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ORIGINAL ARTICLE

Preparation of Patient Doses of [^{177}Lu]Lu-DOTATATE and [^{177}Lu]Lu-PSMA-617 with Carrier Added (CA) and No Carrier Added (NCA) ^{177}Lu

Raviteja Nanabala[1,2] · Maroor Raghavan Ambikalmajan Pillai[1] · Buvaneswari Gopal[2]

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Abstract

Purpose [^{177}Lu]Lu-DOTATATE and [^{177}Lu]Lu-PSMA-617 used for targeted radionuclide therapy are very often prepared in the hospital radiopharmacy. The preparation parameters vary depending upon the specific activity of the ^{177}Lu used. The aim of this study was to develop optimized protocols to be used in the nuclear medicine department for the preparation of patient doses of the above radiopharmaceuticals.

Method ^{177}Lu (CA and NCA) were used for radiolabeling DOTATATE and PSMA-617. Parameters studied are ^{177}Lu of different specific activity and different peptide concentrations and two different buffer systems. Paper and thin layer chromatography systems were used for estimating the radiochemical yield as well as radiochemical purity. Solid-phase extraction was used for the purification of the labeled tracers.

Results [^{177}Lu]Lu-DOTATATE was prepared with CA ^{177}Lu ($n=13$) and NCA^{177}Lu ($n=6$). Four batches each of [^{177}Lu]Lu-PSMA-617 were prepared using CA and NCA ^{177}Lu. Radiochemical yields > 80% and final product with less than < 1% radiochemical impurity could be obtained in all batches which were used for therapy.

Conclusion Robust protocols for the preparation of clinical doses of [^{177}Lu]Lu-DOTATATE and [^{177}Lu]Lu-PSMA-617 were developed and used for the preparation of clinical doses. The quality of the SPECT images of both the tracers are consistent with the expected uptake in respective diseases.

Keywords [^{177}Lu]Lu-DOTATATE · [^{177}Lu]Lu-PSMA-617 · Neuroendocrine tumours · Prostate cancer · Targeted radionuclide therapy

Introduction

[^{177}Lu]Lu-DOTATATE and [^{177}Lu]Lu-PSMA-617 are two therapeutic radiopharmaceuticals the use of which is increasing in nuclear medicine [1–4]. Lutetium-177 has a half-life of 6.74 days, emits β^- particles with maximum energy of 498 keV and having a maximum range of 3 mm in the tissue making it one of the best radionuclides for targeted therapy [5]. Lutetium-177 also emits 208 keV (11%) and 103 keV (6%) gamma photons which are useful for single-photon emission computed tomography (SPECT) imaging [6].

The precursor for radiolabeling with ^{177}Lu is prepared by conjugating a metal chelating agent to a targeting biomolecule either directly or through a linker molecule. Being a Lewis hard acid exhibiting + 3 metal chemistry, Lu as well as other lanthanides form stable complexes with macrocyclic amine-carboxylate chelating agents [7–9]. DOTA((1,4,7,7,10-Tetraazacyclododecane-1,4,7,10-tetrayl) tetraacetic acid) is the most favoured chelator for making ^{177}Lu radiopharmaceuticals [10, 11].

There are two routes for the production of ^{177}Lu in the reactor [12, 13]. The most commonly used route is by irradiation of enriched ^{176}Lu target which undergoes

✉ Buvaneswari Gopal
gbhuvaneswari@vit.ac.in

Raviteja Nanabala
ravi.teja131@gmail.com

Maroor Raghavan Ambikalmajan Pillai
pillai.m.r.a@gmail.com

[1] Molecular Cyclotrons Private Limited, Puthuvype, Ernakulam, Kerala 682508, India

[2] Department of Chemistry, School of Advanced Sciences, Vellore Institute of Technology University, Vellore, Tamil Nadu 632014, India

$^{176}Lu(n,\gamma)^{177}Lu$ nuclear reaction. The specific activity of the product formed varies widely depending on the enrichment of the ^{176}Lu target, the neutron flux of the reactor as well as the irradiation time. The post-processing decay also results in significant loss of specific activity. The main advantage of this production route is that the nuclear reaction has a neutron capture cross-section of 2065 barns, and hence, large quantities of the product can be prepared by irradiation of small amounts of target material [14]. The specific activity of CA ^{177}Lu can vary up to 60 Ci/mg depending on the flux of the reactor and irradiation conditions. The specific activity of ^{177}Lu prepared in the Dhruva reactor, India, having a neutron flux of 1.8×10^{14} n.cm^{-2} s^{-1} is ~ 15–30 Ci/mg at the time of dispatch [15].

The second route of production of ^{177}Lu is by irradiating ^{176}Yb-enriched target. It undergoes the nuclear reaction $^{176}Yb(n, \gamma)^{177}Yb$ and the ^{177}Yb formed decays to ^{177}Lu with a half-life of 1.91 h [16]. The ^{177}Lu prepared through this route is no carrier added (NCA) because of the absence of any other isotopes of lutetium. Theoretical specific activity of 110 Ci/mg can be obtained in the case of NCA ^{177}Lu. However, a complete separation from the target ytterbium is needed as Yb is an equally good complexing metal with the chelates used for making bioconjugates. Hence, the actual specific activity of ^{177}Lu formed could be lower if Yb is also considered.

The cost of the ^{177}Lu vary significantly depending on its route of production, NCA being far more expensive than CA ^{177}Lu. This has a greater implication in a hospital radiopharmacy as option to choose the radiopharmaceutical is given to the patients based on their affordability. There is demand for both the options, and hence, the radiopharmacist should be well trained to make the preparation using both NCA and CA ^{177}Lu. The latter protocol being more complex than the former as the specific activity vary from batch to batch. This paper describes the development of robust protocols for the preparation of [^{177}Lu]Lu-DOTATATE and [^{177}Lu]Lu-PSMA-617 which can be adapted by nuclear medicine departments wanting to start this mode of therapy.

Materials and Methods

Sodium ascorbate, ascorbic acid, sodium acetate, and ethanol used were of pharmaceutical grade and purchased from Sigma Aldrich. Millipore water, 0.22-micron filters, TLC plates and Whatman 3 MM paper were purchased from Merck. C18 light cartridges were procured from Waters, India. DOTATATE and PSMA-617 were purchased from ABX Advanced Biochemical Compounds, Germany.

NCA ^{177}Lu is obtained from ITM, Germany, whereas CA ^{177}Lu was obtained from the Board of Radiation and Isotope Technology (BRIT), Mumbai. Usually 250 mCi (9.25 GBq) of ^{177}Lu is ordered per patient dose. Both the manufacturers supply the product as [^{177}Lu]$LuCl_3$ in dilute hydrochloric acid at pH ~ 2. No further processing is done with the [^{177}Lu]$LuCl_3$ and directly used for the preparation of the radiopharmaceuticals. The specific activity at reference time is quoted by the vendors and used for calculating the Lu metal content. In the case of CA ^{177}Lu, decay is applied to calculate the specific activity at the time of preparation of the radiopharmaceutical, whereas for CA ^{177}Lu the quoted specific activity is used which is ~ 10–20% lower than the theoretical specific activity. The specification sheet also contains other metal contamination. The activity content is measured prior to the preparation of the radiopharmaceutical in a dose calibrator in ^{177}Lu window.

Preparation of Reagents

DOTATATE or PSMA-617 is dissolved in Millipore water to get microgram per milliliter concentration. Aliquots of 100 µL (100 µg) is dispensed in 1.5 mL centrifuge tubes and stored at − 20 °C. We used two buffer systems. Buffer 1 (B1) was prepared by dissolving 410 mg (5 mM) of sodium acetate and 500 mg (2.8 mM) of ascorbic acid in 10 mL of Millipore water. Buffer 2 (B2) was prepared by dissolving 800 mg (4 mM) of sodium ascorbate and 200 mg (1.13 mM) of ascorbic acid in 10 mL of millipore water.

Ethanol (70%) for elution of the solid-phase cartridge was prepared by mixing 14 mL of ethanol with 6 mL of Millipore water. Mobile phase for thin layer chromatography (TLC) was prepared as follows: 1.47 g of trisodium citrate in 25 mL of water, 500 µL of concentrated HCl was added, and the solution was made up to 50 mL. Mobile phase for paper chromatography (PC) was prepared by mixing acetonitrile and water in 1:1 ratio.

All operations involving handling of radioactivity was done inside a fume hood spread with adsorbent sheets and inside a clean radiopharmacy. Double gloves were worn during the entire operation.

Preparation of [^{177}Lu]Lu-DOTATATE/PSMA-617

A 10-mL clean sterile glass vial was used as the reaction vessel. The required quantity of DOTATATE/PSMA-617 was transferred to the vial using micropipette. Two hundred microliters of buffer was added to the peptide tube and again transferred to the reaction vial. This was followed by addition of 800 µL of buffer to the reaction tube. [^{177}Lu]$LuCl_3$ was withdrawn from the container and transferred to the reaction vial using a micropipette. The pH of the solution was measured by taking a small drop in a pH paper and confirmed to be 4.5–5.0. The reaction mixture was heated for 20 min at 95 °C in a water bath. A small aliquot of the reaction mixture was withdrawn using a clean capillary and

PC and TLC were done to estimate the radiochemical yield. If the radiochemical yield was less than 80% another 100 µL (100 µg) of peptide/inhibitor ligand was added to the reaction mixture and heated for another 20 min. The radiochemical yield was again estimated by PC and TLC.

Purification and Aseptic Filtration

A C18 light cartridge was used for the purification of both [^{177}Lu]Lu-DOTATATE and [^{177}Lu]Lu-PSMA-617. The C18 light cartridge was first conditioned by passing 5 mL of 70% ethanol prior to the usage. The crude product was withdrawn using 5-mL syringe and injected through the cartridge and the effluent was collected in a sealed 10-mL vial marked "waste." After that, the outlet of the cartridge was connected to a 0.22-micron syringe filter which was connected to a sealed 10-mL vial marked "product" through a syringe. One milliliter of 70% ethanol was passed through the cartridge and the product was collected in a 10-mL sterile glass vial followed by another 5 mL of 0.9% saline. The radioactivity contents of the product and waste vials were measured in a dose calibrator. The cartridge as well as the syringe filter and tubes were neatly contained in a plastic bag and the radioactivity was measured.

Estimation of Radiochemical Yield and Purity

TLC was performed with 1 × 10 cm ITLC-SG plate as stationary phase and developed with trisodium citrate buffer as mobile phase. PC was carried with 1 × 10 cm Whatman 3MM chromatography paper as stationary phase and acetonitrile: water (1:1) as mobile phase. The TLC/PC strips were cut into four equal pieces and radioactivity measured either in a dose calibrator or in a NaI(Tl) scintillation counter.

Patient Studies

Dose Administration

Before administering [^{177}Lu]Lu-DOTATATE, the patient is pre-medicated by slow intravenous injection of 8 mg ondansetron followed by 8 mg dexamethasone shortly before starting amino acid infusion. Hemodynamic parameters such as blood pressure and pulse are recorded. One thousand milliliters of amino acid infusion (lysine 22.3 mg/mL and arginine 8.0 mg/mL) is started 30 min before the [^{177}Lu]Lu-DOTATATE infusion and continued till 3.5 h after the termination of [^{177}Lu]Lu-DOTATATE infusion. Amino acids administration was not done for [^{177}Lu]Lu-PSMA-617.

The radiopharmaceuticals are diluted in normal saline solution to a volume of 20 mL and infused by indwelling IV catheters for approximately 30 min. The line was flushed with normal saline after the completion of infusion. One thousand milliliters of 0.9% normal saline was administered IV for hydration before and after radiopharmaceutical administration. The patients were admitted in an isolation therapy ward and discharged as per regulatory protocol.

Image Acquisition

Siemens SPECT with medium energy collimator with the 208 keV was used for acquiring [^{177}Lu]Lu-DOTATATE/PSMA-617 images. Whole-body scanning was done for 30 min and spot views of the upper abdomen or all involved sites were acquired at three time points, which was on days 1, 4, and 7 days post-injection.

Siemens PET-CT was used to image the patients pre- and post-therapy to estimate disease burden as well as response to therapy. ^{68} Ga-DOTATATE was used for PET-CT imaging of neuroendocrine tumor patients. Prostate cancer patients were imaged with ^{68} Ga-PSMA-11 which uses an inhibiter ligand which is different from PSMA-617. The activity infused varied from 2.2 to 3.5 mCi (81–129 MBq). Whole-body scans were acquired for 5 bed positions of 3 min each. Parameters of CT scan were current 240 mA and voltage 130 kVp and slice thickness of 3 mm.

Results

Radiochemical Yield and Purity Estimation

A dual chromatography system was used for the analysis of radiochemical yield and purity. In TLC, the [^{177}Lu]Lu-DOTATATE and [^{177}Lu]Lu-PSMA-617 stayed at the point of spotting while free ^{177}Lu moved to the solvent front (Fig. 1). In PC, free ^{177}Lu stayed at point of spotting and [^{177}Lu]Lu-DOTATATE and [^{177}Lu]Lu-PSMA-617 moved to solvent front (Fig. 2). These chromatographic techniques were used for estimation of radiochemical yield during preparation based on which the decision to add additional peptide/ligand was taken. After cartridge purification, PC and TLC were repeated. The radiochemical purity of the purified radiotracers prepared using both CA and NCA ^{177}Lu was always > 99% for all the batches.

[^{177}Lu]Lu-DOTATATE with CA ^{177}Lu

The results of the production 13 batches of [^{177}Lu]Lu-DOTATATE with CA ^{177}Lu are summarized in Table 1. The specific activity of ^{177}Lu at the time of preparation of the radiopharmaceutical varied from 11.6 to 27 Ci/mg (0.43–1.03 GBq/µg). The average specific activity was 20 ± 4.34 Ci/mg. Depending on the specific activity of the $^{177}LuCl_3$, adjusting peptide concentration in every batch was required to get

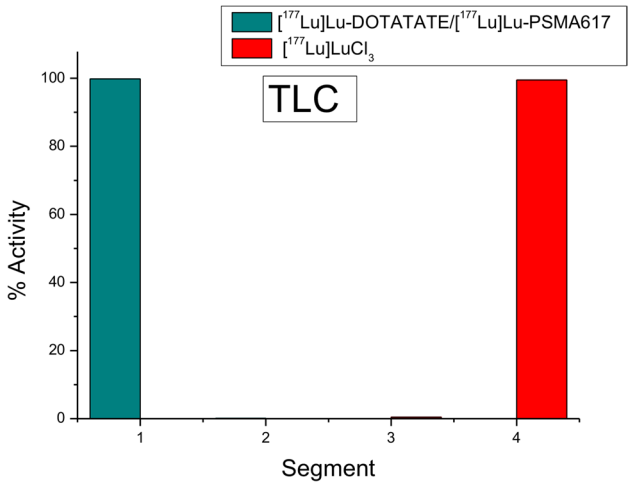

Fig. 1 TLC-SG pattern of [^{177}Lu]Lu-DOTATATE/[^{177}Lu]Lu-PSMA-617 developed with citrate buffer. Both the tracers remain at the point of spotting whereas $^{177}LuCl_3$ moves to the solvent front

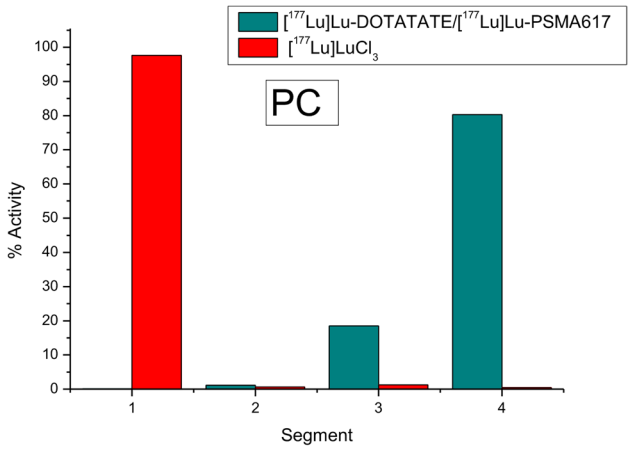

Fig. 2 PC pattern of [^{177}Lu]Lu-DOTATATE/[^{177}Lu]Lu-PSMA617 in Whatman No.1 paper developed with 50% acetonitrile in water. $^{177}LuCl_3$ remains at the point of spotting whereas the labelled peptide/ligand moves towards the solvent front

maximum radiochemical yield. The average content of ^{177}Lu used was 0.07 ± 0.02 µmol and the peptide concentration was 0.22 ± 0.06 µmol. Hence, the M:L ratio varied from 2.38 to 4.94 with an average of 3.06 ± 0.72 in 13 batches.

In buffer 1, sodium acetate/ascorbic acid was used in the first three batches of [^{177}Lu]Lu-DOTATATE, and in buffer 2, sodium ascorbate/ascorbic acid was used in subsequent batches. Both the buffers worked equally well. Ascorbic acid being vitamin C is biologically more acceptable and hence buffer 2 was used in all subsequent batches (batches 4–13).

Figure 3 shows the radiochemical yields obtained in different batches while using CA ^{177}Lu. The radiochemical yields were 83.1 ± 6.9%. While in most batches it was > 80%, in two batches, it was < 70%. As a usual practice, further peptide could have been added but not done as the amount of peptide was already above the limit of mCi/µg as the cut of limit put in many studies [17].

Cartridge purification was found essential to remove free ^{177}Lu from the reaction mixture. After cartridge purification, the radiochemical purity of the product, [^{177}Lu]Lu-DOTATATE was 100% as no free ^{177}Lu was detected either in TLC or PC. The radioactivity associated with the product, waste, cartridge, and Millipore filter were measured to see the loss of activity during production. The results are summarized in Fig. 4. The product retention in the reaction vessel was minimum. Cartridge, filter, and the tubing's retained 2–5% activity. The activity in the waste vial corresponded to free ^{177}Lu and varied from batch to batch. One important observation was that the cartridge purification is essential while using CA ^{177}Lu to ensure the preparation of high purity radiolabeled [^{177}Lu]Lu-DOTATATE.

In addition to the 13 batches reported in Table 1, there was four failed batches while using CA ^{177}Lu the results of which are presented in Table 2. The radiochemical yields were poor and varied 4–22%. Further addition of the peptide did not help in the improvement of radiochemical yield. Cartridge purification was employed for all these batches. Most of the activity was seen in the waste. Despite using an average L:M ratio of 3.68 ± 0.57, the radiochemical yields were very low. The radiochemical purity after cartridge purification was < 40% and the product prepared was not used for patient administration. These results are presented here to emphasize the fact that irrespective of following a well-established protocol some batches can fail depending upon the quality of ^{177}Lu used and the radiopharmacist need to put extra care to see that poor-quality products are not injected to patients. It is often a dilemma to report a batch failure as it results in significant financial loss to the hospital and discomfort to the patient.

[^{177}Lu]Lu-DOTATATE with NCA ^{177}Lu

Results of the six batches of the preparation of [^{177}Lu]Lu-DOTATATE with NCA $^{177}LuCl_3$ are summarized in Table 3. In buffer 2, ascorbic acid/ sodium ascorbate buffer (pH 4.5–5) was used in all batches. The activity used was in the range of 216–240 mCi (8.2–8.9 GBq) in five batches. One of the batches was done for two patients, and hence, double the activity was used. The amount of peptide used was 150 µg (0.1 µmol) in the first two batches. However, the reaction yields were not quantitative. Hence, 200 µg (0.14 µmol) was used in all subsequent batches. One of the batches was for 2 patient doses and used 475 mCi (17.6 GBq) and 400 µg of DOTATATE was used. The radiochemical yields while using NCA ^{177}Lu was 92.8 ± 4.9 which was more consistent than production with CA ^{177}Lu (Table 3).

Table 1 Preparation of [^{177}Lu]Lu-DOTATATE using CA $^{177}LuCl_3$

Batch number	$^{177}LuCl_3$ (mCi)	Specific activity (mCi/μg)	Concentration of ^{177}Lu (μmol)	Concentration DOTATATE (μmol)	Ligand to metal ratio [L:M]	[^{177}Lu]Lu-DOTATATE (mCi)
1	318	25	0.07	0.17	2.43	254
2	250	21	0.07	0.17	2.61	224
3	414	23	0.10	0.35	3.48	284
4	237	16	0.08	0.24	2.96	169
5	222	16	0.08	0.24	3.16	198
6	249	20	0.07	0.21	3.02	201
7	229	19	0.07	0.17	2.57	200
8	245	17	0.08	0.17	2.19	202
9	218	27	0.04	0.15	3.47	182
10	260	23	0.06	0.23	3.65	212
11	206	22	0.05	0.26	4.94	180
12	225	17	0.07	0.17	2.38	199
13	201	12	0.10	0.28	2.86	182

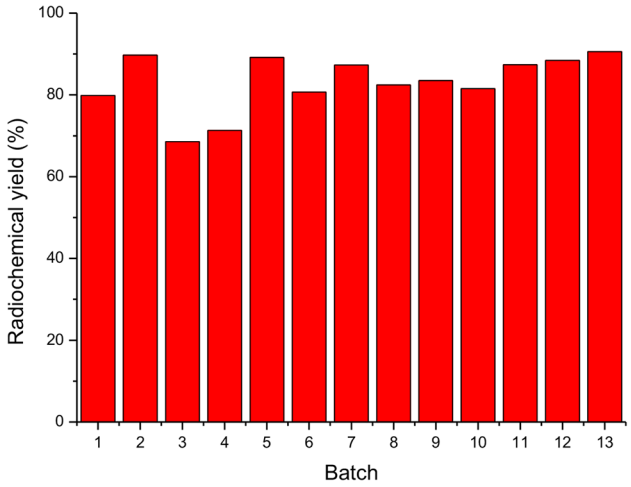

Fig. 3 Radiochemical yields of thirteen batches of [^{177}Lu]Lu-DOTATATE prepared using CA ^{177}Lu

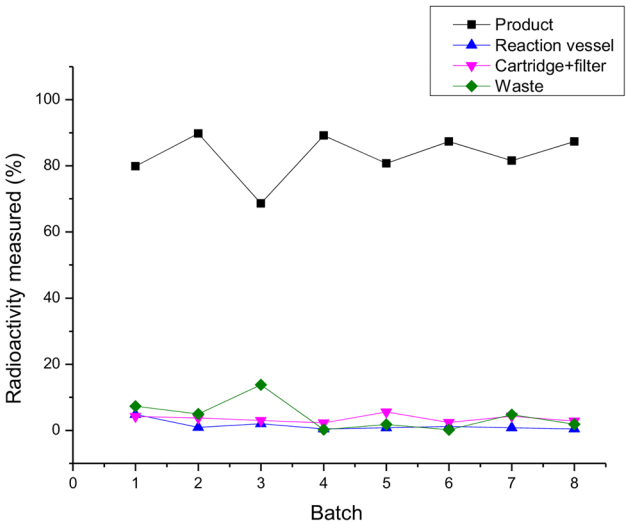

Fig. 4 Radioactivity fractionation as product, waste, cartridge + filter and reaction vial in 8 batches of [^{177}Lu]Lu-DOTATATE

The optimized protocol worked well except in the case of one batch when the radiochemical yield was < 80% despite the fact that M:L ratio was ~ 4.5. However, peptide concentration was not increased instead purification was done to remove the unreacted [^{177}Lu]$LuCl_3$. The lower radiochemical yield in this batch could be because of other metal contamination in the CA ^{177}Lu supplied, though the same was not reflected in the specification sheet of the product.

Cartridge purification removed unreacted [^{177}Lu]$LuCl_3$ and the radiochemical purity of [^{177}Lu]Lu-DOTATATE with NCA ^{177}Lu was always ~ 100% as free ^{177}Lu was not detected in both in TLC and PC.

[^{177}Lu]Lu-PSMA-617 Preparation

Results of the preparation of [^{177}Lu]Lu-PSMA-617 with CA ^{177}Lu are summarized in Table 4. The radiochemical yields were 83 ± 6%. The ligand to metal ratio used varied from 2.11 to 5.36 and the radiochemical yield during reaction varied from 74 to 87%. Cartridge purification was done in all batches. The radiochemical purity of the product recovered was ~ 100% after cartridge purification.

The results of the production of [^{177}Lu]Lu-PSMA-617 with NCA ^{177}Lu is summarized in Table 5. The reaction was always done with 100 μg (0.1 μmol) of PSMA-617.

Table 2 Results of failed batches of [^{177}Lu]Lu-DOTATATE using CA ^{177}Lu

Batch No	$^{177}LuCl_3$ (GBq)	Specific activity (mCi/µg)	Concentration of ^{177}Lu (µmol)	Concentration DOTATATE (µmol)	Ligand to metal ratio [L:M]	Product (mCi)	Waste (mCi)	Radiochemical yield %
1	225	23	0.05	0.24	3.21	50	148	22
2	224	23	0.05	0.17	3.23	14	187	6
3	200	23	0.05	0.17	3.62	9	194	4
4	211	22	0.07	0.23	3.38	20	165	9

Table 3 Details of or production of [^{177}Lu]Lu-DOTATATE using NCA ^{177}Lu

Batch number	$^{177}LuCl_3$ (mCi)	Specific activity (mCi/µg)	Concentration of ^{177}Lu (µmol)	Concentration DOTATATE (µmol)	Ligand to metal ratio [L:M]	Radiochemical yield (%)
1	239	100	0.01	0.10	7.74	96
2	222	98	0.01	0.10	8.17	94
3	240	87	0.02	0.14	8.90	83
4	216	88	0.01	0.14	10.05	97
5	217	87	0.01	0.14	9.89	92
6	476	88	0.03	0.28	9.12	95

Table 4 Radiochemical yield of [^{177}Lu]Lu-PSMA-617 using CA ^{177}Lu

Batch No	$^{177}LuCl_3$ (mCi)	Specific activity of ^{177}Lu (mCi/µg)	Concentration of ^{177}Lu (µmol)	Concentration of PSMA-617 (µmol)	Ligand/metal ratio [L:M]	Radiochemical yield (%)
1	148	23	0.04	0.19	5.36	86
2	428	23	0.14	0.29	2.11	87
3	213	17	0.06	0.19	2.98	86
4	290	19	0.06	0.19	2.93	74

Data of the first three batches were included in an earlier publication [9]. Data of one more batch done subsequently is added and presented for the sake of completion in this paper

Table 5 Summarized details of preparation of [^{177}Lu]Lu-PSMA-617 using NCA ^{177}Lu

Batch No	$^{177}LuCl_3$ (mCi)	Specific activity of ^{177}Lu (mCi/µg)	Concentration of ^{177}Lu (µmol)	Concentration of PSMA-617 (µmol)	Ligand/metal ratio [L:M]	Radiochemical yield
1	215	89	0.01	0.1	7.0	99
2	125	90	0.01	0.1	12.2	94
3	239	81	0.02	0.1	5.8	91
4	232	84	0.02	0.2	12.3	98

In one batch (batch no. 4), further addition of ligand was needed as the radiochemical yield was about 75% only which subsequently increased after addition of peptide. The radiochemical yield in all batches were > 98%, and hence, cartridge purification was not done for these batches.

Imaging

SPECT images of [^{177}Lu]Lu-DOTATATE (B) and [^{177}Lu]Lu-PSMA-617 (D) are given in Fig. 5. ^{68} Ga-DOTATATE (A) and ^{68} Ga-PSMA-11 (C) of the same patients are also

given in Fig. 5. The tracer uptake patterns are concordant with PET-CT images. All the patients treated showed significant regression of tumour with multiple doses of [^{177}Lu]Lu-DOTATATE/PSMA-617(results are not given here).

Discussion

Though a late entrant in the nuclear medicine filed, ^{177}Lu has already proven to be one of the most widely used radionuclide for targeted radionuclide therapy [18]. Many factors helped in its growth which included the ease of preparation of the radionuclide, convenient half-life, decay properties, and the easily amenable metallic chemistry which enabled radiolabeling with a wide range of targeting molecules. [^{177}Lu]Lu-DOTATATE was the first ^{177}Lu therapeutic radiopharmaceutical which was used for the treatment of neuroendocrine tumours (NETs) [18]. NETs being a relatively uncommon cancer, the number of patients who could benefit from lutetium therapy was limited. The use of [^{177}Lu]Lu-PSMA-617 for the treatment of prostate cancer opened an opportunity to address a major cancer [19]. Both [^{177}Lu]Lu-DOATATATE and [^{177}Lu]Lu-PSMA-617 have the advantage that these radiopharmaceuticals use small molecules as targeting vectors. The molecular weight of DOTATATE is 1435 Daltons and that of PSMA-617 is 1042 Daltons. While using small molecular weight carrier molecules the targeting is fast and non-targeted radiopharmaceuticals are excreted from the body very quickly. This improves the radiation dosimetry significantly as larger quantities of radioactivity can be injected which gives higher radiation dose to the target without exceeding dose limits to other organs and tissues [20]. The clinical use of both [^{177}Lu]Lu-DOATATATE and [^{177}Lu]Lu-PSMA-617 are increasing rapidly [21]. Several other ^{177}Lu radiopharmaceuticals are now used in clinical setups [22, 23]. All these radiopharmaceuticals use small molecular weight peptides or inhibitor molecules as targeting vectors.

Lutetium-177 is prepared using nuclear reactors in many countries across the world and the quality of ^{177}Lu vary drastically depending on the production procedure followed in each reactor. The two independent routes of production followed either using enriched ^{176}Lu or enriched ^{176}Yb yield CA or NCA ^{177}Lu, respectively. NCA ^{177}Lu is preferred from a radiochemists point of view as the specific activity is high and remains constant despite decay. This makes the preparation of the radiopharmaceutical easier as a set protocol can be followed from batch to batch to get consistent yields of the finished products. The specific activity of the radiopharmaceutical prepared is also high which is clinically desirable as saturation of the receptors or enzymes in the cancer cells will not happen. For example, as per an IAEA clinical protocol, the specific activity limit for [^{177}Lu]Lu-DOTATATE is 1 mCi/μg [17]. However, while using CA ^{177}Lu a major problem is the varying specific activity of the product delivered by the vendor. This is because the neutron flux, irradiation time, post-bombardment radioactive decay, etc. vary significantly from batch to batch of the production of CA ^{177}Lu. Nevertheless, CA product is sometimes preferred because of the lower cost of production which is also translated in the cost of the finished product [24]. While using CA ^{177}Lu, it is important to adjust the amount of

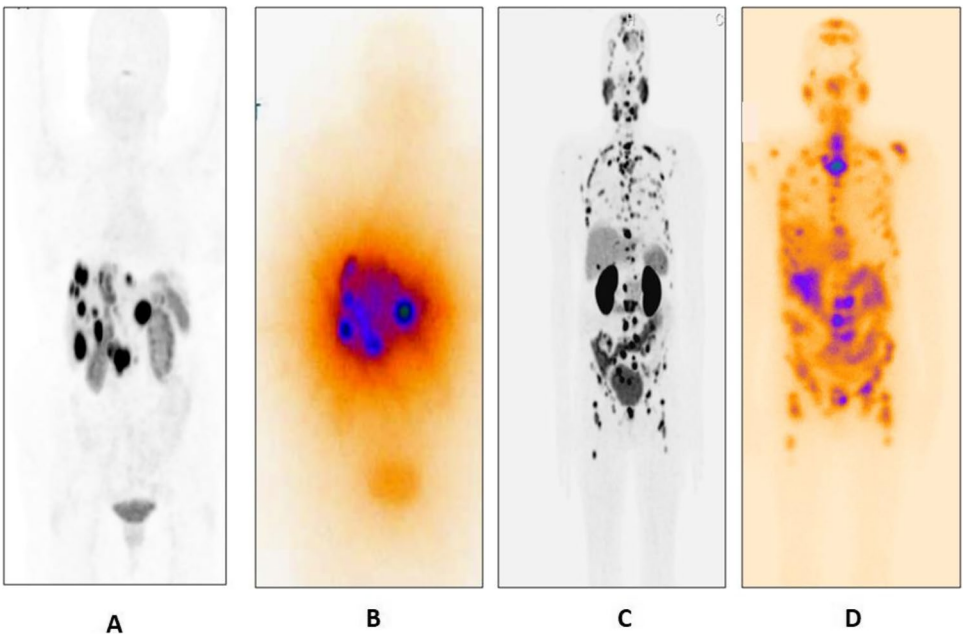

Fig. 5 **A** MIP image of ^{68} Ga-DOTATATE PET-CT of a patient injected with 3.5 mCi (130 MBq) activity. **B** Whole-body SPECT image of a patient administered with 178 mCi (6.6 GBq) of [^{177}Lu]Lu-DOTATATE. **C** MIP image of ^{68} Ga-PSMA-11 PET-CT of a patient administered with 4.05 mCi (120 MBq) activity. **D** Whole-body SPECT image of a patient administered with 190 mCi (7.03 GBq) of [.^{177}Lu]Lu-PSMA-617

peptide/inhibitor ligand used during every batch of production. It is sometimes necessary to increase the concentration of peptide/ligand to get higher radiochemical yields.

While both DOTATATE and PSMA-617 use DOTA as the chelating agent, there could be difference in the reaction kinetics because of the difference in the conjugation methodology used to attach the chelate to the peptide/inhibitor molecule. In the case of DOTATATE, DOTA is directly conjugated to the peptide without a linker molecule (Fig. 6) [25]. Whereas in the case of PSMA-617, the chelate is added through an eight-carbon linker molecule (Fig. 7) [26]. The complexation chemistry while using DOTATATE could be different from PSMA-617 because of the proximity of other amino acids having donor atoms. Hence, the protocol for each radiopharmaceutical will vary depending on the molecule used for labeling and hence need to be carefully optimized.

Estimation of the radiochemical yield as well as the radiochemical purity is an important aspect while preparation of radiopharmaceuticals in a hospital radiopharmacy. Radio HPLC is the preferred analytical technique for quality control of radiopharmaceuticals as it can distinguish not only between the product and free ^{177}Lu but can also separate other species, if any present. However, most of the hospital radiopharmacies are not equipped with HPLC. Also, it is important to have fast analytical methods to quickly ascertain the radiochemical purity. PC and TLC are mainly relied in hospital radiopharmacy for QC of finished radiopharmaceuticals. It is preferred to have at least two

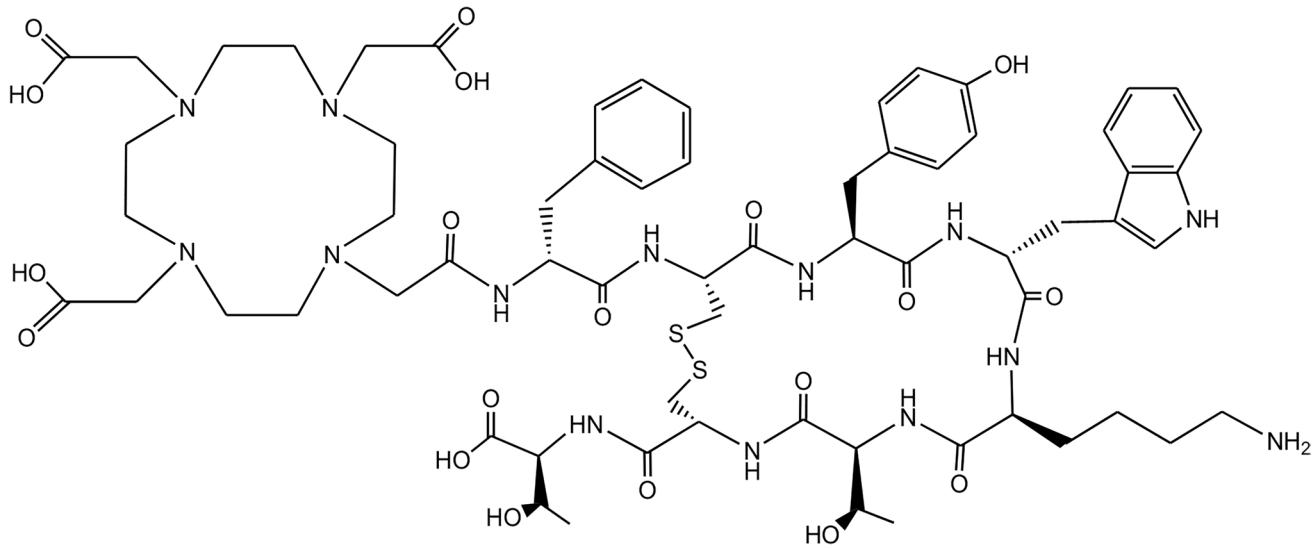

Fig. 6 Structure of DOTATATE used for [^{177}Lu]Lu-DOTATATE preparation

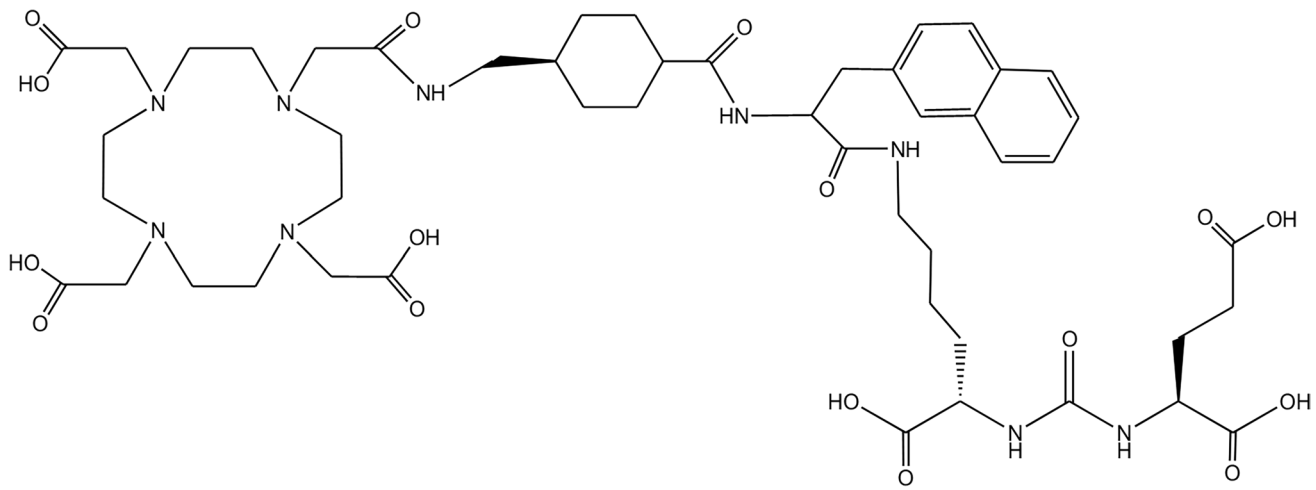

Fig. 7 Structure of PSMA-617 used for [^{177}Lu]Lu-PSMA-617 preparation

chromatography systems wherein the product and impurity have opposite movements. It is important to note that at the end of the complexation reaction ^{177}Lu might not be present as $^{177}LuCl_3$ but could be in some weak complex with the buffers used and hence could have similar movements as the labeled peptide in some solvent systems. In PC using Whatman 1 paper using acetonitrile: water (1:1) as mobile phase, [^{177}Lu]Lu-DOTATATE and [^{177}Lu]Lu-PSMA-617 remain at the point of spotting, whereas free ^{177}Lu moves to the solvent front. In ITLC-SG developed in acidified trisodium citrate solution, both [^{177}Lu]Lu-DOTATATE and [^{177}Lu]Lu-PSMA-617 moved to the solvent front, whereas free ^{177}Lu remained at the point of spotting. By combining the two chromatographic systems, it was possible to estimate the radiochemical yields as well as radiochemical purity. Cartridge purification step also acted as an additional test for estimation of the radiochemical yield. If needed, it can also be used for estimation of radiochemical purity.

In the present studies, preparation of the radiopharmaceuticals with CA ^{177}Lu needed higher technical skill. Our procedure was to initially adapt a metal to ligand ratio ~ 1:2.5 to facilitate complexation. If the radiochemical yields were less than 90% additional peptide/inhibitor ligand was added and reaction continued. However, we aimed to minimize the ligand concentration such that a minimum specific activity of mCi/μg was obtained for the finished radiopharmaceuticals. However, in certain batches of DOTATATE, we had to supplement with additional peptide.

The pH of the reaction is highly crucial. Lutetium-177 is supplied as chloride salt in dilute hydrochloric acid solution and is stable. Whereas at basic pH, Lu is likely to hydrolyse and will not complex with chelates. The desired pH reported for lutetium complexation with DOTA chelate is 4.5–5.0. This is achieved by having a suitable buffer system. The use of ammonium acetate/gentisic acid buffer was reported for the preparation of [^{177}Lu]Lu-DOTATATE [26]. The same buffer system was also reported to be used in freeze-dried kits also [27]. Other buffers reported are ascorbic acid/sodium hydroxide and ascorbic acid/sodium acetate in an automated module and freeze-dried kits for [^{177}Lu]Lu-DOTATATE [28]. We used two buffer systems, sodium acetate/ascorbic acid and sodium ascorbate/ascorbic acid. The molarity was adjusted such that the buffer will have sufficient buffering capacity and the pH is maintained at ~ 5 when $^{177}LuCl_3$ is added. In our experience, both the buffer systems worked well. However, we preferred to use sodium ascorbate/ascorbic acid system as ascorbic acid being vitamin C is biologically acceptable.

The purification step using solid-phase cartridge is crucial as demonstrated with the failed batches. Though both TLC and PC showed good complexation yields in these batches, the product recovery after cartridge purification was very low. Similar problem was reported from users who used activity from the same batches of CA^{177}Lu. Cartridge purification also removed other ingredients including buffer salts from the final product. The product eluted with 70% ethanol is diluted with 0.9% saline solution prior to injection to patients. These product solutions were stable for extended hours and days; however, it was used immediately after preparation.

The clinical studies showed that both the radiopharmaceuticals have the desired uptake and the SPECT images obtained from ^{177}Lu radiopharmaceuticals are concordant with the PET-CT images using corresponding ^{68} Ga radiopharmaceuticals.

Conclusion

Preparation of ^{177}Lu radiopharmaceuticals using radionuclide and chelate conjugated biomolecules is practiced in many nuclear medicine departments across the world. The technical skill needed for the preparation of these radiopharmaceuticals are much higher than what is needed for the formulation of radiopharmaceuticals using freeze-dried kits and generator eluted ^{99m}Tc or ^{68} Ga. The varying specific activity of CA ^{177}Lu is a major issue during the preparation of the radiopharmaceuticals. Robust protocols able to provide radiopharmaceuticals with high radiochemical purity was developed for both CA and NCA ^{177}Lu. The protocols developed for the preparation of [^{177}Lu]Lu-DOTATATE and [^{177}Lu]Lu-PSMA-617 and reported in this paper can be adapted by nuclear medicine departments wanting to start these targeted therapies.

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Data Availability Contact the corresponding author for data requests.

Declarations

Conflict of Interest Raviteja Nanabala, Maroor Raghavan Ambikalmajan Pillai, Buvaneswari Gopal declare that they have no conflict of interest.

Ethical Statement The study was approved by the institutional review board of Doctors Diagnostic and Nuclear Medicine Research Centre (DDNMRC) and informed consent was obtained from all individual participants included in the study. All procedures performed in studies

involving human participants were in accordance with Helsinki declaration as revised in 2103 and its later amendments.

Consent for Publication Not applicable.

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