversion from (1) initial unfavorable to favorable CTC count at 12 weeks (2) and detectable to undetectable CTC count at 12 weeks. We reported both these measures.

Synthesis of ^{225}Ac -DOTA-J591 (^{225}Ac -J591)

The humanized monoclonal antibodies—huJ591 (5 mg/mL, 25 mg/vial) and DOTA-HuJ591 (10 mg/mL, 1.2 mL/vial)—were manufactured under good manufacturing practice conditions as previously published. ^{225}Ac (18.5–37 MBq) was purchased from the Oak Ridge National Laboratories (ORNL) of the Department of Energy and supplied as a radiochemical grade ^{225}Ac nitrate dry residue in a 2-mL glass vial. The parent ^{225}Ac is measured when it is in secular equilibrium with its daughters (at least 6 hours after reactions or sample preparations). ^{225}Ac activity was measured in a dose calibrator, previously calibrated using Ac-225 activity from ORNL.

For radiolabeling studies, ^{225}Ac nitrate is first converted to ^{225}Ac chloride by dissolving the dry residue in 0.2 M hydrochloric acid (metal-free) to achieve a concentration of 370 MBq/mL. ^{225}Ac chloride (10–18 MBq/0.050 mL) was first mixed with 2 M

tetramethyl ammonium acetate (0.05–0.125 mL) and L-ascorbic acid solution (2–5 mg) to bring the pH of ^{225}Ac solution to 5.5–6.0. To this solution, DOTA-huJ591 mAb (3 mg/in 0.3 mL) was added. The mixture was gently mixed and incubated at 37°C for 1–2 hours. At the end of incubation, 10 mM diethylenetriamine pentaacetate (DTPA) solution (0.05 mL) was added to stop the labeling reaction and convert the unbound ^{225}Ac to ^{225}Ac -DTPA. ^{225}Ac -huJ591 mAb is purified from any unreactive Ac-225 using the PD-10 gel filtration column, using physiologic saline solution containing 2% human serum albumin. The purified product (4–5 mL) was sterilized and filtered using 0.2 μm membrane sterilizing filter into a sterile, pyrogen free vial. The radiochemical purity (RCP) was determined using instant thin layer chromatography-silica gel and 10 mM ethylenediaminetetraacetic acid solution. The chromatograms were scanned for radioactivity distribution only after the parent-daughter equilibrium was reached. The drug product was released for clinical studies only after quality control testing if all acceptance criteria were met, including appearance, radionuclide identity/purity, RCP, radionuclide concentration, pH, specific activity, endotoxin level, and filter integrity.

Preclinical Studies

Twenty-five Bagg Albino (BALB/c) mice were administered escalating radioactivity doses (1, 2, 3, 4, or 5 KBq) of ^{225}Ac -J591 or control (phosphate buffered saline [PBS]). All mice receiving ^{225}Ac -J591 were alive and healthy at 6 weeks (Appendix Fig A1A).

In a radioimmunotherapy (RIT) dose-escalation study, six cohorts of four nude BALB/c mice were implanted with LNCaP xenografts. After implantation and 10 days after engraftment, escalating doses (62.5, 125, 187.5, 250, or 312.5 KBq/kg) of ^{225}Ac -J591 or control (PBS) were administered. Mouse weight and tumor volume were serially measured. Mice receiving control and the lowest dose of ^{225}Ac -J591 had significant growth of tumor by 2 weeks and had to be sacrificed by day 55, whereas tumor burden was controlled in those receiving higher radioactivity doses of ^{225}Ac -J591 (Appendix Fig A1B).

Predicted Dosimetry

Dose-escalation strategy was based on ^{89}Zr -DF0-J591 studies in patients with prostate cancer, with additional information stemming from ^{177}Lu -J591 and ^{111}In -J591 experience. On the basis of positron-emission tomography (PET) studies ^{89}Zr -J591,^{14,35} the uptake in blood, liver, kidney, and lung was determined. ^{225}Ac dosimetry (Appendix Table A3) for select organs was estimated assuming that the relative biologic effectiveness for alpha emitters is equal to 5 (data provided by Dr O'Donoghue, Memorial Sloan Kettering Cancer Center). The predicted dose-limiting organs for RIT with ^{225}Ac -J591 are red marrow followed by liver, kidney, and lung. Assuming that the dose limit for red marrow is 2 Gy, the maximum predicted activity that can be administered (per cycle) is about 6.06 MBq or 160 μCi .

Patient-Reported Outcomes

Despite a high proportion with at least one AE, there were no meaningful changes in the study population when comparing total Functional Assessment of Cancer Therapy-Prostate (FACT-P) scores, individual FACT-P subscales, or Brief Pain Inventory-Short Form at baseline compared with week 12, noting that there was a drop-off in responses at week 12 (Appendix Table A2). Of the 12 with stability or numerical improvement in FACT-P, 10 (80%) had a $>50\%$ PSA decline versus two of 10 (30%) with numerical worsening of scores having a $>50\%$ PSA decline.

Multivariable Analysis

Multivariable analysis of variables associated with outcome (PSA response, progression-free survival, and OS) included clinical variables (grouped as Cancer and Leukemia Group B nomogram¹⁸ prognostic categories), PSMA imaging (using PSMA PET standardized uptake value (SUV)/max values compared with liver SUVmean as previously published³⁶), previous exposure to ^{177}Lu -PSMA, and administered radioactivity of ^{225}Ac -J591. Results are displayed in Appendix Table A3.

Post Hoc Analysis of Patients With Liver Metastases

Four radiographically evaluable patients had liver metastases at baseline (three with measurable liver metastases detected on computed tomography [CT], one with subcenter liver metastases detected only by PSMA PET at baseline and retrospectively evaluable on CT). As patients with liver metastases are of interest, a post hoc analysis was performed with details in Appendix Table A4.

TABLE A1. Predicted ^{225}Ac -J591 Dosimetry

Organ	Radiation Dose		^{225}Ac -J591 to Be Injected ^a		Maximum Radiation Dose
	Gy/MBq	Gy/MBq	MBq	μCi	Organ, Gy
Bone marrow	0.066	0.33	6.06	165	2
Liver	0.591	2.955			17.9
Kidney	0.382	1.91			11.6
Lung	0.079	0.395			2.39

NOTE. RBE = 5.

Abbreviation: RBE, relative biological effectiveness.

^a ^{225}Ac -J591 maximum activity to deliver 2 Gy to bone marrow.**TABLE A2.** Patient-Reported Outcome Differences Between Baseline and Week 12

Characteristic	No.	Median Difference (IQR)
FACT-P total	19	-4 (-13 to 12)
Prostate cancer subscale	19	3 (-4 to 6)
Physical well-being	19	-1.0 (-4.7 to 1.5)
Social/family well-being	19	0.0 (-1.0 to 1.5)
Emotional well-being	19	0.0 (-2.5 to 1.0)
Functional well-being	19	-1.0 (-5.0 to 2.0)
BPI pain	19	0.0 (-1.0 to 0.6)
BPI interference	14	0.0 (-0.4 to 3.3)

Abbreviations: BPI, Brief Pain Inventory; FACT-P, Functional Assessment of Cancer Therapy-Prostate.

TABLE A3. Multivariable Analyses

Characteristic	PSA Response		Progression-Free Survival		Overall Survival	
	OR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
CALGB group ¹⁸	0.45 (0.06 to 2.91)	.4	1.52 (0.44 to 5.24)	.5	3.94 (1.09 to 14.2)	.04
PSMA PET imaging score ³⁶	1.38 (0.18 to 12.6)	.8	1.07 (0.28 to 4.04)	>.9	1.13 (0.41 to 3.06)	.8
Previous ^{177}Lu -PSMA	0.97 (0.14 to 7.71)	>.9	0.71 (0.23 to 2.17)	.6	1.89 (0.65 to 5.49)	.2
^{225}Ac dose	2.89 (0.48 to 19.9)	.3	0.98 (0.32 to 2.95)	>.9	2.10 (0.76 to 5.84)	.2

Abbreviations: CALGB, Cancer and Leukemia Group B; HR, hazard ratio; OR, odds ratio; PET, positron-emission tomography; PSA, prostate-specific antigen; PSMA, prostate-specific membrane antigen.

TABLE A4. Liver Metastasis Subset Analysis

Site of Metastases (imaging modality)	Liver Metastasis PSMA PET Uptake (SUVmax)	Normal Liver PSMA PET Uptake (SUVmean)	Liver Metastasis Change With Treatment	PSA Change With Treatment	CTC Count Change With Treatment
Liver (CT)	5.87	5.13	3.3 → 5.7 cm	34% increase	40 → not collected
Bone (bone scan and PET)					
Lymph node (PET)					
Liver (CT and PET)	15.21	3.18	2.9 → 2.1 cm	17% decrease	14 → 0
Bone (bone scan and PET)					
Lymph node (CT and PET)					
Liver (CT and PET)	6.71	1.33	2.4 → 1.3 cm	53% decrease	118 → 34
Bone (bone scan and PET)					
Lymph node (CT and PET)					
Liver (PET)	90.56	3.45	Decrease in subcentimeter size (retrospectively seen on CT after PET)	88% decrease	232 → 139
Bone (bone scan and PET)					
Lymph node (PET)					

Abbreviations: CT, computed tomography; CTC, circulating tumor cell; PET, positron-emission tomography; PSA, prostate-specific antigen; PSMA, prostate-specific membrane antigen; SUV, standardized uptake value.

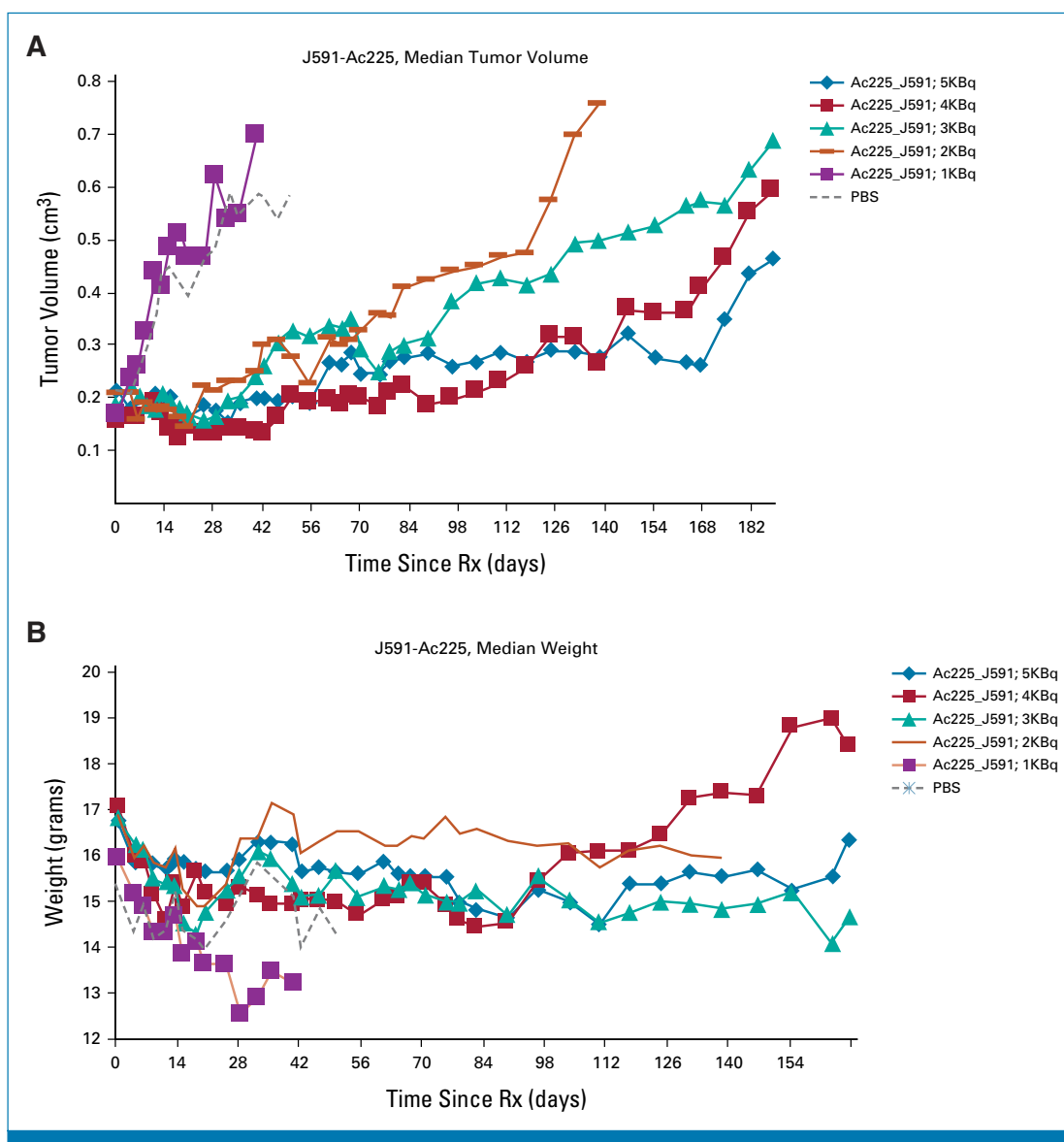


FIG A1. (A) Healthy mice study with ^{225}Ac -J591. (B) Dose-escalation radioimmunotherapy study with ^{225}Ac -J591.