α-Labeling of J591, an Antibody Targeting Prostate-Specific Membrane Antigen: The Technique and Considerations from the First Dedicated Production Lab at an Academic Institution in the United States

Kritika Subramanian1, Judith Stangl-Kremser2, Lady Sawoszczyk1, Vasilios Avlonitis1, Andrew Gernerd1, Kyla Nixon1, Michael Zgaljardic3, Scott Tagawa4, Neil Bander2, and Joseph R. Osborne1

1Division of Molecular Imaging and Therapeutics, Department of Radiology, Weill Cornell Medicine, New York, New York; 2Department of Urology, Weill Cornell Medicine, New York, New York; 3Department of Medical Health Physics, Weill Cornell Medicine, New York, New York; and 4Division of Medical Oncology, Department of Oncology, Weill Cornell Medicine, New York, New York

The protein expression of the prostate-specific membrane antigen correlates with unfavorable or aggressive histologic features of prostate cancer, resulting in use as a diagnostic PET imaging radiotracer and therapeutic target. Here, we discuss the methods to develop 225Ac-DOTA-J591, an α-labeled compound targeting an extracellular epitope of prostate-specific membrane antigen, which is currently being studied in early clinical trials. In addition, we review quality control, radiation safety measures, and clinical considerations before administration of this radioimmunotherapeutic agent.

Key Words: radiochemistry; radionuclide therapy; radiopharmaceuticals; 225Ac; PSMA; α-particle

DOI: 10.2967/jnmt.122.265166

Prostate-specific membrane antigen (PSMA) is a multifunctional transmembrane protein expressed on the surface of prostate cancer cells. Protein expression levels are higher in poorly differentiated carcinomas and metastases (1–3). Receptor binding induces internalization of the ligand into the tumor cell (4). Hence, PSMA is an optimal target for both prostate cancer imaging and therapeutic intervention. At our institution, we have an investigational-new-drug application (application 11,613) for radiolabeled J591 with β-emitters. This humanized monoclonal antibody (mAb) has been demonstrated to be safe and efficacious in phase I and II clinical trials (NCT001950039 and NCT03276572) (5–8). To date, the only Food and Drug Administration–approved PSMA-targeted therapy is 177Lu-vipivotide tetraxetan (Pluvicto; Advanced Accelerator Applications) as third-line therapy for metastatic castration-resistant prostate cancer (9).

Unlike β-emitters, α-emitters characterized by high energy (5–9 MeV) (10) and a short pathlength (50–100 mm (10) and ~100 μm in tissue (11)) have demonstrated anticancer potential by reducing tumor burden and serum prostate-specific antigen levels (12–15). The prototypical α-emitting particle used for radiotherapeutics is 225Ac, which has a half-life of 10 d (10).

Currently, a phase I dose-escalation trial of 225Ac-DOTA-J591 in patients with metastatic castration-resistant prostate cancer is underway (7), with promising preliminary results (7,8). In this work, we present methods for producing this radioimmunotherapeutic, particularly in the context of implementation in the first dedicated α-labeling lab at an academic institution. We discuss technical and production details, quality control tests, radiation safety measures, and therapeutic administration considerations.

TECHNICAL AND PRODUCTION DETAILS

Biochemistry of 225Ac-DOTA-HuJ591

In essence, HuJ591 is conjugated with a DOTA bifunctional chelating agent that undergoes radiolabeling with 225Ac-nitrate. This IgG1 with humanized Vh and VL regions specifically targets the extracellular domain of PSMA. 225Ac-DOTA-HuJ591 has a molecular weight of about 147 kDa and is formulated as a single-dose intravenous injection in a sterile, pyrogen-free isotonic saline solution.

Production Assembly Steps

A 37 MBq (1.0 mCi) activity of 225Ac-nitrate residue is supplied in a 2-mL glass vial (Department of Energy, Oak Ridge National Laboratory). Figure 1 summarizes the production assembly mechanism.

A 30-mL 10 durable grade (10 DG) desalting disposable column is washed twice with 15 mL of 3% hydrogen peroxide in a laminar flow hood. Thirty minutes later, the column is washed twice with 15 mL of sterile water for injection...
Add 100 μL of 0.2 M HCl to 0.037 GBq 225Ac vial. Wait for 30 min.

Add 50 μL of solution to reaction vial (assuming 0.037 GBq of 225Ac was initially provided in the vial).

Add 75-125 μL of 2 M TMAA buffer, 25 ± 5 μL of ascorbic acid, and 300 μL (3 mg) DOTA-HuJ591 mAb to the vial.

Gently mix and incubate at 37°C for 1-2 h.

Perform instant thin layer chromatography for labelling efficiency.

Add 50 μL DTPA to the vial. Incubate for 10 min at 37°C.

Extract the first eluate into a sterile vial (Fraction-1).

Rinse reaction vial with 1.5 mL of 2% HSA in sterile saline. Reload Fraction-1 eluate and collect.

Elute with 4 mL of 2% HSA in sterile saline and collect Fraction-2.

Remove 4 mL of Fraction-2 and transfer into FDP vial through sterile filter.

Rinse filter with 1.0 mL of saline-HSA solution.

Measure 225Ac-DOTA-HuJ591 mAb activity every 1-2 h for 6-8 h.

Store 225Ac-DOTA-J591 mAb in a 10 mL sterilized vial.

FIGURE 1. Steps for production assembly of 225Ac-J591. DG = durable grade; DTPA = diethylenetriaminepentaacetic acid; FDP = final drug product; HSA = human serum albumin; SDP = sterile drug product; SWFI = sterile water for injection; TMAA = triethylammonium acetate.

and twice with sterile 0.9% saline with 2% human serum albumin. This column is prepared for purification of the radiolabeled mAb. Within the same laminar flow hood, a 20-gauge sterile needle is attached to a 0.2-μm sterile filter and inserted into a pyrogen-free sterile drug product vial and the final drug product vial.

Using aseptic technique in a laminar flow hood, 100 μL of 0.2 M hydrogen chloride are added to the 225Ac vial for 30 min; 3,700-5,550 kBq (100-550 μCi) are removed and added to the reaction vial; and 75-125 μL of 2 M triethylammonium acetate buffer, 25 ± 5 μL of ascorbic acid, and 3 mg of DOTA-HuJ591 mAb (300 ± 15 μL) are subsequently added to the vial. The DOTA labeling of the HuJ591 mAb is performed at an outside facility. After gentle mixing of the vial, it is incubated at 37°C for 1-2 h. Instant thin-layer chromatography with silica gel is performed to determine labeling efficiency, which must be more than 50% to be acceptable for further processing.

Diethylenetriaminepentaacetic acid solution, 50 μL, is added to the vial and incubated for an additional 10 min at 37°C. The reaction mixture is loaded onto the prepared desalting column, and the eluate is collected into a sterile vial (fraction 1). The reaction vial is rinsed with 1.5 mL of 2% human serum albumin in sterile saline and reloaded in the column, and the eluate in the fraction 1 vial is collected. Fraction 2 is eluted with 4 mL of sterile saline with 2% human serum albumin into a sterile tube. Using a sterile syringe, 4 mL of fraction 2 are removed and transferred through a sterile filter into the final drug product vial. The filter is rinsed with 1.0 mL of saline-human serum albumin solution. The activity of 225Ac-DOTA-HuJ591 mAb over the next 6-8 h is measured at intervals of 1-2 h.

The 225Ac-DOTA-J591 mAb injection is stored in a 10-mL sterilized vial sealed with gray butyl rubber septa crimped closed with an aluminum stopper (Hollister Stier Laboratories or Allergy Laboratories) and certified as sterile and apyrogenic.

TABLE 1

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Values or range of acceptance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Colorless, clear, free from</td>
</tr>
<tr>
<td></td>
<td>particulate matter</td>
</tr>
<tr>
<td>Assay</td>
<td>0.4–2.8 MBq (11–76 μCi)/mL</td>
</tr>
<tr>
<td>Specific activity</td>
<td>0.67–4.67 MBq (18–126 μCi)/mg</td>
</tr>
<tr>
<td>pH</td>
<td>5–8</td>
</tr>
<tr>
<td>Radiochemical purity</td>
<td>225Ac-DOTA-J591 &gt; 95%</td>
</tr>
<tr>
<td>Test for endotoxins</td>
<td>&lt;35 endotoxin units/mL</td>
</tr>
<tr>
<td>Bubble test</td>
<td>Pi &gt; Pm, where Pm = ψ</td>
</tr>
<tr>
<td>Sterility test</td>
<td>Test started within 24 h</td>
</tr>
</tbody>
</table>

Pi = pressure at which bubbles appear; Pm = minimum acceptable bubble point pressure; ψ = pound-force per square inch.
quality assurance tests are performed and assembled in a report to ensure that each batch meets the institutional acceptance criteria for batch release (Table 1).

The final drug product must be colorless after being swirled in the glass vial, suggesting no contamination. Instant thin-layer chromatography with silica gel and 10 mM ethylenediaminetetraacetic acid solution determines the radiochemical purity of the drug batch, which must be more than 95% (Fig. 2). The labeling efficiency before ethylenediaminetetraacetic acid elusion, remarkable for high concentration of $^{225}\text{Ac}$ bound to protein (green peak) and small percentage of unbound $^{225}\text{Ac}$, which has formed into daughter compounds of francium and bismuth (red peaks). Two hours after elusion, higher concentration of daughter compounds is noted whereas peak concentration of $^{225}\text{Ac}$ bound to protein is stable. Because of shorter half-lives of daughter compounds, their presence is negligible at 24 h after elution, validating chemical purity of final product.

The bubble point test in a nondestructive test evaluating filter integrity. It determines the minimum pressure required to force liquid out of the filter pores, which is an indirect way of measuring the pore diameter and, in essence, any defects in the filter that may permit bacterial penetration. The pressure at which bubbles appear must be greater than the minimum acceptable bubble point pressure as defined by the manufacturer. Fluid thioglycolate medium and soybean casein digest medium were used to evaluate the sterility of the injection drug product by transferring 0.1-mL samples of $^{225}\text{Ac}$-DOTA-J591 to the medium and incubating entire 5-mL batch of the final drug product (35 endotoxin units/mL) (Fig. 4).

The bubble point test in a nondestructive test evaluating filter integrity. It determines the minimum pressure required to force liquid out of the filter pores, which is an indirect way of measuring the pore diameter and, in essence, any defects in the filter that may permit bacterial penetration. The pressure at which bubbles appear must be greater than the minimum acceptable bubble point pressure as defined by the manufacturer. Fluid thioglycolate medium and soybean casein digest medium were used to evaluate the sterility of the injection drug product by transferring 0.1-mL samples of $^{225}\text{Ac}$-DOTA-J591 to the medium and incubating entire 5-mL batch of the final drug product (35 endotoxin units/mL) (Fig. 4).

The bubble point test in a nondestructive test evaluating filter integrity. It determines the minimum pressure required to force liquid out of the filter pores, which is an indirect way of measuring the pore diameter and, in essence, any defects in the filter that may permit bacterial penetration. The pressure at which bubbles appear must be greater than the minimum acceptable bubble point pressure as defined by the manufacturer. Fluid thioglycolate medium and soybean casein digest medium were used to evaluate the sterility of the injection drug product by transferring 0.1-mL samples of $^{225}\text{Ac}$-DOTA-J591 to the medium and incubating entire 5-mL batch of the final drug product (35 endotoxin units/mL) (Fig. 4).

The bubble point test in a nondestructive test evaluating filter integrity. It determines the minimum pressure required to force liquid out of the filter pores, which is an indirect way of measuring the pore diameter and, in essence, any defects in the filter that may permit bacterial penetration. The pressure at which bubbles appear must be greater than the minimum acceptable bubble point pressure as defined by the manufacturer. Fluid thioglycolate medium and soybean casein digest medium were used to evaluate the sterility of the injection drug product by transferring 0.1-mL samples of $^{225}\text{Ac}$-DOTA-J591 to the medium and incubating entire 5-mL batch of the final drug product (35 endotoxin units/mL) (Fig. 4).

The bubble point test in a nondestructive test evaluating filter integrity. It determines the minimum pressure required to force liquid out of the filter pores, which is an indirect way of measuring the pore diameter and, in essence, any defects in the filter that may permit bacterial penetration. The pressure at which bubbles appear must be greater than the minimum acceptable bubble point pressure as defined by the manufacturer. Fluid thioglycolate medium and soybean casein digest medium were used to evaluate the sterility of the injection drug product by transferring 0.1-mL samples of $^{225}\text{Ac}$-DOTA-J591 to the medium and incubating entire 5-mL batch of the final drug product (35 endotoxin units/mL) (Fig. 4).

The bubble point test in a nondestructive test evaluating filter integrity. It determines the minimum pressure required to force liquid out of the filter pores, which is an indirect way of measuring the pore diameter and, in essence, any defects in the filter that may permit bacterial penetration. The pressure at which bubbles appear must be greater than the minimum acceptable bubble point pressure as defined by the manufacturer. Fluid thioglycolate medium and soybean casein digest medium were used to evaluate the sterility of the injection drug product by transferring 0.1-mL samples of $^{225}\text{Ac}$-DOTA-J591 to the medium and incubating entire 5-mL batch of the final drug product (35 endotoxin units/mL) (Fig. 4).

The bubble point test in a nondestructive test evaluating filter integrity. It determines the minimum pressure required to force liquid out of the filter pores, which is an indirect way of measuring the pore diameter and, in essence, any defects in the filter that may permit bacterial penetration. The pressure at which bubbles appear must be greater than the minimum acceptable bubble point pressure as defined by the manufacturer. Fluid thioglycolate medium and soybean casein digest medium were used to evaluate the sterility of the injection drug product by transferring 0.1-mL samples of $^{225}\text{Ac}$-DOTA-J591 to the medium and incubating entire 5-mL batch of the final drug product (35 endotoxin units/mL) (Fig. 4).

The bubble point test in a nondestructive test evaluating filter integrity. It determines the minimum pressure required to force liquid out of the filter pores, which is an indirect way of measuring the pore diameter and, in essence, any defects in the filter that may permit bacterial penetration. The pressure at which bubbles appear must be greater than the minimum acceptable bubble point pressure as defined by the manufacturer. Fluid thioglycolate medium and soybean casein digest medium were used to evaluate the sterility of the injection drug product by transferring 0.1-mL samples of $^{225}\text{Ac}$-DOTA-J591 to the medium and incubating entire 5-mL batch of the final drug product (35 endotoxin units/mL) (Fig. 4).

The bubble point test in a nondestructive test evaluating filter integrity. It determines the minimum pressure required to force liquid out of the filter pores, which is an indirect way of measuring the pore diameter and, in essence, any defects in the filter that may permit bacterial penetration. The pressure at which bubbles appear must be greater than the minimum acceptable bubble point pressure as defined by the manufacturer. Fluid thioglycolate medium and soybean casein digest medium were used to evaluate the sterility of the injection drug product by transferring 0.1-mL samples of $^{225}\text{Ac}$-DOTA-J591 to the medium and incubating entire 5-mL batch of the final drug product (35 endotoxin units/mL) (Fig. 4).

The bubble point test in a nondestructive test evaluating filter integrity. It determines the minimum pressure required to force liquid out of the filter pores, which is an indirect way of measuring the pore diameter and, in essence, any defects in the filter that may permit bacterial penetration. The pressure at which bubbles appear must be greater than the minimum acceptable bubble point pressure as defined by the manufacturer. Fluid thioglycolate medium and soybean casein digest medium were used to evaluate the sterility of the injection drug product by transferring 0.1-mL samples of $^{225}\text{Ac}$-DOTA-J591 to the medium and incubating entire 5-mL batch of the final drug product (35 endotoxin units/mL) (Fig. 4).
either at 37°C (fluid thio-glycolate medium) or at room temperature (soybean casein digest medium) up to 14 d. Growth in the medium through visual inspection is recorded on the third, seventh, and 14th days after culturing.

225Ac-J591 can be stored at 4°C–8°C for up to 4 h after production, which also represents the time window for therapeutic use of the produced dose.

RADIATION SAFETY

Lead-shielded waste containers and sharps disposal containers should be close to where the 225Ac work is performed. A spill kit must be present in the room. An absorbent pad must be placed under the production and distribution area for 225Ac, including the laminar hood and glove box.

If the staff needs to leave the room, their feet and hands must be checked using a survey meter (e.g., Ludlum model 14C). Similarly, when tagging is complete, the hands, feet, and body of each individual need to be evaluated for radiation contamination. Baseline serologies evaluating hepatic and renal function must be reviewed before administration of the radioimmuno-therapeutic agent. Not much uptake is seen in the marrow by default, but it is imperative to also review blood counts. PSMA PET/CT imaging may also be used to evaluate the baseline tumor burden before administration of the 225Ac-J591 (45 kBq/kg). After the treatment course, currently performed at our institution as 2 sessions 6 wk apart, repeat imaging helps guide clinicians in planning further management (Fig. 5).

CLINICAL CONSIDERATIONS

Current guidelines require that individuals with metastatic castration-resistant prostate cancer who have refractory disease or are unwilling to undergo other commercially available prostate cancer therapies should be considered for experimental treatment with 225Ac-J591. 225Ac-J591 is metabolized by the liver, as well as excreted by the kidney and the bowel. Therefore, baseline serologies evaluating hepatic and renal function must be reviewed before administration of the 225Ac-J591 (45 kBq/kg). After the treatment course, currently performed at our institution as 2 sessions 6 wk apart, repeat imaging helps guide clinicians in planning further management (Fig. 5).

DISCUSSION

In this paper we have explained an optimized technique that meets all federal recommendations and safety guidelines for producing 225Ac-DOTA-HuJ591 at an academic institution.
In addition, we have discussed the clinical considerations before therapeutic administration. Multiple considerations involving safety precautions must be taken to ensure that radiation exposure to the staff is minimalized. In addition, several quality control tests must be performed on each production batch to ensure that there is no compromise in the quality of the radioimmunotherapeutic.

Numerous benefits are associated with the production of α-emitting radioligand therapy at the administering institution. There are no delays associated with outside manufacturing and delivery, thereby reducing logistic expenses. The measures for quality control indicated on the quality assurance reports are reassuring to the administering and treating physicians. Individuals may receive their therapeutic administration, enhancing patient care.

Currently, our α-theranostics laboratory produces solely 225Ac-DOTA-HuJ591. However, with time, more actinium-bound radioligand therapies may be incorporated into the portfolio for application in a wider range of malignancies.

CONCLUSION

This paper provides the assembly methods for local production of 225Ac-DOTA-HuJ591 at an academic institution, for which there are many considerations and advantages.

KEY POINTS

**QUESTION:** What are the considerations in developing an α-labeled compound targeting PSMA?

**PERTINENT FINDINGS:** This paper discusses quality control, radiation safety measures, and clinical considerations before administration of 225Ac-DOTA-HuJ591.

**IMPLICATIONS FOR PATIENT CARE:** Reduced logistic expenses and enhanced quality control measures by the administering institution reassure the treating physician and the patient on the quality of the radioimmunotherapeutic being administered. Imaging can also be performed at the same institution as the administration, enhancing patient care.

DISCLOSURE

No potential conflict of interest relevant to this article was reported.

ACKNOWLEDGMENTS

We thank Dr. Shankar Vallabhajosula for his guidance on developing the methods and implementing them at our institution.

REFERENCES