In Vitro Characterization of Radiolabeled Monoclonal Antibodies Specific for the Extracellular Domain of Prostate-specific Membrane Antigen

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ABSTRACT

Prostate-specific membrane antigen (PSMA) is a well-characterized cell surface antigen expressed by virtually all prostate cancers (PCaS). PSMA has been successfully targeted in vivo with 111In-labeled 7E11 monoclonal antibody (mAb; ProstaScint; Cytogen, Princeton, NJ), which binds to an intracellular epitope of PSMA. This work reports the in vitro characterization of three recently developed mAbs that bind the extracellular domain of PSMA (PSMAext). Murine mAbs J415, J533, and J591, and 7E11 were radiolabeled and evaluated in competitive and saturation binding studies with substrates derived from LNCaP cells. J415 and J591 were conjugated to 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid labeled with 111In. The uptake and cellular processing of these antibodies were evaluated in viable LNCaP cells. All four mAbs could be labeled with 111In up to a specific activity of 350 MBq/mg with no or little apparent loss of immunoreactivity. Competition assays revealed that J415 and J591 compete for binding to PSMAext antigen. J533 bound to a region close to the J591 binding epitope, but J533 did not interfere with J415 binding to PSMA. mAb 7E11 did not inhibit the binding of J415, J533, or J591 (or vice versa), consistent with earlier work that these latter mAbs bind PSMAext, whereas 7E11 binds the intracellular domain of PSMA. Saturation binding studies demonstrated that J415 and J591 bound with a similar affinity (Kd 1.76 and 1.83 nM), whereas J533 had a lower affinity (Kd 18 nM). In parallel studies, all four mAbs bound to a similar number of PSMA sites expressed by permeabilized cells (1,000,000–1,300,000 sites/cell). In parallel studies performed with viable LNCaP cells, J415, J533, and J591 bound to a similar number of PSMA sites (i.e., 600,000–800,000 sites/cell), whereas 7E11 bound only to a subpopulation of the available PSMA sites (95,000 sites/cell). This apparent binding of 7E11 to viable cells can be accounted for by a 5–7% subpopulation of permeabilized cells produced when the cells were trypsinized and suspended. Up to five DOTA chelates could be bound to either J415 or J591 without compromising immunoreactivity. A comparison of the cellular uptake and metabolic processing of the 111In- and 131I-labeled antibodies showed a rapid elimination of 111In from the cell and a high retention of 131I. All four mAbs recognized and bound to similar numbers of PSMAexpressed by ruptured LNCaP cells (i.e., the exposed intracellular and extracellular domains of PSMA). By comparison to J415 and J591, J533 had a lower binding affinity. Both J415 and J591 recognized and bound to the same high number of PSMA by intact LNCaP cells. By contrast, 7E11 bound to fewer sites expressed by intact LNCaP cells (i.e., the exposed extracellular domain of PSMA). Both J415 and J591 are promising mAbs for the targeting of viable PSMA-expressing tissue with diagnostic and therapeutic metallic radionuclides.

INTRODUCTION

PCa is the most frequently diagnosed cancer and the second most common cause of cancer mortality in United States males (1). Many groups have studied mAbs for in vivo diagnosis and therapy of PCa (3–8), but the only successful application to date has been the targeting of PSMA for in vivo imaging. PSMA is a type II membrane protein that is expressed by virtually all PCaS (9, 10). Unlike other prostate-related antigens, such as prostate-specific antigen, prostatic acid phosphatase, and prostate secretory protein, PSMA is an integral membrane protein, and therefore, it is not appreciably released into the circulation. PSMA expression has been shown to be up-regulated in both poorly differentiated (11), advanced PCaS (9) and after androgen-deprivation therapy (12). Interestingly, PSMA is expressed on the tumor vascular endothelium of other carcinomas and sarcomas (13, 14) but not on normal vascular endothelium, making it also potentially useful as an antibody-mediated diagnostic and therapeutic target across the full spectrum of solid tumors.

Currently, an 111In-labeled form of the 7E11 murine mAb is approved by the Food and Drug Administration (Prostascint) for the clinical detection of recurrent and metastatic prostate cancer in soft tissue (15). 7E11 mAb binds to the intracellular portion (NH2 terminus) of the PSMA antigen and, as such, does not bind viable cells (13, 14). It is believed that successful imaging with Prostascint results from mAb binding to antigen expressed in dead or dying cells within some tumor sites (16, 17). Early clinical trials using 90Y-labeled 7E11 resulted in no objective or biochemical (prostate-specific antigen) remissions (7).

Recently, a series of mAbs to PSMAext has been characterized and reported (13, 14, 18). In this current study, we report on the in vitro evaluation of radiolabeled forms of these antibodies against PSMAext and the selection of interesting candidates for in vivo evaluation of their diagnostic and therapeutic potential.

MATERIALS AND METHODS

Murine mAbs J415, J533, and J591 were produced as described earlier (13). Purified 7E11 was generously provided by Dr. Gerald P. Murphy (Pacific Northwest Research Foundation, Seattle, WA). 131I and 111In were purchased from Norton International (Kanata, Ontario, Canada). 90Y was purchased from New England Nuclear (Boston, MA). DOTA was purchased from Macro cyclics, Inc. (Richardson, TX).

LNCaP cells (American Type Culture Collection, Rockville, MD) were grown in RPMI 1640, supplemented with 10% FCS, at a temperature of 37°C in an environment containing 5% CO2. Prior to use, the cells were trypsinized, counted, and suspended in serum-free medium. LNCaP cells were permeabilized by adding methanol at −80°C to the cells. The cells were maintained at −20°C for 20 min before the methanol was removed, and the cells were rehydrated by washing four times with PBS (with 5 mM Ca2+ and 5 mM Mg2+) over 20 min.

Cell membranes were prepared by lysing the cells with a Polytron in a...
hypotonic buffer [1 mm Na₂CO₃ (pH 7.4) with 1 mm EDTA and 1 mm phenylmethylsulfonyl fluoride]. Large fragments were removed by centrifuging at 2000 × g. The supernatant was centrifuged at 150,000 × g for 2 h, and the pellet membranes were resuspended in PBS, aliquoted, and frozen at −70°C until required.

Radioiodination

The four murine mAbs were radiolabeled with 131I using the Iodogen (1,3,4,6-tetrachloro-3a,4a-diphenylglycoluril) method (19). Briefly, 10-ml glass tubes were coated with 50 μg of Iodogen by adding 100 μl of a 0.5 mg/ml solution of Iodogen (Pierce, Rockford, IL) in chloroform. The chloroform was removed by blowing a gentle stream of sterile nitrogen into the tube for 30 min before the tubes were sealed and stored in the dark. The iodination reaction was initiated by adding between 4 and 40 MBq of 131I (0.01 M NaOH) to 0.08 mg of mAb in 0.1 ml of ice-cold PBS. This reaction mixture was allowed to react for 5 min on ice before being loaded onto a 10-ml Biogel-P6 column (Bio-Rad Laboratories, Hercules, CA) equilibrated with 1% BSA in PBS. Once the reaction mixture was loaded onto the column, it was washed with 2 ml of 1% BSA PBS before the main 131I-labeled mAb fraction was eluted with 2 ml of 1% BSA PBS. The amount of free iodine in the 131I-labeled mAb preparations was evaluated using instant TLC with a silica gel impregnated glass fiber support and a mobile phase of isotonic saline. Briefly, a mixture composed of 20 μl of 131I-InCl₃ (300 MBq), 0.01 M HCl, and 400 μl of DOTA-J591 (4 mg/ml; 0.3 M NH₄OAc, pH 7.0) was allowed to react at 37°C for 20 min. The reaction mixture was then separated on a 10-cm Biogel-P6 column equilibrated with 4 × 10 ml of sterile 1% HSA in PBS. After the reaction mixture was loaded onto the column, it was washed with an additional 5 ml of 1% HSA PBS before the main 111In-DOTA-J591 fraction was eluted with 3 ml of 1% HSA PBS. A similar procedure was used for radiolabeling with 90Y, but an incubation time of 5 min was used, and the labeling mixture included 50 mm ascorbic acid.

J415 and J591 antibodies were modified with DOTA by an analogous method to that used by Lewis et al. (20). This method uses the direct coupling of one of the four carboxylic acid groups of DOTA to the primary amines present in the protein structure (Fig. 1). Twenty-five mg of antibody were concentrated in a AM-30,000 Microsep centrifugal concentrator (Pall Filtron, Northborough, MA) and washed with 5 × 4 ml of 1% DTPA (pH 5.0) over a period of 24 h. The antibody buffer was then changed to 0.1 M phosphate (pH 7.0) using the same centrifugal technique. An active ester of DOTA was formed with each of the radioiodinated antibodies using either LNCaP tumor sections or membranes derived from LNCaP cells. Acetone fixed and frozen 10-mm sections of LNCaP tumors. The immunoreactivity of the 131I- and 111In-labeled mAbs preparations was assessed by the method of Lindmo et al. (21), which extrapolates the binding of the radiolabeled antibody at an infinite excess antigen. Briefly, six test solutions were prepared (in duplicate) and contained 20,000 cpm of the radioiodinated antibody, and increasing amounts of membranes were prepared from LNCaP cells in a total test volume of 250 μl of PBS (0.2% BSA, pH 7.4). The solutions were incubated at 37°C for 45 min prior to being filtered through a glass membrane filter and washed with ice-cold 10 mM Tris-0.9% NaCl buffer. Filters were counted in a gamma counter with standards representing the total radioactivity added. Data were then plotted as the reciprocal of the substrate concentration (X axis) against the reciprocal of the fraction bound (Y axis). The data were then fitted according to a least squares linear regression method using Origin software (Microcal Software, Inc., Northampton, MA). The Y intercept gave the reciprocal of the immunoreactive fraction. A similar method using intact or permeated LNCaP cells and centrifugal isolation of the cells gave the same results.

Assay of Binding Site Number

DOTA-J591 conjugate concentration was assayed by determining the UV absorption at 280 nm. Two 50-μl aliquots of DOTA-J591 were mixed with either 20 or 40 μl of a 1.30 mm solution of IntCl (0.01 M HCl) spiked with a tracer amount of 111In. The mixture was incubated at 37°C for 16 h and then analyzed by ITLC, using a silica gel-impregnated glass fiber 10-cm strip (ITLC-SG, Whatman), and an eluant of 1% DTPA (pH 6.0). Antibody-bound activity remains at the origin, and free In⁹⁰ moves with the solvent front as an [In-DTPA]²⁻ complex. The relative amounts of In¹¹¹ and In-DOTA-J591 were determined by cutting the ITLC strip at a Rf of 0.5 and counting the two halves with a Na(Tl)I detector. The number of binding sites was calculated by considering the molar reaction ratio between In and DOTA-mu-J591, and the observed ratio of 111In and 111In-DOTA-mu-J591 was detected.

111In and ⁹⁰Y Labeling of DOTA Conjugate

Radioiodination of DOTA-J591 with 111In was achieved by adding the radiouclide (in dilute HCl) to the amonium acetate-buffered DOTA-J591. Briefly, a mixture composed of 20 μl of 111InCl₃ (300 MBq), 0.01 M HCl, and 400 μl of DOTA-J591 (4 mg/ml; 0.3 M NH₄OAc, pH 7.0) was allowed to react at 37°C for 20 min. The reaction mixture was then separated on a 10-cm Biogel-P6 column equilibrated with 4 × 10 ml of sterile 1% HSA in PBS. After the reaction mixture was loaded onto the column, it was washed with an additional 5 ml of 1% HSA PBS before the main 111In-DOTA-J591 fraction was eluted with 3 ml of 1% HSA PBS. A similar procedure was used for radiolabeling with ⁹⁰Y, but an incubation time of 5 min was used, and the labeling mixture included 50 mm ascorbic acid.

The radiolabeled DOTA-J591 preparations were determined using the ITLC method with a silica gel-impregnated glass fiber support and a mobile phase of 1% DTPA (pH 5.5). A portion of the radiolabeled DOTA-J591 was spotted on a 10-cm ITLC-SG strip and developed in 1% DTPA (pH 5.5). Once the solvent front had reached the end of the strip, it was removed from the solvent and cut at a Rf of 0.5. The two portions were assayed for radioactivity, and the radiochemical purity was determined using the equation described earlier.

Chelate Stability Studies

111In-labeled DOTA-J591 and DTPA-7E11 were mixed with an equal volume of 50 mM DTPA and maintained at 37°C. Periodically, samples were removed and spotted on a 10-cm ITLC-SG strip and developed in 1% DTPA (pH 5.5). Once the solvent front had reached the end of the strip, it was removed from the solvent and cut at a Rf of 0.5. The two portions were assayed for radioactivity, and the amount of intact chelate was determined using the equation described earlier.

Binding Studies

Immunoreactivity. The immunoreactivity of the 131I- and 111In-labeled mAb preparations was assessed by the method of Lindmo et al. (21), which extrapolates the binding of the radiolabeled antibody at an infinite excess antigen. Briefly, six test solutions were prepared (in duplicate) and contained 20,000 cpm of the radioiodinated antibody, and increasing amounts of membranes were prepared from LNCaP cells in a total test volume of 250 μl of PBS (0.2% BSA, pH 7.4). The solutions were incubated at 37°C for 45 min prior to being filtered through a glass membrane filter and washed with ice-cold 10 mM Tris-0.9% NaCl buffer. Filters were counted in a gamma counter with standards representing the total radioactivity added. Data were then plotted as the reciprocal of the substrate concentration (X axis) against the reciprocal of the fraction bound (Y axis). The data were then fitted according to a least squares linear regression method using Origin software (Microcal Software, Inc., Northampton, MA). The Y intercept gave the reciprocal of the immunoreactive fraction. A similar method using intact or permeated LNCaP cells and centrifugal isolation of the cells gave the same results.

Competitive Binding Studies. Competitive binding studies were performed with each of the radioiodinated antibodies and the four unlabeled antibodies using either LNCaP tumor sections or membranes derived from LNCaP tumors. Acetone fixed and frozen 10-μm tumor sections were soaked in Tris buffer [170 mm (pH 7.4), with 2 mm CaCl₂ and 5 mm KCl] for 15 min and then washed with Tris buffer (170 mm, pH 7.4). The sections were then

1) Preparation of active ester

2) Conjugation

Fig. 1. Two-step conjugation of DOTA to free amine displayed by either J415 or J591. The first step used N-hydroxysuccinimide and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide to create an active ester with DOTA. In the second step, the unpurified active ester is allowed to react with the monoclonal antibody.
incubated with the radioiodinated antibodies in the presence of 100 mM concentrations of each of the unmodified mAbs for 1 h at 4°C. Sections were washed three times with PBS (0.2% BSA) and once with Tris buffer (170 mM, pH 7.4) prior to being fixed with acetone and exposed with BioMax film (Kodak). The assay using the membranes typically used 50 μg of membranes, 10 fmol of iodinated antibody, and amounts of competing antibody from 0.25 fmol to 25 pmol in a 250-μl volume of PBS (0.2% BSA). Membranes were isolated as described above, and data were analyzed by a least squares regression method and Origin software (Microcal Software, Inc.) was used to determine the IC50.

Saturation Binding Studies. Saturation binding studies were performed with each of the radioiodinated antibodies using substrates of intact and permeated LNCaP cells. Briefly, 10 test solutions were prepared (in duplicate) and incubated with increasing amounts of LNCaP cells. Total/bound data are then plotted as a function of the reciprocal antigen concentration. The Y intercept gives the reciprocal of the immunoreactivity. The assay, when performed with either intact LNCaP cells (■) or LNCaP cell membranes (●), gives the same immunoreactivity of ~80% for this labeled mAb.

Internalization and Cellular Processing of J415 and J591

LNCaP cells were plated in 8-cm2 Petri dishes and allowed to grow until confluent. One μCi of either the 131I- or 111In-labeled forms of J415, J591, or 7E11 (~0.1–0.2 μg) were added to cells and allowed to incubate for 1 h. The medium was then removed, and the cells were washed once with fresh media. One ml of fresh medium was added, and the cells were incubated for up to 2 days at 37°C. Triplicate samples were periodically removed, and the medium was isolated. Surface bound activity was stripped and collected with an ice-cold acid wash (100 mM acetate, 100 mM glycine, pH 3.0). The cells were then treated with 1 ml of a 1% solution of Triton X-100 (containing 5 μg/ml of each of antipain, pepstatin, and leupeptin as well as 1 mM phenylmethylsulfonyl fluoride) and kept at on ice for 20 min. The resultant suspension was then centrifuged, and the three samples were counted with a gamma counter. The medium and supernatants were also analyzed by ITLC and size exclusion HPLC to determine the amounts of free iodide produced or the size of the radioactive species created.

RESULTS

Radioiodination and Quality Control. The radioiodination yield for the four mAbs was typically 70–80%, and the amounts of free iodine in the purified mAbs was <0.3%. Specific activities of 350 MBq/mg were routinely achieved. The immunoreactivities of the 131I-labeled mAbs were determined by extrapolating the binding of a fixed amount of 131I-labeled mAb to an infinite amount of PSMA (Lindmo method; Fig. 2). This method gave immunoreactivities of >75% for all mAbs tested. When labeling conditions were increased to produce specific activities >350 MBq/mg, the immunoreactivity was compromised.

An average of five DOTA molecules could be randomly conjugated to J591 and J415, with little apparent loss of immunoreactivity. Conjugation of an average of eight DOTA molecules to J591 resulted in a 20% reduction in immunoreactivity. A 90% incorporation of 111In could be achieved within 15 min. A 90% incorporation of 90Y could be achieved within 5 min. Using the DOTA-J591 conjugate with an average of five DOTA molecules attached, specific activities of 280 MBq 111In/mg DOTA-J591 and 360 MBq 90Y/mg DOTA-J591 were achieved.

111In-Chelate Stability Studies. A direct comparison of the chelate stability of 111In-DTPA-7E11 and 111In-DOTA-J591 showed that 111In was lost from DTPA-7E11 with an apparent half-life of 11 h, whereas the DOTA chelate had an apparent half-life exceeding 1000 h (Fig. 3).

Competitive Binding Studies: Membranes. Radiolabeled J415 could be displaced from binding to LNCaP cell membranes by both J415 and J591 but not J533 (Fig. 4A). The J415 mAb had an mean IC50 of 1.5 nM (±0.9; n = 6), and J591 had a mean IC50 of 6.6 nM (±4.5; n = 6). Similarly, 131I-labeled J533 could be displaced by J533 and J591 but not by J415 (Fig. 4B). In these studies, J533 had a mean IC50 of 2.3 nM (±1.5; n = 3), and J591 had a mean IC50 of 1.7 nM (±1.3; n = 3). Finally, 131I-labeled J591 could be displaced by J415, J533, and J591 (Fig. 4C). The observed IC50 was 1.3 nM (±0.9; n = 6) for J415, 7.7 nM (±5.5; n = 6) for J533, and 3.1 nM (±1.5; n = 6) for J591. The 7E11 mAb did not inhibit the binding of J415, J533, or J591 (or vice versa). These data are consistent with earlier data (11) that J415, J533, or J591 bind to the extracellular domain of PSMA, whereas 7E11 binds to the intracellular domain of PSMA (13).

Saturation Binding Studies. The saturation binding curves generated were characteristic of high affinity binding of an antibody to a single class of antigen. These studies, performed with intact LNCaP cells (Fig. 5), demonstrated that J415 and J591 bound with a similar...
affinity ($K_d$ 1.76 ± 0.69 and 1.83 ± 1.21 nM), whereas J533 had a lower affinity ($K_d$ 18 ± 5 nM). In parallel studies, all four mAbs bound to a similar number of PSMA sites expressed by permeabilized cells (1,000,000–1,300,000 sites/cell). In parallel studies performed with viable LNCaP cells, J415, J533, and J591 bound to a similar number of PSMA sites (i.e., 600,000–800,000 sites/cell). In contrast, 7E11 specifically bound to only 10–15% of the PSMA sites expressed by apparently intact LNCaP Cells ($K_d$, 6.69 nM); but when the cells were deliberately ruptured (Fig. 6), 7E11 bound to a similar number of antigen sites as the other three mAbs. In parallel studies, using 131I-labeled J591, permeabilized cells expressed about twice the amount of PSMA as intact LNCaP cells, suggesting that not all available PSMA is simultaneously expressed on the cell surface.

**Internalization and Cellular Processing of J415 and J591.** Both 131I-labeled J415 and J591 demonstrated a poor cellular retention of...
radioactivity (Fig. 7). For both mAbs, a biexponential curve fit of the data showed that ~10% of the radioactivity was released from the cells with an apparent half-life of 1 h, and the remaining 90% was released into the medium with apparent half-lives of 31 and 38 h for J415 and J591, respectively. In parallel studies, 131I-labeled J415 consistently showed a faster release of radioactivity than 131I-labeled J591. Little or no activity (<1%) was associated with the Triton X-100 (or NaOH) insoluble cell pellet. Analysis of the Triton X-100 soluble fractions indicated that there were no appreciable amounts of free 131I present (<1%). HPLC and TLC analysis of the culture medium showed that a large iodinated species, which corresponded to the same size as the intact mAbs, was being released from the cells, but this never amounted to >10% of the total activity, and after 4–6 h, no further release of this radioactive species was observed. The predominant metabolite of the iodinated mAbs found in the cell medium had the same HPLC and TLC elution profile as free 131I. HPLC and TLC elution profile of free 131I showed the rapid formation of two groups of metabolites (based on molecular size). One group of metabolites achieved a maximum concentration after 1–2 h, after which it began to steadily decline. The second group of metabolites, however, demonstrated an ever-increasing intracellular concentration. This second metabolite did not behave the same as 111In3+ (HPLC or TLC), but rather it had a similar molecular weight as an 111In-DOTA or an 111In-DOTA-amino acid fragment. The first metabolite had a molecular weight between that of the intact mAb and the second metabolite (Mw ~10,000–30,000).

The uptake rates of 111In-labeled J415, J591, and 7E11 by LNCaP cells showed a similar initial uptake rate for J415 and J591, which was 10–20 times faster than that of 7E11 (Fig. 9). However, by 4 h after the addition of the radioactivity, the cells treated with 111In-DOTA-J591 have incorporated and retained more activity than those treated with 111In-DOTA-J415.
DISCUSSION

To understand the characteristics of and to select the best imaging/therapeutic agent, we studied binding characteristics and retention rates of the variously labeled mAbs using the LNCaP cell line, which expresses PMSA (Table 1).

The initial labeling of the three mAbs with $^{131}I$, up to a specific activity of 350 MBq/mg, resulted in little or no apparent loss of immunoreactivity. Similarly, the conjugation of up to an average of five DOTA chelates per mAb enabled specific activities of up to 280 MBq $^{111}In$/mg DOTA-J591 with no apparent loss of immunoreactivity. Site-specific modification of the antibody is sometimes required when this type of random DOTA coupling results in loss of immunoreactivity attributable to the presence of a lysine residue in the antigen binding domain. High specific activities are often required for accurate mAb characterization and particularly when large amounts of antigen binding domain. High specific activities are often required for accurate mAb characterization and particularly when large amounts of the radiotherapeutic agent are administered to a patient. J451 and J591 could be modified with sufficient DOTA to produce high specific activity $^{111}In$- and $^{90}Y$-labeled mAbs for both in vitro binding studies and eventual in vivo studies.

Early approaches to labeling mAbs with radiometals used DTPA, which in its dicyclic anhydride form could be conveniently coupled to mAbs (22). Unfortunately, this simple coupling chemistry produced a more labile chelate than bifunctional forms of the same unconjugated DTPA chelator (23). Macrocyclic chelators have shown even higher kinetic stability (24), but they are even more time consuming to chemically synthesize (25). DOTA can be coupled directly to mAbs using simple chemistry and commercially available materials (20).

The reaction kinetics for $^{111}In$ and DOTAs are longer than for DTPA, but an incubation period of 15 min can give high labeling yields. The DOTA chelator was immensely superior to DTPA in its ability to tightly chelate $^{111}In$ in the presence of an excess of competing ligand. This is in agreement with other studies (20, 26) and could be modified with sufficient DOTA to produce high specific activity $^{111}In$- and $^{90}Y$-labeled mAbs for both in vitro binding studies and eventual in vivo studies.

In conclusion, both J415 and J591 have similar nanomolar affinities to PMSA as $^{7}E11$. Similarly, these two mAbs are far more readily bound and were internalized by live LNCaP cells than $^{7}E11$. The $^{111}In$-labeled DOTA conjugates are able to associate more radioactivity with LNCaP cells than the comparable iodinated forms. The $^{111}In$-labeled DOTA conjugates are also more stable to loss of $^{111}In$ than DTPA-$^{7}E11$. These findings make DOTA-J415 and DOTA-J591 attractive candidates for further evaluation as either diagnostic or radiotherapeutic agents in patients with various cancers that express PMSA.

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