natera | Signatera™
Residual disease test (MRD)

Optimize immunotherapy treatment decisions with a tumor-informed ctDNA assay

Signatera™: personalized, tumor informed ctDNA monitoring with pan-tumor Medicare coverage for immunotherapy response

Prognostic of disease recurrence and progression[1,2]

Signatera™ ctDNA status at baseline is prognostic of disease recurrence and progression across multiple tumor types

Early predictor of treatment benefit

Changes in ctDNA as early as week 6 is a strong predictor of PFS and OS outcomes

Optimized for longitudinal monitoring

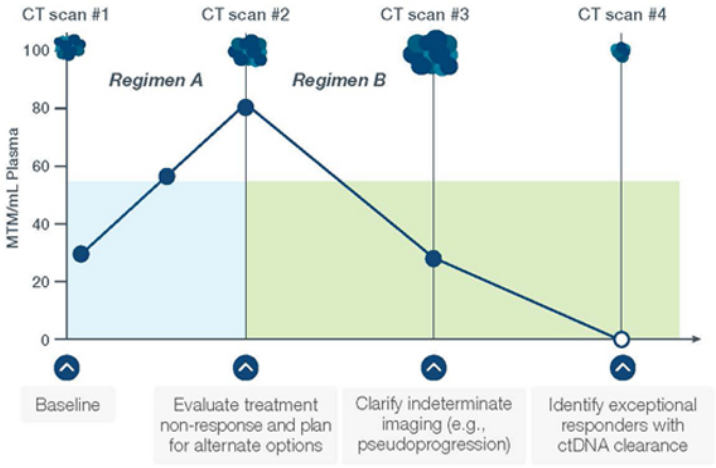
Signatera™ measures real time fluctuations in ctDNA dynamics that can be used to help assess changes in disease burden over time

Established Medicare coverage

Signatera™ is covered by Medicare for immunotherapy response monitoring across all tumor types and stages of cancer

Signatera™ quantifies ctDNA over time to help answer critical questions during immunotherapy treatment

- Is the treatment working?
- Should treatment be changed or escalated?
- Is the cancer actually progressing?
- Is the patient an exceptional responder?



Learn more at **natera.com/signatera-iomonitoring-clinicians**

References

1. Bratman SV, Yang SYC, Iafolla MAJ, et al. Personalized circulating tumor DNA analysis as a predictive biomarker in solid tumor patients treated with pembrolizumab. *Nature Cancer.* 2020;1(9):873 881. **2.** Powles T, Assaf ZJ, Davarpanah N, et al. ctDNA guiding adjuvant immunotherapy in urothelial carcinoma. *Nature*. 2021.

13011 McCallen Pass, Building A Suite 100 | Austin, TX 78753 | natera.com

natera™

Original Article

Phase 1/2 Study of Fractionated Dose Lutetium-177–Labeled Anti–Prostate-Specific Membrane Antigen Monoclonal Antibody J591 (^{177}Lu-J591) for Metastatic Castration-Resistant Prostate Cancer

Scott T. Tagawa, MD [1,4,5]; Shankar Vallabhajosula, PhD[2]; Paul J. Christos, DrPH, MS[3,5]; Yuliya S. Jhanwar, MD[2]; Jaspreet S. Batra, MD[4]; Linda Lam, BA[1]; Joseph Osborne, MD, PhD[2]; Himisha Beltran, MD[1,5,6]; Ana M. Molina, MD[1,5]; Stanley J. Goldsmith, MD[2]; Neil H. Bander, MD[4,5]; and David M. Nanus, MD[1,4,5]

BACKGROUND: Prostate cancer is radiosensitive. Prostate-specific membrane antigen (PSMA) is selectively overexpressed on advanced, castration-resistant tumors. Lutetium-177–labeled anti-PSMA monoclonal antibody J591 (^{177}Lu-J591) targets prostate cancer with efficacy and dose-response/toxicity data when delivered as a single dose. Dose fractionation may allow higher doses to be administered safely. **METHOD:** Men with metastatic castration-resistant prostate cancer refractory to or refusing standard treatment options with normal neutrophil and platelet counts were enrolled in initial phase 1b dose-escalation cohorts followed by phase 2a cohorts treated at recommended phase 2 doses (RP2Ds) comprising 2 fractionated doses of ^{177}Lu-J591 2 weeks apart. ^{177}Lu-J591 imaging was performed after treatment, but no selection for PSMA expression was performed before enrollment. Phase 2 patients had circulating tumor cell (CTC) counts assessed before and after treatment. **RESULTS:** Forty-nine men received fractionated doses of ^{177}Lu-J591 ranging from 20 to 45 mCi/m^2 ×2 two weeks apart. The dose-limiting toxicity in phase 1 was neutropenia. The RP2Ds were 40 mCi/m^2 and 45 mCi/m^2 ×2. At the highest RP2D (45 mCi/m^2 ×2), 35.3% of patients had reversible grade 4 neutropenia, and 58.8% of patients had thrombocytopenia. This dose showed a greater decrease in prostate-specific antigen (PSA) levels and longer survival (87.5% with any PSA decrease, 58.8% with >30% decrease, 29.4% with >50% decrease; median survival, 42.3 months [95% confidence interval, 19.9-64.7]). Fourteen of 17 (82%) patients with detectable CTCs experienced a decrease in CTC count. Overall, 79.6% of patients had positive PSMA imaging; those with less intense PSMA imaging tended to have poorer responses. **CONCLUSION:** Fractionated administration of ^{177}Lu-J591 allowed higher cumulative radiation dosing. The frequency and depth of PSA decrease, overall survival, and toxicity (dose-limiting myelosuppression) increased with higher doses. ***Cancer* 2019;125:2561-2569**. © *2019 American Cancer Society*.

KEYWORDS: prostate cancer, prostate-specific membrane antigen, radioimmunotherapy, monoclonal antibody.

INTRODUCTION

Prostate cancer is radiosensitive, and different forms of radiation have been validated for treatment, including bone-targeting radioisotope therapy.[1-3] However, unsealed radiation sources (^{89}Sr, ^{153}Sm, ^{223}Ra) target sites of increased bone metabolism rather than directly targeting tumors. Consequently, the antitumor effects derive from indirectly targeting only bone metastases; there are no effects on soft tissue or visceral metastases. As such, a method of targeting radiation-emitting particles directly to tumor cells in bone and soft tissue offers potential advantages.

Prostate-specific membrane antigen (PSMA) is an ideal prostate cancer target because: 1) its expression is highly specific, 2) it is expressed by approximately 90% of PCs and 3) we have shown that PSMA functions as an internalizing cell surface receptor.[4,5] We have previously demonstrated that the anti-PSMA monoclonal antibody J591 can be radiolabeled and delivered in a single dose with efficacy in patients with metastatic castration-resistant prostate cancer (mCRPC), but with dose-limiting myelosuppression.[6-11]

Corresponding author: Scott T. Tagawa, MD, Weill Cornell Medicine, Division of Hematology & Medical Oncology, 525 East 68th Street, Box 403, New York, NY 10065; stt2007@med.cornell.edu

[1]Division of Hematology and Medical Oncology, Department of Medicine, Weill Cornell Medicine, New York, New York; [2]Division of Nuclear Medicine, Department of Radiology, Weill Cornell Medicine, New York, New York; [3]Department of Healthcare Policy and Research, Weill Cornell Medicine, New York, New York; [4]Department of Urology, Weill Cornell Medicine, New York, New York; [5]Meyer Cancer Center, Weill Cornell Medicine, New York, New York; [6]Dana Farber Cancer Institute, Boston, Massachusetts.

We thank Lauren Emmerich, Jyothi Sreekumar, Irene Karpenko, Muhammad J. Niaz, Amy Hackett, and John P. Leonard for helpful contributions.

Additional supporting information may be found in the online version of this article.

DOI: 10.1002/cncr.32072, **Received:** November 16, 2018; **Revised:** February 7, 2019; **Accepted:** February 11, 2019, **Published online** April 23, 2019 in Wiley Online Library (wileyonlinelibrary.com)

The degree of antitumor response after administration of targeted radionuclides depends on several variables, especially total (ie, cumulative) radiation dose to the tumor, dose rate, and tumor radiosensitivity.[12] Bone marrow is the dose-limiting organ in radioimmunotherapy.[12,13] Dose fractionation is a practical strategy for decreasing the dose to bone marrow while increasing the cumulative radiation dose to the tumor at an optimal dose rate.[14-16] Here we report safety and efficacy data for a phase 1b/2a dose-escalation study of fractionated dose lutetium-177–labeled anti-PSMA monoclonal antibody J591 (^{177}Lu-J591) in patients with metastatic CRPC.

PATIENTS AND METHODS

Adults with progressive metastatic CRPC were eligible. Any number of previous regimens was allowed except for systemic beta-emitting therapy. Additional entry criteria included Eastern Cancer Cooperative Group performance status 0-2, absolute neutrophil count ≥2000/mm^3, platelet count ≥150,000/mm^3, serum bilirubin level ≤1.5× the upper limit of normal, aspartate aminotransferase level ≤2× the upper limit of normal, and serum creatinine level ≤2.5 mg/dL. This registered study (NCT00538668) was approved by the Weill Cornell Institutional Review Board and the Human Research Protection Office of the US Army Medical Research and Material Command and monitored by the Weill Cornell Medicine Data Safety Monitoring Board. All patients provided written informed consent.

Treatment

Preparation and quality control of ^{177}Lu-J591 was performed as described previously (details are provided in the Supporting Information).[9,11] Patients received 2 doses of ^{177}Lu-J591 2 weeks apart without premedication (dose levels are provided in Supporting Table 1). When dose-limiting toxicity (DLT) and the maximum tolerated dose without growth factor were determined,[17] we simultaneously observed a dose–response relationship in the phase 2 single-dose study[11] and lack of dose-limiting thrombocytopenia with fractionated dosing. Phase 2 patients were then enrolled in the recommended phase 2 dose (RP2D) cohorts in a staged design.

Complete blood counts were monitored at least weekly for 8 weeks. Filgrastim/pegfilgrastim (but not sargramostim) was allowed in phase 2. Chemistry and PSA were monitored at least every 4 weeks. Circulating tumor cell (CTC) count was assessed (CellSearch) at baseline and at 4-6 weeks in phase 2.

No patient selection based on PSMA expression was performed. Planar gamma camera imaging was obtained after ^{177}Lu-J591 infusion. Radiolabeled J591 images were compared with baseline bone scintigraphy and cross-sectional imaging; semiquantitative PSMA expression analysis was performed with a visual score as described previously.[11] Briefly, visual scoring was performed by 2 independent radiologists and was scored as follows: 0, no uptake; 1, weakly positive; 2, definitely positive; 3, equal intensity to liver; 4, greater uptake than liver.

Statistical Plan

The primary endpoint of the phase 1 portion was determination of DLT and RP2D. A modified 3+3 dose-escalation design was employed. DLT was defined as described previously (Supporting Information).[9,11] As above, after completion of the phase 1 portion of the study, an amendment was approved to enroll 2 cohorts in phase 2. Secondary endpoints included proportion with PSA decrease, overall survival, and treatment-emergent adverse events according to Common Terminology Criteria for Adverse Events version 4.

Phase 2 incorporated Simon's 2-stage minimax design, assuming a 10% level of significance and 80% power. If <2 of the first 9 evaluable patients did not experience more than a 30% decrease in PSA, enrollment would be terminated. If ≥2 of the first 9 evaluable patients had a decrease in PSA of >30%, additional accrual proceeded to the target sample size of 16. The new regimen is worthy of further testing if ≥5 of 16 patients had a decrease in PSA of >30%, yielding a 0.80 probability of a positive result if the true 30% PSA decrease proportion is ≥40% and a 0.90 probability of a negative result if the true 30% PSA decrease proportion is <15%.

Kaplan-Meier survival analysis was used to estimate overall survival (OS). Descriptive statistics were performed to characterize the study sample. Fisher's exact test was used to compare PSA decrease response proportions between dose cohorts and between low (0-1) and high (2-4) PSMA visual score. A log-rank test was employed to compare OS among dose cohorts. *P* values were 2-sided, with statistical significance evaluated at the 0.05 alpha level. Analyses were performed using SAS version 9.4 (SAS Institute) and STATA version 15.0 (StataCorp).

RESULTS

In the phase 1 dose-escalation phase, 28 patients were treated between August 2007 and April 2010.[17] An additional 21 patients were treated between June 2010 and August 2014 in phase 2 cohorts. Baseline demographics

TABLE 1. Baseline Patient Characteristics (N = 49)

Characteristic	Value
Age, y, median (range)	72 (52-93)
Gleason sum, n (%)	
6	5 (10.2)
7	15 (30.6)
8	11 (22.4)
9	18 (36.7)
PSA, ng/mL, median (range)	44.9 (1.9-766.5)
Sites of metastases, n (%)	
Bone	42 (85.7)
Lung	13 (26.5)
Liver	6 (12.2)
Lymph node	29 (59.2)
Other	1 (2.0)
ECOG performance status, n (%)	
0	7 (14.3)
1	38 (72.6)
2	4 (8.2)
LDH, U/L, median (range)	192 (99-625)
Hemoglobin, g/dL, median (range)	12.7 (10-16.3)
Alkaline phosphatase, U/L, median (range)	79 (22-490)
CALGB prognostic group, n (%)	
High	28 (57.1)
Intermediate	17 (34.7)
Low	4 (8.2)
Previous therapies, n (%)	
Docetaxel	18 (36.7)
Abiraterone/Enzalutamide	9 (18.4)
Radium-223	1 (2.0)
Sipuleucel-T	5 (10.2)
Radiation	34 (69.4)

Abbreviations: CALGB, Cancer and Leukemia Group B; ECOG, Eastern Cancer Cooperative Group; PSA, prostate-specific antigen; LDH, lactate dehydrogenase.

TABLE 2. Treatment-Emergent Toxicities

CTCAE Toxicity	Grade 1-2	Grade 3	Grade 4	Total
Nonhematologic				
Fatigue	23 (46.9)			23 (46.9)
Hypersensitivity (ie, infusion reaction)	18 (36.7)			18 (36.7)
Weight loss	17 (34.7)	1 (2)		18 (36.7)
Nausea	13 (26.5)			13 (26.5)
Pain, localized	9 (18.3)	4 (8.2)		13 (26.5)
AST (SGOT)	10 (20.4)			10 (20.4)
Dyspnea	9 (18.4)			9 (18.4)
Anorexia	8 (16.3)			8 (16.3)
Edema, limb	7 (14.3)			7 (14.3)
Constipation	6 (12.2)			6 (12.2)
Diarrhea	6 (12.2)			6 (12.2)
Insomnia	6 (12.2)			6 (12.2)
Urinary frequency	6 (12.2)			6 (12.2)
Cough	5 (10.2)			5 (10.2)
Pain, joint	4 (8.2)	1 (2)		5 (10.2)
Epistaxis	4 (8.2)			4 (8.2)
Anxiety	3 (6.1)			3 (6.1)
Depression	3 (6.1)			3 (6.1)
Dizziness	3 (6.1)			3 (6.1)
ALT (SGPT)	2 (4.1)			2 (4.1)
Confusion	2 (4.1)			2 (4.1)
Creatinine	2 (4)			2 (4)
Headache	2 (4.1)			2 (4.1)
Memory loss	2 (4.1)			2 (4.1)
Pain, abdomen NOS	2 (4.1)			2 (4.1)
Weakness	2 (4.1)			2 (4.1)
Arrhythmia	1 (2)			1 (2)
Chest pain	1 (2)			1 (2)
Dry mouth	1 (2)			1 (2)
Dyspepsia	1 (2)			1 (2)
Flu-like symptoms	1 (2)			1 (2)
Hematuria	1 (2)			1 (2)
Hypertension	1 (2)			1 (2)
Numbness	1 (2)			1 (2)
Petechiae/purpura	1 (2)			1 (2)
Rash/desquamation	1 (2)			1 (2)
Thromboembolism (DVT)	0	1 (2)		1 (2)
Hematologic				
Platelets	16 (32.7)	11 (22.4)	19 (38.8)	46 (93.9)
Neutrophils (ANC)	17 (34.7)	12 (24.5)	11 (22.4)	40 (81.6)
Febrile neutropenia		1 (2)		1 (2)
Leukocytes (total WBC)	17 (34.7)	15 (30.6)	6 (12.2)	38 (77.6)
Hemoglobin	21 (42.9)	5 (10.2)	1 (2)	27 (55.1)

Abbreviations: ALT, alanine aminotransferase; ANC, absolute neutrophil count; AST, aspartate aminotransferase; CTCAE, Common Terminology Criteria for Adverse Events; DVT, deep vein thrombosis; NOS, not otherwise specified; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase; WBC, white blood count.
All data are presented as n (%).

are provided in Table 1. Phase 1b patients received between 20 and 45 mCi/m^2 (0.74-1.67 GBq/m^2) per dose. No DLT occurred in cohorts 1-5 [up to 40 mCi/m^2 (1.48 GBq/m^2) ×2] with 1 subject withdrawing from treatment after only 1 dose with grade 2 infusion reaction (complete adverse events are listed in Table 2 and are described below). In cohort 6 [45 mCi/m^2 (1.67 GBq/m^2) ×2], 2 subjects experienced grade 4 neutropenia lasting >7 days (without fever). As described in Patients and Methods, the 40 and 45 mCi/m^2 ×2 cohorts were declared RP2D levels (with allowance for growth factor in phase 2) and expanded, with both cohorts meeting criteria to move forward to stage 2 (16 and 17 subjects at each dose level).

Antitumor Effects and Survival

Overall, 8 (16.3%) patients experienced a ≥50% best decrease in PSA from baseline, 16 (32.7%) patients experienced a ≥30% decrease, and 27 (55.1%) patients experienced any decrease in PSA without need for repeat value for confirmation (Table 3). At the higher RP2D, 87.5% of patients had some PSA decrease, 58.8% had a >30% decrease, and 29.4% had a >50% best PSA decrease. Each subject's best PSA response is depicted in Figure 1. A dose–response relationship was observed, with a higher frequency of any, ≥30%, and ≥50% best PSA decreases with higher doses. The time to progression ranged from 7 to 81.3 weeks, with a median time to progression of 16.7 weeks (95% confidence interval [CI], 14.4-21). Of the 23 patients with measurable disease at baseline, 14 (60.8%) had stable disease and 6 (26.1%) had progressive disease according to Response Evaluation Criteria in Solid Tumors (RECIST); 3 patients withdrew

TABLE 3. Dose Comparison

	Cohorts				
			RP2D (Cohorts 5-6)		
	Total	Low Dose (Cohorts 1-4)	Total	5	6
Dose, mCi/m^2 (×2)	20-45	20-35	40-45	40	45
n	49	16	33	16	17
PSA decrease, %					
Any decrease	55.1	37.5	66.7	50	87.5
>30%	32.7	12.5	42.4	25	58.8
>50%	16.3	6.3	25	12.5	29.4
Median survival, mo (95% CI)	23.6 (15.0-32.2)	14.6 (9.9-19.4)	27.8 (11.1-44.3)	19.6 (9.5-29.8)	42.3 (19.9-64.7)
Platelet grade, %					
3	22.4	6.3	30.3	31.3	29.4
4	38.8	12.5	36.4	43.8	58.8
Platelet transfusion, %	28.6	0	42.4	31.3	53
Neutropenia grade, %					
3	24.5	18.8	27.2	31.3	23.5
4	22.4	0	33.3	31.3	35.3
GCSF use, %	12.2	0	18.2	25	11.8

Abbreviations: CI, confidence interval; GCSF, granulocyte stimulating factor; PSA, prostate-specific antigen.

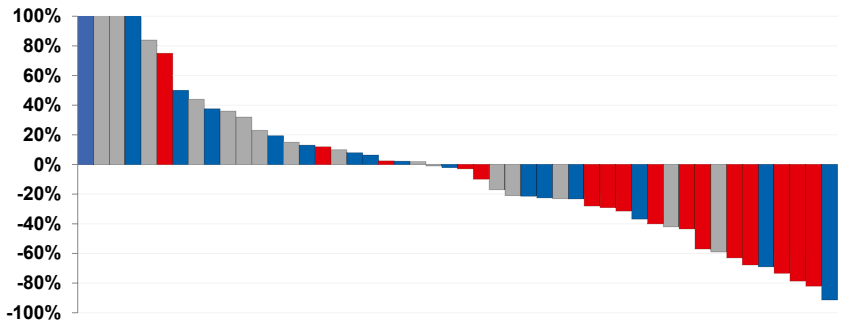


Figure 1. PSA waterfall plot showing each patient's best PSA response. Patients treated with lower doses of ^{177}Lu-J591 (20-35 mCi/m^2 ×2 [cohorts 1-4]) are indicated in light gray; patients who received recommend phase 2 doses are indicated in blue (40 mCi/m^2 ×2 [cohort 5]) or red (45 mCi/m^2 ×2 [cohort 6]).

from treatment and received alternative therapy before follow-up imaging.

Overall survival for the entire treated population was 23.6 months (95% CI, 15.0-32.2) and was significantly longer in the higher-dose cohorts, including a median OS of 42.3 months (range, 19.9-64.7) in the 45 mCi/m^2 ×2 cohort (Fig. 2, Table 3). Because additional drugs were approved during and after the phase 2 portion of the study, survival analysis was conducted using the Cancer and Leukemia Group B (CALGB) prognostic nomogram and accounting for receipt of life-prolonging therapy (defined as docetaxel, sipuleucel-T, cabazitaxel, abiraterone, enzalutamide, radium-223) after ^{177}Lu-J591. CALGB prognostic scores tended to associate with survival ($P = .07$) and subsequent receipt of life-prolonging therapy led to longer OS ($P = .002$). On multivariate analysis, administered dose was associated with longer OS when controlling for CALGB prognostic score and receipt of subsequent life-prolonging therapy, with a hazard ratio for death of 0.53 (95% CI, 0.28-1.01; $P = .053$).

Twenty-five patients in the phase 2 portion of the study had evaluable CTC counts at baseline and follow-up. Of these, CTC counts decreased in 14 (56%) patients, remained undetectable in 8 (32%) patients, and increased in 3 (12%) patients (Supporting Fig. 1). Of the 12 patients with unfavorable CTC counts at baseline (≥5), 8 patients had a favorable count at follow-up, 2 patients experienced a decrease, and 2 patients experienced an increase.

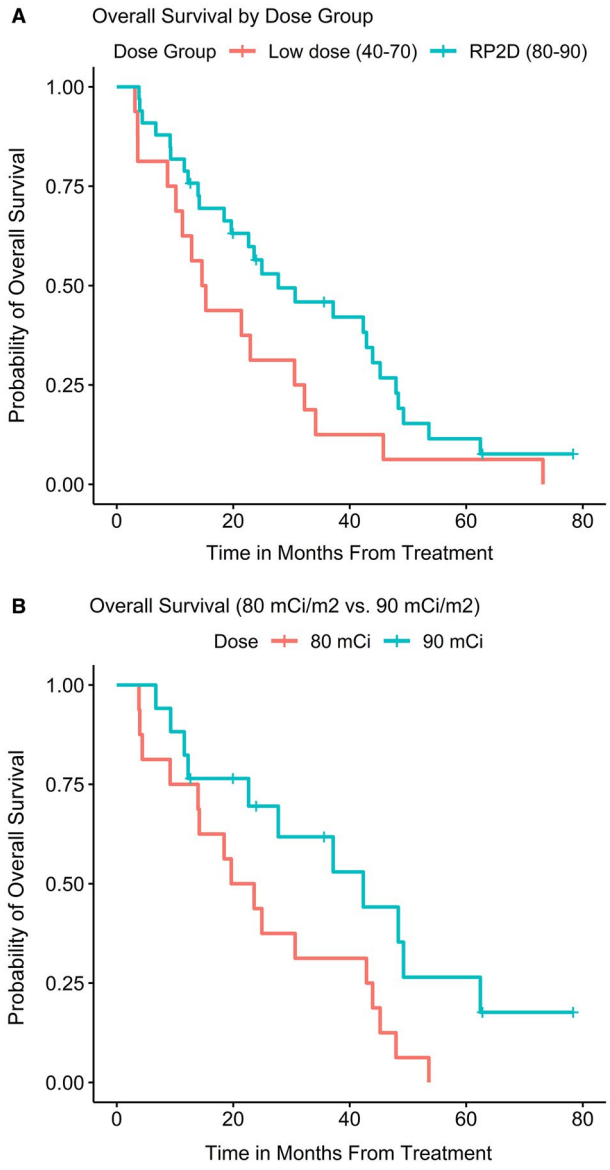


Figure 2. Probability of overall survival by dose received. (A) Low-dose cohorts (40-70 mCi/m^2, cumulative) versus recommend phase 2 dose (RP2D) cohorts. (B) RP2D cohorts (80 and 90 mCi/m^2, cumulative).

Imaging

Thirty-nine of 49 (79.6%) patients had ^{177}Lu-J591 uptake on planar imaging in known sites of disease when compared with baseline computed tomography (CT) and/or magnetic resonance imaging and bone scan images, though those with liver metastases were difficult to assess because of the radiolabeled antibody's hepatic clearance (Fig. 3). PSMA positron emission tomography (PET) imaging was not available at the time of this trial. An analysis of semiquantitative imaging with visual scores[11] and response revealed that patients with visual scores of 0-1 tended to have a lower (21.1%) proportion, with a ≥30% decrease in PSA levels, compared with patients who had visual scores of 2-4 (40.0%) ($P = .17$).

Toxicity

Treatment-emergent toxicities are described in Table 2. As expected, myelosuppression was dose-limiting, with the majority experiencing hematologic toxicity (all-grade anemia in 55.1%, neutropenia in 81.6%, thrombocytopenia in 93.9%). Grade 4 thrombocytopenia occurred in 19 (38.8%) patients, with a platelet nadir at day 41, lasting a median of 6 days (range, 2-23 days); 14 patients received platelet transfusions (median number of transfusions, 2 [range, 1-7]). There were no high-grade hemorrhagic episodes. Fourteen of 19 (73.7%) patients experienced complete platelet recovery within a median of 26 days. Three patients experienced recovery to grade 1, all with concurrent progressive disease by PSA. Two patients with partial platelet count recovery (ie, an increase from nadir) and a subsequent decrease in platelets had concurrent PSA rises and significant prostate cancer infiltration with scant nondysplastic hematopoietic elements on bone marrow biopsy. Eleven (22.4%) patients experienced grade 4 neutropenia up to 17 days in duration (median, 7 days [range, 1-17 days]); 1 patient had febrile neutropenia. Six (12.2%) patients received filgrastim or pegfilgrastim. Hematologic toxicity was greater in the 45 mCi/m^2 ×2 cohort (Table 3), with significantly more platelet transfusions and grade 4 neutropenia. Prior receipt of chemotherapy, radiation, sites of metastatic disease, and amount of uptake on PSMA imaging were not associated with toxicity.

Without premedication, 18 (36.7%) patients experienced transient, reversible infusion reactions consisting of feelings of warmth (with or without temperature changes), cold (without episodes of hypothermia), flushing, rigors, or elevation of blood pressure. One patient with grade 2 reaction after the first dose withdrew from treatment. Twenty-three (46%) patients had low-grade fatigue, 13 (26.5%) patients had low-grade nausea, and 10 (20.4%) patients experienced transient grade 1-2 aminotransferase elevation.

DISCUSSION

Because prostate cancer frequently metastasizes to bone and is radiosensitive, it is not surprising that bone-targeted radioisotopes are useful[1-3]; however, targeting the tumor directly has obvious potential benefits,

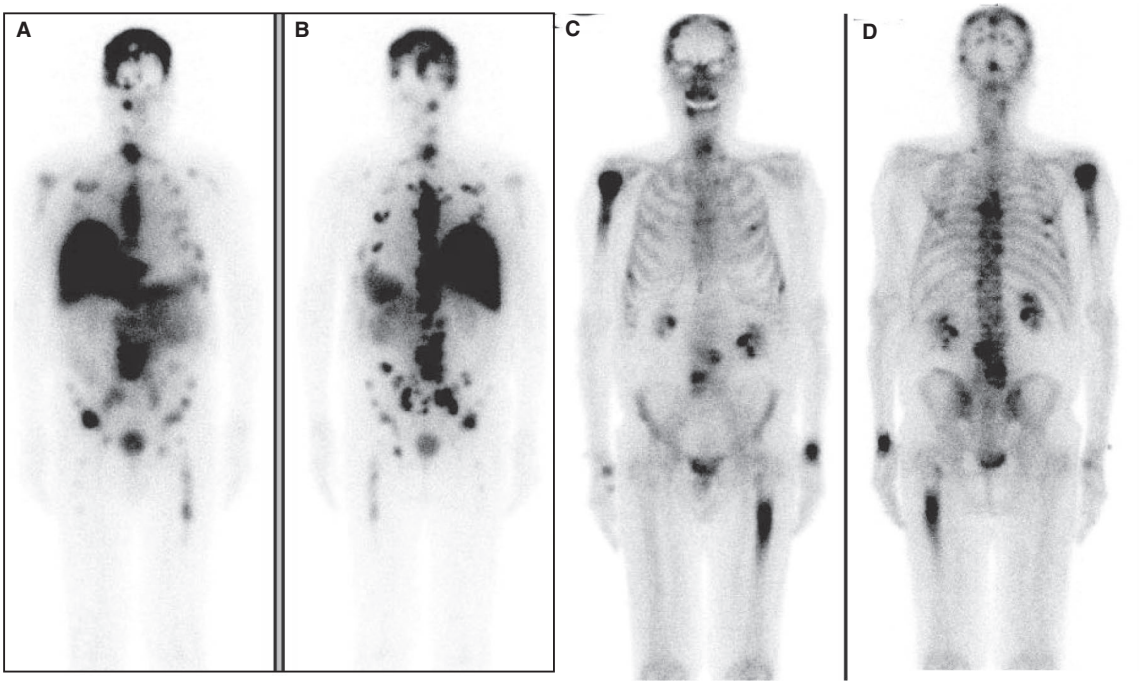


Figure 3. (A, B) ^{177}Lu-J591 imaging. Anterior (A) and posterior (B) total body images were obtained via dual head gamma camera of sites of uptake 7 days after ^{177}Lu-J591 administration. (C, D) ^{99m}Tc-MDP bone scan. Anterior (C) and posterior (D) images of pretreatment bony metastases are shown. (Note: Radiolabeled antibody was partially cleared via the liver, resulting in nonspecific ^{177}Lu localization.).

and PSMA is an ideal tumor target. In addition, PSMA expression increases as androgen receptor (AR) signaling is dysregulated or inhibited, so resistance to AR-targeted therapies such as abiraterone and enzalutamide results in upregulated PSMA expression.[18-20] We showed the ability to target prostate cancer with radiolabeled J591 and demonstrated efficacy with a dose–response effect with ^{177}Lu-J591, with dose-limiting thrombocytopenia.[6-8,10,11]

In this study, we report that a higher cumulative dose of ^{177}Lu can be delivered by dose fractionation with acceptable toxicity. Of note, dose fractionation is conceptually different than retreatment, aiming to deliver a higher cumulative amount of radiation in a single cycle without allowing for tumor repopulation between doses. Our prior dose–response relationship was confirmed, with greater decreases in PSA and longer OS with higher total administered doses. This is consistent with prior dose–response data in large radiation studies.[21,22] However, there is also a clear dose–toxicity relationship. Fortunately, the timing of myelosuppression is predictable and is generally short-term and self-limited; it is manageable with growth factors or transfusion when necessary with uncommon bleeding or infection, and this fractionated dose regimen may be safely delivered with concurrent docetaxel chemotherapy.[23] Many observers have commented that myelosuppression will limit further development of monoclonal antibody-based radioimmunotherapy. In the present study, we detail the severity, duration, and consequences of our hematologic adverse events, include an analysis of subsequent therapy with our OS results, and are comfortable with the proportion of patients with high-grade thrombocytopenia and the need for platelet transfusions. Furthermore, we have published our long-term follow up of 150 patients treated with ^{177}Lu-J591 or ^{90}Y-J591, with all patients having complete neutrophil count recovery and 97.3% with platelet count recovery to grade 0-1; ^{177}Lu-J591 radioimmunotherapy did not prevent subsequent chemotherapy.[24]

Targeting of PSMA with radiopharmaceuticals has increased with the use of PSMA ligands, initially for imaging and subsequently for treatment. Although no direct comparisons can be made due to different patient populations with access to different additional treatments and different methodology, it is of interest to put our study into current context. Most radioligand publications have been retrospective, without clearly defined entry criteria, treatment regimens, or follow-up. The largest published experience is a retrospective 12-center case series of 145

patients with mCRPC treated with ^{177}Lu-PSMA-617, which appears to provide evidence of efficacy and safety, but many patients did not have data for response or toxicity assessment.[25]

More recently, results of the first prospective study of ^{177}Lu-PSMA-617 were published.[26] Australian investigators enrolled 43 men with mCRPC and treated 30 (excluding 7 [16%] due to imaging results). Following PSMA and FDG PET/CT, patients received 4-8 GBq of up to 4 planned doses of ^{177}Lu-PSMA-617. Seventeen (57%) patients had a >50% decrease in PSA levels, with 97% experiencing at least some decrease; 82% of patients with measurable disease had an objective response according to RECIST. The median PFS was 7.6 months, and the median OS was 13.5 months. In this prospective study, as expected with rigorous monitoring, adverse events were more common than previously reported retrospectively. Xerostomia (grade 1-2) occurred in 87% of patients, fatigue in 53% of patients, and nausea in 50% of patients, in addition to other adverse events in smaller proportions. All-cause grade 3/4 anemia occurred in 23% of patients, neutropenia in 7% of patients, and thrombocytopenia in 27% of patients, though attributable toxicity was lower. An additional prospective study has been reported in abstract form using the same drug, but with escalated doses administered in a single fractionated cycle with similar preliminary results.[27]

The selective high prevalence of PSMA expression makes it a target of interest in prostate cancer. Importantly, the biodistribution of radiolabeled anti-PSMA monoclonal antibody differs from that of radioligand therapy due to different mass and pharmacokinetics. Although the DLT of radiolabeled antibodies is myelosuppression (with non1specific bone marrow exposure over many days as a high blood flow organ), PSMA expression in normal tissues (including renal tubules and salivary glands) is not targeted due to a combination of luminal location of PSMA expression, intervening tight junction barriers, and antibody mass. The long circulation times of monoclonal antibody–based radioimmunotherapy is a disadvantage when it comes to myelosuppression, but is advantageous for continuous delivery and uptake of radionuclides by tumor over many days. In contrast to the high incidence of low-grade xerostomia in prospective studies of ^{177}Lu-targeted radioligand therapy, we have not observed this phenomenon (nor targeting of salivary glands by imaging).[6-8,10,11,24,28] Renal toxicity is a potential late side effect of PSMA radioligand therapy.[29] No long-term follow-up has been performed with PSMA-targeted radioligand therapy, but we have not observed any significant acute or delayed nephrotoxicity in our studies with many years of follow-up.[6-8,10,11,24]

Approximately 90% of prostate cancer expresses PSMA, but the incidence of AR and PSMA negative tumors may be increasing and heterogeneity exists.[30,31] We have not selected patients/tumors based on PSMA expression primarily so we would be able to evaluate response across the full spectrum of PSMA expression levels. In our prior single-dose ^{177}Lu-J591 study, we observed a trend toward lower likelihood of response in those with poorer uptake on planar or single-photon emission computed tomography imaging in both retrospective (^{177}Lu) and prospective (^{111}In) imaging cohorts (before the availability of PSMA PET). In this study as well, patients with low visual scores had a lower likelihood of response, although there were still some responses in patients with no or minimal uptake. Patient selection is an ongoing issue to be addressed in current and future studies of anti-PSMA therapeutics, and it is possible that response rates will be higher in a selected population.

Sequential prospective radiolabeled J591 clinical trials have demonstrated targeting, dose response, and improved ability to deliver higher doses with fractionation. With the recent favorable data on PSMA radioligands confirming the value of PSMA targeting and radionuclide therapy, our current goal is to further optimize this therapeutic approach with a series of early phase trials running in parallel.[27,32] Importantly, several randomized studies are in progress including a phase 3 trial of best standard care with or without ^{177}Lu-PSMA-617 (VISION, NCT03511664), ^{177}Lu-PSMA-617 versus cabazitaxel (TheraP, NCT03392428), and ^{177}Lu-J591 vs ^{111}In-J591 (control) in non-metastatic (M0) CRPC (NCT00859781).

In conclusion, targeting metastatic castration-resistant tumors with radiolabeled anti-PSMA antibody J591 has antitumor efficacy. Our hypothesis that a higher cumulative dose could be safely delivered with a dose-fractionation strategy is confirmed. Myelosuppression remains the DLT, with predictable and manageable neutropenia and thrombocytopenia. A relationship exists between administered dose and efficacy (PSA decrease and OS) as well as toxicity.

FUNDING SUPPORT

This work was supported by US Department of Defense grant W81XWH-04-1-0267; the Prostate Cancer Foundation Young Investigator Award; National Institutes of Health grants ULI RR024996, 1-KL2-RR024997-01, and PTBF5405; the David H. Koch Foundation; the Robert Dow Foundation; and the Lawrence and Carol Zicklin Charitable Trust.

CONFLICT OF INTEREST DISCLOSURES

Neil H. Bander is an inventor of patents assigned to the Cornell Center for Technology Licensing for the J591 antibody described in this article. He is also a paid consultant for and holds equity in BZL Biologics, LLC, the company to which these patents were licensed for further research and development. The other authors have no relevant disclosures.

AUTHOR CONTRIBUTIONS

Scott T. Tagawa: conceptualization, methodology, investigation, resources, writing (original draft), writing (review and editing), visualization, supervision and funding acquisition. **Shankar Vallabhajosula:** conceptualization, methodology, investigation, resources, writing (review and editing), and funding acquisition. **Paul J. Christos:** methodology, formal analysis, data curation, writing (original draft). **Yuliya S. Jhanwar:** investigation and visualization, writing (review and editing). **Jaspreet S. Batra:** conceptualization, methodology, writing (original draft), visualization, data curation, project administration. **Linda Lam:** resources, data curation, project administration, writing (review and editing). **Joseph Osborne:** methodology, investigation and visualization, writing (review and editing). **Himisha Beltran:** conceptualization, methodology, investigation, resources, writing (review and editing), visualization, supervision. **Ana M. Molina:** conceptualization, methodology, investigation, resources, writing (review and editing), visualization, supervision. **Stanley J. Goldsmith:** investigation and visualization, writing (review and editing). **Neil H. Bander:** conceptualization, methodology, investigation, resources, writing (original draft), writing (review and editing), visualization, supervision and funding acquisition. **David M. Nanus:** conceptualization, methodology, investigation, resources, writing (original draft), writing (review and editing), visualization, supervision and funding acquisition.

REFERENCES

1. Porter AT, McEwan AJ, Powe JE, et al. Results of a randomized phase-III trial to evaluate the efficacy of strontium-89 adjuvant to local field external beam irradiation in the management of endocrine resistant metastatic prostate cancer. *Int J Radiat Oncol Biol Phys*. 1993;25:805-813.
2. Sartor O, Reid RH, Hoskin PJ, et al. Samarium-153-Lexidronam complex for treatment of painful bone metastases in hormone-refractory prostate cancer. *Urology*. 2004;63:940-945.
3. Parker C, Nilsson S, Heinrich D, et al. Alpha emitter radium-223 and survival in metastatic prostate cancer. *N Engl J Med*. 2013;369:213-223.
4. Silver DA, Pellicer I, Fair WR, Heston WD, Cordon-Cardo C. Prostate-specific membrane antigen expression in normal and malignant human tissues. *Clin Cancer Res*. 1997;3:81-85.
5. Liu H, Rajasekaran AK, Moy P, et al. Constitutive and antibody-induced internalization of prostate-specific membrane antigen. *Cancer Res*. 1998;58:4055-4060.
6. Bander NH, Trabulsi EJ, Kostakoglu L, et al. Targeting metastatic prostate cancer with radiolabeled monoclonal antibody J591 to the extracellular domain of prostate specific membrane antigen. *J Urol*. 2003;170:1717-1721.
7. Morris MJ, Divgi CR, Pandit-Taskar N, et al. Pilot trial of unlabeled and indium-111-labeled anti-prostate-specific membrane antigen antibody J591 for castrate metastatic prostate cancer. *Clin Cancer Res*. 2005;11:7454-7461.
8. Milowsky MI, Nanus DM, Kostakoglu L, Vallabhajosula S, Goldsmith SJ, Bander NH. Phase I trial of yttrium-90-labeled anti-prostate-specific membrane antigen monoclonal antibody J591 for androgen-independent prostate cancer. *J Clin Oncol*. 2004;22:2522-2531.
9. Bander NH, Milowsky MI, Nanus DM, Kostakoglu L, Vallabhajosula S, Goldsmith SJ. Phase I trial of 177lutetium-labeled J591, a monoclonal antibody to prostate-specific membrane antigen, in patients with androgen-independent prostate cancer. *J Clin Oncol*. 2005;23:4591-4601.
10. Tagawa ST, Beltran H, Vallabhajosula S, et al. Anti-prostate-specific membrane antigen-based radioimmunotherapy for prostate cancer. *Cancer*. 2010;116(4 suppl):1075-1083.
11. Tagawa ST, Milowsky MI, Morris M, et al. Phase II study of Lutetium-177-labeled anti-prostate-specific membrane antigen monoclonal antibody J591 for metastatic castration-resistant prostate cancer. *Clin Cancer Res*. 2013;19:5182-5191.
12. Larson SM, Carrasquillo JA, Cheung NK, Press OW. Radioimmunotherapy of human tumours. *Nat Rev Cancer*. 2015;15:347-360.
13. Ali AM, Dehdashti F, DiPersio JF, Cashen AF. Radioimmunotherapy-based conditioning for hematopoietic stem cell transplantation: Another step forward. *Blood Rev*. 2016;30:389-399.
14. DeNardo GL, Schlom J, Buchsbaum DJ, et al. Rationales, evidence, and design considerations for fractionated radioimmunotherapy. *Cancer*. 2002;94(4 suppl):1332-1348.
15. Shen S, Duan J, Meredith RF, et al. Model prediction of treatment planning for dose-fractionated radioimmunotherapy. *Cancer*. 2002;94(4 suppl):1264-1269.
16. O'Donoghue JA, Sgouros G, Divgi CR, Humm JL. Single-dose versus fractionated radioimmunotherapy: model comparisons for uniform tumor dosimetry. *J Nucl Med*. 2000;41:538-547.
17. Tagawa ST, Vallabhajosula S, Osborne J, et al. Phase I trial of fractionated-dose 177lutetium radiolabeled anti-prostate-specific membrane antigen (PSMA) monoclonal antibody J591 (177Lu-J591) in patients (pts) with metastatic castration-resistant prostate cancer (metCRPC). *J Clin Oncol*. 2010;28(15 suppl):4667-4667.
18. Wright GL Jr, Grob BM, Haley C, et al. Upregulation of prostate-specific membrane antigen after androgen-deprivation therapy. *Urology*. 1996;48:326-334.
19. Evans MJ, Smith-Jones PM, Wongvipat J, et al. Noninvasive measurement of androgen receptor signaling with a positron-emitting radiopharmaceutical that targets prostate-specific membrane antigen. *Proc Natl Acad Sci U S A*. 2011;108:9578-9582.
20. Meller B, Bremmer F, Sahlmann CO, et al. Alterations in androgen deprivation enhanced prostate-specific membrane antigen (PSMA) expression in prostate cancer cells as a target for diagnostics and therapy. *EJNMMI Res*. 2015;5:66.
21. Kuban DA, Tucker SL, Dong L, et al. Long-term results of the M. D. Anderson randomized dose-escalation trial for prostate cancer. *Int J Radiat Oncol Biol Phys*. 2008;70:67-74.
22. Zietman AL, Bae K, Slater JD, et al. Randomized trial comparing conventional-dose with high-dose conformal radiation therapy in early-stage adenocarcinoma of the prostate: long-term results from Proton Radiation Oncology Group/American College of Radiology 95-09. *J Clin Oncol*. 2010;28:1106-1111.
23. Tagawa ST, Whang YE, Kaur G, et al. Phase I trial of docetaxel/prednisone plus fractionated dose radiolabeled anti-prostate-specific membrane antigen (PSMA) monoclonal antibody ^{177}lu-J591 in patients with metastatic, castration-resistant prostate cancer (mCRPC). *J Clin Oncol*. 2014;32(suppl 5). doi:10.1200/jco.2014.32.15_suppl.5064
24. Tagawa ST, Akhtar NH, Nikolopoulou A, et al. Bone marrow recovery and subsequent chemotherapy following radiolabeled anti-prostate-specific membrane antigen monoclonal antibody j591 in men with metastatic castration-resistant prostate cancer. *Front Oncol*. 2013;3:214.
25. 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.
26. Hofman MS, Violet J, Hicks RJ, et al. [^{177}Lu] PSMA 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.
27. Tagawa ST, Vallabhajosula S, Jhanwar Y, et al. Phase I dose-escalation study of fractionated dose ^{177}Lu-PSMA-617 for progressive metastatic castration resistant prostate cancer (mCRPC). *Ann Oncol*. 2018;29(suppl 8). doi:10.1093/annonc/mdy284.008
28. Pandit-Taskar N, O'Donoghue JA, Durack JC, et al. A phase I/II study for analytic validation of 89Zr-J591 immunoPET as a molecular imaging agent for metastatic prostate cancer. *Clin Cancer Res*. 2015;21:5277-5285.
29. Kiess AP, Minn I, Vaidyanathan G, et al. (2S)-2-(3-(1-carboxy-5-(4-211At-astatobenzamido)pentyl)ureido)-pentanedioic acid for PSMA-targeted alpha-particle radiopharmaceutical therapy. *J Nucl Med*. 2016;57:1569-1575.

30. Tosoian JJ, Gorin MA, Rowe SP, et al. Correlation of PSMA-Targeted (18)F-DCFPyL PET/CT findings with immunohistochemical and genomic data in a patient with metastatic neuroendocrine prostate cancer. *Clin Genitourin Cancer*. 2017;15:e65-e68.
31. Beltran H, Wyatt AW, Chedgy EC, et al. Impact of therapy on genomics and transcriptomics in high-risk prostate cancer treated with neoadjuvant docetaxel and androgen deprivation therapy. *Clin Cancer Res*. 2017;23:6802-6811.
32. Miyahira AK, Pienta KJ, Morris MJ, et al. Meeting report from the prostate cancer foundation PSMA-directed radionuclide scientific working group. *Prostate*. 2018;78:775-789. doi:10.1002/pros.23642