Despite intense efforts to develop radiotracers to detect cancers or monitor treatment response, few are widely used as a result of challenges with demonstrating clear clinical use. We reasoned that a radiotracer targeting a validated clinical biomarker could more clearly assess the advantages of imaging cancer. The virtues and shortcomings of measuring secreted prostate-specific antigen (PSA), an androgen receptor (AR) target gene, in patients with prostate cancer are well documented, making it a logical candidate for assessing whether a radiotracer can reveal new (and useful) information beyond that conferred by serum PSA. Therefore, we developed $^{89}$Zr-labeled 5A10, a novel radiotracer that targets “free” PSA. $^{89}$Zr-5A10 localizes in an AR-dependent manner in vivo to models of castration-resistant prostate cancer, a disease state in which serum PSA may not reflect clinical outcomes. Finally, we demonstrate that $^{89}$Zr-5A10 can detect osseous prostate cancer lesions, a context where bone scans fail to discriminate malignant and nonmalignant signals.

SIGNIFICANCE: This report establishes that AR-dependent changes in PSA expression levels can be quantitatively measured at tumor lesions using a radiotracer that can be rapidly translated for human application and advances a new paradigm for radiotracer development that may more clearly highlight the unique virtues of an imaging biomarker.
INTRODUCTION

The serendipitous discovery that $^{18}$F-fluorodeoxyglucose uptake reflects aberrant c-KIT signaling (and predicts response to imatinib therapy) in gastrointestinal stromal tumors has greatly simplified patient management (1) and, more generally, underscores the promise of molecular imaging in oncology. However, deliberately engineering radiotracers to achieve similar success in other tumors has proven challenging, resulting in the high attrition rate of novel radiotracers in the clinic.

The target of a radiotracer necessarily frames its potential context of use (i.e., detection, response indicator), and candidates are often selected on the basis of preclinical evidence pointing to an upregulation in cancer. In this regard, it can be challenging to appropriately evaluate novel radiotracers in patients without a thorough appreciation of the pathobiologic mechanism of target upregulation. Therefore, we reasoned that a radiotracer targeting a well-studied, tumor-specific clinical biomarker reflective of oncogenic pathway activation could more rapidly document the unique advantages of studying patient response with a cognate noninvasive imaging tool.

To establish this concept, we selected prostate-specific antigen (PSA) based on the large body of research highlighting the virtues and shortcomings of measuring the secreted forms of this protein in prostate cancer (2–4). Originally, it was hoped that measuring total concentrations of PSA in serum might revolutionize prostate cancer management by allowing early detection of subclinical disease and precise monitoring of residual disease after therapy. This hope was based on the fact that PSA is expressed almost exclusively by prostate epithelia and, as an androgen receptor (AR) target gene, its expression reflects AR pathway activity. Years of work to clinically validate this biomarker have revealed certain limitations. For example, despite strong associations between metastasis or death from prostate cancer and PSA levels in blood (5), the inability to distinguish PSA produced by normal versus malignant prostate tissue limits its general use in primary screening. Moreover, apart from a few contexts in which a dramatic reduction confirms successful therapeutic intervention (e.g., postradical prostatectomy), interpreting changes in serum PSA levels in response to therapy has been problematic. At first glance, this may seem surprising because PSA expression is tightly coupled to AR signaling. However, the ability to detect PSA in serum not only requires expression, but also secretion and leakage into the circulation—two processes that are very poorly understood (2). Also, it is well documented that only a very small percentage of intratumoral PSA is secreted into the perivascular space (6), and the rate-determining step to serum circulation is undefined. These considerations raise the possibility that a noninvasive tool measuring tumor-associated PSA expression could more clearly reflect AR-driven changes in PSA expression.

PSA is initially produced as a catalytically active serine protease [“free” PSA (fPSA)], and subsequent to its release into the perivascular space, it is rapidly and irreversibly converted to noncatalytic forms [“complexed” PSA (7, 8)]. We therefore reasoned that 5A10, a monoclonal antibody that specifically recognizes an epitope adjacent to the catalytic cleft of PSA (9–11), and therefore selectively binds fPSA, could in principle target tumor-associated PSA. In considering radiolabeling strategies, we noted that our recent studies with $^{89}$Zr-labeled monoclonal antibodies (mAb) yielded high-contrast images of tumors with low radiotracer uptake in normal tissues (12–14). Based on these observations, we prepared $^{89}$Zr-labeled SA10.

RESULTS

We conjugated $^{89}$Zr to SA10 with the chelator desferrioxamine B through a previously established synthetic route (See Methods and Supplementary Fig. S1) (12, 14). To determine the affinity of $^{89}$Zr-SA10 for PSA, competition binding assays were conducted, and the bioconjugation of SA10 resulted in virtually no loss of affinity for purified PSA (Supplementary Fig. S2).

We began in vivo studies by administering $^{89}$Zr-SA10 to intact male mice inoculated with subcutaneous xenografts of LNCaP-AR [a PSA-positive prostate cancer model derived from parental LNCaP overexpressing wild-type AR (15)]. This model was chosen because the magnitude of PSA production and circulation in mouse serum closely approximates that observed in patients with advanced disease (Supplementary Table S1). Tissues were harvested at multiple time points postinjection to determine the kinetics of radiotracer biodistribution (Fig. 1A). Peak tumor-associated activity was observed 24 hours postinjection (19.5% ± 4.9% ID/g, tumor-to-muscle ratio: 22.80 ± 18.6), and with few exceptions, little $^{89}$Zr-SA10 accumulation was observed in host tissues (Fig. 1A; Supplementary Fig. S3, and Supplementary Tables S2 and S3). Positron emission tomography (PET) studies showed a region of contrast at the tumor, supportive of the biodistribution data (Fig. 1B). Using 100-fold excess of unlabelled SA10 to compete $^{89}$Zr-SA10 uptake, we confirmed the specificity of the biologic interaction between $^{89}$Zr-SA10 and LNCaP-AR (Fig. 1C, Supplementary Fig. S4, and Supplementary Tables S4 and S5). Moreover, there was little incorporation of the nonspecific radiotracer $^{89}$Zr-labeled mouse IgG3 in LNCaP-AR at 24 hours postinjection (Fig. 1C, Supplementary Fig. S5, and Supplementary Tables S6 and S7). As expected, PC3 xenografts, an AR- and PSA-negative model of human prostate cancer, showed little avidity for $^{89}$Zr-SA10 at 24 hours postinjection (Fig. 1C, Supplementary Fig. S6, and Supplementary Table S8). Finally, subcutaneous CWR22Rv1 xenografts were avid for $^{89}$Zr-SA10, another AR- and PSA-positive prostate cancer model (Fig. 1C, Supplementary Fig. S6, and Supplementary Table S9).

We next asked whether $^{89}$Zr-SA10 could detect androgen-regulated elevations in fPSA expression. Castrated male mice were inoculated with LNCaP-AR, and after tumor formation, animals received no manipulation or a surgically implanted subcutaneous testosterone pellet. $^{89}$Zr-SA10 was administered 7 days postmanipulation, and biodistribution studies were conducted 24 hours postinjection. $^{89}$Zr-SA10 localization was significantly higher in LNCaP-AR xenografts exposed to testosterone compared with control (Fig. 1D, Supplementary Fig. S7, and Supplementary Table S10). Intratumoral PSA levels
also increased, as expected (Supplementary Table S11). Similar results were observed with CWR22Rv1 xenografts (Supplementary Fig. S8 and Supplementary Tables S12 and S13). Collectively, these results show that $^{89}\text{Zr}$-SA10 can faithfully reflect intratumoral AR signaling.

We next tested if pharmacologic inhibition of AR can be quantified in vivo with $^{89}\text{Zr}$-SA10 PET using the anti-androgen MDV3100, whose clinical activity is correlated with responses in the LNCaP-AR model (16, 17). Castrated male mice were inoculated with subcutaneous LNCaP-AR xenografts, and tumor-bearing mice were randomized into groups receiving a daily oral gavage of vehicle or MDV3100 at 10, 40, or 80 mg/kg. Seven days postinitiation of treatment, $^{89}\text{Zr}$-SA10 was administered, and biodistribution studies were conducted 24 hours postinjection (Fig. 2A, Supplementary Fig. S9, and Supplementary Table S14). As expected, this course of therapy inhibited tumor growth and PSA protein in the tumor (Supplementary Fig. S10 and Supplementary Table S15). Accordingly, tumor-associated $^{89}\text{Zr}$-SA10 was significantly decreased by a 40- and 80-mg/kg dose of MDV3100 (Fig. 2A). Statistically significant changes in tumor-associated $^{89}\text{Zr}$-SA10 were observed by PET between groups of animals receiving a daily oral gavage of vehicle or 80 mg/kg MDV3100 (Fig. 2B and C and Supplementary Table S16). In addition, a significant ST16 increase in tumor-associated $^{89}\text{Zr}$-SA10 was observed in a separate treatment arm of mice receiving subcutaneous testosterone pellets. Notably, the fold change in tumor-associated $^{89}\text{Zr}$-SA10 closely mirrored the fold change in expression of intratumoral PSA. For example, the testosterone pellet resulted in an approximate 2.5-fold increase in tumor-associated $^{89}\text{Zr}$-SA10 (Supplementary Table S10), very close to the approximate 2.6-fold increase in PSA expression in the LNCaP-AR tumors (Supplementary Table S11). Collectively, these results highlight the ability of $^{89}\text{Zr}$-SA10 to measure pharmacologically triggered changes in intratumoral AR signaling.

One of the challenges in evaluating experimental therapies like MDV3100 is assessing therapeutic effects on individual metastatic lesions in patients with diffuse disease (18).
FIGURE 2. $^{89}$Zr-SA10 detects pharmacologic inhibition of AR in vivo. A, biodistribution data from castrated male mice bearing LNCaP-AR xenografts show that MDV3100 inhibits localization of $^{89}$Zr-SA10 to tumor. Animals were treated with vehicle, or the indicated dose of MDV3100 for 7 days, at which time $^{89}$Zr-SA10 was injected, and animals were harvested for biodistribution studies 24 hours postinjection. $^*P < 0.01$ for the 40 mg/kg and 80 mg/kg dose of MDV3100 compared to vehicle or 10 mg/kg MDV3100. B, representative transverse (Trans.) and coronal PET slices of intact male mice bearing LNCaP-AR xenografts on the right flank and imaged with $^{89}$Zr-SA10 24 hours postinjection after manipulation with a subcutaneous testosterone (Test.) pellet or a daily oral gavage of vehicle (Veh.) or MDV3100 (80 mg/kg) for 7 days. Clear visual differences in tumor-associated $^{89}$Zr-5A10 can be seen between the groups. Arrows indicate the position of the tumor (T) and the murine liver (L). C, region-of-interest analysis of the tumors from the PET study shows statistically significant changes in tumor-associated $^{89}$Zr-5A10. $^*P < 0.01$ compared with vehicle. $^{**}P < 0.05$ compared with vehicle. Error bars represent the standard deviation from mean.

Although bone is the most common site of metastatic spread in prostate cancer (19), metastases remain particularly challenging to characterize, because the most widely used nuclear medicine technologies (e.g., $^{99m}$Tc-MDP, $^{18}$F-NaF) do not directly image the tumor but, rather, target nearby normal bone repair (20). Therefore, these scans cannot distinguish between malignant and nonmalignant disease (e.g., injury or degenerative joint disease). Furthermore, response to androgen deprivation therapy cannot be efficiently assessed because resolution of tumor-induced bone repair can lag clinical response by months or years.

With these considerations in mind, we asked if $^{89}$Zr-SA10 can more clearly distinguish a skeletal prostate cancer lesion. Osseous tumors were established in intact male mice through injection of LNCaP-AR in the tibia of the left hindlimb, and tumor development was confirmed by MRI after 7 weeks (Supplementary Figs. S11 and S12). PET/computed tomography (CT) studies showed high contrast in the tumor-bearing hindlimb compared with the contralateral limb (Fig. 3A). Consistent with this observation, coregistered PET/MRI images showed an alignment of the PET and MRI contrast in the tibia (Fig. 3B and Supplementary Fig. S13). Postmortem autoradiography of a surgically excised tibia showed that positron emissions from $^{89}$Zr-SA10 coaligned with the topography of the LNCaP-AR lesion defined by histology (Supplementary Fig. S14). To further confirm that $^{89}$Zr-SA10 does not crossreact with bone remodeling, bone fractures were induced surgically in a separate cohort of mice by puncturing the tibia in the right hindlimb. Both $^{18}$F-NaF and $^{99m}$Tc-MDP readily localized to the site of repair, whereas $^{89}$Zr-SA10 did not (Fig. 3C). Collectively, these results highlight the unique specificity of $^{89}$Zr-SA10 for prostate cancer tumors in the bone.

**DISCUSSION**

In this report, we show that changes in the expression of PSA, an AR-regulated, prostate-specific gene, can be measured noninvasively with the novel radiotracers $^{89}$Zr-SA10. In support of its suitability for quantifying AR signaling in castration-resistant prostate cancer, $^{89}$Zr-SA10 readily localized to multiple AR- and PSA-positive prostate cancer models and quantitatively measured declines in PSA synthesis induced by androgen therapy in a clinically validated xenograft model of castration-resistant prostate cancer. Because $^{89}$Zr-SA10 specifically targets prostate cancer cells rather than the nonmalignant skeletal pathologies that phosphoryl the changes induced by cancers on bone scans, this radiotracer offers the opportunity for more accurate staging and better treatment selection. Owing to the abundant expression of PSA also in benign pathologies of the prostate (2), unambiguously detecting prostate cancer lesions within the prostate gland with $^{89}$Zr-SA10 may be challenging. In this respect, the most exciting immediate clinical application for $^{89}$Zr-SA10 is likely the opportunity to study AR-driven tumor activity in individual lesions in a heterogeneous disease to enhance the clinical assessment of advanced disease.

We previously reported that changes in prostate-specific membrane antigen (PSMA), a cell surface protein whose expression is suppressed by AR, can also serve as a noninvasive marker for imaging of AR signaling (21). Although PSMA imaging with PET is attractive because several human-ready targeting agents already exist (22, 23), PSMA expression is not prostate specific and the clinical impact of AR-directed therapy on PSMA expression is not known (24). Also, a formal comparison in LNCaP-AR xenografts showed that $^{89}$Zr-SA10 PET resulted in a more compelling change in tumor localization posttherapy than $^{89}$Zr-J391, a radiotracer derived from a humanized monoclonal antibody to PSMA (Supplementary Fig. S15).
The precise mechanism by which $^{89}$Zr-SA10 allows visualization of AR- and fPSA-positive prostate cancer models is unclear. Our data and published work suggest the following model. Before secretion, fPSA exists as a proteolytically active protein (25) and then is transiently present in the pericellular space before sequestration by extracellular-binding proteins that preclude recognition by SA10. We propose that visualization of prostate cancer cells by $^{89}$Zr-SA10 is dependent on this ability of SA10 to recognize fPSA in this unique context. Future studies are required to refine our understanding of the biologic basis of localization of $^{89}$Zr-SA10 to tumor in situ, which may also further enhance our understanding of the pathobiologic mechanism(s) of fPSA secretion and processing. Regardless of the mechanism, the molecular imaging tool presented here could have near-term clinical impact, particularly because the dosimetry of other $^{89}$Zr-labeled mAbs has been determined to be favorable for humans and will soon be examined in clinical trials (26).

**METHODS**

**Preparation of Zirconium-89**

Zirconium-89 was produced through the $^{89}$Y(p,n)$^{89}$Zr transmutation reaction on an EBCO TR19/9 variable-beam energy cyclotron (EBCO Industries, Inc.) in accordance with previously reported methods (27). $^{89}$Zr-oxalate was isolated in high radiochemical purity > 99.9% with an effective specific activity of 195 to 497 MBq/μg (5.27–13.31 mCi/μg).

**Preparation of Radiolabeled Constructs**

Desferrioxamine B (DFO) was conjugated to antibodies using the following protocol. Antibody (in phosphate-buffered saline) was added to a centrifuge vial and the pH adjusted to 9.5 to 10.0 with Na$_2$CO$_3$(aq.). Four equivalents of [Fe(N-succDFO-TFP)] were added, and the reaction was conducted at room temperature for 1 hour. The pH of the reaction was then adjusted to 3.9 to 4.2 by the slow addition of 0.25 M H$_2$SO$_4$(aq.), and a 10-fold excess of ethylenediaminetetraacetic acid disodium salt was added. The reaction was incubated in a water bath at 38°C for 1 hour. The DFO-conjugated antibody was purified by size-exclusion chromatography (Sephadex G-25 M, PD-10 column; GE Healthcare).

Typical radiolabeling reactions were conducted according to previously reported methods (12) used for labeling monoclonal antibodies with $^{89}$Zr. Briefly, $^{89}$Zr-oxalate [429 MBq (11.6 mCi)] in 1.0 M oxalic acid (250 μL) was adjusted to pH 7.1 to 7.7 with 1.0 M Na$_2$CO$_3$(aq.). DFO-conjugated SA10 [500 μL, 2.0 mg/mL (1.0 mg of protein), in sterile saline] was added and the reaction was mixed gently. The reaction was incubated at room temperature for between 1 and 2 hours and progress was monitored with instant thin-layer chromatography (ITLC; diethylene triamine pentaacetic acid, 50 mM, pH 7). After 2 hours, crude radiolabeling yields and RCP were typically > 80% to 90%. $^{89}$Zr-SA10 was purified by using spin-column centrifugation [4 mL total volume, > 30 kDa particle retention (Amicon Ultra-4; Millipore), washed with 4×3 mL sterile saline]. The radiochemical purity of the final $^{89}$Zr-SA10 (formulation: pH 5.5–6.0; < 500 μL; sterile saline) was measured by ITLC and size-exclusion chromatography. In the ITLC experiment, the $^{89}$Zr-SA10, $^{89}$Zr-IgG, and $^{89}$Zr-DFO remain at the baseline ($R_1 = 0$), whereas $^{89}$Zr$^{4+}$(aq.) ions and the complex $^{89}$Zr-diethylenetriaminepentaacetic acid (DTPA) elute with the solvent front ($R_1 = 1.0$). The final radiochemical yield of the purified $^{89}$Zr-SA10 was typically > 70% and the product was formulated in sterile saline with a radiochemical purity > 99% (n = 5) and a specific activity of 195.0 ± 8.0 MBq/mg (5.27 ± 0.2 mCi/mg) of protein. Supplementary Figure S1 shows a typical radio-ITLC chromatogram of the crude and purified (formulated) $^{89}$Zr-SA10.
Cell Lines

PC3 and CWR22Rv1 were purchased from American Type Culture Collection, and AR and PSA expression was annotated by immunoblot and ELISA. The cell lines were cultured according to the manufacturer’s instructions. LNCaP-AR was previously developed and reported by the Sawyers laboratory (15). The cell line was authenticated for AR overexpression and PSA expression by immunoblot.

Animal Studies

All animal experiments were conducted in compliance with institutional guidelines at Memorial Sloan-Kettering Cancer Center. Male CB-17 severe combined immunodeficient (SCID) mice (6–8 weeks old) were obtained from Taconic Farms, Inc.; LNCaP-AR, CWR22Rv1, and PC3 tumors were inoculated in the right flank by subcutaneous injection of 1.0 × 10⁶ cells in a 200-μL cell suspension of a 1:1 v/v mixture of media with Matrigel (Collaborative Biomedical Products, Inc.). Tumors developed after 3 to 7 weeks. MDV3100 was dissolved in dimethyl sulfoxide (DMSO) so that the final DMSO concentration when administered to animals would be 5%. The formulation of the vehicle is 1% carboxymethyl cellulose, 0.1% polysorbate 80, and 5% DMSO. MDV3100 or vehicle was administered daily by gavage. Tumor volume (V/mm³) was estimated with caliper measurements.

Preparation of Osseous Tumor Grafts and Bone Fracture Model

Before surgery, castrated male SCID mice were anesthetized with ketamine, and an incision was made in the left hindlimb. The tibia was punctured using a bone drill, and 1 × 10⁴ cells (CWR22Rv1 or LNCaP-AR) were injected into the cavity. The puncture was closed with bone wax, the incision sutured, and animals received a palliative dose of carprofen (5 mg/kg) once daily for 3 days post surgery. Tumor development was followed with bioluminescence imaging and confirmed with MRI. The bone fracture model was prepared similar to the osseous tumor model, excluding injection of cells and application of bone wax.

Biodistribution Studies

Biodistribution studies were conducted to evaluate the uptake of ⁸⁹Zr-SA10 in human prostate cancer xenograft models. Mice received ⁸⁹Zr-SA10 [1.11–1.85 MBq (30–50 μCi), 5.7–9.5 μg of protein, in 200 μL sterile saline for injection] through intravenous tail-vein injection (t = 0 hour). Animals (n = 4–5 per group) were euthanized by CO₂ asphyxiation at 1, 4, 12, 24, 48, 72, 96, and 120 hours postinjection and blood was immediately harvested by cardiac puncture. Sixteen tissues (including the tumor) were removed, rinsed in water, dried in air for 5 minutes, weighed, and counted on a gamma-counter for accumulation of ⁸⁹Zr radioactivity. The tumor tissues were partitioned for biodistribution or PSA ELISA. The mass of ⁸⁹Zr-SA10 formulation injected into each animal was measured and used to determine the total number of counts per minute by comparison to a standard syringe of known activity and mass. Count data were background- and decay-corrected and the tissue uptake [measured in units of percent injected dose per gram (% ID/g)] for each sample was calculated by normalization to the total amount of activity injected.

Small-Animal Positron Emission Tomography Imaging

PET imaging experiments were conducted on a micro-PET Focus 120 scanner (Concorde Microsystems). In repeated studies (n = 4) mice were administered formulations of ⁸⁹Zr-SA10 [10.4–12.6 MBq [280–340 μCi], 53.1–64.5 μg of protein, in 200 μL sterile saline for injection] through intravenous tail-vein injection. Approximately 5 minutes before recording PET images, mice were anesthetized by inhalation of 1% to 2% isoflurane (Baxter Healthcare)/oxygen gas mixture and placed on the scanner bed. PET images were recorded at various time points between 1 and 120 hours postinjection. List-mode data were acquired using a γ-ray energy window of 350 to 750 keV and a coincidence timing window of 6 nanoseconds. For all static images, scan time was adjusted to ensure a minimum of 20 million coincident events were recorded. Data were sorted into 2-dimensional histograms by Fourier rebinning, and transverse images were reconstructed by filtered back-projection into a 128 × 128 × 63 (0.72 × 0.72 × 1.3 mm) matrix. The reconstructed spatial resolution for ⁸⁹Zr was 1.9 mm full-width half-maximum at the center of the field of view. The image data were normalized to correct for non-uniformity of response of the PET, dead-time count losses, positron branching ratio, and physical decay to the time of injection, but no attenuation, scatter, or partial-volume averaging correction was applied. An empirically determined system calibration factor [in units of (mCi/mL)/(cps/voxel)] for mice was used to convert voxel count rates to activity concentrations. The resulting image data were then normalized to the administered activity to parameterize images in terms of percentage injected dose per gram. Manually drawn 2-dimensional regions of interest were used to determined the maximum and mean percentage injected dose per gram (decay corrected to the time of injection) in various tissues. Images were analyzed by using ASIPro VM™ software (Concorde Microsystems).

Coregistered Positron Emission Tomography/Computed Tomography

CT images were acquired on a small-animal Siemens/CTI microCAT II (Siemens Medical Solutions) scanner with an 8.5-cm axial by 5.0-cm transaxial field of view. Coregistered PET/CT images were recorded and mapped to a matrix in accordance with previously reported methods (28).

Magnetic Resonance Imaging

Mouse prostate MR images were acquired on a Bruker 4.7-T Biospec scanner operating at 200 MHz and equipped with a 400 mT/m inner diameter 12-cm gradient coil (Bruker Biospin MRI GmbH). A custom-built quadrature birdcage resonator with inner diameter of 32 mm was used for radiofrequency excitation and acquisition (Stark Contrast MRI Coils Research, Inc.). Mice were anesthetized with oxygen and 1% isoflurane gas. Animal breathing was monitored by using a small-animal physiologic monitoring system (SA Instruments, Inc.). T2-weighted scout images along 3 orthogonal orientations were first acquired for animal positioning. The T2-weighted fast spin-echo rapid acquisition with relaxation enhancement (RARE) sequence was used to acquire axial mouse pelvic images with a slice thickness of 0.8 mm and field of view 30 mm × 34 mm with a spatial resolution of 117 × 133 μm. The following acquisition parameters—TR = 4.5 s, TE = 40 ms, RARE factor 8, and an acquisition time of 20 minutes—were used.

PSA Detection in Serum and Tumor Tissues

Free PSA and total PSA were measured with a dual-label immunofluorometric assay (DELFI A Prostatex™ PSA Free/Total PSA; Perkin-Elmer Life Sciences) according to the manufacturer’s recommendations. This assay measures free PSA and complexed PSA in an equimolar fashion (29), and the crossreactivity of PSA-ACT for free PSA is less than 0.2% (10). The lower limits of detection are 0.05 μg/L for total PSA [coefficient of variation (CV) = 5.0% at 2.32 μg/L] and 0.04 μg/L for free PSA (CV = 5.9% at 0.25 μg/L). For detection, the 1235 automatic immunoassay system from Perkin-Elmer Life Sciences was used. Complexed PSA concentrations were calculated by subtracting free PSA from total PSA.

Affinity Tests of ⁸⁹Zr-Labeled SA10

The capture mAb H117 was immobilized onto microtiter plates through physical adsorption by using low-fluorescence Maxisorp

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strips (Nunc). The wells were coated with 1 μg of the mAb H117 in 100 L of buffer containing 0.2 mol/L NaH₂PO₄ buffer overnight at 37°C. Coated wells were washed twice with DELFIA wash solution and were then saturated for 3 hours at room temperature with 300 μL of a solution containing DTPA-treated bovine serum albumin (1 g/L), sorbitol (60 g/L), diazolidinyl urea (1 g/L), and 50 μmol of NaH₂PO₄. After saturation, the wells were aspirated, dried, and stored at 4°C in sealed plastic bags with desiccant until use.

Using a fresh sample of ²⁰³Zr-SA10, affinity assays were conducted by adding a solution of PBS (25 μL, 47.7 ng/mL) in 100 μL of DELFIA assay buffer to each well from the previously prepared plates. After incubation for 1 hour at room temperature, the solution was aspirated, and the wells were washed twice with assay buffer. The binding assay was initiated with the addition of 200-μL aliquots of assay buffer containing 20 μg of ²⁰³Zr-SA10 and 0.0, 0.002, 0.02, 0.2, 0.5, 1, 2, 4, 6, 8, or 10 μg of unlabeled SA10. All reactions were conducted in duplicate. The reactions were incubated, with slow shaking, for 2 hours, at which time the wells were aspirated and rinsed 4 times with DELFIA wash solution. The bound activity was determined in a NaI (TI) well counter (Perkin Elmer 2480 Automatic Gamma Counter).

**Autoradiography and Tissue Histology**

After mice were euthanized, the tibia, including the tumor, was surgically excised and embedded in optimal cutting temperature compound (Miles, Inc.) and snap-frozen on dry ice in a cryomold. Sets of 10 contiguous 5-μm-thick tissue sections were cut using a Microm HM500 cryostat microtome (Microm International) and arrayed onto poly-L-lysine-coated glass microscope slides. Tissue sections were fixed in 10% phosphate-buffered formalin for 5 minutes, washed twice, air-dried, and stained with hematoxylin and eosin (H&E). Stained tissue sections were placed in a film cassette against a Fuji film BAS-MS2325 imaging plate (Fuji Photo Film Co.) to acquire digital autoradiograms. The slides were exposed for 48 hours, approximately 168 hours after injection of ²⁰³ZrCl₃ or ²⁰³Zr-SA10. Exposed phosphor plates were read by a Fujifilm BAS-1800II bio-imaging analyzer (Fuji Photo Film Co.) generating digital images with 50-μm pixel dimensions. Digital images were obtained with an Olympus BX60 System Microscope (Olympus America, Inc.) equipped with a motorized stage (Prior Scientific, Inc.). Subsequently, H&E images were acquired to the same resolution as the DAR data. DAR images were manually aligned to the H&E images using rigid planar transforms.

**Statistical Analyses**

Data were analyzed by using the unpaired, 2-tailed Student’s t test. Differences at the 95% confidence level (P < 0.05) were considered to be statistically significant.

**Disclosure of Potential Conflicts of Interest**

C.L. Sawyer is a co-inventor of MDV3100 and owns stock in the company (Medivation) that is developing the drug for prostate cancer treatment. H. Lilja is a co-inventor of IPAS assays for in vitro diagnostics in blood (Arctic Partners). This article does not make any claims about the efficacy of MDV3100 or the diagnostic value of IPAS measurements in the blood; it merely uses MDV3100 and IPAS measurements in serum as tools to evaluate the SA10 antibody-based radiotracer described herein.

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