

Lutetium-177 Therapeutic Radiopharmaceuticals: Linking Chemistry, Radiochemistry, and Practical Applications

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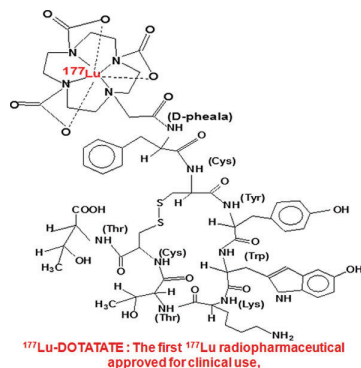
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Received: March 27, 2014

Published: April 13, 2015



ACS Publications

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DOI: 10.1021/cr500171e
Chem. Rev. 2015, 115, 2934–2974

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1. INTRODUCTION

Interest in the use of radionuclides for treatment of various diseases has a long history and parallels the isolation of radium by Marie and Pierre Curie in the early part of the 20th Century. The availability of radium generated widespread enthusiasm and was considered as a potential medicine for many incurable diseases. Success evaded such attempts until radioactive phosphorus (^{32}P) prepared at the University of California Berkeley cyclotron was found to be effective for treating polycythemia rubra vera, a myeloproliferative disease characterized by overproduction of red blood cells. The introduction of iodine-131 (^{131}I) as a radioactive medicine for treatment of thyroid cancer in 1946 saw the successful application of radionuclides in medicine. The clinical use of radioiodine (^{131}I) therapy is a key example of molecular nuclear medicine, and this therapeutic radionuclide continues to be used in many technologies focused on cancer treatment where no competing replacement is envisaged in the near future.

Several radionuclides which decay by emission of beta particles (β^-), alpha particles (α), or Auger (AE) and conversion electrons (CE) are under both radiopharmaceutical development and clinical evaluation as potential therapeutic radionuclides. Among the radionuclides suggested for targeted therapy, research with ^{177}Lu -based radiopharmaceuticals has demonstrated spectacular growth in recent years. Less than 10 papers were published on the development of lutetium-177 (^{177}Lu)-labeled radiopharmaceuticals in the last century, whereas more than 500 publications have appeared in the last 14 years, demonstrating the increasing interest in the use of this therapeutic radionuclide. Monoclonal antibodies, peptides, phosphonate ligands, particulates, steroids, and other small molecules have been radiolabeled with ^{177}Lu for

the development of a wide variety of therapeutic radiopharmaceuticals. The success of treating patients suffering from neuroendocrine tumors with ^{177}Lu -labeled DOTA-Tyr³-octreotate (DOTA-TATE), a somatostatin analogue peptide, is the single most important example that has contributed to the worldwide interest and growth of ^{177}Lu as a therapeutic radionuclide.¹

Although decay properties are an important consideration for selection of a therapeutic radionuclide, the success of using any radioisotope as an integral part of a radiopharmaceutical depends on the feasibility for production in high activity levels with acceptable quality and the ability for transportation to nuclear medicine facilities, which are generally distant from production centers. As discussed later in this review, ^{177}Lu has many advantages compared to other therapeutic radionuclides for the potential treatment of several types of cancers. Radionuclidic characteristics of ^{177}Lu such as the energies and abundance of the emitted β^- particles and gamma photons and its half-life make it suitable for use as a therapeutic radionuclide for targeting small primary tumors and metastatic sites.

Although the clinical efficacy of several ^{177}Lu radiopharmaceuticals has been demonstrated using "in-house" formulations, at present there are no ^{177}Lu -labeled radiopharmaceuticals with regulatory approval for routine clinical use. A number of clinical trials using ^{177}Lu radiopharmaceuticals are also in progress in many countries; however, it is expected that approved ^{177}Lu radiopharmaceuticals will be commercially available in the near future.

This paper is the first published review on ^{177}Lu radiopharmaceuticals and summarizes the developments in this emerging important field. This comprehensive review on ^{177}Lu radiopharmaceuticals covers research from 1960 and begins with an introduction on radiopharmaceuticals used in nuclear medicine with a goal to orient the reader to the importance of this field. Lutetium is the last member of the lanthanide family, and its chemistry plays an important role in the preparation of radiopharmaceuticals that are stable in vivo. Various bifunctional chelating agents (BFCA) that are used for tagging ^{177}Lu with carrier vectors are discussed. The review also covers the production aspects of ^{177}Lu in detail and its different production methods. The comparative advantages and disadvantages of the two major reactor production routes are elaborated. Research which led to the development of different ^{177}Lu radiotracers is provided, and this review also describes the results of promising clinical studies that have been conducted with ^{177}Lu radiopharmaceuticals.

1.1. Radiopharmaceutical Overview

Radioactive drugs (radiopharmaceuticals) used in nuclear medicine, oncology, interventional radiology/cardiology, and related specialties involve the use of unsealed radioactive sources—as opposed to the use of sealed radioactive sources in radiation oncology. Radiopharmaceuticals are radiolabeled molecules designed to target tissues and processes in vivo and are used in either diagnostic or therapeutic applications. Unlike the well-established applications of nonradioactive drugs, diagnostic radiopharmaceuticals contain very small doses of the active ingredients and are not pharmacologically active. On the other hand, therapeutic radiopharmaceuticals generally possess a significant concentration of active ingredient which can induce pharmacological changes. Radiopharmaceuticals are designed to measure a physiological event (imaging) or for the treatment of a malady (therapy). In the case of therapeutic applications, such as

the use of ^{177}Lu -labeled pharmaceuticals discussed in this review, the therapeutic effects results from the radiotoxicity induced by the emission of particulate radiations. Radiopharmaceuticals are manufactured under current Good Manufacturing Practices (cGMP) with specific regulations and must be of adequate purity for human administration.

The major tools employed for diagnosis are imaging modalities, which include both planar and tomographic imaging technologies such as single-photon emission computed tomography (SPECT) and positron emission tomography (PET). Radionuclides that primarily emit gamma photons or positrons that can have a high abundance of photon emissions are used in diagnostic nuclear medicine. Radiopharmaceuticals used for therapeutic applications, in contrast, contain radionuclides that decay by particulate emission (alpha, beta, or Auger electrons), and the decay energy is deposited at the target sites to kill cancerous or other diseased cells.^{2,3} The design of radiopharmaceuticals involves radionuclide attachment to the targeting molecule either directly or via the use of a bifunctional chelating agent (BFCA). An example of a direct radiolabeling is the radioiodination of the phenolic group of the amino acid tyrosine in a biological vector. In the case of radiolabeling through a chelator, a radionuclide is complexed with the donor atoms of a BFCA. These targeting molecules are often peptides, antibodies, antibody fragments, or small molecules that are receptor specific and peptidomimetics or nonpeptide receptor ligands. Often a proven conventional drug is selected as a lead molecule to be developed into a radiopharmaceutical by incorporating an appropriate radionuclide useful for diagnosis or therapy. An important challenge is to maintain the molecular targeting characteristics of the modified molecule, since even subtle structural changes in molecules that act as the vector can often result in loss of targeting properties. The design of a radiopharmaceutical for a specific application therefore must take into consideration the properties of both the targeting carrier molecule as well as the radionuclide.

1.2. Role of Molecular Nuclear Medicine

The distinct advantage of nuclear medicine is its application using novel biomarkers for the study of biochemical processes at the cellular level. These techniques can delineate changes in cellular function at a stage much earlier than the manifestation of anatomical changes or the onset of clinical symptoms. This unique and important strength is referred to as molecular nuclear medicine.⁴ Many nuclear medicine imaging techniques measure flow, but localization of the radiopharmaceutical can also provide information to visualize and map the biological processes, such as cell growth or cell destruction leading to biochemical changes occurring in living systems. An example is the widespread use of ^{18}F -labeled fluorodeoxyglucose (^{18}F FDG or simply FDG) where a glucose analogue radiolabeled with ^{18}F , a positron-emitting radionuclide, is injected into a patient followed by imaging with positron emission tomography (PET) instrumentation. The images obtained permit the visualization of abnormal cellular metabolism and proliferation as glucose, and thereby FDG is taken up by diseased cells more than in normal cells. The clinical introduction of dual imaging modalities such as PET/CT (computed tomography) and more recently PET/MRI (magnetic resonance imaging) permits simultaneous measurement of both cellular metabolic processes as well as anatomical details. The increasing availability of these technologies has led to a new era of accurate mapping of cellular processes occurring in cancer and other diseases, which range from detection, staging,

treatment planning, and finally disease management. These studies not only aid in assessing treatment response for the use of chemotherapeutic agents but also help in distinguishing cells which are proliferating owing to angiogenesis from normal cells.

Molecular nuclear medicine has thus attained the present status due to a gradual evolutionary process which includes the unique capability for noninvasive assessment of physiological processes occurring in vivo by following radiopharmaceutical metabolism. The advances in imaging techniques have expanded opportunities, paving the way for nuclear medicine investigators to obtain two-dimensional images of the whole body. Subsequent improvements brought about by the introduction of new and improved radiotracers as well as new imaging techniques have enabled the acquisition of tomographic (i.e., three-dimensional) images of coronary blood flow and related tissue function.

Understanding changes at the molecular and cellular level provides vital clues for evaluating the effectiveness of a clinical treatment strategy. This information, in turn, has a major impact on understanding disease and its detection and progression, deciding individualized treatment, and consequently developing suitable drugs. The concept of molecular nuclear medicine provides a "window" to visualize the biochemical processes occurring in vivo.⁵ Functional radionuclide imaging helps in following the pathology in individual patients and provides a means to tailor clinical management, contributing to the conceptualization of the new era of "personalized medicine". The relatively well-established, unique advantages of molecular nuclear medicine over conventional techniques such as ultrasound (US), CT, and MRI are the opportunities to provide unique physiological and molecular information in the fields of oncology, cardiology, and neurology.

2. TARGETED THERAPY

In contrast to the use of diagnostic applications in nuclear medicine, radiopharmaceuticals designed for therapy are agents which deliver therapeutic doses of particulate and ionizing radiation to the diseased sites.^{2,3,6} As the term implies, targeted therapy is a treatment modality using agents which act as molecular vectors for transporting/targeting radionuclides to specific biological sites. Agents intended for use in targeted therapy are endowed with target specificity due to the presence of the carrier molecule with specific affinity for targeted sites. Examples include monoclonal antibodies which target specific antigens or peptides which target specific receptors that are overexpressed on cancerous tissues. Therapeutic efficacy is accomplished by inducing cytotoxicity to the tumor cells to arrest further proliferation. The most desirable features of a therapeutic radiopharmaceutical are the ability to deliver sufficient radiation dose to the target, to retain the radiopharmaceutical or the metabolite carrying the radionuclide at the site of interest, and to ensure rapid clearance of radioactivity from nontargeted tissues and organs. Target specificity is ensured by identifying a suitable target-seeking molecule and radiolabeling it with the radionuclide without compromising biological targeting.

There are several challenges involved in the development of therapeutic radiopharmaceuticals which arise from the required balance between specific in vivo targeting properties to the sites of interest with simultaneously less accumulation and more rapid clearance of radioactivity from nontarget sites. The possibilities of designing new radiopharmaceuticals arise from the evaluation of a large number of therapeutic radionuclides with widely

different radionuclidic characteristics and also the availability of a large library of molecular targeting vectors.

2.1. Therapeutic Radionuclides

While designing a radiopharmaceutical for a particular therapeutic application, the choice of the appropriate radionuclide constitutes a prime determinant.^{2,3,7–9} The major criteria for choice of a radionuclide for therapeutic use include the radionuclidic half-life, the type, energy, and branching ratio of particulate radiation, as well as photon abundance and energies. It is important to match the physical half-life of the radionuclide with the biological half-life of the carrier molecule used. Other important considerations include the availability of convenient and high-yield chemical strategies for stable attachment of the radionuclide to the carrier molecule, specific activity (activity/mass), radionuclidic and chemical purity, production feasibility, and cost. There are a large number of radionuclides which show potential use for the development of therapeutic radiopharmaceuticals. While it is difficult to select any one radionuclide as ideal or the best suited for therapy, a few will have more desirable properties than others for a desired application. A summary of key radionuclides which exhibit nuclear decay characteristics of interest for various in vivo therapeutic applications is given in Table 1.¹⁰

On the basis of the nature of the emitted radiation, radionuclides can be classified as α -particle emitters, β^- -particle emitters, conversion electron (CE) emitters, or Auger electron emitters (AE). Auger electrons are emitted by radionuclides that decay by electron capture (EC) or internal conversion (IC). The decay creates a vacancy in an inner atomic shell which is filled by electrons cascading down from higher shells leading to a cascade of electron transitions with the emission of characteristic X-ray photons or Auger, Coster–Kronig, or super Coster–Kronig monoenergetic electrons. These electrons are distinguished on the basis of the shells involved with the transition and are often collectively referred to as Auger electrons.

Many of the radionuclides also emit γ rays after emission of either α or β^- particles, and some metastable radionuclides emit only γ photons. Each type of particle emission used for targeted therapy with unsealed sources has different linear energy transfer (LET) values and different ranges in soft tissue (Figure 1). LET is the measure of the energy transferred to the medium as an ionizing radiation passes through it and is used to quantify the effect of ionizing radiation on the medium such as a biological specimen. The LET values depend on both the nature of the radiation as well as on the material through which the particulate radiation passes. Particulate emissions such as α and β^- particles have high LET, whereas gamma and X-rays have low LET. High LET results in higher radiation damage to the biological systems and is quantified by the term “relative biological effect” (RBE). The RBE is the ratio of biological effectiveness of one type of ionizing radiation relative to another, given the same amount of absorbed energy. Among nuclear radiation, the RBE of alpha particles is the highest, followed by β^- particles and γ rays. The higher the RBE, the more damaging the radiation for the same absorbed energy. Radiations having higher LET, and hence high RBE, are necessary for inducing therapeutic effects. Pure gamma emitters are hence not useful for therapeutic applications in nuclear medicine, whereas high intensity γ radiation is used in sealed sources. Among the three other particulate emissions, α particles have the highest LET and are hence capable of producing the maximum RBE.¹¹ Radionuclides which emit α particles are effective where it is advantageous to use particulate

Table 1. Summary of Key Therapeutic Radionuclides of Current Interest¹⁰

radionuclide	half-life	particulate energy in keV	principal γ energy in keV (% abundance)
β^- -particle emitters			
¹¹¹ Ag	7.450 days	1036	342 (6.7)
⁷⁷ As	38.83 h	682	239 (1.6)
¹⁹⁸ Au	2.695 days	1372	411 (95.5)
¹⁹⁹ Au	3.139 days	452	158 (36.9)
⁶⁷ Cu	61.83 h	577	184 (48.7)
¹⁶⁵ Dy	2.33 h	1286	94 (3.6)
¹⁶⁹ Er	9.400 days	351	nil
¹⁵⁹ Gd	18.48 h	970	58 (26.2)
¹⁶⁶ Ho	26.83 h	1854	80 (6.2)
¹³¹ I	8.020 days	970	364 (81.2)
¹⁷⁷ Lu	6.734 days	498	208 (11.0)
³² P	14.26 days	1710	nil
¹⁰⁹ Pd	13.70 h	1115	88 (3.6)
¹⁴⁹ Pm	53.08 h	1071	285 (2.8)
¹⁴² Pr	19.13 h	2162	nil
¹⁸⁶ Re	90.64 h	1069	137 (8.6)
¹⁸⁸ Re	16.98 h	2120	155 (14.9)
¹⁰⁵ Rh	35.36 h	567	318 (19.2)
⁴⁷ Sc	3.345 days	600	159 (68.0)
¹⁵³ Sm	46.27 h	808	103 (28.3)
⁸⁹ Sr	50.53 days	1496	nil
¹⁶¹ Tb	6.88 days	518	74 (10.2)
⁹⁰ Y	64.10 h	2282	nil
¹⁷⁵ Yb	4.185 days	470	396 (6.5)
α -particle emitters			
²²⁵ Ac	10.0 days	5935	99 (3.5)
²¹¹ At	7.21 h	5982	687 (0.25)
²¹² Bi	60.55 min	6207	727 (11.8)
²¹³ Bi	45.59 min	5982	439 (27.3)
Auger electron emitters			
¹²⁵ I	59.40 days	12.24	35 (6.68)
¹¹¹ In	2.80 days	6.75	245 (94)
⁶⁷ Ga	3.26 days	6.26	93 (39.21)
conversion electron emitter			
¹¹⁷ Sn	13.6 days	127, 129	159 (86)

^aFor β^- particles the maximum β^- energy is mentioned. Auger electron energy is the average kinetic energy of Auger and Coster–Kronig electrons emitted per decay.

radiation with a range of only a few cell diameters, such as the use of ²¹³Bi for therapy of leukemia cancer cells in the vascular system.¹² An emerging clinical application of an alpha emitter (²²³Ra), such as Alpharadin, is for the treatment of cancer metastases in the skeleton.¹³ Alpha particles deposit their energy over a short range (40–100 μ m) and produce high-density ionizations along the tracks they traverse.¹⁴ As a result, α particles are capable of producing significant cellular damage by inducing double-stranded DNA breakage while delivering minimum radiation damage to nontargeted tissues. For oncologic applications, α -particle emitters are more compatible for use in the treatment by rapid localization in blood-borne cancers and tumors with small diameters and where their localization within the tumor is homogeneous and crossfire to surrounding cells is not an issue.¹⁵ One of the challenges for broader use of α -particle therapy is the lack of large-scale availability of suitable radionuclides. In addition, the short tissue range and short

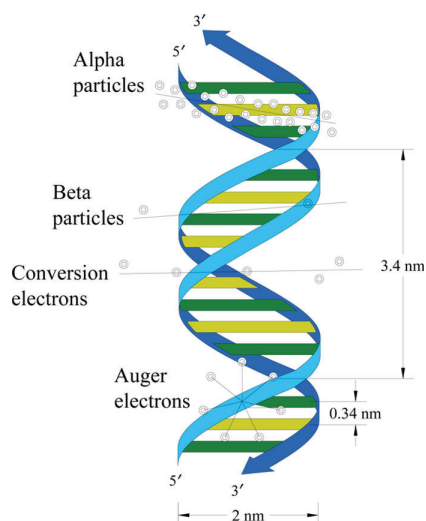


Figure 1. Cartoon illustration showing the interaction of different types of particulate radiation with DNA. Path ranges in the figure are not drawn to scale.

half-life generally require rapid targeting. The ranges of the different ionizing radiations along the tracks as they pass through the biological system are $\alpha = 50\text{--}100\ \mu\text{M}$, $\beta^- = 0.2\text{--}15\ \text{mm}$, Auger electrons = a few nanometers, $\gamma =$ several centimeters. The ionization densities (mean energy deposition/path length ($\text{keV}/\mu\text{m}$)) are $\alpha = 80\text{--}300$, $\beta^- = 0.2$, and $\text{AE} = 4\text{--}26$.³

Radionuclides that decay by emission of β^- particles and conversion electrons have been most extensively used for a broad series of radiotherapeutic applications because of their availability and suitability to treating large tumor volumes. These applications include cancer therapy, treatment of rheumatoid arthritis (synovectomy), arterial restenosis therapy, nonmelanoma skin cancer, etc.^{2,3} β -Particle emitters produce a nearly homogeneous radiation dose distribution even though generally their deposition is heterogeneously distributed in target tissues.¹⁶ As an example, most neoplasia consist of a heterogeneous distribution of stromal (structural), normal parenchymal (functional) cells, and tumor cells. Although the tumor cells may express a specific receptor, due to differential blood flow and other barriers, all cells will not be accessible by the targeting agent and hence will not receive the tracer. This makes the “cross-fire” effect particularly important for larger tumors.

Auger electrons are monoenergetic, low-energy electrons emitted during the decay of certain radionuclides by internal conversion (IC) or electron capture (EC) processes. Auger electrons have very short range in soft tissues and deposit their energy over subcellular dimensions. If targeted to the cell nucleus, high radiotoxicity is exhibited in the immediate vicinity of the DNA decay site.^{17,18} Although Auger electrons actually have very low energy (20–500 eV), a high LET-like response is achieved due to their short range (1–10 nm).¹⁹ However, Auger electron emitters must generally be targeted into the cell nucleus. The cytotoxicity, and hence the therapeutic efficacy, will be much less even when these radionuclides are present in the cytoplasm or on the surface of the target cells. Early studies have indicated that ¹¹¹In–Octreotide, an Auger-electron-emitting radiopharmaceutical, has low therapeutic efficacy where the tumor size becomes an important factor for the success of such therapy.²⁰ The disadvantage of the peptides radiolabeled with hard beta emitters such as ⁹⁰Y is the dose-limiting toxicity to the kidneys.

While efforts with promising Auger electron emitters are currently being focused on the design of therapeutic radiopharmaceuticals, the design of agents which demonstrate the high targeting required for effective in vivo therapy remains a challenge.

2.2. Theranostics: Use of Diagnostic/Therapeutic Radionuclide Pairs

The term “theranostics” was coined to describe the combined use of a diagnostic tool that assists in the selection of the most appropriate therapeutic tool for treatment of a specific disease.^{21,22} The concept of theranostics, also known as “theranosis”, is utilized to tailor the therapy in a specific patient following the complete diagnosis of the disease, thus introducing the concept of personalized medicine. Nuclear medicine offers an ideal opportunity for theranostics since the dose of a diagnostic agent can be augmented to obtain a therapeutic effect. The advantage of this modality is the ability to perform imaging using SPECT/CT or PET/CT to provide the necessary pretherapy information on biopharmacokinetics and to guide the dosimetry focused on limiting dose to a critical organ or tissue.²³ The information thus obtained is used for defining the maximum tolerated dose (MTD). If the imaging results then warrant it, it is generally considered safe and appropriate to follow up with dose-ranging experiments to allow targeted molecular therapy using a higher dose of the same radiopharmaceutical. These factors are especially important for being able to perform individualized imaging as well as therapy with the same radiopharmaceutical, in the same patient.

A typical example of a theranostic radionuclide which emits both gamma photons as well as particulate radiation is ¹³¹I, which has been used for many years in low doses for the diagnosis and staging of thyroid cancer using gamma imaging.²⁴ Subsequently, large doses of ¹³¹I are administered for thyroid ablation therapy. Examples of radionuclides which have theranostic potential are given in Table 2.

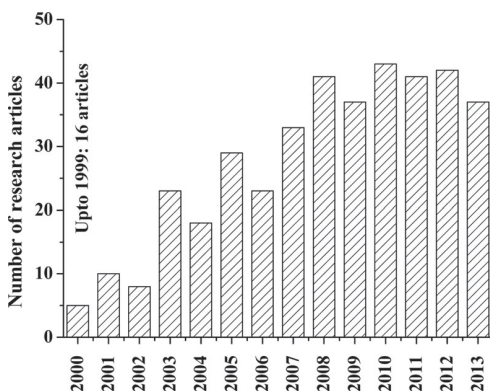
3. EVOLUTION OF ¹⁷⁷Lu RADIOPHARMACEUTICALS

The first clinical use of ¹⁷⁷Lu was reported by Anderson et al. in 1960 when three patients suffering from myelomatosis were treated by intravenous injection of ¹⁷⁷Lu as lutetium chloride and/or citrate.²⁵ Results of these clinical studies were not promising since the patients did not show long-term survival but reported mild pain relief. No subsequent publication on ¹⁷⁷Lu appeared until Keeling et al. in 1988 reported a study on the uptake of ¹⁷⁷Lu hydroxyapatite (HA) particles to investigate the mechanism of uptake on bone minerals by in vitro techniques.²⁶ Schlom et al. in 1991 reported ¹⁷⁷Lu radiolabeling of the CC49 murine monoclonal antibody that recognizes the tumor-associated glycoprotein 72 (TAG-72).²⁷ Ando et al. reported the preparation and biological evaluation of ¹⁷⁷Lu–EDTMP (EDTMP = ethylenediaminetetramethylene phosphonic acid) as a bone palliating agent which was followed by another independent report by Solla et al., who applied this agent in patients.^{28,29} The broader potential use of ¹⁷⁷Lu as a therapeutic radionuclide was, however, established with the use of ¹⁷⁷Lu–DOTATATE (DOTA = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid; TATE = tyrosine-3-octreotate), a radiopharmaceutical which targets neuroendocrine tumors.^{1,30} Radiolabeling of several lead molecules has been more recently reported, and the potential application of ¹⁷⁷Lu as a therapeutic radionuclide is expanding, as seen from the increase of publications since the beginning of the past decade (Figure 2).

Table 2. Key Examples of Theranostic Radionuclides Which Emit Both Gamma Photons as Well as Particulate Radiation¹⁰

radionuclide ^a	half-life	$E_{\beta/\max}$ in keV (% abundance)	E_{γ} in keV (% abundance)
¹⁶⁶ Ho	26.83 h	1854 (50.0) 1774 (48.7)	81 (6.2)
¹³¹ I	8.02 days	606 (89.9) 333 (7.27)	364 (81.2) 636 (7.27)
¹⁷⁷ Lu	6.73 days	498 (78.6) 385 (9.1) 176 (12.2)	208 (11.0) 113 (6.4)
¹⁸⁶ Re	90.64 h	1069 (80) 932 (21.5) 581 (5.78)	137 (8.6)
¹⁸⁸ Re	16.98 h	2120 (71.1) 1965 (25.6)	155 (14.9)
¹⁰⁵ Rh	35.36 h	567 (75) 260 (5.2) 247 (19.7)	306 (5.13)
¹⁵³ Sm	46.27 h	808 (17.5) 705 (49.6) 635 (32.2)	103 (28.3) 70 (5.25)
¹⁶¹ Tb	6.88 days	593 (10.0) 567 (10.0) 518 (66.0) 461 (26.0)	74 (9.8) 49 (14.8)
¹⁷⁰ Tm	128.4 days	968 (76) 884 (24)	84 (3.2) 52 (2.2) 51 (1.3) 59 (0.9)
¹⁷⁵ Yb	4.185 days	470 (86.5), 73 (10.2)	396 (6.5), 282 (3.1)

^aRadionuclides with beta and gamma emissions and suitable for therapy are listed.

**Figure 2.** Number of publications with ¹⁷⁷Lu related to nuclear medicine (data from Pubmed, Medline, and Scopus).

The rapid growth of interest in ¹⁷⁷Lu as a therapeutic radionuclide can be attributed to its favorable nuclear characteristics and amenable chemistry, leading to stable products that show good in vivo characteristics. However, the single most important factor which contributes to the increased interest and use of ¹⁷⁷Lu in nuclear medicine is its ease of production in high activity levels with high specific activity in many existing nuclear reactors worldwide.³¹

4. LUTETIUM CHEMISTRY

4.1. Inorganic Chemistry of Lu

Lutetium is the last member of the lanthanide series, with 71 electrons arranged in the [Xe]4f¹⁴5d¹6s² configuration. During chemical reaction, Lu atoms lose the two outermost electrons as well as the only 5d electron, thereby generating a +3 metal cationic species. Chemically, lutetium is a typical lanthanide since its only common oxidation state is +3, observed in its oxide, halides, and other compounds. The Lu atom is the smallest lanthanide, due to the lanthanide contraction phenomenon, which explains several important properties of Lu, including the highest metallic hardness and density. Unlike other lanthanides which are categorized in the f block of the periodic table, Lu could also be considered as the first element of the d block in the sixth period because it has a completely filled 4f orbital containing 14 electrons. In its most stable +3 oxidation state, Lu has empty s, p, and d orbitals and a closed shell of f orbitals. Electrons in f orbitals are incapable of bond formation since they are tightly bound due to high effective nuclear charge and are not influenced by ligands surrounding the metal ion. Thus, the hard Lewis acid chemistry of Lu³⁺ is mostly governed by the empty s, p, and d orbitals. Due to the completely filled f orbital, the ionic radius of Lu³⁺ is the smallest (86.1 pm) among the lanthanides, and as a consequence, the number of ligands that may be placed around Lu³⁺ are limited. The coordination number is mostly dictated by the reciprocal repulsions between the various ligands without any relevant influence attributable to the (s, p, and d) orbitals involved in bond formation.

Lutetium salts as well as their aqueous solutions, with the common exception of the iodide, are colorless and form white crystalline solids upon drying. While the nitrate, sulfate, and acetate salts are water soluble and crystallize with water molecules to form hydrates, the oxide, hydroxide, fluoride, carbonate, phosphate, and oxalate are insoluble in water.³² In its metallic state lutetium is slightly unstable in air at standard conditions and burns readily at 150 °C to form lutetium oxide, which is known to adsorb water and carbon dioxide. Lutetium can thus be used to remove water and carbon dioxide vapors from closed systems.³³ Lutetium reacts with cold water slowly, the reaction being more rapid at higher temperature, forming lutetium hydroxide.³⁴ Soluble lutetium trihalide salts are formed in reaction with halogens with the exception of LuF₃, which is insoluble. Reaction of lutetium with weak acids and dilute sulfuric acid yields the colorless nine-coordinated aqua complex [Lu(H₂O)₉]³⁺. Lutetium forms complexes with coordination numbers of 6, 7, 8 and 9.

4.2. Isotopes of Lutetium

Naturally occurring lutetium exists as the stable isotope ¹⁷⁵Lu (97.41%) together with the radioisotope ¹⁷⁶Lu (2.59%), which is very long lived ($T_{1/2} = 3.78 \times 10^{10}$ years). More than 50 radionuclides have been produced, including 23 nuclear isomers ranging in mass number from 150 to 184.³⁵ Among them, the long-lived radionuclides are ¹⁷⁴Lu ($T_{1/2} = 3.31$ years) and ¹⁷³Lu ($T_{1/2} = 1.37$ years).^{10,35} The remaining radionuclides have half-lives of less than 9 days, and the majority of them have half-lives of less than 30 min. Radionuclides lighter than stable ¹⁷⁵Lu decay via electron capture or positron emission to produce isotopes of ytterbium. The heavier radionuclides decay primarily via β^- emission, producing hafnium isotopes. Among the 18 metastable states of Lu, the most stable are ^{177m}Lu ($T_{1/2} = 160.4$ days) and ^{174m}Lu ($T_{1/2} = 142$ days). These half-lives are longer than half-

lives of the ground states of the corresponding Lu radionuclides.³⁵ The range of atomic weights of Lu vary from 149.973 (¹⁵⁰Lu) to 183.961 (¹⁸⁴Lu). The radionuclides of Lu with half-lives ≥ 1 min are listed in Table 3.

Table 3. Radionuclides of Lu with Half-Lives ≥ 1 min

radionuclide	half-life	decay mode	daughter products
¹⁶² Lu	1.37 min	β^+	¹⁶² Yb
^{162m} Lu	1.5 min	β^+ IT (rare)	¹⁶² Yb, ¹⁶² Lu
¹⁶³ Lu	3.97 min	β^+	¹⁶³ Yb
¹⁶⁴ Lu	3.14 min	β^+	¹⁶⁴ Yb
¹⁶⁵ Lu	10.74 min	β^+	¹⁶⁵ Yb
¹⁶⁶ Lu	2.65 min	β^+	¹⁶⁶ Yb
^{166m} Lu	1.41 min	EC (58%) IT (42%)	¹⁶⁶ Yb ¹⁶⁶ Lu
¹⁶⁷ Lu	51.5 min	β^+	¹⁶⁷ Yb
¹⁶⁸ Lu	5.5 min	β^+	¹⁶⁸ Yb
^{168m} Lu	6.7 min	β^+ (95%) IT (5%)	¹⁶⁸ Yb ¹⁶⁸ Lu
¹⁶⁹ Lu	34.06 h	β^+	¹⁶⁹ Yb
¹⁷⁰ Lu	2.012 days	β^+	¹⁷⁰ Yb
¹⁷¹ Lu	8.24 days	β^+	¹⁷¹ Yb
¹⁷² Lu	6.70 days	β^+	¹⁷² Yb
^{172m} Lu	3.7 min	IT	¹⁷² Lu
¹⁷³ Lu	1.37 years	EC	¹⁷³ Yb
¹⁷⁴ Lu	3.31 years	β^+	¹⁷⁴ Yb
^{174m} Lu	142 days	IT (99.38%) EC (62%)	¹⁷⁴ Lu ¹⁷⁴ Yb
¹⁷⁵ Lu	stable	believed to undergo α decay to ¹⁷¹ Tm	
¹⁷⁶ Lu	3.78×10^{10} y	β^-	¹⁷⁶ Hf
^{176m} Lu	19 h	β^- (99.9%) EC (0.095%)	¹⁷⁶ Hf ¹⁷⁶ Yb
¹⁷⁷ Lu	6.647 days	β^-	¹⁷⁷ Hf
^{177m} Lu	160.44 days	β^- (78.3%) IT (21.7%)	¹⁷⁷ Hf ¹⁷⁷ Lu
¹⁷⁸ Lu	28.4 min	β^-	¹⁷⁸ Hf
^{178m} Lu	23.1 min	β^-	¹⁷⁸ Hf
¹⁷⁹ Lu	59 h	β^-	¹⁷⁹ Hf
¹⁸⁰ Lu	5.7 min	β^-	¹⁸⁰ Hf
¹⁸¹ Lu	3.5 min	β^-	¹⁸¹ Hf
¹⁸² Lu	2.0 min	β^-	¹⁸² Hf

^aData taken from ref 35.

4.3. Bifunctional Chelating Agents (BFCAs) for Lu Complexation

A typical bifunctional chelating molecule used for radiopharmaceutical agents consists of an organic molecule which possesses a chelating moiety located at one terminus of the agent and an active functionality such as an aromatic isothiocyanate group or an activated ester located at the other end of the molecule. The synthetically versatile activated group facilitates chemical conjugation with functional groups such as $-\text{NH}_2$, $-\text{COOH}$, or any other suitable pendant moiety located in the target specific biomolecule. A linker moiety may also be incorporated between the chelator and the targeting vector to influence the pharmacokinetic properties of the conjugate. These linker groups, which are usually hydrocarbon chains (CH_2)_n,

polyethylene glycol, or polypeptide linkers, can alter the pharmacokinetics and biodistribution by changing the overall charge and hydrophilicity, i.e., the solubility of the resultant species intended for use as a drug.^{36–40}

Since the most stable oxidation state of Lu is 3+, the chemistry of such hard, trivalent cations in solution is characterized by the strong tendency to form complexes with hard donor atoms such as O, F[−], and N. The coordination number is usually 8 or 9, and the resultant complexes are thermodynamically very stable with both acyclic and cyclic polyaminopolycarboxylate-type ligands that have 8 or 9 donor atoms. Among the ligands, the macrocyclic DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) ligand forms complexes of lanthanides (Ln) with significantly high thermodynamic stability and kinetic inertness. The DOTA complexes are generally kinetically more inert than the complexes with DTPA (diethylenetriaminepentaacetic acid).^{41–44} Although both DOTA and DTPA complexes have been widely used for chelating radiometals for the development of radiopharmaceuticals, some of the DTPA complexes have been shown to dissociate and release metals under physiological conditions. This unfavorable aspect is important when considering suitability for targeted radiopharmaceutical applications. The details of the molecular structure of Lu with macrocyclic ligands are not readily available; however, they are expected to follow the general lanthanide behavior. The reported DOTA complexes of Ln³⁺ have capped square antiprism geometry where the basal plane is occupied by four amine nitrogens of the macrocycle, the capped plane is occupied by four carboxylate oxygens of the carboxylic residues, and the capping position is occupied by a water molecule (Figure 3).^{45,46}

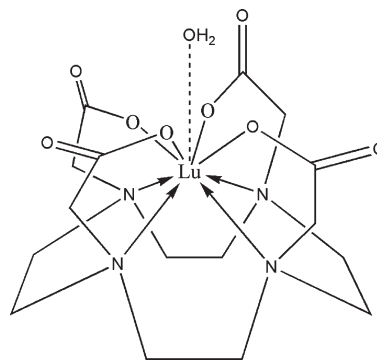


Figure 3. Representative structure of the Lu–DOTA complex.

In aqueous solution, the relative orientation of the carboxylic side arms and the macrocyclic ring results in two coordination isomers that differ in the torsion or twist angle between the basal N₄ and the capped O₄ squares. While a torsion angle of $\sim 39^\circ$ defines a square antiprismatic structure, an angle of $\sim 29^\circ$ defines a twisted square antiprismatic coordination geometry. These coordination isomers can interconvert by arm rotation or ring inversion.⁴⁷ Lanthanide(III) chelates of other DOTA derivatives also exist in the two interconverting diastereomeric forms of the square antiprism and twisted square antiprism. The ratio of the two forms depends on the size of the lanthanide cation as well as the ligand stereochemistry.^{48,49} Lutetium forms stable complexes with several ligands, and coordination numbers of 6, 7, 8, and 9 are reported.^{50,51} The log stability constants of some of the bifunctional chelating agents (Figure 4) have been reported (Table 4).⁵² DOTA is the most widely used BFCAs for Lu and

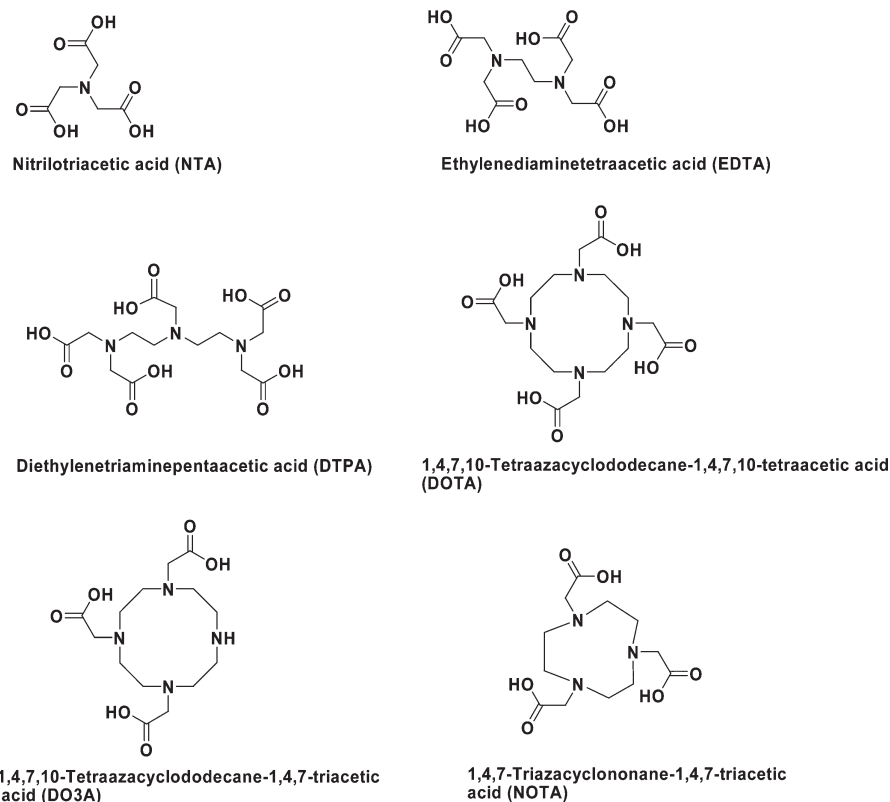


Figure 4. Common bifunctional chelating agents (BFCAs) used for Lu^{3+} .

forms the $\text{Na}[\text{Lu}(\text{DOTA})(\text{H}_2\text{O})]_4\text{H}_2\text{O}$ nine-coordinated complex with a stability constant of about 25.4.

Table 4. Common Chelating Agents of Lu^{3+} and log Stability Constants⁵²

chelating agent	log stability constant
diethylenetriaminepentaacetic acid (DTPA)	12.5
ethylenediaminetetraacetic acid (EDTA)	19.8
nitrilotriacetic acid (NTA)	22.4
1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA)	25.4
1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid (DO3A)	23.0
1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA)	15.3

4.4. Radiolabeling of Bifunctional Chelators with ^{177}Lu

The radiolabeling or complexation of ^{177}Lu with the DOTA-biomolecule conjugate constitutes an important aspect for the preparation of ^{177}Lu -labeled radiopharmaceuticals based on antibodies, peptides, and several other small molecules. Lutetium follows the general chemistry of lanthanide ions (Ln^{3+}) with DOTA derivatives, and hence, this ligand can be taken advantage of for radiolabeling studies. Direct radiolabeling of the chelating ligands such as polyaminopolyposphonates is also followed for the preparation of radiopharmaceuticals for bone pain palliation. As in all complexation reactions a variety of parameters including pH, temperature, ligand-to-metal ratio, and choice of buffer are important attributes which require careful optimization for obtaining stable complexes with $^{177}\text{Lu}^{3+}$. Despite very high stability constants, the rate of formation of Lu^{3+} complexes with

DOTA and other macrocyclic ligands is very slow, especially at the low concentrations of Lu and chelating agents used in radiopharmaceutical preparations. As indicated by kinetic measurements, the first step in the formation of lanthanide complexes with DOTA derivatives involves the rapid formation of a mono- or diprotonated intermediate, and the rate-determining step is the base-assisted rearrangement of this intermediate.⁵³ In some cases, formation of the protonated intermediates has been observed by UV-vis, luminescence, and NMR spectroscopies. The slow rate of complexation of DOTA-based ligands with ^{177}Lu becomes a significant disadvantage for preparation of DOTA-based radiopharmaceuticals. For this reason, heating becomes essential in most cases in order to increase the rate of the chelation process. Generally, heating at 95 °C for 25–30 min is performed in order to achieve near quantitative labeling yields for the preparation of ^{177}Lu -DOTA-conjugated somatostatin analogues.^{54–56} DOTA-affibodies have been radiolabeled with ^{177}Lu under heating at lower temperature (60 °C) to obtain high-efficiency labeling.^{57,58} In all these radiolabeling procedures, pH also plays a critical role with the rate of complex formation increasing with the hydrogen ion concentration. However, above pH 6, lanthanide cations form insoluble hydroxides, making the optimal pH for radiolabeling between 5 and 6, which is usually maintained by using sodium acetate or ammonium acetate buffers. Radiolabeling procedures are generally conducted with several-fold higher excess of ligand compared to the radiometal. For this reason, only a small fraction of the available chelator sites are labeled and the large excess of ligand prevents the formation of insoluble metal-hydroxo species through the rapid formation of intermediary complexes. This also allows complexation at higher pH values (pH 7–8).⁵⁹

High specific activity of the radiolabeled agent is of prime importance for the design of therapeutic radiopharmaceuticals since the target sites and density for attachment of the radiopharmaceuticals are usually limited, especially for those therapeutic agents which bind to specific receptors. This is not the case for agents such as ^{177}Lu -based phosphonates where the targeting mechanism does not involve binding with limited expression of receptors on tumor tissue.

The presence of metal ion contaminants also adversely affects the specific activity of the target-specific radiopharmaceuticals by competing with Lu^{3+} for complexing the BFCA in the conjugate.⁶⁰ Hence, all solvents and reagents used should be of very high purity. A significant amount of ^{177}Hf metal ions will be present as the decay product of ^{177}Lu in the radionuclide preparation. However, Hf^{4+} has low affinity for DOTA and therefore does not affect the chelation of Lu with DOTA.⁵⁴

It is pertinent to note that in some studies the high-affinity binding of Ln(III) ions with certain peptide sequences has been reported, which could possibly interfere with the complexation of the peptide–DOTA conjugate. However, since the thermodynamic stability of the DOTA chelates with Lu is several orders of magnitude higher than that of the peptide–metal complexes, such a possibility does not pose a problem.^{61,62}

The therapeutic radiopharmaceuticals have very high radioactive concentration, and hence, radiolytic degradation of the product is often seen. Ascorbic acid or gentisic acid is often added to the preparation; they act as free radical scavengers and provide better stability for the products.⁶³

5. LUTETIUM-177 RADIONUCLIDE

5.1. Radionuclidic Characteristics of ^{177}Lu

A simplified decay scheme which accounts for the major β^- -particle and γ -photon emissions from ^{177}Lu is summarized in Figure 5, and data are summarized in Table 5. Figure 6 depicts

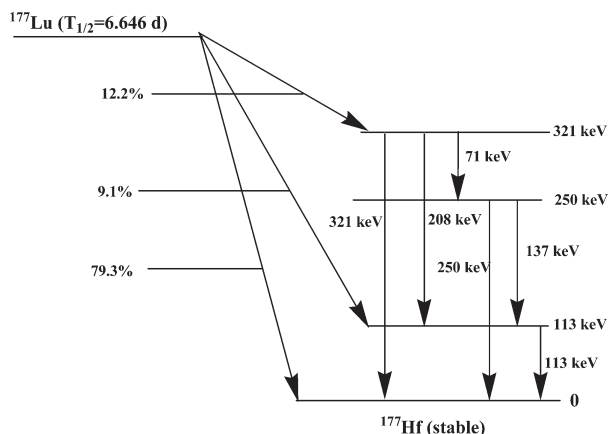


Figure 5. Simplified decay scheme of ^{177}Lu accounting for the major β^- particles and γ photons emitted by ^{177}Lu .

Table 5. Major Beta and Gamma Emissions of ^{177}Lu ¹⁰

β^- emissions (abundance)	γ emissions (abundance)
498 keV (79.3%)	321.3 (0.219%)
380 keV (9.1%)	249.7 (0.2120%)
176 keV (12.2%)	208.37 (11.00%)
	112.95 (6.40%)
	71.65 (0.15%)

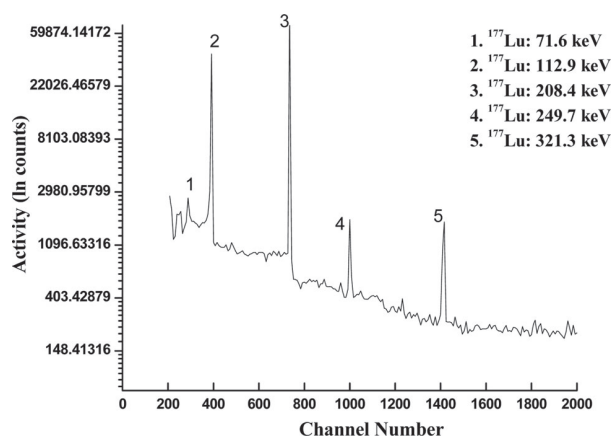


Figure 6. Gamma-ray spectrum of pure ^{177}Lu taken using a high-purity germanium (HPGe) detector coupled to a multichannel analyzer.

the gamma-ray spectrum of ^{177}Lu obtained using a high-purity germanium (HPGe) detector. The abundance and energy of the 208 and 113 keV photon peaks are suitable for imaging by single-photon emission computed tomography (SPECT). The ability to obtain scintigraphic images provides an opportunity to evaluate the targeting, pharmacokinetics, and excretion behavior of ^{177}Lu -labeled radiopharmaceuticals which, in turn, aids in estimation of the adsorbed radiation dose by tumors and other organs.

5.2. Feasibility of Production of ^{177}Lu in Therapeutic Quantities

Apart from its favorable radionuclidic characteristics, such as the low-energy β^- emission and a favorable half-life of 6.647 days, the opportunity to produce high specific activity and high multi-Curie activity levels of ^{177}Lu in many available research reactors is one of the important aspects which makes ^{177}Lu an attractive radionuclide for targeted therapy. The activity of a radionuclide produced by irradiation of a target material with neutrons in a nuclear reactor is calculated by using the formula

$$A = N\sigma\phi(1 - e^{-\lambda t})$$

where A = activity of the radionuclide produced, N = number of target atoms, σ = cross-section of the nuclear reaction, ϕ = neutron flux during irradiation, λ = decay constant of the radionuclide produced, and t = time of irradiation.

Thus, the levels of radioactivity produced from irradiation in a nuclear reactor are dependent on a variety of factors which include the number of target atoms irradiated, the thermal neutron flux of the reactor, the cross-section of that particular nuclear reaction, the irradiation time, and the half-life of the radionuclide being produced. The term $(1 - e^{-\lambda t})$ is referred to as the saturation factor and approaches unity when the irradiation period is 5–6 half-lives of the radionuclide being produced, and this irradiation period is required to obtain the maximum activity from a particular system.

In radionuclide production, the neutron activation cross-section (σ) plays an important role. The amount of radionuclide formed is directly proportional to the cross-section, as is the specific activity of a “directly” produced radionuclide. The cross-section is a constant for a given neutron energy and varies from millibarn (1 barn = 10^{-24} cm²) to several thousands of barn for different nuclear reactions. The thermal neutron capture cross-section (σ) of the ^{176}Lu [$^{176}\text{Lu}(n,\gamma)^{177}\text{Lu}$] capture reaction is

~2065 barn, which is the highest encountered among all (n,γ) produced radionuclides presently used for any medical application including radionuclide therapy. The high neutron capture cross-section of the reaction ensures that ^{177}Lu can be produced with adequately high SA for radionuclide therapy applications even while using moderate flux reactors. The high cross-section also ensures that there will be no constraints in the large-scale production of ^{177}Lu , and hence, it is expected that increasing global demand of ^{177}Lu in the coming years can be easily accommodated. The long half-life of ^{177}Lu provides a logistical advantage for facilitating supply to institutions distant from the reactor site.

5.3. Theranostic Potential of ^{177}Lu

The low-abundance emission of gamma photons [$E_\gamma = 113$ (6.4%) and 208 keV (11%)] from the decay of ^{177}Lu can be used for imaging, thereby providing a theranostic opportunity for use of this therapeutic radionuclide. The essential requirement of theranostics which enables nuclear medicine for determining initial localization and to perform pretherapy imaging is possible using ^{177}Lu . By administration of only tracer levels (i.e., subtherapeutic activity) of the ^{177}Lu -based radiopharmaceutical, preclinical dosimetric studies have been carried out in patients. These studies are described in detail in section 8.

5.4. Lutetium-177 as a Replacement of ^{131}I for Nonthyroid Applications

Iodine-131 has been a successful radionuclide for targeted therapy and in use for the last 69 years. The wide availability of ^{131}I was mainly due to its ease of production for worldwide supply. Iodine-131 accumulates in the follicular cells of the thyroid gland through the sodium iodide symporter (NIS) protein.⁶⁴ Due to this unique biological mechanism of transportation to the thyroid, ^{131}I will continue to be used in the treatment of thyroid cancer and hyperthyroidism. However, the emission of high-energy gamma photons in high abundance emitted by ^{131}I (636 (7.2%), 364 (81.7%), and 284 keV (6.14%)) provides undue radiation burden to nontarget organs as well as to individuals coming in contact with the patients. Table 6 provides a comparison of the radionuclides characteristics and production details of ^{131}I and ^{177}Lu .⁶⁵ Considering the close similarity in

their radionuclides characteristics, ^{177}Lu can be considered as a metallic analogue of ^{131}I and could be used instead of ^{131}I for some radiotherapeutic applications.

Iodine-131 is produced either by irradiation of natural tellurium in a nuclear reactor, through the $^{130}\text{Te}(n,\gamma)^{131}\text{Te} \rightarrow ^{131}\text{I}$ reaction, or by nuclear fission. Natural Te consists of the three isotopes, viz. ^{126}Te , ^{128}Te , and ^{130}Te , all of which capture neutrons with the simultaneous production of ^{127}I (inactive) and ^{129}I ($T_{1/2} = 5 \times 10^5$ years), in addition to ^{131}I during reactor irradiation. The presence of the inactive and long-lived radioiodine significantly reduces the specific activity of ^{131}I , and only about 20% of the total iodine atoms is present as ^{131}I . The relative levels of ^{131}I continue to reduce after production because of radioactive decay. Iodine-131 is one of the major fission products during nuclear fission of ^{235}U or other heavy elements and is produced in ~6% yield. Other isotopes of iodine, including ^{127}I and ^{131}I , are also formed during nuclear fission, and the achievable isotopic abundance through this route is also only ~20%. As discussed below, ^{177}Lu having specific activity similar to or greater than that of ^{131}I can be prepared in many of the existing nuclear reactors.

6. PRODUCTION OF ^{177}Lu

Although ^{177}Lu can also be produced by charged particle acceleration using a cyclotron, neutron irradiation in a nuclear reactor is the most practical and cost-effective route. Radioactivity yields by charged particle acceleration route are much lower, and the process is more expensive, thus making this method an impractical one. In a nuclear reactor, ^{177}Lu can be prepared by neutron activation using direct activation of enriched ^{176}Lu or through the indirect route by activation of ^{176}Yb followed by β^- decay to ^{177}Lu . Details of production of ^{177}Lu through different routes are described in the following sections.

6.1. Cyclotron Production of ^{177}Lu

The scope of ^{177}Lu production via the accelerator route is limited. Hermanne et al. studied the deuteron-induced reaction on natural Yb for generation of carrier-free Lu isotopes using a stacked-foil activation technique.⁶⁶ Excitation functions have been reported for the generation of ^{170}Lu , $^{171\text{m}}\text{Lu}$, $^{172\text{m}}\text{Lu}$, ^{172}Lu , ^{173}Lu , ^{174}Lu , and ^{177}Lu . A maximum cross-section of 217 mb was reported for the production of ^{177}Lu with a deuteron energy of 12 MeV. Use of enriched ^{176}Yb could yield ^{177}Lu free from other coproduced lutetium radionuclides such as ^{170}Lu , ^{171}Lu , ^{172}Lu , ^{173}Lu , and ^{174}Lu . An efficient separation yielding ^{177}Lu free from Yb contamination constitutes an important requirement for this production route to be useful. Medvedev et al. reported the feasibility of production of ^{177}Lu via activation of natural hafnium or tantalum targets using high-energy protons. The largest cross-sections (~20 mb) were found for the Hf target at 195 MeV. Coproduction of other Lu isotopes will reduce the product SA.⁶⁷ In another study, the cyclotron production of high specific activity ^{177}Lu using deuteron-induced nuclear reactions $^{176}\text{Yb}(d,p)^{177}\text{Yb} \rightarrow ^{177}\text{Lu}$ (Yb targets of natural isotopic compositions) has been reported.⁶⁸ It has been shown that an energy of 11 MeV is required to avoid the production of $^{177\text{m}}\text{Lu}$ (below detectable limit) as is evidenced by recording the gamma spectrum after 1 year from the EOB showing no peaks corresponding to $^{177\text{m}}\text{Lu}$. However, the main disadvantage of the method is that the activity of ^{177}Lu produced is about one-tenth of the activity that can be produced by a nuclear reactor following the "indirect" route as the cross-section for the (d,p) reaction is much less. This reduces the commercial viability of these production methods. From the

Table 6. Comparison of the Radionuclides Characteristics and Production of ^{131}I and ^{177}Lu ⁶⁵

characteristics	iodine-131	lutetium-177
half-life	8.02 days	6.647 days
beta energy (%)	608 keV (89.9%)	498 keV (78.6%),
	330 keV (7.27%)	385 keV (9.1%),
	250 keV (2.1%)	176 keV (12.2%)
β^- range _{max} (mm)	~2 mm	<2 mm
gamma energy (%)	636 keV (7.2%)	113 keV (6.4%)
	364 keV (81.7%)	208 keV (11%)
	284 keV (6.14%)	
production	^{235}U (n,f) $^{130}\text{Te}(n,\gamma)^{131}\text{Te} \rightarrow ^{131}\text{I}$	$^{176}\text{Lu}(n,\gamma)^{177}\text{Lu}$ $^{176}\text{Yb}(n,\gamma)^{177}\text{Yb} \rightarrow ^{177}\text{Lu}$
specific activity	up to ~20% isotopic abundance; ^{129}I and ^{127}I are present	up to ~70% (direct) and 100% (indirect) isotopic abundance
chemistry	halogen	metal chemistry (+3)
principal use	targeted therapy, mainly thyroid and others	targeted therapy other than thyroid

data reported thus far, cyclotron irradiation is not envisaged to be an economical route for the production of the high activity levels of ^{177}Lu required for therapy.

6.2. Reactor Production of ^{177}Lu

Two independent routes using nuclear reactors are followed for the production of ^{177}Lu (Figure 7). Nuclear data for the

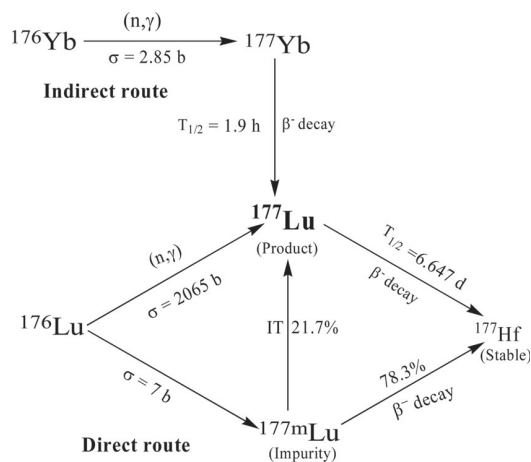
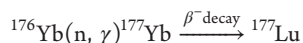


Figure 7. Two independent reactor routes for production of ^{177}Lu .

production of ^{177}Lu from these indirect and direct routes are given in Table 7. As the availability and cost of ^{177}Lu will be significantly influenced by the production route and subsequent processing technologies, the advantages vis-a-vis disadvantages of the two reactor routes are discussed.

6.2.1. Indirect Route for Production of ^{177}Lu . The logistics of production of ^{177}Lu in a research reactor have been described by Pillai et al.⁷⁰ and Knapp et al.^{71–73} In the indirect production route, the short-lived ^{177}Yb is produced by neutron capture of enriched ^{176}Yb . Decay of ^{177}Yb provides no carrier added (NCA) ^{177}Lu which is then separated by suitable radiochemical processes.^{74–76}



The major advantages of this route are that it provides NCA ^{177}Lu and the product formed is free from $^{177\text{m}}\text{Lu}$, the long-lived radionuclidic impurity. Radiochemical separation of ^{177}Lu from the irradiated Yb_2O_3 target is challenging due to the similarity in the chemistry of the two adjacent members of the lanthanide series. A clean separation process is essential in this mode of production since microgram quantities of ^{177}Lu need to be separated from gram quantities of irradiated Yb target. A variety of methods based on different types of analytical separation techniques, such as solvent extraction, column chromatography,

and a combination of these methods, have been employed successfully to separate ^{177}Lu from the irradiated Yb.^{77–83} Although most of the methods reported in the literature involve only laboratory-scale production of ^{177}Lu , some of the authors have described production of high levels of ^{177}Lu activity employing this route. A two-step electrochemical separation method based on an electrochemical cell containing a mercury pool cathode for the separation of NCA ^{177}Lu from Yb was also reported.⁸⁴ This method was scaled up to the 18.5 GBq (500 mCi) level, and the ^{177}Lu thus obtained was proven to be suitable for biomedical application. Radiochemical processing can be conveniently carried out 24 h after the end of irradiation (EOI), since the ^{177}Yb ($T_{1/2}$ 1.9 h) formed will completely decay to ^{177}Lu during this period. Natural Yb targets can also be used in this method instead of enriched ^{176}Yb targets. However, this will result in the production of substantially higher activity levels of ^{175}Yb , which will increase the radiation dose during processing. Moreover, some natural Lu isotopes will also be produced as decay products, which will reduce the specific activity of ^{177}Lu produced. By using enriched ^{176}Yb , NCA ^{177}Lu is produced and hence the product SA should approach the theoretical specific activity of ~ 110 Ci (4.07 TBq)·mg^{−1}. A major advantage of the indirect route, apart from the preparation of a high specific activity product, is the absence of the long-lived metastable $^{177\text{m}}\text{Lu}$ isotope, which is coproduced via the direct route. In spite of the above advantages, the indirect production route suffers from some disadvantages. The thermal neutron capture cross-section value for the $^{176}\text{Yb}(n, \gamma)^{177}\text{Yb}$ nuclear reaction is only 2.85 barn; therefore, the ^{177}Lu production yields by this route are significantly less than the direct route based on target mass. The target must be irradiated in a high-flux reactor in order to provide adequate production yields and lead to efficient utilization of the enriched target. Figure 8 illustrates the calculated production yields at different thermal neutron flux values.

The saturation yield of ^{177}Lu occurs after 5–6 half-lives of ^{177}Lu , and hence, irradiation of the targets must be conducted over a time period of several weeks. The production of ^{177}Lu through the indirect route will significantly increase the cost of the radionuclide as the production involves the use of an enriched target, larger irradiation volumes, and longer irradiation times and the need for elaborate radiochemical processing capabilities. However, these factors do not significantly contribute in the case of production of larger batch size and the high cost can be compensated. Large quantities of ^{176}Yb -enriched targets will be required for the preparation of ^{177}Lu in usable quantities for therapy. The use of enriched targets with low-activation cross-sections is not economical for isotope production, as a significant fraction of the target does not undergo neutron capture and must be carefully recovered. The radiochemical processing adopted in the indirect route must meet stringent requirements for complete removal of ytterbium.

Table 7. Nuclear Data for ^{177}Lu and ^{177}Yb Production⁶⁹

radionuclide		half-life	activation cross-section, barn, σ_{th}	resonance integral, I_{∞}	burn-up cross-section, σ
target	product				
^{175}Lu	^{176}Lu	3.73×10^{10} years	6.9	610	
	$^{176\text{m}}\text{Lu}$	3.66 h	16.2	550	
^{176}Lu	^{177}Lu	6.73 days	2065	1087	1000
	$^{177\text{m}}\text{Lu}$	160.4 days	7	4.7	± 100
^{176}Yb	^{177}Yb	1.911 h	2.85	6.3	3.18

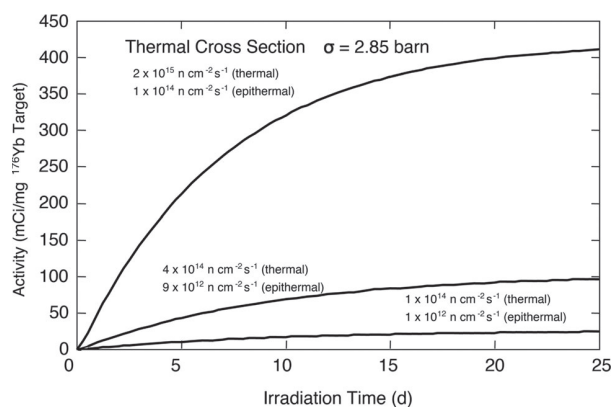


Figure 8. Calculated yields of ^{177}Lu by the indirect route by irradiating enriched ^{176}Yb in reactors having different thermal neutron flux values.

The presence of Yb in ^{177}Lu will reduce the apparent specific activity of the product since both Lu and Yb are expected to have similar reaction kinetics with most of the currently available ligands used for chelation, such as DOTA. No carrier added ^{177}Lu is prepared on a large scale following some of the above methods, is currently commercially available, and is preferred by some groups.

6.2.2. Direct Route for Production of ^{177}Lu . In the direct route of production, ^{176}Lu undergoes neutron capture to provide ^{177}Lu by the $^{176}\text{Lu}(n,\gamma)^{177}\text{Lu}$ nuclear reaction. The cross-section for ^{176}Lu thermal neutron capture is ~ 2065 barn, and there is also an epithermal neutron resonance integral with a value of ~ 1087 barn, which increases the production yields as well as specific activity. Its excitation function (cross-section) variation with neutron energy for the $^{176}\text{Lu}(n,\gamma)^{177}\text{Lu}$ reaction is shown in Figure 9.

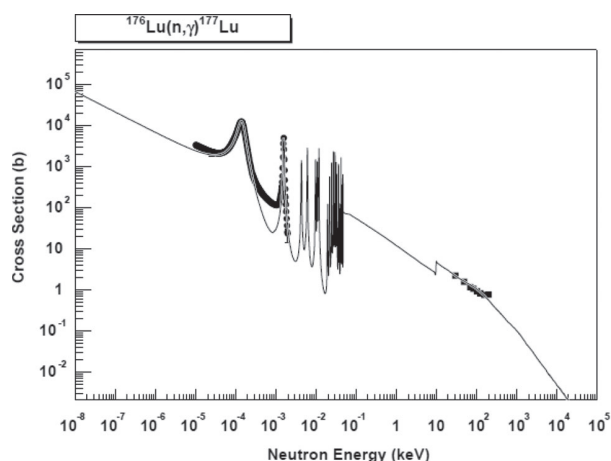


Figure 9. Excitation function (cross-section) variation with neutron energy for $^{176}\text{Lu}(n,\gamma)^{177}\text{Lu}$. Reprinted with permission from ref 69. Copyright 1997 International Atomic Energy Agency. IAEA data bank.

The direct production route has two major advantages, which include the very high thermal neutron cross-section and the added advantage that very high activity levels of ^{177}Lu can be produced using highly enriched targets irradiated in even medium-high flux research reactors ($>1 \times 10^{14} \text{ n cm}^{-2} \text{ s}^{-1}$). Specific activity values as high as $>70 \text{ Ci}$ (2.59 TBq) mg^{-1} can be

obtained at a thermal neutron flux of $\sim 2 \times 10^{15} \text{ n cm}^{-2} \text{ s}^{-1}$.⁸⁵ In addition, radiochemical processing of the target is very simple as it involves dissolution of the target with dilute acid with no subsequent processing or purification prior to dispensing.

Although the natural abundance of ^{176}Lu is only 2.6%, highly enriched ($>80\%$ ^{176}Lu) lutetium targets are available at reasonable cost. The target consumption is very low, typically 25–100 μg of ^{176}Lu per Ci (37 GBq) of ^{177}Lu produced, depending on the reactor thermal neutron flux and the irradiation time. The use of enriched targets irradiated in such medium flux reactors ($>1 \times 10^{14} \text{ n cm}^{-2} \text{ s}^{-1}$) could provide specific activity values greater than 20 Ci (740 GBq) mg^{-1} (theoretical specific activity $\approx 110 \text{ Ci}$ (4.07 TBq) mg^{-1}) resulting in isotopic abundance greater than 20%. The high ^{176}Lu reaction cross-section for the $^{176}\text{Lu}(n,\gamma)^{177}\text{Lu}$ reaction also calls for careful optimization of the irradiation time, which varies from 1 to 3 weeks depending upon the neutron flux available in the reactor.⁷⁰ Figure 10 shows the estimated production rates of ^{177}Lu by reactor irradiation of enriched ^{176}Lu as a function of time at different thermal neutron flux values.

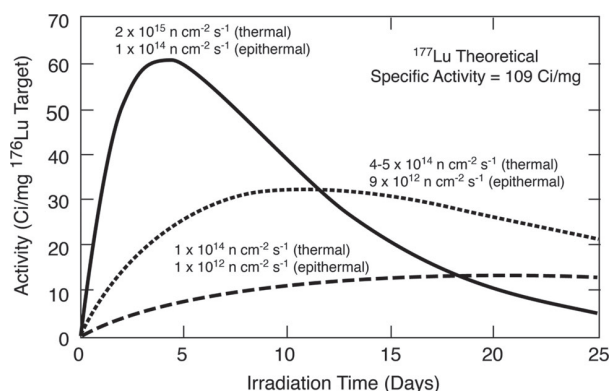


Figure 10. Estimated production rates of ^{177}Lu by irradiation of enriched (100%) ^{176}Lu as a function of time at different thermal neutron flux.

Medium-high specific activity, 25–35 Ci (925–1295 GBq) mg^{-1} , ^{177}Lu could be prepared by irradiating highly enriched ^{176}Lu in medium-flux research reactors. This corresponds to 23–32 atom % of ^{177}Lu . A maximum specific activity of $\sim 40 \text{ Ci}$ (1480 GBq) mg^{-1} was achieved when irradiation was carried out with an 82% enriched target at a thermal neutron flux of $1 \times 10^{14} \text{ n cm}^{-2} \text{ s}^{-1}$ for 21 days, which corresponds to $\sim 36\%$ of the theoretical specific activity. Another report on the production of ^{177}Lu via the (n,γ) route in high-flux reactors has mentioned a specific activity of 76 Ci (2812 GBq) mg^{-1} , which is 69% of the theoretical specific activity of ^{177}Lu .⁸⁵

6.2.3. Coproduction of $^{177\text{m}}\text{Lu}$ as Radionuclidic Impurity. The direct production route also results in formation of a small amount of long-lived metastable $^{177\text{m}}\text{Lu}$ in activity levels that depend on the duration of irradiation as well as the neutron flux.⁷⁰ Irradiation of the target at higher flux as well as for longer time periods results in higher $^{177\text{m}}\text{Lu}$ yields. In most irradiation conditions, the level of $^{177\text{m}}\text{Lu}$ formed is relatively low due to the low cross-section (7 barn) for the $^{176}\text{Lu}(n,\gamma)^{177\text{m}}\text{Lu}$ nuclear reaction and the long $^{177\text{m}}\text{Lu}$ half-life ($T_{1/2} = 160.5$ days). Figure 11 gives an estimate of the activity of $^{177\text{m}}\text{Lu}$ at different thermal neutron flux values and for different irradiation periods. At very high flux values and long irradiation times, substantial

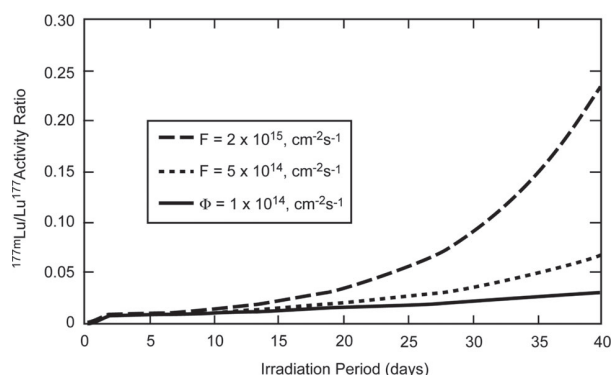


Figure 11. Calculated $^{177\text{m}}\text{Lu}/^{177}\text{Lu}$ ratios as a function of the irradiation period and thermal neutron flux. Reprinted with permission from ref 85. Copyright 2006 International Atomic Energy Agency.

activity levels of $^{177\text{m}}\text{Lu}$ are estimated to be formed; however, long irradiation times at high flux are not required, since the production of ^{177}Lu reaches a maximum after a few days of irradiation.

The level of $^{177\text{m}}\text{Lu}$ measured in ^{177}Lu prepared in medium-flux research reactors has been experimentally measured to be $<0.02\%$.⁷⁰ Since $^{177\text{m}}\text{Lu}$ is an isotopic impurity, its accumulation occurs in the same tissues targeted with the Lu radiopharmaceutical, where its residence time will be more prolonged because of its much longer half-life. If the targeting agent is metabolized, free $^{177\text{m}}\text{Lu}$ might be excreted or become redistributed to bone. In both cases the radiation dose delivered will be low compared to the overall radiation dose from the radiopharmaceutical. However, the presence of $^{177\text{m}}\text{Lu}$ in ^{177}Lu is considered a problem in certain countries with respect to management of the waste generated in nuclear medicine departments. A curie of ^{177}Lu used in a clinic will contain a few μCi of $^{177\text{m}}\text{Lu}$ as waste. The containment and postdecay release of such low-activity waste needs to be managed by suitable design of delay tanks or waste management facilities.⁸⁶

6.3. Logistical Advantages in Distribution of ^{177}Lu and Cost Factors

A major advantage in the use of ^{177}Lu worldwide as a therapeutic radionuclide is its availability in high activity levels, with adequate specific activity, prepared using many of the operating medium-high flux reactors around the world. With careful optimization of the irradiation time and by using highly enriched targets, one could easily produce ^{177}Lu with specific activity $> 20 \text{ Ci (740 GBq)} \cdot \text{mg}^{-1}$ at EOB while using reactors with neutron flux $> 1 \times 10^{14} \text{ n} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$. It is worthwhile to compare this specific activity of ^{177}Lu with that of ^{131}I , as the latter radionuclide is widely accepted for the radiolabeling of many small molecules and antibodies. The production routes of ^{131}I , either through neutron activation or by fission, do not produce isotopic abundances of more than 20%.

Although NCA ^{177}Lu with a theoretical specific activity of $\sim 110 \text{ Ci (4.07 TBq)} \cdot \text{mg}^{-1}$ can be prepared by the indirect route, the cost of production is very high. There are several reasons for these high production costs, including (i) the cost involved in the use of the enriched ^{176}Yb target, (ii) lower production yields due to the low production cross-section ($\sigma = 2.85 \text{ barn}$), and (iii) the necessity for elaborate radiochemical target processing and target material recovery for reuse. Large quantities of ^{176}Yb -enriched target (unlike the ^{176}Lu target used for the direct route) are

needed for the preparation of ^{177}Lu in usable quantities for therapy. The use of enriched targets having low activation cross-sections is generally not economical for radioisotope production, as a significant part of the target will be wasted or has to be carefully recovered. For these reasons, use of the direct route of ^{177}Lu production is of interest in several countries. The preference for use of the NCA ^{177}Lu produced by the indirect route is generally based on the need for the highest specific activity for special targeting strategies and issues associated with waste handling, as discussed earlier.

The second factor responsible for interest in the broad distribution of ^{177}Lu -based radiopharmaceuticals is the comparatively longer half-life of ^{177}Lu . The 6.647 day half-life of ^{177}Lu is not a limitation for in vivo use and at the same time offers distinct logistic advantages for distribution. The minimum decay loss encountered during distribution of the ^{177}Lu to members of the user community, remotely located from the site of production, indirectly contributes to making ^{177}Lu comparatively cost effective. This favorable logistic feature has contributed to the rapid growth of the interest in and use of ^{177}Lu as a therapeutic radionuclide in several regions of the world.^{87,88} There are several manufacturers now providing CA and NCA ^{177}Lu suitable for the preparation of radiopharmaceuticals.

7. REVIEW OF ^{177}Lu -LABELED MOLECULAR CARRIERS AS POTENTIAL RADIOPHARMACEUTICALS

A number of ^{177}Lu -labeled agents have been evaluated in preclinical settings, of which a few have entered the clinical arena. Carrier molecules such as inorganic chelating agents, peptides, antibodies, steroids, and other small molecules have been evaluated for the development of targeted agents for radiotherapy. An overview of the research efforts that have envisaged and conceptualized the use of ^{177}Lu for potential applications in radiotherapy is given below.

7.1. Monoclonal Antibodies

Paul Ehrlich's concept that tumors overexpressing certain antigens can be targeted using specific antibodies is more than 100 years old.⁸⁹ However, the technique could not be adapted until Koehler and Milstein prepared monoclonal antibodies (mAb) through the hybridoma technique.⁹⁰ When monoclonal antibodies are labeled with specific radionuclides, they serve as targeted molecular vehicles for the delivery of the radionuclides to the tumor sites.^{91,92} This forms the essence of radio-immunoimaging (RII) and radioimmunotherapy (RIT). As a therapeutic modality, its success is dependent on the principal interdependent factors such as the specificity and affinity of the antibody, the antigen concentration in the tumor, and the radiotoxicity of the radionuclide.

Like all other modes of radionuclide therapy, the selection of radionuclides for RIT also depends on factors such as the half-life of the radionuclide and the energy of the particulate emissions. Radionuclides with half-lives of a few days are preferred for RIT to match with the biological uptake of antibodies. Yttrium-90 and iodine-131 are the more commonly used radionuclides for labeling of most monoclonal antibodies for radioimmunotherapy. Both radionuclides have good therapeutic efficacy due to a significant radiation dose, which is delivered as they undergo beta decay. The longer ^{177}Lu half-life (6.647 days) compared to the 2.7 day half-life for ^{90}Y offers an advantage in terms of the logistics of production of the labeled antibody and planning the treatment. Additionally, due to the low energy of the β^- particles of ^{177}Lu , the bone marrow toxicity is also lower. The longer half-life

Table 8. Examples of ^{177}Lu -Labeled MoAbs Studied for Radioimmunotherapy

antibody	targeting antigen	type of cancer targeted
Rituximab ⁹⁹	CD 20	non-Hodgkin's lymphoma
Anti-L-CAM ^{100,101}	L1 cell adhesion protein	neuroblastoma, renal, and ovarian
Anti tenascin (Ch81C6) ¹⁰²	tenascin	brain tumor
Anti-VEGF (VG76e) ¹⁰³	vascular endothelial growth factor	
CC-49 ^{104–113}	tumor-associated antigen (TAG-72)	colon, ovarian, adenocarcinoma etc.
CC-49 single-chain Fv construct ¹¹⁴	tumor-associated antigen (TAG-72)	several cancers such as colon, ovarian and adenocarcinoma.
CC-49 single-chain Fv construct (pretargeting) ^{115–117}	tumor-associated antigen (TAG-72)	colon, ovarian, adenocarcinoma, etc.
Cetuximab ¹¹⁸	EGFR	several targets
cG250 ¹¹⁹	RCC	renal cell carcinoma
7E11 ¹²⁰	PSMA	prostate cancer
Epratuzumab (hLL2) ¹²¹	CD 22	non-Hodgkin's lymphoma
huA33 ¹²²	antigen 33 (A 33)	colorectal cancer
hu3S193 ^{123,124}	PSMA	prostate cancer
J-591 ^{125–131}	prostate-specific membrane antigen	prostate cancer
MOv18 ¹³²	α -isoform of folate receptor	prostate cancer
Pertuzumab ^{133,134}	HER-2, tyrosine kinase receptor	breast cancer and others
RS-7 ¹³⁵	epithelial glycoprotein	small cell lung carcinoma
Trastuzumab ¹³⁶ (Herceptin)	HER 2	breast cancer
U36 ¹³⁷	CD44v6	head and neck squamous cell carcinoma

reduces the dose rate while providing the same dose over a period of time with lower activity, as well as helping to eliminate the nontargeted antibody from circulation before it delivers the bulk of its radiation dose to nontargeted tissues. As a result, a major fraction of the radionuclide decays after the levels in the blood are reduced. Similarly, a greater fraction of the total decays occurs after the peak targeting of the antibody. By using the γ -rays emitted by ^{177}Lu , scintigraphy can be performed to quantify the localization of the radioimmunoconjugate. Lutetium-177 emits β^- particles with a maximum energy of 497 keV and an average energy of 133 keV, which translates into the ability to deposit radiation doses into cells at approximately 12 cell diameters from a cell at which the immunoconjugate is targeted. This represents a maximum cell–interaction potential of approximately 50 cell diameters. While this shorter range of ^{177}Lu is not as effective as ^{90}Y in terms of dose delivery to larger tumors, radiation from ^{177}Lu may be sufficient for many small solid tumors. On the contrary, the less penetration in soft tissue will be beneficial in limiting the radiation delivered to surrounding normal cells. The lower renal toxicity of ^{177}Lu over that of ^{90}Y is an advantageous feature which has provided impetus to the development of ^{177}Lu -based monoclonal antibodies (mAbs).

Currently, there are several mAbs approved by the U.S. Food and Drug Administration (FDA) for immunotherapy of a variety of cancer types. These products include Rituximab (for B-cell lymphomas), Trastuzumab (for breast cancer), Alemtuzumab (for chronic lymphocytic leukemia), Cetuximab (colorectal, head, and neck cancers), and Bevacizumab (for colorectal cancers). The two radiolabeled mAbs approved by the FDA for radioimmunotherapy (RIT) are based on anti-CD20 antibodies, Zevalin (labeled with ^{90}Y) and Bexxar (labeled with ^{131}I). Both of these agents have demonstrated significant antitumor response following a single treatment in patients with B-cell lymphomas, resulting in more than 40–70% complete response lasting 3–7 years.⁹³ However, the manufacture and marketing of Bexxar has been discontinued.

There are several studies describing the radiolabeling of monoclonal antibodies with ^{177}Lu for the development of therapeutic radiopharmaceuticals, and at least three products have been administered in humans, and their efficacy has been

evaluated. Table 8 summarizes those monoclonal antibodies which have been labeled with ^{177}Lu and evaluated in biological systems. Most of these studies have used DOTA as the chelating moiety where the coupling group to the antibody was attached to either the N atom of the DOTA framework (e.g., DOTA-NHS ester) or the C atom on the main carbocyclic backbone of the DOTA moiety (e.g., pSCN-Bn-DOTA). It has been documented that the ligand structure and physical characteristics, such as thermodynamic stability, dissociation rates, and serum stability, play an important role in controlling the in vivo dissociation of radiolabeled coordination complexes. Such studies were carried out with a series of different BFCA's with different backbone substitution in DTPA ligands. The correlation of the in vivo stability of conjugated coordination complexes with that of the aforementioned physical parameters provides important clues toward the screening of radiolabeled agents intended for use as radiopharmaceuticals.

Each reported study entails rigorous standardization of the protocols for conjugation, which include variation of the mAb concentration, antibody BFCA ratio, pH, temperature (room temperature or 37 °C), and reaction time. The chelate-to-antibody ratio is critical to retain the immunoreactivity of the monoclonal antibody postlabeling.

There are a few methods reported for the determination of the average number of chelates per antibody molecule, and the availability of these methods is relevant to the development of ^{177}Lu -labeled mAbs to estimate the number of protein-bound chelating groups.^{94–96} Dadachova et al. developed a spectrophotometric method in which the titration of the blue-colored 1:1 Pb(II)–arsenazo complex with the DOTA chelating agent is performed, wherein a color change occurs upon transchelation of the Pb(II) from the arsenazo to the DOTA, which is monitored at 656 nm.⁹⁵ The assay is performed at ambient temperature within 20 min without any interference from interactions between the protein and the Pb(II)–arsenazo complex. Lu et al. used matrix-assisted laser desorption/ionization time-of-flight (MALDI-ToF) mass spectrometry for the determination of the average number of DOTA attached to the mAb huJ591.⁹⁷ A comprehensive review by Liu et al. has analyzed the critical aspects governing the development of clinically useful agents for

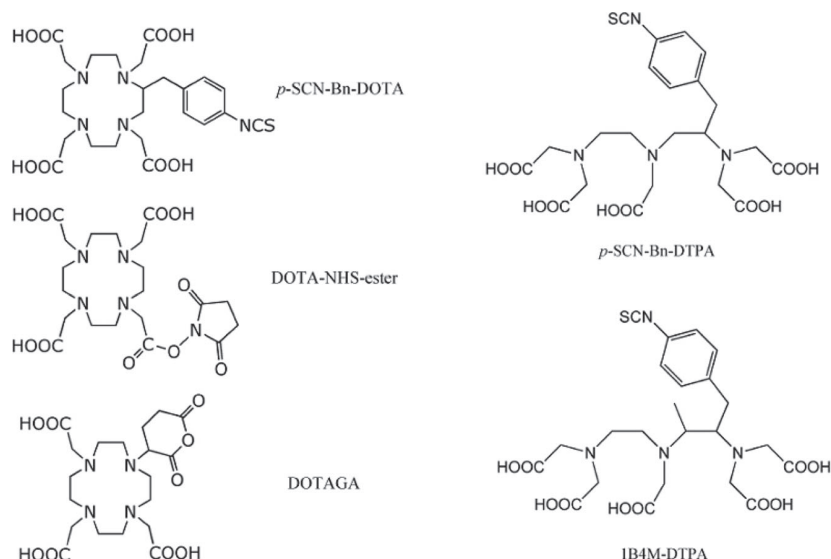


Figure 12. BFCAs used for conjugation of mAbs for RIT.

radiotherapy using different BFCAs.³⁷ Figure 12 illustrates the structures of some key BFCAs used for preparation of radiolabeled monoclonal antibodies. The importance of in vivo inertness of the radiometal as a primary determinant in preclinical studies of a radiopharmaceutical has been evaluated using DTPA chelates (Figure 13) and Y(III) metal ions.⁹⁸

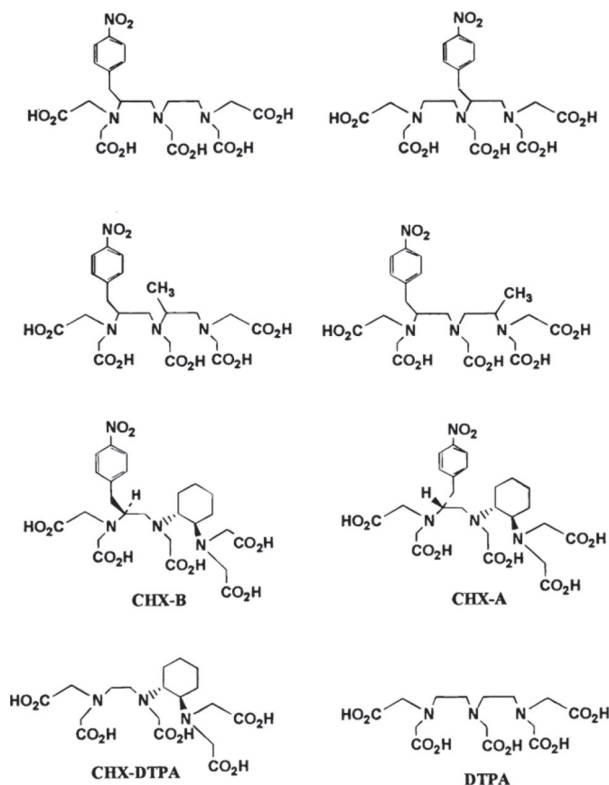


Figure 13. Functionalized DTPA ligands with one site available for antibody conjugation. Reprinted with permission from ref 98. Copyright 1998 American Chemical Society.

The following sections discuss the details of some of the monoclonal antibodies radiolabeled with ^{177}Lu .

7.1.1. Anti CD-20. Rituximab is a chimeric mouse–human monoclonal antibody which selectively binds to the B-lymphocyte antigen CD20, a hydrophilic transmembrane antigen expressed in B-lymphocytes and on about 90% of B-cell non-Hodgkins lymphomas (NHL). The CD20 antigen regulates the early steps in the activation process of cell cycle initiation and differentiation. Free CD20 antigen is not detectable in circulating blood and is neither shed nor internalized from the cell surface once bound to the antibody. Anti CD20 antibodies have been used in several studies, and there are two registered radiopharmaceuticals using the anti CD20 antibody. These are Zevalin (^{90}Y –ibritumomab) and Bexaar (^{131}I –tositumomab). The anti CD20 antibody (rituximab) was modified via thiourea bonds formed between $\epsilon\text{-NH}_2$ groups of lysine residues on the antibody molecule and the isothiocyanate-benzyl-DOTA.⁹⁹ The conjugate was labeled with ^{177}Lu to a specific activity of 444 MBq (12 mCi)·mg^{−1}. Using a pH of 9.0 for the coupling reaction and rituximab at a concentration of 10 mg·mL^{−1} resulted in a conjugate with an approximate ratio of one DOTA molecule to one rituximab molecule, which was considered insufficient for prompt labeling with the required levels of the ^{177}Lu radiometal. Increasing the pH from 9.0 to 9.5 and the concentration of antibody from 10 to 100 mg·mL^{−1} resulted in a conjugate with multiple DOTA molecules per antibody molecule [(DOTA)₄–rituximab]. In this study a comparative immunoreactivity with cells of the lymphoma LVB1 cell line was estimated by FACS assay using a (DOTA)₄–rituximab immunoconjugate and commercially available rituximab. The immunoreactivity was not found to be compromised by the conjugation of the antibody with DOTA. However, when the ratio of DOTA molecules to antibody molecules was increased to 8:1 so as to form [(DOTA)₈–rituximab], the immunoreactivity was drastically reduced to 50%, indicating that conjugation of eight DOTA molecules per rituximab molecule affects the binding sites of the antibody. This study also reported the development of a ready-to-use kit for radiolabeling studies. The (DOTA)₄–rituximab “kit” has been shown to be efficacious in labeling with ^{90}Y and ^{177}Lu , with

complete retention of immunoreactivity of the antibody–conjugate.

7.1.2. Anti-L1-CAM. The L1 cell adhesion protein is overexpressed in tumors such as neuroblastomas, renal cell carcinomas, ovarian carcinomas, and endometrial carcinomas and represents a target for tumor therapy with anti-L1-CAM antibody chCE7. In an optimization study reported by Knogler et al.,¹⁰⁰ the DOTA chelator-to-antibody ratio for the glycosylated variant of the anti-L1-CAM antibody chCE7 (chCE7agl) was varied such that a high specific activity ¹⁷⁷Lu-labeled conjugate with low liver uptake could be obtained. The ¹⁷⁷Lu–DOTA-labeled anti-L1-CAM antibody was evaluated in vitro. The BFCA used in anti-L1-CAM antibody labeling was 3-(*p*-nitrobenzyl)-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetate-triglycyl-L-*p*-isothiocyanato-phenylalanine (Figure 14). The results showed

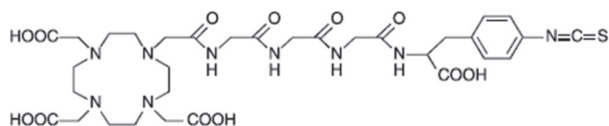


Figure 14. BFCA used for conjugation with anti-L1-CAM antibody chCE7. Reprinted with permission from ref 127. Copyright 2005 American Association for Cancer Research.

that the number of DOTA chelators coupled to the glycosylated variant of the anti-L1-CAM antibody chCE7 (chCE7agl) should not exceed 12 for labeling with ¹⁷⁷Lu.¹⁰⁰ A higher substituted chCE7agl cannot be labeled to increase SA, and the immunoconjugate exhibits rapid clearance from the blood and accumulates in the liver. Divalent fragments of this internalizing antibody were also labeled with ¹⁷⁷Lu, and the tumor and tissue uptake in nude mice with SK-N-BE2c xenografts was evaluated.¹⁰¹

7.1.3. ch81C6. The chimeric antibody antitenascin (ch81C6) was labeled with ¹⁷⁷Lu via the macrocyclic ligand NHS–DOTA and MeO–DOTA (Figure 15).¹⁰² The ¹⁷⁷Lu-labeled immunoconjugate was evaluated for binding to tenascin and evaluated in athymic mice bearing subcutaneous D54 MG human glioma xenografts. A comparison of the biodistribution of ¹²⁵I-labeled ch81C6 versus ¹⁷⁷Lu-labeled-ch81C6-1B4M and ch81C6-MeO–DOTA (1B4M = 2-(4-isothiocyanatobenzyl)-6-

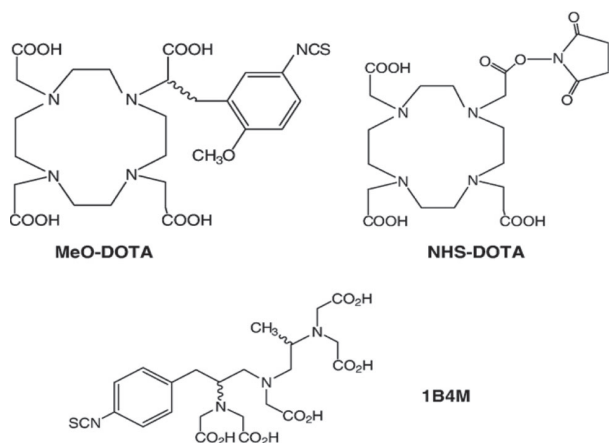


Figure 15. Bifunctional chelates MeO–DOTA, NHS–DOTA, and 1B4M used for conjugation. Reprinted with permission from ref 102. Copyright 2007 Elsevier.

methyldiethylene-triaminepentaacetic acid (1B4M–DTPA)), was carried out in the same animal model. MeO–DOTA was found to have promise as a bifunctional chelate for labeling ⁸¹C6 mAbs with ¹⁷⁷Lu.

7.1.4. Anti-VEGF. Vascular endothelial growth factor (VEGF) is one of the molecules responsible for regulating angiogenesis, a phenomenon observed in many cancers. The VG76e, an anti-VEGF monoclonal antibody, was labeled with ¹⁷⁷Lu using *p*-SCN-Bz-DOTA and CHX-A''–DTPA as chelators with high labeling yield and stability.¹⁰³ Biodistribution and gamma scintigraphic studies were carried out using the ¹⁷⁷Lu-labeled immunoconjugate. The in vivo behavior in normal and tumor-bearing mice showed promise, suggesting that these agents can be used for tumor targeting.

7.1.5. CC-49. The first reported and most exhaustively studied^{104–114} monoclonal antibody for radiolabeling with ¹⁷⁷Lu is CC-49, a murine monoclonal antibody specific to tumor-associated glycoprotein 72 (TAG 72), which is expressed on several major cancers. Extensive research was performed to design novel genetically engineered monoclonal antibodies which could achieve high tumor uptake and rapid blood clearance, thereby enhancing the therapeutic index of intraperitoneal radioimmunotherapy. In this connection, a novel humanized anti-TAG-72 monoclonal antibody (HuCC49DeltaCH₂) has been described, which localized in subcutaneous xenograft tumors and exhibits rapid plasma clearance.¹¹¹ The TAG 72 antigen is expressed on adenocarcinomas of the gastrointestinal tract, ovarian and endometrial carcinomas, nonsmall cell lung adenocarcinoma, pancreatic carcinomas, and mammary carcinomas. CC49 is a second-generation TAG72-specific antibody, and in one reported method,¹⁰⁵ ¹⁷⁷Lu was precomplexed with *p*-aminobenzyl DOTA and then activated with thiophosgene, followed by reverse-phase cartridge purification prior to conjugation with the CC49 monoclonal antibody. Conjugation of ¹⁷⁷Lu–DOTA with the mAb occurs through its aryl isothiocyanate group with the lysine –NH₂ group of the monoclonal antibody. Size exclusion chromatography was used to purify the labeled antibody from the unconjugated ¹⁷⁷Lu–DOTA complex.

In another study, radiolabeling of the Fab fragment of CC49 was carried out using PA (*p*-aminobenzyl) DOTA as the bifunctional chelating agent.¹¹¹ The combination of chemotherapy with RIT was found to be well tolerated, with bone marrow suppression as the dose-limiting toxicity. Preclinical studies were carried out with coinjection of the amino acid to enhance renal clearance. No further studies have been subsequently reported with this agent.

The ¹⁷⁷Lu-labeled tetravalent single-chain Fv construct [Sc(Fv)₂] of the CC49 monoclonal antibody and intact CC49 IgG that recognizes tumor-associated glycoprotein-72 (TAG-72) has also been prepared.¹¹⁴ TAG-72 is expressed by a majority of human adenocarcinomas and absent in most normal tissues. The BFCA used for conjugation was (S)-1-*p*-isothiocyanatobenzyl-diethylenetriamine pentaacetic acid (ITCB–DTPA or SCN–Bz–DTPA). In this report, a comparative study of biodistribution, blood clearance, and tumor targeting with ¹⁷⁷Lu–CC49 was studied in athymic mice bearing tumor xenografts.¹¹⁴ The product showed less renal uptake, fast clearance, but equivalent tumor uptake with respect to ¹⁷⁷Lu–CC49. Use of the pretargeting approach by administering single-chain Fv constructs of monoclonal antibodies linked to streptavidin, followed by the administration of ¹⁷⁷Lu–DOTA–biotin, was also suggested.^{115–117}

7.1.6. Cetuximab. Radiolabeling of Cetuximab, a chimeric IgG1 mAb, against epidermal growth factor (EGFR) with ^{177}Lu through different bifunctional chelating agents was attempted, and in vitro stability studies have been reported.¹¹⁸ Cetuximab was conjugated with either *p*-isothiocyanatobenzyl-DOTA (*p*-SCN-Bz-DOTA) or *p*-isothiocyanatobenzyl-DTPA (*p*-SCN-Bz-DTPA). PET images after coinjection of ^{89}Zr and ^{177}Lu antibody conjugates showed similar biodistribution.

7.1.7. cG250. Brouwers et al. reported the relative merits of ^{177}Lu , ^{90}Y , ^{186}Re , and ^{131}I in radiolabeling G250 (mAb cG250), a chimeric monoclonal antibody.¹¹⁹ This antirenal cell cancer mAb was radiolabeled with ^{177}Lu using cyclic diethylene triamine pentaacetic acid anhydride (cDTPA), isothiocyanatobenzyl-DTPA (SCN-Bz-DTPA), or 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA). Biodistribution and therapeutic efficacy were studied in nude mice bearing SK-RC-52 human RCC xenografts. In radioimmunotherapy (RIT) experiments at maximum tolerated dose, tumor growth was delayed most effectively by cG250 labeled with ^{177}Lu compared to ^{90}Y - and ^{186}Re -labeled antibodies. ^{177}Lu -cG250 was also found to be superior to ^{131}I -cG250. Radiolabeling with ^{177}Lu and ^{90}Y led to higher radiation doses to the tumor and served as better candidates than the conventionally used ^{131}I for RIT with cG250 in patients with renal cell carcinoma (RCC). The most likely reason for the higher efficacy of ^{177}Lu -cG250 is attributed to the optimal radiation characteristics for small tumors and a physical half-life that matches the mAb pharmacokinetics.

7.1.8. 7E11. Prostate-specific membrane antigen is a transmembrane glycoprotein highly expressed in many prostate cancers and can be targeted with radiolabeled antibodies for treatment of this disease. The 7E11 mAb is used in the ProstaScint agent (^{111}In -7E11), which is an approved immunodiagnostic agent. The ^{177}Lu labeling of the antibody was carried out using DOTA as the chelator for conjugation.¹²⁰ Pharmacokinetic and biodistribution studies of ^{177}Lu -7E11 in lymph node cancer of prostate (LNCaP) xenograft mice were performed, and ^{177}Lu -7E11 showed uptake and retention in mice bearing prostate tumors, thereby indicating its potential toward development as a radioimmunotherapeutic agent for prostate cancer.

7.1.9. hLL2 (Epratuzumab). The hLL2 (epratuzumab) mAb is a humanized mAb which internalizes and is directed against the CD22 antigen. It was labeled with ^{177}Lu and administered to nude mice with subcutaneous human lymphoma xenografts. Tumor uptake of ^{177}Lu -hLL2 was found to be higher than the tumor uptake of ^{131}I - and ^{186}Re -hLL2.¹²¹ For labeling with ^{177}Lu , isothiocyanatobenzyl-diethylenetriaminepentaacetic acid (ITC-DTPA) was used as the chelator.

7.1.10. huA33. The A33 (huA33) mAb is a potential targeting agent against colorectal carcinoma since the A33 antigen is expressed in >95% in all colorectal cancers, both in primary and in metastatic tumors. The huA33 antibody was labeled with ^{177}Lu using CHXA''DTPA as the chelator. Biodistribution studies were carried out in nude mice with colorectal SW1222 tumor xenografts, showing highly favorable biodistribution with high tumor uptake, indicating that the conjugate may be suitable for radioimmunotherapy of colorectal cancer.¹²²

7.1.11. Hu3S193. The first study to investigate the in vitro efficacy of ^{177}Lu -hu3S193, demonstrating that ^{177}Lu -hu3S193 mediates apoptosis in prostate cancer cells, was reported by Kelly et al.¹²³ Radiolabeling of hu3S193 mAb with ^{177}Lu was achieved using the C-functionalized *trans*-cyclohexyldiethylenetriamine-

pentaacetic acid (CHX-A''-DTPA) bifunctional chelator. The ^{177}Lu -labeled anti-Lewis Y mAb (hu3S193) was studied in mice bearing prostate cancer xenografts. The in vivo biodistribution and tumor localization of ^{177}Lu -hu3S193 was evaluated in mice bearing established DU145 tumor xenografts, and the results of the biodistribution studies demonstrated specific targeting of DU145 prostate cancer xenografts by this agent, which was found to cause specific and dose-dependent inhibition of growth of tumors in prostate cancer.

7.1.12. J-591. Cancer of the prostate is one of the major cancers in men, and prostate-specific membrane antigen (PSMA) is a well-established and well-characterized cell-surface antigen. It is a 100 kDa type II transmembrane glycoprotein and expressed by all prostate cancers. The density of PSMA increases in higher grade cancers, metastasis, and hormone refractory prostate cancer. Among solid tumors, prostate cancer is often amenable to radioimmunotherapy since it is relatively radio-sensitive and its predilection for forming small foci in the marrow and lymph nodes makes its target antigen readily available to circulating antibodies.¹²⁴ The 19 amino-acid cytoplasmic domain of this peptide contains a novel MXXXL internalization motif, resulting in its internalization and endosomal recycling, which makes PSMA an ideal antigen for targeting.^{125–127} Prostate cancer often metastasizes and is the focus of several diagnostic and therapeutic strategies. Earlier studies with the ^{111}In -DOTA-J591-labeled antibody demonstrated more rapid blood clearance with significant uptake in PSMA-positive human LNCaP tumors in mice models.¹²⁸ Similarly, ^{131}I -labeled J591 showed 20 times higher uptake in PSMA-positive LNCaP tumors compared to that in PSMA-negative PC3 and DU145 tumor xenografts.¹²⁹ The above studies suggested the utility of J591 as a suitable candidate for radioimmunotherapy. The monoclonal antibody J591 binds to the extracellular domain of PSMA with high affinity (1 nM). Lutetium-177 labeling of the monoclonal antibody J591 was carried out using DOTA as bifunctional chelating agent.^{130,131}

7.1.13. MOv18. The mouse monoclonal antibody MOv18 is directed against the α -isoform of the folate receptor (FR), which is a glycoprotein expressed in some normal tissues but significantly overexpressed on the cell membrane of ovarian carcinomas. The pharmacokinetics and long-term therapeutic efficacy of the ^{177}Lu -radiolabeled analogue were studied in a xenografted mouse model. The ^{177}Lu -MOv18 was able to eradicate small-size tumor masses expressing the antigen.¹³² The bifunctional chelator chosen in this study for conjugation with the MoAb was *p*-isothiocyanatobenzyl-DOTA.

7.1.14. Pertuzumab. Pertuzumab is a monoclonal antibody against HER-2 which targets the dimerization subdomain of HER-2, a tyrosine kinase receptor that is overexpressed in several carcinomas, especially breast cancer. Lutetium-177 labeling of pertuzumab was carried out by conjugation with isothiocyanatobenzyl-CHX-A''-DTPA as the BFCA.^{133,134} In a preliminary study, to investigate whether pertuzumab retains HER-2 targeting capacity after labeling with ^{177}Lu and to establish the findings, in vitro and in vivo experiments were carried out. The ^{177}Lu -labeled agent showed favorable targeting properties in BALB/c mice with HER-2-overexpressing xenografts. The absorbed dose estimate to the tumor was five times higher than the absorbed dose in blood and more than seven times the absorbed dose in any other normal organ. The results showed efficacy of the radiolabeled antibody and are particularly encouraging. In one study, transplanted SKOV-3 cells known to be radioresistant have been used for in vitro studies.

Table 9. Examples of Regulatory Peptides Which Are over Expressed in Human Tumors^a 138–140

peptide receptor	subtypes	tumor types
A-MSH	α -MSH	melanoma
ANP	ANP A-C	neuroblastoma
bombesin/ GRP	BB1 (NMB-R), BB2 (GRP-R), BB3, BB4	prostate, breast, pancreas, gastric, colorectal, small cell lung cancer SCLC, colon, breast, glioblastoma, prostate
endothelin	ET A,B	breast, ovary, lung cancer, MTC
exendin	GLP-1	insulinomas, gastrinomas, pheochromocytomas, para-gangliomas, and medullary thyroid carcinomas
gastrin/CCK	CCK1,2	MTC, SCLC, pancreatic, astrocytoma, stromal ovarian cancer
LNRH	LNRH-R	breast, prostate, ovarian cancer
neurotensin	NT1–3	exocrine pancreatic tumors, small cell lung cancer, neuroblastoma, colonic cancer
NPY	Y1–6	breast cancer, neuroblastoma
oxytocin	oxytocin	glial tumors, neuroblastoma, breast, and endometrial cancers
somatostatin	sstr _{1–5}	neuroendocrine tumors (gastroenteropancreatic tumors), non-Hodgkins lymphoma, paraganglioma, carcinoids, breast, brain, renal, small cell lung cancer, medullary thyroid cancer melanoma, breast cancer
substance P	NK1	glioblastoma, astrocytoma, MTC, breast cancer, intra- and peritumoral blood vessels, medullary thyroid cancer, small cell lung cancer, gastrointestinal stromal tumor, stromal ovarian cancer, astrocytomas

^aMTC = medullary thyroid carcinoma, SCLC = small cell lung cancer, α -MSH = alpha melanocyte stimulating hormone, BB = bombesin, NK = neurokinin, NT = neurotensin, ET = endothelin, ANP = atrial natriuretic peptide, LNRH = luteinizing hormone-releasing hormone, NPY = neuropeptide Y, GLP = gastrin-releasing peptide.

Experimental therapy showed that ¹⁷⁷Lu–pertuzumab delayed tumor progression compared with controls.¹³⁴

7.1.15. RS7. The tumor targeting and therapeutic efficacy of ¹⁷⁷Lu-labeled monoclonal antibody RS7 (antiepithelial glycoprotein-1) was evaluated in a human nonsmall cell lung carcinoma xenograft model. In this study reported by Stein et al., the potential of ¹⁷⁷Lu-labeled RS7 was compared with that of RS7 labeled with ⁹⁰Y and a residualizing form of ¹³¹I. DOTA was used as the chelator for preparation of the immunoconjugate.¹³⁵ ¹⁷⁷Lu–RS7 was shown to be an effective radioimmunoconjugate for radioimmunotherapy of even relatively large tumor xenografts. With its radiophysical properties similar to those of ¹³¹I, coupled with its facile and stable attachment to mAb, ¹⁷⁷Lu promises to be an alternative to ¹³¹I and a complement to ⁹⁰Y in radioimmunotherapy. Therapy was performed in nude mice with subcutaneous Calu-3 xenografts using the maximal tolerated dose (MTD) of ¹⁷⁷Lu–DOTA–RS7 (10.2 MBq [275 μ Ci]). Complete regression of the tumors, with an initial mean tumor volume of 0.24 cm³, was observed with ¹⁷⁷Lu–DOTA–RS7 doses ranging from 5.6 to 9.3 MBq (150–250 μ Ci) per nude mouse, with no significant difference in response rate noted between the doses in this range. The conclusion of the studies was that ¹⁷⁷Lu–RS7 is an effective radioimmunoconjugate for radioimmunotherapy.

7.1.16. Trastuzumab. The Trastuzumab monoclonal antibody, commonly known as Herceptin, is used in treating breast cancer and was radiolabeled with ¹⁷⁷Lu through the DOTA-N-hydroxy succinimide ester as the chelating group. In vitro quality control tests were performed as a first step in the development of this radiopharmaceutical.¹³⁶ The immunoreactivity and toxicity of the complex were tested on the MCF7 breast cancer cell line, and at a concentration of 1.9 nM, 90 $\pm$ 5% of the cells were killed. The results are promising for further evaluation in animals and possibly in humans as a new radiopharmaceutical for use in radioimmunotherapy against breast cancer.

7.1.17. U36. The chimeric mAb U36 monoclonal antibody, which is specific to the CD44v6 antigen, was labeled with ¹⁷⁷Lu, and its uptake, retention, and affinity were determined using an automated ligand binding assay.¹³⁷ The method described the interactions of the ¹¹¹In- and ¹⁷⁷Lu-labeled monoclonal antibody, mAb U36, with the CD44v6 antigen. Uptake studies with varying

specific activity of the chimeric mAb U36 and with an irrelevant antibody for the CD44v6 receptor verified the reliability of the method as well as the specificity of the antibody–receptor binding.

7.2. Peptides

Nearly all cancers have overexpression of specific receptors on the tumor surface, which forms the basis of peptide receptor radionuclide therapy (PRRT).^{138,139} The most widely employed modality of PRRT uses somatostatin analogues for targeting somatostatin receptors, which are overexpressed in neuroendocrine cancer. Other examples are the integrin receptors ($\alpha_v\beta_3$) present on activated endothelial cells of neovasculature as well as on different types of growing tumors including osteosarcomas, neuroblastomas, glioblastomas, melanomas, lung and breast carcinomas, cholecystokinin receptors for small cell lung carcinoma and medullary thyroid carcinoma, epidermal growth factor receptors for glioma, and gastrin-releasing peptide/bombesin receptors for prostate and breast cancers. Other key examples of peptides which target tumor overexpressed antigens include bombesin, cholecystokinin/gastrin, glucagon-like-peptide-1 (GLP-1)/exendin, and arginine-glycine-aspartic acid (RGD), which have been evaluated and resulted in identifying a number of radiolabeled peptide probes with promising potential. In this regard, a variety of peptides have been radiolabeled with ¹⁷⁷Lu with an aim to developing radiopharmaceuticals for radionuclide therapy.

The most important peptides of interest for targeting tumor cells for radionuclide therapy are the physiologically occurring regulatory peptides, which are also referred to as neuropeptides.¹³⁹ The great majority of these peptides mediate their regulatory functions by binding to the membrane-bound G protein-coupled receptors. While physiologically different peptide receptors are found in a variety of tissues, including the brain, they can be highly overexpressed in primary human cancers. Table 9 summarizes the most important regulatory peptide receptors which are expressed in human tumors.

A variety of strategies and optimized protocols for efficient labeling of peptides with clinically relevant radionuclides for radiopharmaceutical development have been reviewed.¹⁴¹

7.2.1. Somatostatin Analogues. Somatostatin (SST) has a short plasma half-life (~3 min) owing to rapid enzymatic

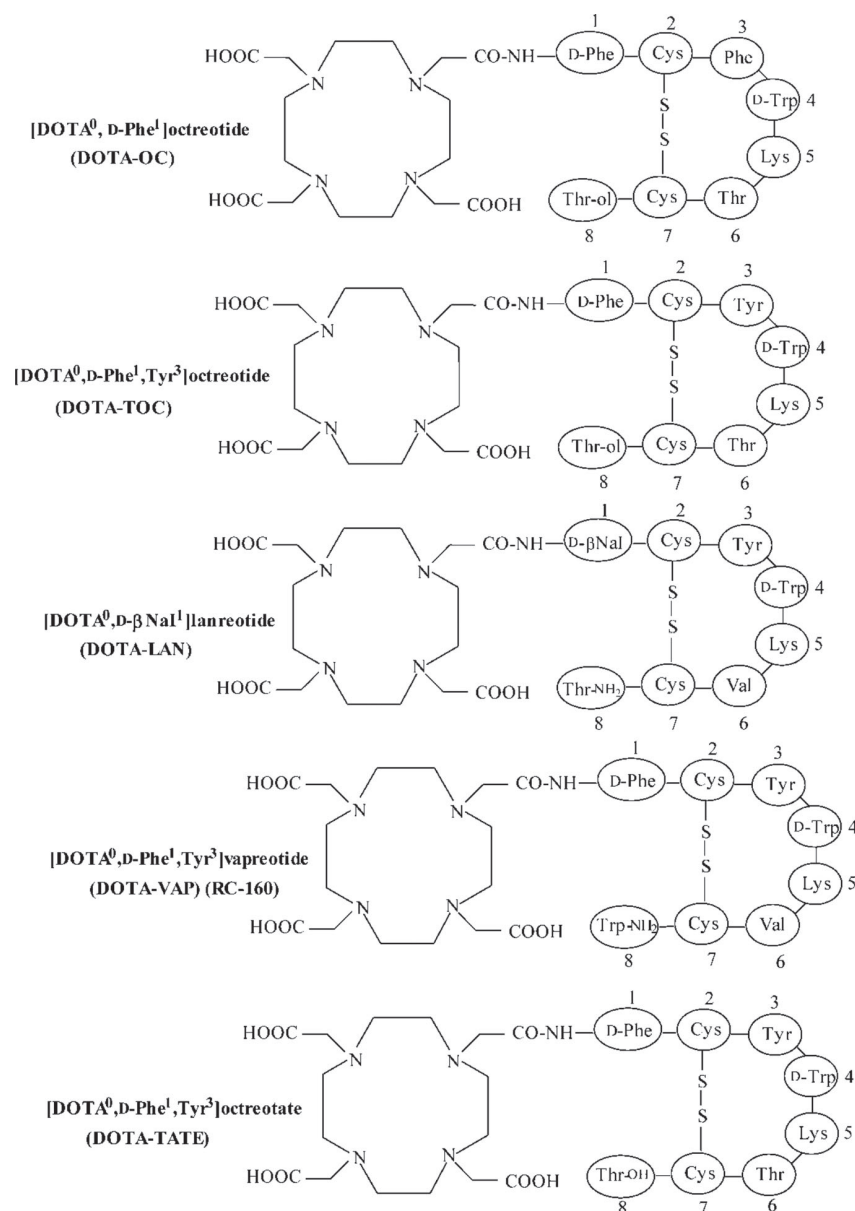


Figure 16. Examples of ^{177}Lu -labeled-DOTA-somatostatin analogs used in PRRT. Reprinted with permission from ref 138. Copyright 2012 Ivyspring.

degradation by endogenous peptidases. This short time period prior to deactivation by hydrolysis would preclude the use of the radiolabeled natural peptides for effective targeting. Synthetic SST peptide analogs (Figure 16) that are more resistant to enzymatic degradation have thus been prepared by molecular modification but with preservation of most of the biological activity of the original SST peptide. For example, the “Octreotide” analogue was developed by introduction of physiologically inactive D-amino acids, decreasing the ring size with retention of the active sequence, the 4-amino acid motif (Phe-D-Trp-Lys-Thr), of the native SST-14 involved in receptor binding. These smaller peptides have a significantly longer plasma half-life as compared to endogenous SST. The lanreotide and vapreotide (RC-160) synthetic SST analogues have also been studied, in which the cyclic forms preserved via a disulfide bond have been found to possess enhanced metabolic stability.

Somatostatin analogues are by far the most exhaustively studied therapeutic peptides in nuclear medicine using PRRT.¹⁴²

A high density of somatostatin receptors (sstr) is found in neuroendocrine tumors such as pituitary adenoma, pancreatic islet cell tumor, carcinoid, pheochromocytoma, paraganglioma, medullary thyroid cancer (MTC), and small cell lung carcinoma (SCLC). In addition, tumors of the nervous system including meningioma, neuroblastoma, and medulloblastoma also express high densities of somatostatin receptors. These tumors are commonly classified as gastroentero-pancreatic neuroendocrine tumors (GEP-NETs). Several other tumors classically not originating from the neuroendocrine or neural cells can also express somatostatin receptors.¹⁴² These include renal cell cancer, breast cancer, lymphoma, hepatocellular cancer, prostate cancer, sarcomas, and gastric cancer. Several subtypes of somatostatin receptors (sstr_{1–5}) are known, and the most

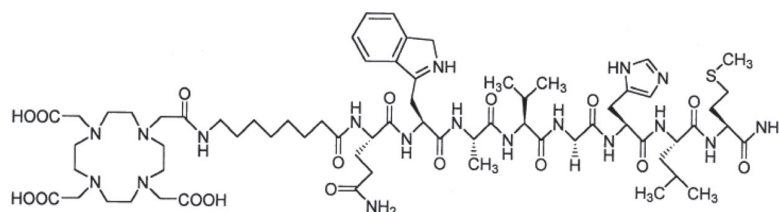


Figure 17. Structure of DOTA-8-Aoc-BBN[7–14]NH₂. Reprinted with permission from ref 164. Copyright 2003 Elsevier.

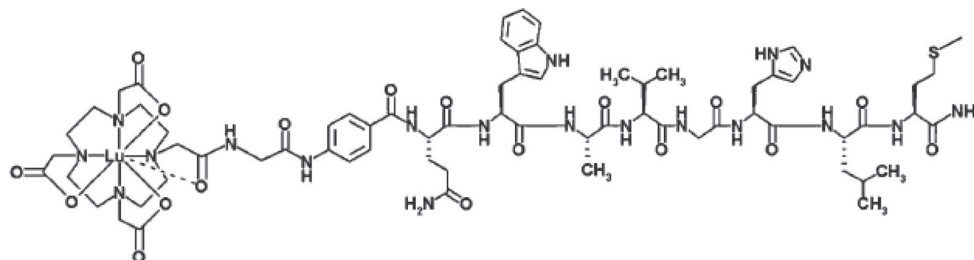


Figure 18. Structure of ^{177}Lu -AMBA. Reprinted with permission from ref 157. Copyright 2006 Society of Nuclear Medicine and Molecular Imaging (SNMMI).

commonly encountered receptor in most of these tumors is the sstr_2 subtype. While somatostatin has similar affinity for most of the receptor subtypes, the modified somatostatin analogue peptides vary in subtype affinity. Peptides having affinity to the sstr_2 subtype are thus far the most successful in the treatment of GEP-NETs. Most of these peptides show high affinity toward the sstr_2 subtype and show no binding to other receptor subtypes, with the exception of Lanreotide, which shows affinity also for the sstr_5 subtype. GEP-NETs are usually slow growing and have been treated with somatostatin analogues, which results in a lengthening of the time for tumor progression.^{143–146} In a study reported by Ginj et al.,¹⁴⁷ it has been shown that antagonists labeled many more sites than agonists. Somatostatin antagonist radiotracers therefore may be preferable over agonists for the in vivo targeting of sstr_2 - or sstr_3 -expressing tumors. Peptides used for PRRT require modification by conjugation with bifunctional chelating agents (BFCA) for radiolabeling with metallic radionuclides. DOTA is thus far the most widely used BFCA for labeling peptides with ¹⁷⁷Lu. However, conjugation of DOTA to the peptides as well as minor modifications of the peptides can affect binding affinity. DOTA-lanreotide exhibits reduced binding affinity for sstr_2 but shows affinity for sstr_3 -expressing tumors. Hence, tumors overexpressing the sstr_3 could be treated using this radiotracer. ¹⁷⁷Lu–lanreotide was prepared and evaluated in cell culture, but no human studies have yet been reported.¹⁴⁸ DOTA-[Tyr³]octreotate (DOTA-TATE) has twice the affinity with sstr_2 when compared with somatostatin.¹⁴⁹ The replacement of Phe³ in Octreotide by Tyr³ (TOC) leads to improved sstr_2 affinity, while C-terminal introduction of Thr (TATE) for Thr(ol) (TOC) results in a further improvement of the sstr_2 affinity. DOTA-TATE labeled with ⁶⁸Ga for PET imaging and with ¹⁷⁷Lu for targeted radionuclide therapy are routinely used for the management of patients with neuroendocrine tumors.¹⁵⁰

Figure 16 shows the somatostatin-DOTA peptides which have been radiolabeled with ^{177}Lu and used in PRRT.

Several somatostatin analog peptides have been synthesized to extend the biological activity profile to enable the targeting of NET-expressing all-sstr subtypes for diagnostic and therapeutic

applications. [DOTA,¹-Nal³]Octreotide (DOTANOC) is one such peptide where the amino acid in the 3 position of Octreotide is exchanged with a naphthyl residue. DOTANOC has been labeled with ¹¹¹In and used for diagnosis.¹⁵¹ This peptide shows affinity to sstr₃ and sstr₅ in addition to sstr₂-expressing tumors. [¹¹¹In/⁹⁰Y]DOTANOC showed high affinity to sstr₃ and sstr₅ when compared to [¹¹¹In/⁹⁰Y]-DOTATOC. In a patient study using ¹⁷⁷Lu-DOTANOC, higher uptake in normal tissues was observed which resulted in an increase in the whole-body dose.¹⁵² The variation of these results indicated the need and importance of individual patient dosimetry as an example of the growing awareness of the importance of the personalized medicine concept.

7.2.2. Bombesin Analogues. Bombesin (BBN) is a 14 amino-acid peptide originally isolated from skin of the frog *Bombina bombina*.¹⁵³ Gastrin-releasing peptide and neuromedin B are the human counterparts of bombesin and have been found in mammalian tissue.¹⁵⁴ Peptides binding to bombesin receptors are interesting candidates since bombesin receptors are overexpressed in many major neoplasia, including prostate, breast, pancreas, gastrointestinal, and small cell lung cancer. Although a large number of BBN analogs have been successfully synthesized and radiolabeled for tumor imaging and therapy applications, most of the analogs studied exhibit high abdominal accumulation, especially in the pancreas and intestine.^{155–161} A universal bombesin sequence [D-Tyr⁶, β-Ala¹¹, Phe¹³, Nle¹⁴] (6–14) has been developed by Mantey et al.^{162,163} and has high affinity to all the bombesin receptor subtypes, and hence, most of the radiolabeled bombesin peptides are based on bombesin (6–14).

Smith et al.¹⁶⁴ reported the BBN8 [DO3A-CH₂CO-8-amino-octanyl-Q-W-A-V-G-H-L-M-NH₂] conjugate for radiolabeling with ¹⁷⁷Lu (Figure 17) which has the following general structure DOTA-X-Q-W-A-V-G-H-L-M-(NH₂), containing the spacer group (X) ωNH₂(CH₂)₇COOH (8-Aoc). In vivo studies in female PC-3 tumor-bearing SCID (severe combined immunodeficiency) mice showed that a suitable radiolabeled derivative of BBN8 exhibited radiotherapeutic efficacy. The pharmacokinetics of ¹⁷⁷Lu-DOTA-8-Aoc-BBN[7–14]NH₂ have been studied, and the in vitro as well as in vivo targeting

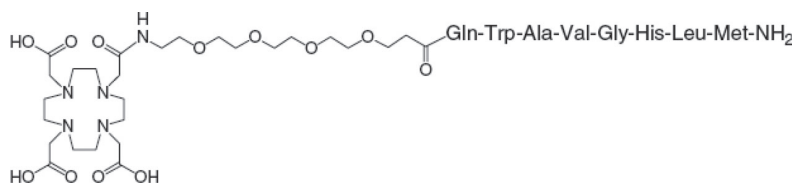


Figure 19. Structure of DOTA-PESIN. Reprinted with permission from ref 171. Copyright 2007 Springer.

abilities of this radiolabeled bombesin analogue for PC-3 human prostate cancer cells were explored. Furthermore, the ability of ^{177}Lu -DOTA-8-Aoc-BBN[7–14] NH_2 to selectively accumulate in tumor xenografts derived from these cells in SCID mice has also been reported.

Lantry et al.¹⁵⁷ developed ^{177}Lu -AMBA as the first radiolabeled BBN analog for therapeutic application. Metabolites of ^{177}Lu -AMBA and their receptor-binding characteristics, bio-distribution routes of excretion, and metabolites have been reported.¹⁶⁵ ^{177}Lu -AMBA is based on BBN (7–14) conjugated to DOTA via a Gly-4-aminobenzoyl spacer for targeting BBN₁ and BBN₂ receptors in human cancers. In another study, an attempt was made to improve the tumor uptake and retention while maintaining the high-affinity binding and desirable pharmacokinetic properties of BBN₈. Toward this goal, the DO3A- $\text{CH}_2\text{CO}-$ chelator and the (Q-W-A-V-G-H-L-M- NH_2) targeting functionality used by Smith et al. was maintained, but the linker was changed to a glycyl-4-aminobenzoic acid in place of the 8-aminooctanoic acid linker used in BBN8.¹⁶⁴ The conjugation of 4-aminobenzyl DOTA to bombesin was carried out to yield AMBA (DO3A- $\text{CH}_2\text{CO}-\text{G}-[4\text{-aminobenzoyl}]-\text{QWAVGHLM}-\text{NH}_2$) (Figure 18) and was radiolabeled with ^{177}Lu .¹⁶⁵ Human prostate cancer cell line PC-3 was used to determine the binding (K_d), retention, and efflux of ^{177}Lu -AMBA. Receptor specificity was determined by in vitro autoradiography in human tissues. Pharmacokinetic and radiotherapy studies were performed in PC-3 tumor-bearing male nude mice. ^{177}Lu -AMBA was shown to demonstrate nanomolar affinity in both human prostate tumor tissue and human PC-3 cells.^{166–169}

In vivo studies with ^{177}Lu -AMBA showed higher activity accumulation (at 1 h as well as 24 h) than ^{177}Lu -BBN₈, and the pancreas is the major nontarget organ which showed accumulation of both the radiolabeled peptides.¹⁶⁵ Treatment with 1–2 doses of ^{177}Lu -AMBA increased the life span of tumor-bearing mice. ^{177}Lu -AMBA was found to demonstrate nanomolar affinity in both human prostate tumor tissue and in the human PC-3 cells. The radiotherapeutic efficacy was studied, and it was reported that an appropriately timed second dose of ^{177}Lu -AMBA increases the efficacy of systemic radiotherapy.¹⁵⁷

Unfortunately, radioinstability may preclude further use of ^{177}Lu -AMBA, which can be overcome by the use of a protection buffer such as ascorbic acid.¹⁶⁸ Biodistribution, pharmacokinetics, bioluminescent imaging, and micro-SPECT/CT imaging of ^{177}Lu -AMBA in PC-3M-luc-C6 luciferase-expressing human prostate tumor-bearing mice has been reported by Liu et al.¹⁶⁹ Results obtained for tumor growth of PC-3 M luc-C6 in SCID mice obtained by direct measurement and bioluminescent imaging showed good correlation. Both biodistribution and micro-SPECT/CT imaging in PC-3M-luc-C6 bearing-tumor mice showed that tumor uptake of ^{177}Lu -AMBA could be retained for 24 h. The results indicated that BLI could be used to monitor tumor growth. High uptake of ^{177}Lu -AMBA in PC-3M-

luc-C6 tumor-bearing mice monitored by micro-SPECT/CT imaging can be used to further evaluate the potential of ^{177}Lu -AMBA therapy for PC-3M-luc-C6 tumors. An important observation is that this agent demonstrates very low kidney uptake and retention, unlike other reported peptide-based radiotherapies, which is often a dose-limiting observation. This agent showed very promising results due to the favorable pharmacokinetics and tumor efficacy in a relevant preclinical model and has also been studied in Phase I clinical trials.¹⁷⁰

In a recent modification of the novel AMBA bombesin analogue DOTA-PEG4-BN(7–14) (DOTA-PESIN), DOTA-PEG4-[Gln⁷-Trp⁸-Ala⁹-Val¹⁰-Gly¹¹-His¹²-Leu¹³-Met¹⁴- NH_2] [PEG = 15-amino-4,7,10,13-tetraoxapentadecanoic acid] (Figure 19) has been reported for radiolabeling with $^{67/68}\text{Ga}$ and ^{177}Lu for diagnosis and radionuclide therapy, respectively, of prostate and other human cancers that overexpress bombesin receptors. When radiolabeled with ^{177}Lu , this conjugate showed high tumor uptake and retention and favorable in vivo pharmacokinetics, which may favor its further evaluation.¹⁷¹

In another study, DOTA-PESIN was radiolabeled with the α -particle emitter ^{213}Bi , and the therapeutic efficacy of this tracer was compared with that of ^{177}Lu -DOTAPESIN in a prostate carcinoma xenograft model.¹⁷² The results showed that α therapy with ^{213}Bi is more efficacious than β^- therapy using ^{177}Lu .

7.2.3. RGD Analogues. The integrin family is a group of transmembrane glycoproteins comprised of 19 α and 8 β subunits, which are expressed in over 25 different α/β heterodimeric combinations on the cell surface. At least 24 distinct integrins are formed by a combination of 18 α and 8 β subunits, such as $\alpha_i\beta_3$, $\alpha_v\beta_3$, $\alpha_5\beta_1$, etc.¹⁷³ Among these, $\alpha_i\beta_3$ has a crucial role in the regulation of tumor growth and metastasis as it is highly expressed on activated and proliferating endothelial cells during tumor angiogenesis and metastasis. The $\alpha_i\beta_3$ integrin binds to the tripeptide Arg-Gly-Asp (RGD), containing components of the extracellular matrix such as vitronectin and fibronectin. Thus, a variety of linear and mostly cyclic RGD-based probes have been developed for monitoring expression of $\alpha_i\beta_3$ in vivo. Integrins play a critical role in several physiological processes including cell proliferation, wound healing, and remodelling of bone. Integrins also contribute to pathological events such as thrombosis, atherosclerosis, tumor invasion, angiogenesis, and metastasis. Among the integrin family, $\alpha_i\beta_3$ (vitronectin receptor or VnR) is studied most extensively for its role in tumor growth, progression, and angiogenesis.¹⁷⁴ The vitronectin receptor has a common RGD (Arg-Gly-Asp) tripeptide sequence, and hence, RGD derivatives when radiolabeled are suitable for targeting $\alpha_i\beta_3$ integrin receptors. The cyclic RGD peptide [Leu¹-Glu²-Glu³-Glu⁴-Glu⁵-Glu⁶-Ala⁷-Tyr⁸-Gly⁹-Trp¹⁰-Met¹¹-Asp¹²-Phe¹³- NH_2] and its multimers have been investigated for developing targeting agents to image neoangiogenesis, and in this respect ^{18}F -cRGD has been evaluated in patients with solid tumors.¹⁷⁵ In a recent review, recent developments in the targeted therapy of integrin receptors

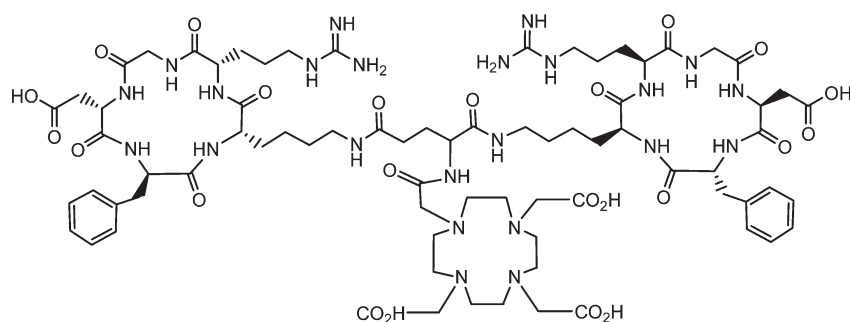


Figure 20. RGD-dimer for radiolabeling with ^{177}Lu .

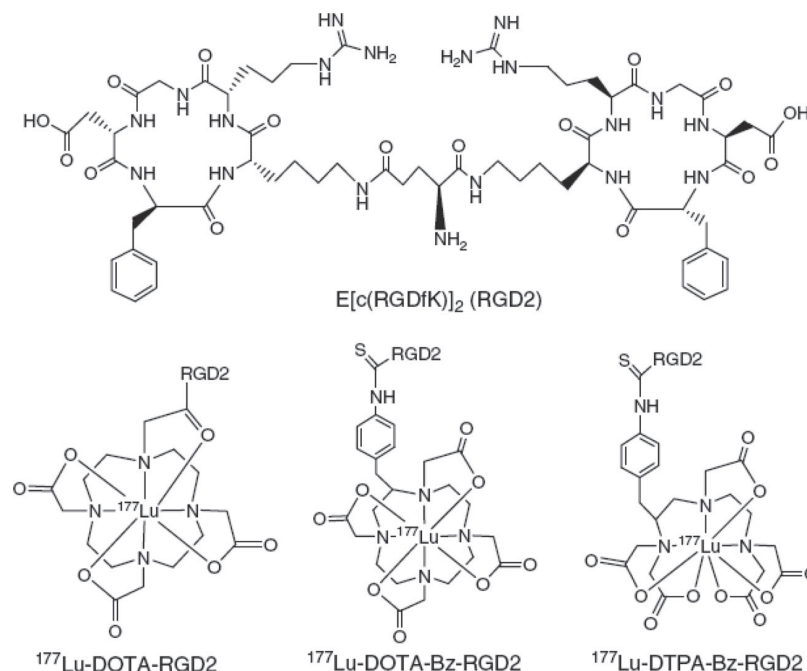


Figure 21. Structures of ^{177}Lu -DOTA-RGD₂, ^{177}Lu -DOTA-Bz-RGD₂, and ^{177}Lu -DTPA-Bz-RGD₂ (RGD₂ = $\text{E}[\text{c}(\text{EGDfK})]_2$). Reprinted with permission from ref 179. Copyright 2010 Springer.

using antibody, peptide, and other ligands have been described.¹⁷⁶

Although a number of radiolabeled RGD peptides have been useful for developing tumor-imaging agents, none have yet been able to achieve the desirable features of a therapeutic agent which requires high tumor uptake and retention together with low uptake in critical nontarget tissues. In one of the reported studies, RGD (c(RGDyK)) was conjugated to 1,4,7-triazacyclononane-*N,N',N''*-triacetic acid (NOTA) and radiolabeled with ^{177}Lu with a labeling efficiency up to 99%. In vivo studies in rats showed moderate tumor uptake ($1.7 \pm 0.33\%$) at 1 h, with the kidneys as the major excretion route.¹⁷⁷ However, the high kidney uptake ($7.56 \pm 0.71\%$) limited its application as a therapeutic agent. Chakraborty et al. recently demonstrated the potential of ^{177}Lu -DOTA-RGD for targeting melanoma tumors in C57BL/6 mice.¹⁷⁸

In another study reported by Shi et al., the three radiolabeled analogs ^{177}Lu -DOTA-RGD₂, ^{177}Lu -DOTA-Bz-RGD₂, and ^{177}Lu -DTPA-Bz-RGD₂ (RGD₂ = $\text{E}[\text{c}(\text{RGDfK})]_2$) (Figure 21) were evaluated as potential therapeutic radiotracers for the treatment of integrin $\alpha_v\beta_3$ -positive tumors.¹⁷⁹ BALB/c nude

mice bearing U87MG human glioma xenografts were used to evaluate the biodistribution characteristics and excretion kinetics of the radiotracers, and no major differences could be observed in their lipophilicity and biodistribution characteristics. In this study, it has been shown that Bz-DTPA constitutes the most desirable BFCA due to its high radiolabeling efficiency and fast kinetics. The radiolabeling occurs at room temperature, while heating and higher concentrations of BFCA are required using DOTA and DOTA-Bz as BFCAs. The authors concluded that the ^{177}Lu -labeled cyclic RGDfK peptides and ^{177}Lu -DTPA-Bz-RGD₂ are potential agents for further investigation toward targeted radiotherapy of integrin $\alpha_v\beta_3$ -positive tumors.

Development of an integrin $\alpha_v\beta_3$ antagonist platform that could be utilized for tumor delivery of diagnostic and therapeutic radionuclides was also attempted.¹⁸⁰ These studies involved development of peptidomimetics having high affinity and selectivity for $\alpha_v\beta_3$ integrin receptors and radiolabeling with various radionuclides. In this study reported by Liu et al., the development of an anaerobic formulation for the routine preparation of ^{90}Y and ^{177}Lu complexes with DOTA-conjugated nonapeptide vitronectin receptor antagonists, including TA 138

(3-sulfon-*N*-[[4,7,10-tris(carboxymethyl)1,4,7,10-tetraazacyclododec-1-yl]acetyl]-*L*-alanyl-*N*-[2-[4-[[[(1*S*)-1-carboxy-2[[[1,4-dihydro-7-[(1*H*-imidazol-2-ylamino)methyl]-1-methyl-4-oxo-3-quinolinyl]carbonyl]amino]ethyl]amino]sulfonyl]-3,5-dimethylphenoxy]-1-oxobutyl]amino]ethyl]-3-sulfo-*L*-alaninamide) (Figure 22), was reported.¹⁸¹ Since both ⁹⁰Y-TA138 and ¹⁷⁷Lu-

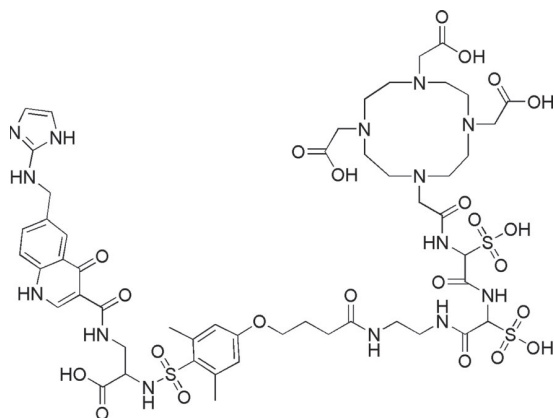


Figure 22. Structure of TA-138. Reprinted with permission from ref 180. Copyright 2001 American Chemical Society.

TA138 are very sensitive to radiolytic degradation, high yield (>95%) and high specific activity complexes can be obtained using strictly anaerobic conditions. The anaerobic formulation described in this study is particularly useful for ⁹⁰Y and ¹⁷⁷Lu labeling of DOTA-conjugated small biomolecules that are sensitive to the radiolytic degradation during radiolabeling. No biological or clinical studies have yet been reported with these complexes.

Cysteine knot miniproteins, also called knottins, are small polypeptides which contain the RGD subset and hence when labeled should bind with integrin receptors.¹⁸² In one of the studies, two knottin peptides, 2.5 D and 2.5 F, were labeled with ¹⁷⁷Lu through DOTA as the bifunctional chelating agent (Figure 23).¹⁸³ Cell binding assays in U87 mg glioma cell lines showed that ¹⁷⁷Lu-labeled peptide binding was blocked by cold c(RGDyK). Biodistribution studies in nude mice bearing human glioblastomas showed low-to-moderate tumor uptake

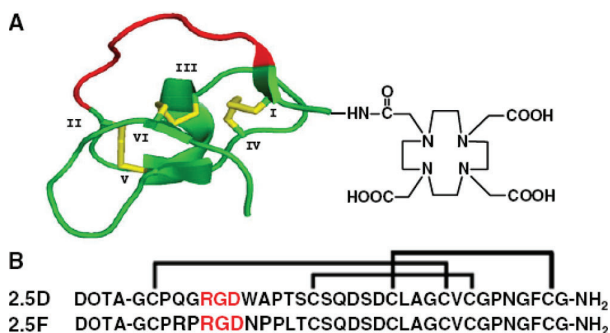


Figure 23. Schematic and sequence of engineered knottin peptides. (A) Cartoon representation of knottin peptide scaffold. Radiometal chelator DOTA was site specifically conjugated to the N-terminal amino group. (B) Amino acid sequence of integrin binding knottins 2.5D and 2.5F with disulfide bonds between Cys1-Cys4, Cys2-Cys5, and Cys3-Cys6. Reprinted with permission from ref 183. Copyright 2011 Springer.

with high levels of the injected activity found in the kidneys, indicating that kidneys will be the dose-limiting organ to carry out cancer-targeted radionuclide therapy.

7.2.4. Substance P. Substance P (SP) is an 11 amino-acid neuropeptide having the amino acid sequence Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH₂ and is known to be a potential member of a family of tachykinins.^{139,140,184} Tachykinins are a group of peptides that share a common -Phe-X-Gly-Leu-Met-NH₂ C-terminal amino acid sequence, where X represents Phe, Ile, or Val. The mammalian family of tachykinins includes substance P, neurokinin A and B, and two N-terminally extended forms of neurokinin A (neuropeptides and gamma). The three tachykinin homologous receptor types are designated as the SP-preferring NK1 receptor, the neurokinin A-preferring NK2 receptor, and the neurokinin B-preferring NK3 receptor. SP acts as the preferential, but not exclusive, endogenous ligand for the NK₁ receptor class. It has been established that SP plays an important role in modulating pain transmission from peripheral and central primary afferents through neurokinin 1 and 2 receptors, and this peptide may also be involved in the pathogenesis of inflammatory diseases. SP receptors are also found in brain, lymphoid tissues, vessels, gut smooth muscle, airway glands, and bronchiolar walls. In receptor autoradiography of tumor specimens *ex vivo*, SP receptors are found to be more abundant than somatostatin receptors on malignant gliomas and medullary thyroid cancer, with lower incidence in nonsmall cell lung cancer and carcinoma of the pancreas. In addition, SP receptors have also been found on peritumoral vessels associated with tumors but rarely found in gastrointestinal tumors or lymphomas. Substance P is able to stimulate the proliferation of malignant tumor cells.¹³⁹ Since NK₁ receptors are consistently overexpressed in all malignant gliomas, the introduction of radiolabeled SP analogues for peptide receptor imaging and radiotherapy can be a focus of interest to characterize and treat those tumors.¹⁸⁵ In addition, radionuclide-based approaches can also eliminate receptor-negative tumor cells not directly targeted by the drug through the range-dependent “cross-fire” effect. Intratumoral and/or intracavitary injection of DOTA-SP represents a potential new tool for the local control of malignant gliomas. For labeling substance P, ⁹⁰Y has been the most widely used radionuclide, but to reduce the cross-fire effect in critically located tumors, the use of ¹⁷⁷Lu and ²¹³Bi, which have much lower radiation path lengths, has been proposed.^{186,187} These receptors are expressed on glioma tumor cells and tumor vessels, and hence, ¹⁷⁷Lu-substance-P could be used for loco-regional therapy of glioma postsurgical intervention.

Radiolabeling of DOTA-conjugated substance P with ¹⁷⁷Lu in high yields (>99%) at optimized conditions has been reported.¹⁸⁸ The preparation remains stable for more than 72 h at 4 °C and 24 h in human plasma. Biodistribution studies have been carried out in nude mice bearing pancreatic tumors (PT). The results show that tumor uptake (~1.0% ID) was observed when compared to normal pancreas (~0.2% ID), suggesting the presence of NK receptors in AR42J pancreatic tumor. The results indicate the viability of this tumor model to predict the specificity of radiolabeled SP to neurokinin receptors (NKr), usually overexpressed in glial malignant brain tumors.¹⁸⁹ Detailed studies have also suggested the importance of prevention of methionine oxidation in substance P during the labeling procedure, which involves addition of methionine to the radiopharmaceutical formulation.

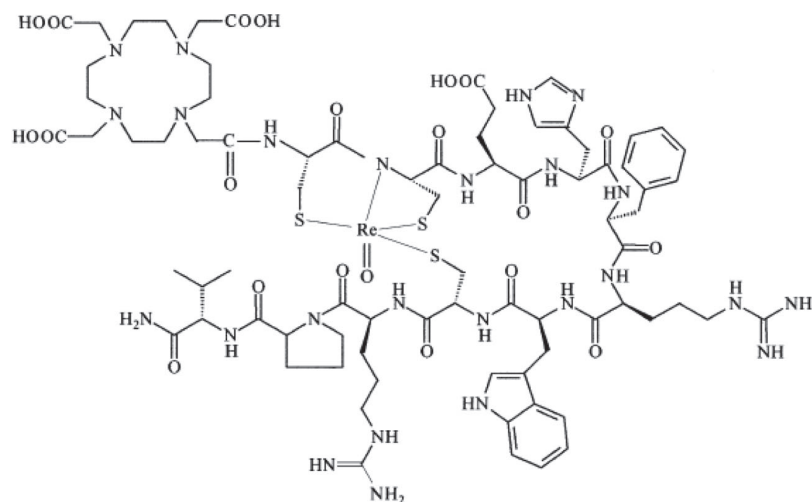


Figure 24. Schematic structure of DOTARE(Arg¹¹)CCMSH ligand for radiolabeling with ¹⁷⁷Lu. Reprinted with permission from ref 191. Copyright 2005 Elsevier.

7.2.5. Other Peptides Studied with ¹⁷⁷Lu. Cholecystokinin (CCK) and gastrin are peptide hormones that have several regulatory functions in the gastrointestinal tract and in the nervous system. Three types of CCK receptors are known which include CCK1 (or CCK-A), having limited expression in humans. The CCK2 (or CCK-B or gastrin receptor) analogue has high affinity for gastrin and CCK and is frequently expressed in human tumors, including medullary thyroid carcinomas, small cell lung cancer, stromal ovarian cancers, astrocytomas, and some gastroenteropancreatic cancers. The CCK2i4sv receptor, which is the splice variant of the CCK2 receptor, is expressed in human colorectal cancers and pancreatic cancers but not in normal colorectal mucosa. The radiolabeled CCK/gastrin analogs are mostly based on CCK8 [D-Asp¹-Tyr²-Met³-Gly⁴-Trp⁵-Met⁶-Asp⁷-Phe⁸-Gly⁹-Trp¹⁰-Met¹¹-Asp¹²-Phe¹³-NH₂] and minigastrin [Leu¹-Glu²-Glu³-Glu⁴-Glu⁵-Glu⁶-Ala⁷-Tyr⁸-Gly⁹-Trp¹⁰-Met¹¹-Asp¹²-Phe¹³-NH₂]. Radiolabeling of the DOTA-minigastrin derivative [His¹-His²-Glu³-Ala⁴-Tyr⁵-Gly⁶-Trp⁷-Nle⁸-Asp⁹-Phe¹⁰-NH₂] with ¹⁷⁷Lu in high yields (~98%) has been reported.¹⁹⁰ However, detailed in vitro and in vivo studies are yet to be studied.

Another peptide studied for ¹⁷⁷Lu labeling is the α -melanocyte-stimulating hormone (α -MSH, Figure 24).^{191–193} It has been shown that α -melanotropin peptide analogs cyclized through rhenium and technetium metal coordination can be designed and characterized.^{194–196} The conclusions derived from these studies reveal the targeting properties of ⁹⁰Y-DOTA-Re(Arg11)CCMSH and ¹⁷⁷Lu-DOTA-Re(Arg11)CCMSH for melanoma and also indicates their potential for the targeted therapy of melanomas. The high renal uptake could be reduced by introducing a negatively charged amino acid (Glu) to the CCMSH peptide sequence, thereby increasing tumor-to-kidney uptake ratios.

The melanoma lesions were visualized in a B16/F1 melanoma-bearing mouse by SPECT/CT images of ¹⁷⁷Lu-DOTA-Re(Arg11)CCMSH, highlighting its potential application as an imaging probe during the process of melanoma treatment. The authors have demonstrated the possible use of ¹⁷⁷Lu-DOTA-Re(Arg11)CCMSH as an imaging probe while carrying out targeted radionuclide therapy as well as its use to perform dosimetric estimates without the need for additional injection of an imaging probe.¹⁹³

A novel DOTA-folate ligand (Figure 25) was prepared by the Cu(I)-catalyzed [3 + 2] cycloaddition of azides and terminal

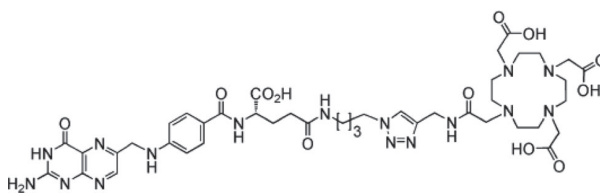


Figure 25. Chemical structure of the DOTA-folate ligand. Reprinted with permission from ref 197. Copyright 2009 Springer.

alkynes, a well-known “click reaction”.¹⁹⁷ In this study, the Cu(I)-catalyzed reaction of folic acid- γ -(4-azido)-butaneamide with an alkyne-derivatized DOTA chelator was carried out to provide the DOTA-folate chelate.

The conjugate was radiolabeled with ¹⁷⁷Lu and its tissue distribution investigated in nude mice bearing KB (human nasopharyngeal carcinoma cell line; CCL-17, expressing folate receptors) tumor xenografts. The results reveal the potential of the radiolabeled folate for therapeutic use as evidenced by its favorable tissue distribution attributable to its hydrophilic properties as well as the combined administration of pemetrexed, an antifolate and a chemotherapeutic, as a kidney protecting agent.

7.3. Bone Pain Palliation Agents

Palliation of bone pain due to skeletal metastases can be effected using radionuclide therapy. For this purpose, treatment with radiopharmaceuticals labeled with β^- /conversion-electron-emitting radionuclides is an effective option and has been widely shown to provide significant improvement in the quality of life of patients.^{198–201} The radioisotopes that have been used for palliative radiotherapy include ³²P [$T_{1/2}$ = 14.26 days, $E_{\beta(\max)}$ = 1.71 MeV] in the form of Na₃³²PO₄, ⁸⁹Sr [$T_{1/2}$ = 50.53 days, $E_{\beta(\max)}$ = 1.49 MeV] in the form of ⁸⁹SrCl₂ (Metastron), ¹⁵³Sm [$T_{1/2}$ = 46.27 h, $E_{\beta(\max)}$ = 0.81 MeV, E_{γ} = 103 keV (28%)] as ¹⁵³Sm-EDTMP (Quadramet), ¹⁸⁶Re [$T_{1/2}$ = 90.64 h, $E_{\beta(\max)}$ = 1.069 MeV, 137 keV (8.6%)] as ¹⁸⁶Re-HEDP and ¹⁸⁸Re [$T_{1/2}$ = 16.9 h, $E_{\beta(\max)}$ = 2.12 MeV, E_{γ} = 155 keV (16%)] as ¹⁸⁸Re-HEDP

(HEDP = hydroxyethylenediphosphonate).^{202–207} While the ¹⁵³Sm- and ⁸⁹Sr-labeled agents have regulatory approval for clinical use in many countries, ³²P is not often used because of the high radiation burden for marrow and ¹⁸⁸Re–HEDP is currently used in clinical trials. ¹⁸⁶Re–HEDP had been available on a physician prescription in Europe, but the breadth of its current use is uncertain.

Lutetium-177 has favorable properties for the development of bone pain palliative agents arising from skeletal metastases. The β^- -particle energies emitted from ¹⁷⁷Lu are adequately low, and hence, the tissue penetration range is considerably lower than beta emission energies for ⁸⁹Sr, ³²P, ¹⁸⁸Re, and ¹⁵³Sm, the currently used radionuclides for bone pain palliation. The low range induces minimum bone marrow suppression on accumulation in skeletal lesions, which may also be an advantage of use of this radiotherapeutic modality, when marrow reserve is limited.

The use of ¹⁷⁷Lu as chloride or citrate was first reported for the treatment of multiple myeloma, which is a primary marrow cancer, related to bone metastasis.²⁵ The first study of the development of ¹⁷⁷Lu bone-seeking radiopharmaceuticals was reported by Ando et al., who described the preparation of ¹⁷⁷Lu–EDTMP and evaluated its biological distribution.²⁸ Solla et al. reported the preliminary clinical use of ¹⁷⁷Lu–EDTMP in a few patients.²⁹ However, the clinical studies were carried out with minimum biological and dosimetry studies in animal models.

In designing suitable radiolabeled agents for palliative care of bone pain due to metastatic skeletal lesions, multidentate polyaminophosphonic acids have been found to be the most promising candidates as carrier ligands owing to their high bone affinity, selective localization in hypermetabolically active skeletal lesions, and ability to form metal chelates with high *in vivo* stability, especially with radiolanthanides.^{203,207–210}

A series of α -aminomethyl phosphonic acids has also been synthesized through the Mannich reaction wherein orthophosphorous acid, 1,2-alkyldiamines, and formaldehyde react in strong acidic medium (Figure 26).^{211,212}

Systematic screening of the ¹⁷⁷Lu complexes of several cyclic and acyclic polyaminopolyposphonate ligands has demonstrated that the cyclic phosphonates are capable of forming complexes at lower ligand-to-metal ratios and with better thermodynamic stability and kinetic inertness.^{208,209} Among the acyclic ligands, better complexation yields have been reported with EDTMP (20:1). In a comparative study of ¹⁷⁷Lu–EDTMP and ¹⁷⁷Lu–DOTMP, both complexes were obtained in near-quantitative yields using significantly low [ligand]:[metal] ratios.²¹³ While biodistribution patterns of both ¹⁷⁷Lu–phosphonate complexes in Wistar rats demonstrated favorable pharmacokinetic features, a similar comparison of biodistribution patterns revealed that the ¹⁷⁷Lu–DOTMP complex exhibits slightly faster blood clearance, marginally lower liver retention, and to some extent more rapid kidney clearance compared to similar values for ¹⁷⁷Lu–EDTMP. On the other hand, ¹⁷⁷Lu–EDTMP showed slightly higher skeletal accumulation compared to ¹⁷⁷Lu–DOTMP throughout the time points studied (Figure 27).

Although both agents exhibited almost similar radiochemical and pharmacokinetic characteristics, their comparative efficacy could only be conclusively decided following extensive human clinical studies.

The published data suggested that EDTMP and DOTMP have the most favorable bone uptake with limited soft tissue localization. DOTMP has the advantage that complexation can

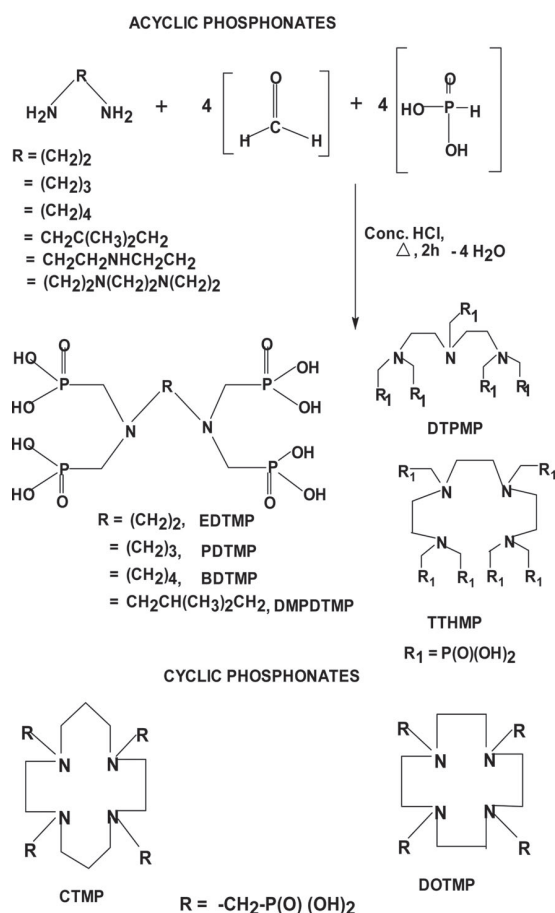


Figure 26. α -Aminomethylphosphonic/phosphonate ligands used for ¹⁷⁷Lu labeling for bone pain palliation studies. Reprinted with permission from ref 211. Copyright 2001 Elsevier.

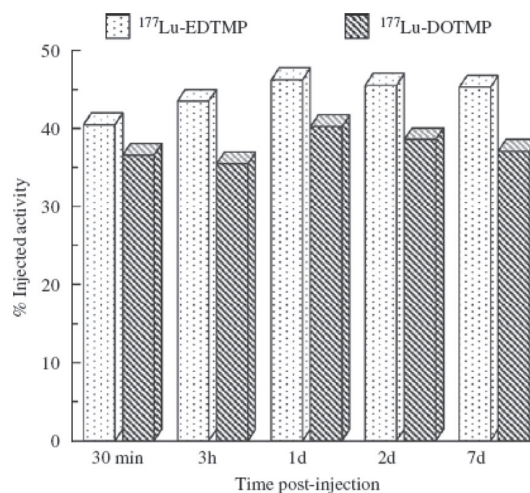


Figure 27. Comparison of skeletal uptakes of ¹⁷⁷Lu–EDTMP and ¹⁷⁷Lu–DOTMP complexes in Wistar rats at different postinjection times. Reprinted with permission from ref 213. Copyright 2008 Elsevier.

be performed using smaller amounts of ligand. In an independent study in four dogs injected with 8.14 MBq·kg^{−1} doses of ¹⁷⁷Lu–DOTMP and monitored for 84 days, no evidence of toxicity in

the bone marrow or vital organs was seen.²¹⁴ Ideally, both EDTMP and DOTMP would be good candidates for clinical trials; however, choice of ^{177}Lu -EDTMP for further clinical development is strengthened from the significant published data for use of EDTMP as the precursor for the clinically approved radiopharmaceutical ^{153}Sm -EDTMP. A detailed preclinical biological evaluation including biodistribution, pharmacokinetics, and toxicity of ^{177}Lu -EDTMP was carried out in multispecies animal models in order to collect data to support human trials.²¹⁵ ^{177}Lu -EDTMP was injected into mice intravenously via a lateral tail vein, and pharmacokinetic parameters were derived from the mean values of biodistribution data acquired at different time points by applying Topfit v2.0 pharmacokinetic software using the noncompartmental model. The results of the biodistribution studies in mice at different time points showed rapid accumulation of activity in bone, peaking at 1 h with values of p.i. $40.82 \pm 3.98\%$ IA in bone. Activity accumulation was primarily observed in the skeleton with rapid renal excretion. The biodistribution, autoradiography, and imaging studies of ^{177}Lu -EDTMP demonstrated that the tracer is taken up almost exclusively by the skeleton, with minimal activity accumulation in any other organ. Multidose studies up to $37 \text{ MBq}\cdot\text{kg}^{-1}$ body weight in dogs did not result in any adverse effects as seen from the biochemical parameters and hematological measurements. In another recent study, preparation, quality control, and pharmacokinetic studies of ^{177}Lu -EDTMP with low specific activity ($80\text{--}135 \text{ mCi}\cdot\text{mg}^{-1}$) were reported with ^{177}Lu produced by irradiating natural Lu_2O_3 .²¹⁶ The tracer could be prepared with high radiochemical purity, exhibited good stability, and showed similar biological uptake to that obtained with the product prepared with high specific activity ^{177}Lu . On the basis of the results, the authors reported their plans for initiating a clinical trial in patients with metastatic bone disease.

Preparation and evaluation of two other tracers, viz. ^{177}Lu -methylene diphosphonate (MDP) and ^{177}Lu -PYP (lutetium-177-labeled pyrophosphate), have been reported.^{217,218} The radiolabeling yields were found to be as high as 99%, and in vitro stability of the complexes was found to be adequate. In vivo distribution studies of ^{177}Lu -PYP in an animal model indicated selective bone accumulation, relatively low uptake in soft tissue (except the liver), and higher skeletal uptake.

7.4. Particulates for Targeted Therapy of Hepatocellular Carcinoma

7.4.1. ^{177}Lu -Labeled Hydroxyapatite. Hydroxyapatite (HA, $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$) is the natural mineral constituent of bone matrix, and HA particles have been evaluated as a potential carrier for developing agents for targeted radiotherapeutic applications in hepatic cellular carcinoma (HCC). HA is biocompatible and biodegradable and can be easily labeled with radiolanthanides. In addition, the biological half-life of the agent is sufficiently long compared to the physical half-lives of radionuclides which have been studied.²¹⁹ HA particles are converted to Ca^{2+} and PO_4^{3-} ions by natural metabolic processes and are eliminated over a period of 6 weeks, thereby providing biocompatibility.^{220,221}

A number of radiolabeled microspheres or particulates, such as ^{90}Y -labeled glass microspheres, ^{90}Y -labeled resin microspheres, ^{166}Ho -labeled polylactic acid (PLA) microspheres, and ^{188}Re -microspheres, have been found to be clinically successful in the treatment of both primary and metastatic liver cancer.^{222–224} Although surgical excision is a viable option for improving the survival status of liver cancer patients, particularly in the cases of

localized tumors, it has limited applicability in metastatic liver cancer. Intra-arterial radionuclidic therapy using a suitable β^- -emitting radionuclide has emerged as a promising alternative to combat primary liver cancer and liver metastases. In this treatment modality, radiotherapeutic agents are administered through the hepatic artery either in the form of radiolabeled particulates of well-defined size or as a lipophilic-radiolabeled viscous liquid. Since the blood supply to the cancerous liver cells is primarily through the hepatic artery and the normal liver parenchyma is oxygenated via the portal vein, these agents deliver the required therapeutic dose to the tumor by being deposited or embolized preferentially in the richly vascularized periphery of the tumor. Lutetium-177-labeled particles have been investigated for use in radiopharmaceutical applications such as for the treatment of inflammatory rheumatoid arthritis (radiation synovectomy, RSV) and for the treatment of primary and metastatic liver cancer.^{225,226} These two applications require the use of HA particles of different size ranges.

^{177}Lu -labeled particulates having a $20\text{--}60 \mu\text{m}$ size range were evaluated for potential radiotherapeutic application in hepatic cellular cancer (HCC).²²⁶ These radiolabeled particulates were prepared with a high radiochemical purity of $\sim 99\%$ under optimized reaction conditions, and the radiolabeled agent showed adequate stability in vitro. The biological behavior of ^{177}Lu -labeled HA particles was studied by both biodistribution and scintigraphic imaging in normal Wistar rats by injecting the preparation directly via the hepatic artery, revealing the slow release of initially accumulated activity from the liver. No appreciable uptake was observed in any of the major organs/tissue, except in the skeleton, where the activity was found to increase gradually, indicating possible release of $^{177}\text{Lu}^{3+}$. Although ^{177}Lu -HA exhibited promising features in radiochemical studies, preliminary bioevaluation studies exhibited suboptimal liver retention and undesirable skeletal uptake, which may render this agent unsuitable for human application.

7.4.2. ^{177}Lu Oxine in Lipiodol. Lipiodol is an iodinated and esterified lipid of poppy seed oil. It was the first X-ray contrast agent and is currently used for trans-arterial embolization because of its high viscosity and lipophilicity.²²⁷ Lipiodol labeled with suitable β^- -emitting radionuclides, such as ^{131}I , ^{188}Re , and ^{90}Y , has been evaluated for targeting liver cancer in both animal models and in human trials.^{228–231} ^{177}Lu -labeled lipiodol, was prepared by a two-step process involving the preparation of a ^{177}Lu -oxine (8-hydroxyquinoline) complex and its subsequent dispersion in lipiodol medium.²³² The ^{177}Lu -oxine complex was prepared in high yields ($\sim 98\%$), and $\sim 95\%$ of the ^{177}Lu activity could be dispersed in lipiodol under the optimized reaction conditions. The radiolabeled preparation was found to exhibit good in vitro stability in human serum. The biological behavior was studied by both in vitro cell binding studies as well as in vivo animal studies involving biodistribution and scintigraphic imaging in both normal and liver-cancer-bearing Wistar rats. In vitro cell uptake studies revealed appreciable uptake in both normal as well as cancerous liver cells. The preparation showed satisfactory initial localization in the liver. However, subsequent leakage of radioactivity and ^{177}Lu uptake in the skeleton would appear to limit the potential of the agent and render it less suitable as a radiopharmaceutical for HCC therapy.

7.5. Particulates for Use in Radiation Synovectomy (RSV)

Rheumatoid arthritis is manifested in pain, joint immobility, and disability and is a major disorder throughout the world. A variety of therapeutic tools are available for treatment of rheumatoid

arthritis, and RSV is a radiotherapeutic modality using β^- -emitting radionuclides which are administered locally by intra-articular injection in the form of colloid or radiolabeled particulates in a 2–10 μm size range.^{233,234} Radionuclide selection is based on the inflamed synovial thickness; thus, a certain beta energy is required for effective ablation, but it should exhibit minimal irradiation of underlying cartilage and bone. The emission of moderate energy β^- particles and other favorable decay characteristics make ^{177}Lu a promising radionuclide for use in radiation synovectomy of small- and medium-sized joints. Hydroxyapatite is one of the preferred particulates for agents intended for radiotherapy applications and can be synthesized easily with the desired particle size.²²¹ Lutetium-177-labeled HA particles have been prepared in high radiochemical purity (~99%), which show good in vitro stability at 37 °C both in saline as well as in human serum.²²⁵ The biological efficacy of the radiolabeled preparation, tested by recording serial scintigraphic images after intra-articular injection of the radiolabeled agent in both normal as well as arthritis-affected knee joints of Wistar rats, showed complete retention of activity within the synovial cavity with no measurable activity leaching out from the joint for a 7 day period of study. The studies indicated the potential of ^{177}Lu -HA as an alternative to ^{169}Er -based colloids used for RSV of small joints.

7.6. Steroids

The use of radiolabeled derivatives of potent estrogens that possess receptor affinity for use as diagnostic agents has been extensively demonstrated.^{235–237} In this area, a plethora of studies have been reported which describe the preparation of estradiol derivatives radiolabeled with ^{77}Br , ^{123}I , ^{125}I , and ^{188}Re .^{238–240}

The relevance of developing potential radiotherapeutic agents based on ^{177}Lu for targeting tumors overexpressing steroidal receptors was realized, and the preparation and evaluation of a ^{177}Lu -labeled estradiol derivative has been reported.²⁴¹ The steroid conjugate (Figure 28) was synthesized via coupling of 6-

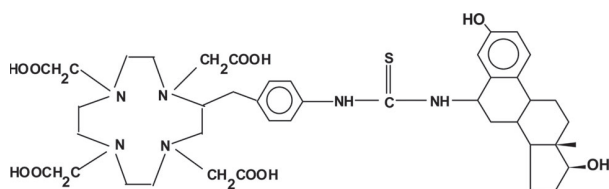


Figure 28. Estradiol–DOTA conjugate. Reprinted with permission from ref 241. Copyright 2005 Elsevier.

amino-17 β -estradiol with a C-functionalized *p*-thiocyanatobenzyl DOTA derivative as the BFCA. For this study, 17 β -estradiol was converted to 6 α -aminoestradiol, which was then conjugated with *p*-thiocyanatobenzyl-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (*p*-NCS-benzyl-DOTA).

The ^{177}Lu -labeled *p*-NCS-benzyl-DOTA-estradiol conjugate exhibited good stability when stored at room temperature and maintained its radiochemical purity to the extent of 77% for a period of 7 days after preparation. The results obtained in binding studies with the MCF-7 human breast cancer cell line, which is known to overexpress estrogen receptors, were promising and indicated specificity of the radiolabeled conjugate toward estradiol receptors. Since the methodology of preparing a suitably derivatized steroid-BFCA (DOTA) conjugate has been demonstrated, the next logical step would be expected to be preparation of the corresponding ^{68}Ga -labeled tracers for evaluation of tumor uptake of these derivatives. On the basis of the tumor uptake of such diagnostic tracers, ^{177}Lu -targeting agents can be developed for therapy of cancers which express steroid-binding receptors. Such products may also find useful applications for treatment of hormone-receptor-positive diseases postsurgery to destroy tumor remnants.

7.7. Porphyrins

Porphyrin derivatives are tumor avid molecular vectors which could be used as potential agents for targeted tumor therapy when radiolabeled with suitable therapeutic radionuclides.²⁴² In this direction, a water-soluble 5,10,15,20-tetrakis[4-carboxymethyleneoxyphenyl]porphyrin derivative was synthesized and conjugated with *p*-aminobenzyl-DOTA (Figure 29).²⁴³ The ^{177}Lu -labeled conjugate exhibited encouraging uptake and retention in biodistribution studies using Swiss mice bearing fibrosarcoma and thymic lymphoma tumors with significant tumor-to-blood and tumor-to-muscle ratios. The scintigraphic studies performed using the tumor-bearing Swiss mice also corroborated similar findings. Preliminary tumor regression studies in this animal model bearing fibrosarcoma and thymic lymphoma tumors showed significant retardation of tumor growth in the treated animals compared to control animals.²⁴⁴

7.8. Nitroimidazoles

The inherent tendency to accumulate nitroimidazole derivatives in hypoxic or anaerobic regions could suggest these compounds as radiosensitizing agents that would enhance the lethal effect of ionizing radiation delivered to hypoxic tissues. Tumor hypoxia makes cells resistant toward chemo and radiation therapy and therefore poses a challenge for cancer management. A DOTA conjugate of 5-nitroimidazole has been radiolabeled with ^{177}Lu . The reported synthetic procedure involved conjugation of *p*-

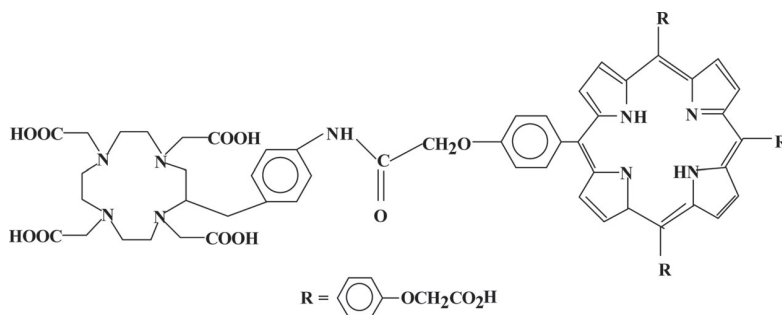


Figure 29. Porphyrin–DOTA conjugate. Reprinted with permission from ref 243. Copyright 2010 Elsevier.

aminobenzyl-DOTA with the $-\text{COOH}$ group of a suitably disposed amino substituent of 2-[*N*-(2'-methyl-5'-nitro)-imidazolyl]ethanoic acid, the carboxylic acid derivative of metronidazole (Figure 30). Synthesis and labeling yields up to 97% have been reported, and the biological behavior of the tracer in tumor-bearing animal models has been studied.²⁴⁵

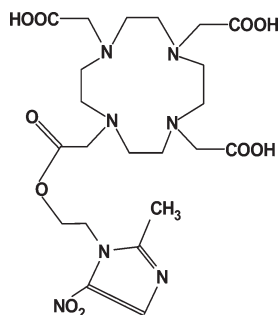


Figure 30. Metronidazole-*p*-aminobenzyl DOTA conjugate.

Preliminary biodistribution studies carried out in Swiss mice bearing fibrosarcoma tumors revealed good tumor uptake with rapid renal clearance and significant tumor-to-blood and tumor-to-muscle ratios.

The preparation of a DOTA conjugate of nitrotriazole has also been reported.²⁴⁶ The nitroimidazole used for the study was [(*N*-2'-(carboxyethyl)-2-(3'-nitro-1'-triazolyl)acetamide], which is the carboxylic acid derivative of Sanazole, and possesses an optimal combination of desired properties including selective toxicity for hypoxic cells and a decreased lipophilicity, which results in decreased neurotoxicity. Radiolabeling of the Sanazole-DOTA conjugate (Figure 31) with ^{177}Lu was achieved under optimized conditions, yielding complexation to an extent of $\sim 98\%$.

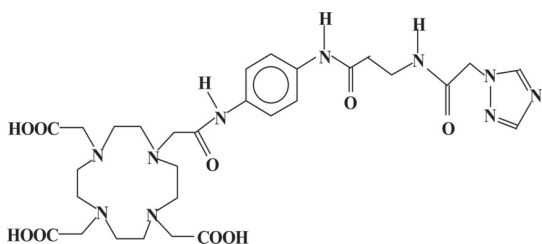


Figure 31. Sanazole derivative-*p*-amino-DOTA-anilide conjugate. Reprinted with permission from ref 246. Copyright 2004 Elsevier.

Bioevaluation studies in Swiss mice bearing fibrosarcoma tumors revealed good tumor uptake with favorable tumor-to-blood and tumor-to-muscle ratios.

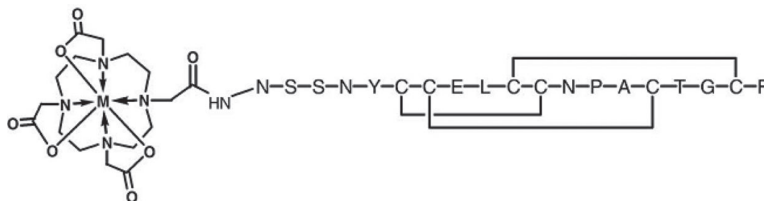


Figure 32. Structure of metalated-DOTA- $\text{F}^{19}\text{-ST}_h$ (1–19). Reprinted with permission from ref 248. Copyright 2006 Elsevier.

7.9. Human *E. coli* Heat-Stable Enterotoxin

The human *E. coli* heat-stable enterotoxin (ST) has a 19 amino-acid sequence designated as $\text{N}^1\text{SSNYCCELCCNPACTGCY}^{19}$ and binds specifically to the guanylate cyclase C (GC-C) receptor, which is present in high density on the apical surface of normal intestinal epithelial cells as well as on the surface of human colorectal cancer cells.²⁴⁷ Giblin et al. reported promising findings with ^{177}Lu -labeled ST analogue conjugated with the DOTA chelating moiety (Figure 32).²⁴⁸ In biodistribution studies carried out in SCID mice bearing T84 human colon cancer tumor xenografts, ^{177}Lu -labeled peptide-DOTA conjugate (DOTA- $\text{F}^{19}\text{-ST}_h$ (1–19)) showed rapid blood clearance, with 90% of injected activity in the urine at 1 h p.i. Uptake of the tracer within tumor xenografts was $1.86 \pm 0.91\% \text{ ID}\cdot\text{g}^{-1}$ at 1 h p.i. with insignificant uptake in any other organ except the kidneys ($2.74 \pm 0.24\% \text{ ID}\cdot\text{g}^{-1}$). At 24 h p.i., 98% ID was excreted into the urine, and localization in tumor was $0.35 \pm 0.23\% \text{ ID}\cdot\text{g}^{-1}$.

7.10. Fullerenes

The potential theranostic use of ^{177}Lu with radiolabeled fullerenes has been recently reported. In a brain tumor mouse model, convection-enhanced intratumoral delivery of a metallofullerene ($\text{f-Gd}_3\text{N@C}_{80}$) labeled with ^{177}Lu via DOTA has been shown to increase median survival from 21 to 52 days, with a prolonged metallofullerene tumor retention that can be visualized with magnetic resonance (MR) imaging.^{249–251} The encapsulation of ^{177}Lu in a fullerene cage as well as its retention within this cage for a period of at least one half-life (6.7 days) of the radionuclide was demonstrated. The conjugation of this agent with an interleukin-13 (IL-13) peptide has also been demonstrated in an attempt to design a targeted agent for mapping receptors in glioblastoma multiforme tumors.^{252–255}

7.11. ^{177}Lu -Labeled Nanoparticles for Targeted Therapy

Nanoparticles (NPs) are useful carriers of drugs to disease sites, and hence, nanomedicine is emerging as a powerful modality. NPs can incorporate various functional moieties on the surface to produce systems with multiple receptor-targeting and multiple therapeutic entities. Gold nanoparticles (AuNPs) absorb in the near-infrared region (NIR) and hence can cause irreversible thermal cellular destruction when they are irradiated using a laser or under a radiofrequency field.²⁵⁶ NPs radiolabeled with ^{177}Lu could represent unique, multifunctional, and target-specific radiopharmaceuticals which may function simultaneously as radiotherapy systems, thermal-ablation systems, and multimodal imaging agents.

Gutierrez et al.²⁵⁷ prepared a multimeric system of ^{177}Lu -labeled AuNPs conjugated to $\text{c}[\text{RGDfK}(\text{C})]$ and compared ^{177}Lu -labeled monomeric and dimeric RGD peptides with respect to the radiation-absorbed dose to $\alpha_v\beta_3$ integrin-positive U87MG tumors in mice. The AuNPs used in these studies were prepared by reducing the salt HAuCl_4 to metallic $\text{Au}(0)$ in aqueous solution, with stabilization by different surfactants. The

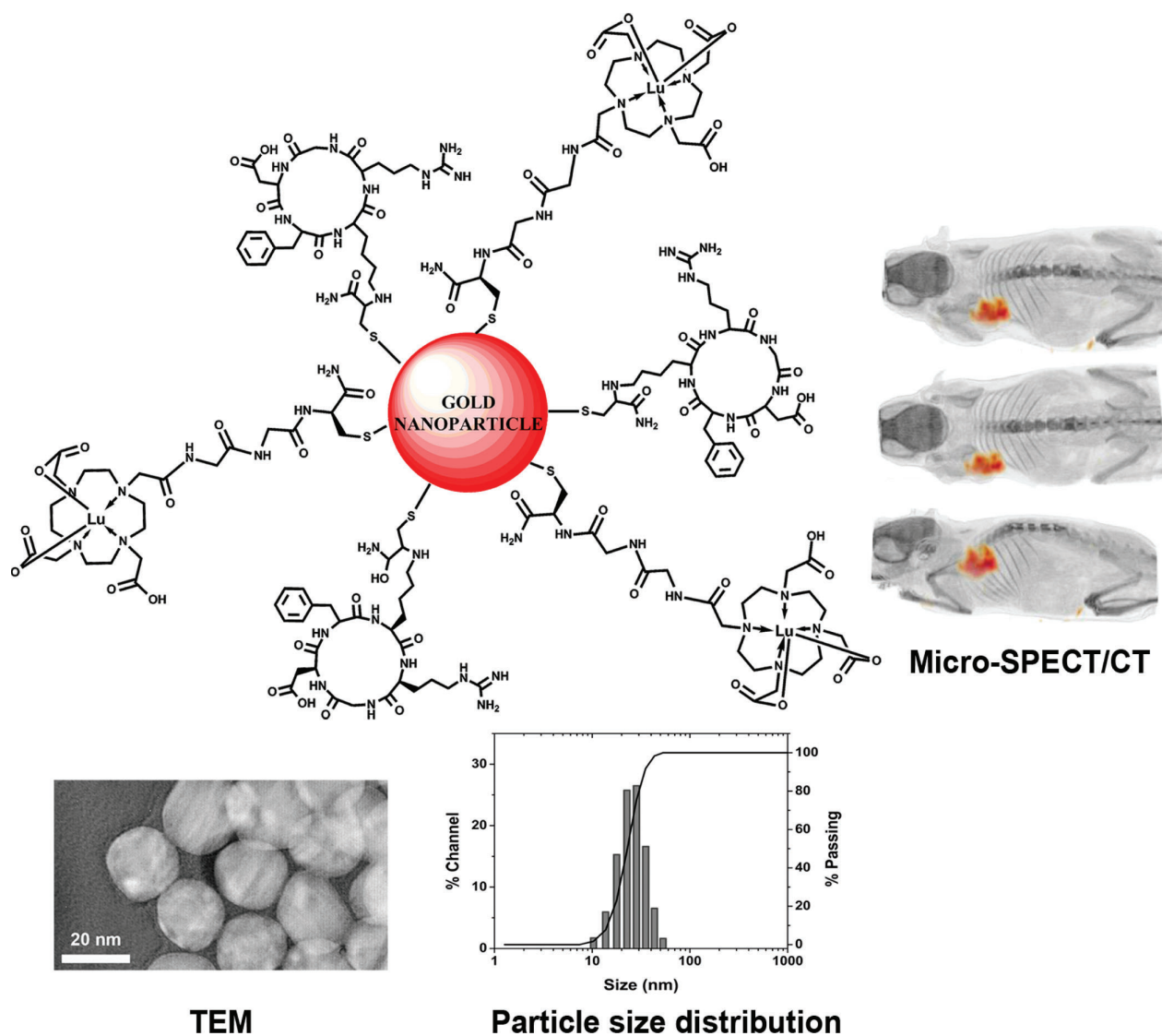


Figure 33. General scheme of the ^{177}Lu -DOTA-GGC-AuNP-c[RGDfK(C)] chemical structure (top left); micro-SPECT/CT images of the radiopharmaceutical at 72 h after intratumoral administration in an athymic mouse bearing a C6 tumor (top right). Morphology of the AuNP (bottom left) and particle size (right). Reprinted with permission from ref 258. Copyright 2014 American Scientific Publishers.

surface of the AuNPs was modified by reaction with thiol groups which act as coupling moieties for the peptide as well as the chelating agent. DOTA-Gly-Gly-Cys (DOTA-GGC) and c-[RGDfK(C)] peptides were synthesized and conjugated to AuNPs by a spontaneous reaction of the thiol groups (Figure 33). TEM, UV-vis, XPS, Raman, and far-IR spectroscopy techniques demonstrated that AuNPs were functionalized with the peptides. To obtain ^{177}Lu -AuNP-c[RGDfK(C)], the ^{177}Lu -DOTA-GGC radiopeptide was first prepared and added to a solution of AuNPs followed by c[RGDfK(C)] at room temperature. Biokinetic studies were accomplished in athymic mice having tumors induced by the intraperitoneal administration of U87MG cells. The ^{177}Lu -absorbed doses per injected activity (per MBq administered) delivered to tumors were 0.36 (multimer), 0.25 (dimer), and 0.10 Gy (monomer). ^{177}Lu -AuNP-c[RGDfK(C)] demonstrated properties suitable for targeted radionuclide therapy of tumors expressing $\alpha(\nu)\beta(3)$ integrins. ^{177}Lu -AuNP-c[RGDfK(C)] was also demonstrated to

significantly decrease glioma tumor progression in mice by combined molecular targeting therapy/radiotherapy. Specifically, the therapeutic response of ^{177}Lu -AuNP-c[RGDfK(C)] in athymic mice bearing $\alpha_v\beta_3$ -integrin-positive C6 gliomas was evaluated. In this study, the radiation-absorbed dose, metabolic activity, histological characteristics, and VEGF gene expression in tumor tissues following treatment with ^{177}Lu -AuNP-c[RGDfK(C)], ^{177}Lu -AuNP, or ^{177}Lu -RGD were reported. The results showed that ^{177}Lu -AuNP-c[RGDfK(C)] delivered the highest tumor radiation-absorbed dose (63 Gy).^{258,259} These results correlated with the observed therapeutic response in which ^{177}Lu -AuNP-c[RGDfK(C)] significantly ($p < 0.05$) slowed tumor progression and tumor metabolic activity and decreased intratumoral vessels and VEGF gene expression. The inhibition of VEGF gene expression involved in angiogenesis indicated a molecular response. MicroPET/CT using [^{18}F]FDG showed a significant decrease in tumor metabolic activity post-treatment. This ^{177}Lu multivalent system can also be used in combination

Table 10. Examples of ^{177}Lu Radiopharmaceuticals in Clinical Use

product	target	biological carrier	application
^{177}Lu -CC-49 ^{105–108,110–113}	tumor-associated antigen (TAG-72)	murine monoclonal antibody specific to tumor associated glycoprotein 72 (TAG 72) MoAb	colon, ovarian, adenocarcinoma
^{177}Lu -J591 ^{125–127}	prostate-specific membrane antigen	PSMA antigen	prostate cancer
^{177}Lu -Rituximab ^{99,263}	CD 20-MoAb	chimeric mouse-human monoclonal antibody	non-Hodgkin's lymphoma
^{177}Lu -DOTATATE ^{145,275–283} Lutathera	somatostatin receptors (subtype 1–5)	peptide-DOTATATE	neuroendocrine cancer
^{177}Lu -EDTMP ^{286,287}	skeletal metastases	ethylenediaminetetramethylene phosphonic acid	metastatic bone pain palliative

with thermoablative therapy using laser heating or an RF field because of its high cell internalization.²⁶⁰

Jimenez-Mancilla et al. prepared a multifunctional system of ^{177}Lu - and $^{99\text{m}}\text{Tc}$ -labeled AuNPs conjugated to Tat(49–57)-Lys3-bombesin ($^{177}\text{Lu}/^{99\text{m}}\text{Tc}$ -AuNP-Tat-BN) and evaluated the radiation-absorbed dose to GRP receptor-positive PC3 tumors in mice.²⁶¹ The ^{177}Lu -absorbed dose per intratumorally injected activity delivered to the PC3 tumors was 7.9 Gy/MBq, and the absorbed dose due to $^{99\text{m}}\text{Tc}$ (mainly Auger electrons) that was delivered to the nuclei was estimated at 0.53 Gy/MBq. Therefore, the $^{177}\text{Lu}/^{99\text{m}}\text{Tc}$ -AuNP-Tat-BN system showed properties suitable for a targeted radionuclide therapy of tumors expressing GRP receptors due to the energy deposition from β -emissions and the Auger and IC electron emissions near the DNA. After laser irradiation, the presence of $^{177}\text{Lu}/^{99\text{m}}\text{Tc}$ -AuNP-Tat-BN internalized in nuclei of PC3 caused a significant increase in the temperature of the medium (46.4 vs 39.5 °C of that without AuNP), resulting in a significant decrease in PC3 cell viability down to 1.3%. After treatment with $^{99\text{m}}\text{Tc}/^{177}\text{Lu}$ -AuNP-Tat-BN, the PC3 cell proliferation was inhibited. The nanosystem exhibited properties suitable for plasmonic photo-thermal therapy and targeted radiotherapy in the treatment of prostate cancer. This radiotherapeutic system can also be used in combination with thermo-ablative therapy and for SPECT/CT imaging.

8. ^{177}Lu RADIOPHARMACEUTICALS IN CLINICAL USE

Radionuclide therapy with therapeutic radiopharmaceuticals often has significant advantages over the use of conventional therapies since it represents specific targeting of cancerous tissue and other diseased tissue while protecting adjacent healthy cells. Clinical investigators have realized the advantages of using ^{177}Lu and initiated the exploitation of its potential in designing agents for a variety of applications, in particular, in the field of oncology. The synergistic effect of the targeting vector with the particulate-emitting radionuclide increases cell killing for effective therapy of cancer and other diseases. Although extensive research with radiolabeled preparations for potential applicability in radiotherapeutics is an ongoing process, a major challenge is the translation of data from preclinical animal studies to human trials.

While there are no approved ^{177}Lu -based radiopharmaceutical products for routine clinical use, several ^{177}Lu -labeled agents are in different stages of development and are expected to advance as radiopharmaceutical products in the future. Examples of ^{177}Lu radiopharmaceuticals which have been evaluated in clinical studies are summarized in Table 10.

8.1. ^{177}Lu -CC49 Monoclonal Antibodies for Adenocarcinoma

A Phase I clinical trial in nine patients with metastatic breast cancer (5 patients), colorectal cancer (3 patients), and lung

cancer (1 patient) involved administration of 10–25 mCi·m^{−2} doses of ^{177}Lu -CC49.¹⁰⁵ Toxicity was limited to bone marrow suppression, which limited dose escalation beyond 25 mCi·m^{−2}. The results of this study suggested that ^{177}Lu -CC49 may be useful in the treatment of hematological malignancies or solid tumors that are primarily localized in the bone or bone marrow. Twelve patients with ovarian cancer that did not respond to chemotherapy underwent a Phase I/II trial by intraperitoneal administration of ^{177}Lu -CC49 with a dose of 10–30 mCi·m^{−2}.¹⁰⁶ The studies did not show any uptake of the antibody by bone marrow that may have induced hematological toxicity. The results compared favorably with intraperitoneal chemotherapy or external beam therapy. In the Phase I/II study with 27 patients, 1 of the 13 patients with measurable disease had a partial response.¹⁰⁷ Seven of nine patients with <1 cm nodules progressed within 21 months, while two remained without progression at 4–5 months. All patients with progression had recurrence in the abdomen. The best chance for prolonged disease-free survival was seen in patients with small-volume disease. A clinical trial in combination with the taxol radiosensitizer was also carried out.¹⁰⁹ Four of the 17 patients with disease measurable by CT had a partial response, whereas 4 out of 27 patients with nonmeasurable disease had progression-free intervals ranging between 18 and 37 months. Long-term survival was seen in five patients with microscopic diseases. Other patients with gross disease showed varying response.^{110–113}

8.2. ^{177}Lu -J591 Monoclonal Antibodies for Prostate Cancer

^{177}Lu -J591 is a radiopharmaceutical possessing the humanized J591 monoclonal antibody targeting prostate-specific membrane antigen (PSMA) radiolabeled with ^{177}Lu leading to the first tumor-specific delivery system which will be able to target radiation to malignant prostate cancer cells. In the first clinical study reported with this agent, ^{177}Lu -DOTA-MoAb J591 was purified by gel filtration and sterilized by membrane filtration and administered in patients in combination with cold antibody. A Phase I clinical trial involving 35 androgen-independent prostate cancer patients using ^{177}Lu -J591 was carried out.^{125–127} The studies provided important information on the potential broader use of ^{177}Lu -J591 in the therapy of hormone refractory prostate cancer. The MTD of ^{177}Lu -J591 was 70 mCi·m^{−2}, which was significantly higher than the MTD of 17.5 mCi·m^{−2} with ^{90}Y -J591. Targeting of the labeled antibody to all soft tissue tumor sites and bone metastasis was observed in 30 patients with positive bone-computed tomography or magnetic resonance imaging. Reduction in prostate-specific antigen (PSA) values of >50% lasting for 3–8 months was seen in four patients, and an additional 16 patients experienced PSA stabilization for a median of 60 days. Fractionation of the dose was found to give higher therapeutic efficacy and resulted in lower myelotoxicity.¹²⁷

The French biotechnology company ATLAB Pharma SAS is in the process of developing a pipeline of targeted anticancer antibodies and other drugs radiolabeled with ^{177}Lu and ^{211}At and has recently consummated an exclusive global licensing agreement with BZL Biologics (Cornell Weill Medical Center, New York, USA). In connection with this project, the novel ^{177}Lu -J591 radiopharmaceutical is being further developed for targeted radiotherapy and is currently in Phase IIB (dose escalation) trials for the treatment of prostate cancer. The specific drug action aims at the unique ability of this radiolabeled agent to eradicate micrometastatic disease at an early stage of cancer progression. It is expected that the current trials will confirm the survival benefits of ^{177}Lu -J591 at the micrometastatic stage of castrate-resistant prostate cancer. The agreement includes exclusive rights to manufacture, develop, and commercialize this radiopharmaceutical product worldwide.²⁶²

8.3. ^{177}Lu -Anti CD-20 Monoclonal Antibody for Non-Hodgkin's Lymphoma

In a preliminary study, ^{177}Lu -DOTA-rituximab was administered in two patients with relapsed non-Hodgkin's lymphoma (NHL), and the studies indicated that the agent was found to be well tolerated, indicating that ^{177}Lu -rituximab could be a good agent for the treatment with few side effects for patients with relapsed NHL.⁹⁹ Forrer et al. recently reported the results of a detailed Phase I/II study in 31 patients with relapsing follicular, mantle cell, and other indolent B-cell lymphomas.²⁶³ In this dose escalation study, 31 patients with histologically confirmed refractory or CD 20 confirmed B-cell lymphoma or other indolent lymphoma were included. All patients received rituximab, 250 $\text{mg}\cdot\text{m}^{-2}$, on days 1 and 8. On day 8, the radiopharmaceutical was injected after infusion of the second dose of rituximab. Different patients received ^{177}Lu -DOTA-rituximab with activity ranging from 740 to 1850 $\text{MBq}\cdot\text{m}^{-2}$, with an escalation dose of 185 $\text{MBq}\cdot\text{m}^{-2}$ in different groups. Dosimetry calculations performed in 20 patients showed a mean whole-body dose of 8.77 $\text{mGy}\cdot\text{MBq}^{-1}$. The mean absorbed dose to the red marrow was found to be 9.70 $\text{mGy}\cdot\text{MBq}^{-1}$. The results indicated that the absorbed dose of radiation for all patients was below 1 Gy to the whole body as well as to the red marrow. No toxicity other than hematologic was observed.

Tumor response was evaluated in 29 of 31 patients. Total responses were observed in 15 of 29 evaluable patients (52%). Six patients (21%) achieved a complete remission, and 9 patients (31%) had partial remission. The overall response rate was 82% (9/11 patients) in patients with follicular lymphoma and 21% (3/14 patients) in patients with mantle cell lymphoma. One patient with follicular lymphoma achieved a tumor regression of 35%, and another patient with mantle cell lymphoma had tumor shrinkage to just below 50%. The response rate for patients without bone marrow involvement at baseline (11/15 patients; 73%) was higher than that for patients with prior bone marrow involvement (4/14 patients; 29%). Patients without prior rituximab treatment had lower response rates (4/9 patients; 44%) than did patients pretreated with rituximab (11/20 patients; 55%). One patient without further bone marrow examination achieved a CR as assessed by imaging. This study demonstrated the feasibility of treatment with ^{177}Lu -DOTA-rituximab and that a single course results in a high rate of tumor response in all lymphoma entities and at the dose levels tested. Hematologic toxicity was temporary and manageable, and nonhematologic toxicity was low. Figure 34 shows the FDG PET of a patient suffering from follicular lymphoma before

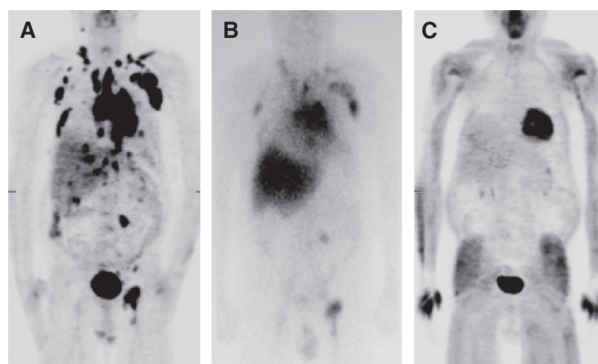


Figure 34. Follicular lymphoma in a 61-year-old patient with relapsing disease with ^{177}Lu -DOTA-rituximab. (A) ^{18}F FDG PET shows disseminated partly bulky relapsing disease. (B) ^{177}Lu scintigram 4 days after ^{177}Lu -DOTA-rituximab depicts distribution of ^{177}Lu in tumor masses. (C) Repeated ^{18}F FDG PET 2 months after radioimmunotherapy shows complete remission. Reprinted with permission from ref 263. Copyright 2013 Society of Nuclear Medicine and Molecular Imaging, Inc.

(Figure 34A) and after (Figure 34C) ^{177}Lu -DOTA-rituximab therapy. Figure 34B (middle) shows a ^{177}Lu scintigram showing the distribution of the radiopharmaceutical in vivo 4 days postadministration. Complete remission is illustrated in Figure 34C in which most tumors have disappeared.

8.4. Combination Therapy

The recent concept of treatment modality using a combination of targeted drugs with radiotherapeutics has been reviewed. In this direction, the monoclonal antibody cetuximab (Erbix) has been approved for the treatment of locally advanced squamous cell cancer of the head and neck in combination with radiotherapy for solid tumors.²⁶⁴

In order to make molecular radiotherapy with radiolabeled molecules more efficacious, a combination therapy using other modalities such as external beam radiation therapy has been proposed. While some studies provide only the theoretical basis in radiobiological models, others have moved ahead to carry out Phase I–II studies aimed at assessing the feasibility and tolerability of the modality in the therapy of tumors, ranging from meningiomas, paragangliomas, non-Hodgkin's lymphomas, bone, brain, hepatic, and breast lesions. The various aspects of this combined modality concept have been reviewed by Cremonesi et al.,²⁶⁵ and the results of clinical studies have been presented with special emphasis on the possibility of effecting an improvement in the therapy options.

Superior antitumor effects of the combination of ^{177}Lu - and ^{90}Y -somatostatin analogs when compared with either ^{90}Y or ^{177}Lu analogues alone in animals bearing tumors of various sizes were demonstrated by De Jong et al.¹⁴⁴ Kunikowska et al. reported a clinical study in patients with disseminated NETs in which a group of 25 patients received ^{90}Y -DOTATATE alone and the second group of 25 patients received a 1:1 mixture of $^{90}\text{Y}/^{177}\text{Lu}$ -DOTATATE.²⁶⁶ The administered activity was based on 3.7 $\text{GBq}\cdot\text{m}^{-2}$ body surface area in 3–5 cycles, with amino acid infusion for nephroprotection. The median survival time in the first group was observed to be 26.2 months, while in the second group it was not reached during the observation period of 54 months. The safety of both protocols was comparable, and the side effects were found to be mild and rare.

8.5. ^{177}Lu -DOTATATE for Neuroendocrine Tumors

Identification of the most optimum radionuclide and the best peptides for PRRT has been evolving and will be based on the results and experience gained from clinical trials. Use of the DOTA-coupled ^{177}Lu -labeled $[\text{DOTA}^0\text{-Tyr}^3]$ octreotide somatostatin analogue has been reported to provide a successful impact on tumor regression and other favorable biological characteristics in animal models.¹⁴⁹ The clinical trials carried out with ^{177}Lu -DOTATATE have been extensively reviewed.^{142,143,267,268} An overall response of 30–38% and a significantly high median overall survival of 48 months have been reported, and quality of life improved significantly after targeted radionuclide therapy with ^{177}Lu -DOTATATE. The kidneys have been reported to be the dose-limiting organ, resulting from the general reabsorption and retention of the small peptides in the negatively charged proximal tubule cells after glomerular filtration. Damage to the kidneys has been reported with peptide radionuclide therapy, especially with ^{90}Y .²⁶⁹ Infusions containing positively charged amino acids such as lysine and arginine are used during therapy in order to reduce the kidney uptake and retention of radiopeptides. A study involving two tumor-bearing rat models has reported that reduction in long-term renal toxicity after administration of high doses of ^{177}Lu -DOTATATE and lysine coinjection could be observed. The conclusions drawn were that the dose fractionation regimen in conjunction with coinjection of lysine had beneficial effects in preventing kidney damage.²⁷⁰

Erion et al.²⁷¹ reported the preparation of ^{177}Lu - $[\text{DOTA}^0\text{-Tyr}^3]$ octreotate and demonstrated tumor regression and survival in a rat model. The unlabeled $[\text{DOTA}^0\text{-Tyr}^3]$ octreotate peptide has 8–9-fold higher affinity for sst_2 subtype receptors as compared to $[\text{DOTA}^0\text{-Tyr}^3]$ octreotide.²⁷² In patient studies, the uptake of ^{177}Lu - $[\text{DOTA}^0\text{-Tyr}^3]$ octreotate was comparable to that of ^{111}In - $[\text{DTPA}^0]$ octreotide for kidneys, spleen, and liver but was 3–4-fold higher for 4/5 tumors.^{273,274} A comparison of ^{177}Lu - $[\text{DOTA}^0\text{-Tyr}^3]$ octreotide and ^{177}Lu - $[\text{DOTA}^0\text{-Tyr}^3]$ octreotate in the same patients showed a 2.1-fold higher uptake with the ^{177}Lu -labeled agent.^{30,274} This property has made ^{177}Lu -octreotate potentially more useful for therapy, as higher tumor-absorbed radiation doses can be delivered. Although the above observation could be patient specific, the current preference is for the use of ^{177}Lu - $[\text{DOTA}^0\text{-Tyr}^3]$ octreotate for GEP-NETs. An additional reason for the above results had been the limited availability of the $[\text{DOTA}^0\text{-Tyr}^3]$ octreotide peptide due to an intellectual property rights (IPR) issue. Although both ^{90}Y - $[\text{DOTA}^0\text{-Tyr}^3]$ octreotate and ^{90}Y - $[\text{DOTA}^0\text{-Tyr}^3]$ octreotide have been successfully used for PRRT, the preference has now been shifted to the use of ^{177}Lu -based agents due to the lower tissue penetration range of ^{177}Lu compared to ^{90}Y . By using ^{177}Lu , higher absorbed doses can be achieved in most tumors with lower doses to dose-limiting organs such as bone marrow and kidney. The cross-fire effects available from ^{177}Lu can extend up to 20 cell diameters, and hence, it is expected that tumors with sufficient receptor density will benefit from ^{177}Lu treatment. Currently, ^{177}Lu -DOTATATE therapy has been reported to have been applied in about 3000 patients, and many more nuclear medicine centers are entering into clinical studies using this agent.^{22,275–283}

In a study carried out in patients with NETs, 2–6 cycles of ^{177}Lu -DOTATATE, with a mean cumulative activity of 6.74–20.1 GBq of administered dose, resulted in a total renal radiation absorbed dose of 4.1–12.5 Gy.²⁷³ All patients were infused with

renal protective amino acids during the administration of the radiopharmaceutical. It was observed in these studies that there was a decline in renal function as measured by GFR. This effect was observed to a larger extent in patients with baseline-impaired renal function than in patients with preserved renal function after PRRT. Hematologic toxicity was found to be relatively rare and could be managed. A mild WBC toxicity was observed because of the radiosensitivity of lymphocytes. In another recent study,²⁸³ the somatostatin receptor expression in noniodine-concentrating metastatic-differentiated thyroid carcinoma was evaluated by ^{68}Ga -DOTATATE PET-CT and $^{99\text{m}}\text{Tc}$ -HYNIC-TOC scintigraphy in a group of 19 patients. The feasibility of ^{177}Lu -DOTATATE therapy with positive ^{68}Ga -DOTATATE PET-CT or $^{99\text{m}}\text{Tc}$ -HYNIC-TOC scintigraphy was investigated in 2 patients. On follow-up after 3 months, a significant fall in serum thyroglobulin level was noted in one of the patients. However, the authors have not drawn any definite conclusions about the therapeutic efficacy of the ^{177}Lu -DOTATATE therapy.

In another study,²⁸⁴ serial pre- and post-therapeutic scans have been used for dose calculation and for predicting therapy doses. The software used for the radionuclide dose calculation employed 4D SPECT/CT image acquisition. Dose was calculated for both therapeutic and preplanning images. The preplanning dose was extrapolated to predict the appropriate therapy dose using the ratio of administered activities. The 3D dose calculation results were also compared with those of the OLINDA code with good correlation. The results indicated that dose planning using pretherapeutic scanning could predict critical organ and tumor doses. In some cases, the prediction resulted in slight overestimations of the final therapy dose.

The outcome of post-PRRT in 74 patients (protocol followed is mean activity of 7.9 GBq per cycle, 4 treatment cycles at standard intervals of 3 months) with gastroenteropancreatic neuroendocrine tumors (GEP NET) has been analyzed with respect to the impact of the K_i -67 index.²⁸⁵ The conclusion drawn shows that the independent predictors of survival were the K_i -67 index, the patient's performance status (Karnofsky performance scale score), the tumor burden, and the baseline neuron-specific enolase level.

Lutathera is the commercial designation of ^{177}Lu -DOTATATE, which is being developed by Advanced Accelerator Applications (AAA) of Saint Genis-Pouilly, France. This radiopharmaceutical has been evaluated over a period of 15 years in major clinical centers in Europe for targeting one of the somatostatin receptors (sstr_2) commonly expressed on most neuroendocrine tumors. A major clinical validation program is being conducted by AAA with the ultimate goal of obtaining FDA approval in the United States for routine clinical use of Lutathera. Toward this goal, 14 major medical centers are participating in a large Phase III trial. The large number of clinical studies carried out in Europe has resulted in the generation of considerable experience and voluminous clinical data demonstrating its safety and therapeutic efficacy. These data have formed the platform for efforts toward obtaining FDA approval.

On the basis of the practical experience gained in several nuclear medicine centers practicing PRRT, "a joint IAEA, EANM, and SNMI practical guidance on peptide receptor radionuclide therapy (PRRNT) in neuroendocrine tumors" was published by Bodei et al.¹

8.6. ^{177}Lu -EDTMP for Bone Pain Palliation

The International Atomic Energy Agency (IAEA) supported the clinical translation of ^{177}Lu -EDTMP through one of its

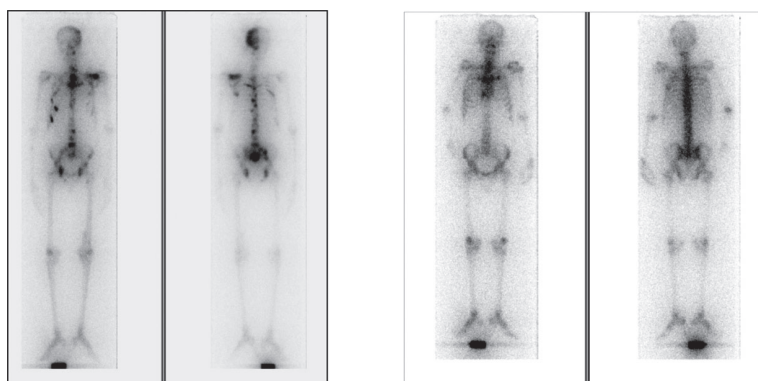


Figure 35. SPECT images with ^{177}Lu -EDTMP in a patient with prostate cancer with metastasis (left two images, anterior and posterior) and in a patient without metastasis (right two images, anterior and posterior) (courtesy C. S. Bal, AIIMS, India, unpublished results).

coordinated research projects (CRP) titled “Evaluation of the biological safety and clinical efficacy of ^{177}Lu -EDTMP for bone pain palliation in metastatic prostate cancer”. While in preliminary evaluation both EDTMP and DOTMP labeled with ^{177}Lu showed favorable biodistribution properties, ^{177}Lu -EDTMP was selected for clinical evaluation as the toxicological data for EDTMP is already well established in connection with the use of ^{153}Sm -EDTMP. It was also reasoned that ^{177}Lu -EDTMP would be more readily approved by regulatory authorities and acceptable to the nuclear medicine community compared to ^{177}Lu -DOTMP. A Phase I/II clinical trial of ^{177}Lu -EDTMP in hormone refractory prostate cancer patients suffering from bone metastasis was conducted. The studies were part of a multicountry initiative, wherein a cold kit of ^{177}Lu -EDTMP was prepared at the Polatom facility in Swierk, Poland, and preclinical animal pharmacokinetics and dosimetry studies were carried out in Hungary.²¹⁵ Preliminary dosimetric estimations in five patients using 185 MBq of ^{177}Lu -EDTMP were done at the All India Institute of Medical Sciences (AIIMS), in New Delhi, India. Representative images obtained 6 days postinjection of 185 MBq of ^{177}Lu -EDTMP are given in Figure 35.

Dose escalation studies of ^{177}Lu -EDTMP were done as part of the above CRP, and results of one such clinical study have been recently reported.²⁸⁶ The study consisted of 16 patients who were divided into low-dose (35 mCi) and high-dose groups (70 mCi). The differences in toxicities and response rates were determined. Parameters such as pain scores, Karnofsky indices, mobility scores, and requirement of analgesic administration were assessed at 0, 2, 4, 6, 8, and 12 weeks after injection of ^{177}Lu -EDTMP to evaluate the therapeutic efficacy. Toxicity was assessed by analyzing hemoglobin, leukocyte, and platelet counts. It has been shown that ^{177}Lu -EDTMP is an effective radiopharmaceutical and can be used as a safe option for palliation of metastatic bone pain in patients with prostate or breast cancer. A dose of 1295 MBq (35 mCi) was sufficient for bone pain palliation therapy, and doses as high as 2590 MBq (70 mCi) were well tolerated. Similar results were obtained in a study from India in 44 patients having skeletal metastasis with primary prostate or breast carcinoma.²⁸⁷ A favorable response was seen in 27 patients (84%) with prostate cancer and in 11 patients (92%) with breast cancer. Nonserious hematological toxicity (grade I/II) was observed in 15 patients (34%), and serious toxicity (grade III/IV) occurred in 10 patients (23%). The authors concluded that ^{177}Lu -EDTMP is safe and effective for bone pain palliation

in patients with metastatic prostate and breast carcinoma. No differences in efficacy or toxicity were observed between patients receiving low-dose (35 mCi) and high-dose (70 mCi) ^{177}Lu -EDTMP. ^{177}Lu -EDTMP was approved for clinical use in India by the Radiopharmaceuticals Committee (RPC), the regulatory body responsible for approval of radiopharmaceuticals, and is commercially available from the Board of Radiation and Isotope Technology (BRIT).

9. CLINICAL STUDIES DEMONSTRATE THE THERANOSTIC POTENTIAL OF ^{177}Lu

The use of ^{177}Lu as a theranostic radionuclide has been recently reported. In a brain tumor mouse model, it has been shown that convection-enhanced intratumoral delivery of a theranostic metallofullerene ($\text{f-Gd}_3\text{N@C}_{80}$ labeled with ^{177}Lu) and via the chelate DOTA (^{177}Lu -DOTA- $\text{f-Gd}_3\text{N@C}_{80}$) increases median survival from 21 to 52 days, with a prolonged metallofullerene tumor retention that can be visualized with magnetic resonance (MR) imaging. In this respect, the radiolabeled nanoplateform represents a true theranostic agent, in which a single compound capable of delivering an effective radiation dose (^{177}Lu) as well as imaging (gadolinium) has been incorporated in the same species.²⁵¹

BPAMD (4-[[bis(phosphonomethyl)-carbamoyl]methyl]-7,10-bis(carboxymethyl)-1,4,7,10-(tetraaza-cyclo-dodec-1-yl)-acetic acid) labeled with ^{68}Ga was used in a first human in vivo study for diagnosis of osteoblastic bone metastases.²⁸⁸ The DOTA-based bisphosphonate ligand BPAMD (Figure 36) is also suitable for complexation with trivalent metallic lanthanide therapeutic radionuclides such as ^{177}Lu . The same ligand thus may be used for diagnosis, dosimetry calculation, therapy, and therapy control via PET/CT. This is yet another example of ^{177}Lu as an isotope for theranostic applications.²⁸⁹

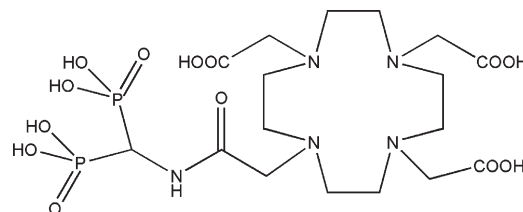


Figure 36. BPAMD ligand for radiolabeling with ^{177}Lu .

In another preliminary study,²⁹⁰ the theranostic potential of ^{177}Lu has been demonstrated in patients with metastatic neuroendocrine tumors. A 10 mCi dose of ^{177}Lu -DOTATATE was injected in 39 patients for diagnostic scanning. Whole body planar images and SPECT-CT images were obtained at 4, 24, and 48 h. The pretherapy scans were also used for dosimetric calculations. The whole body ^{177}Lu -DOTATATE scan with SPECT/CT (Figure 37) demonstrated normal physiological distribution and abnormal increased uptake in all lesions as observed in the contrast CT scan of the patient. The SPECT/CT images demonstrated the heterogeneous versus homogeneous

distribution of receptor uptake and also aided in estimation of tumor volume.

Comparative imaging studies carried out in the same patient indicated that ^{177}Lu -DOTATATE SPECT-CT compares well with ^{111}In -DOTATATE SPECT-CT and ^{68}Ga -DOTATOC PET-CT scans both in sensitivity and in lesion characteristics (Figure 38). This study demonstrated all the metastatic lesions seen in the contrast CT. The pretreatment dosimetry ^{177}Lu DOTATATE whole body diagnostic scans therefore yielded the opportunity to tailor therapies with more accuracy. Fourteen of the total 39 patients were taken ahead for ^{177}Lu -DOTATATE therapy.

9.1. Lutrin

A nonradioactive Lu-texaphyrin molecule (LUTRIN) (Figure 39) has been clinically tested as a photosensitizing agent in cancer therapy.^{291–293} The texaphyrins localize in cancer cells, atherosclerotic plaque, and neovasculature, where they can be activated by various forms of energy, including X-rays, light, and chemotherapeutics, to eliminate diseased tissue. LUTRIN is a lutetium-texaphyrin molecule that is activated by a wavelength of light capable of penetrating deeply through tissues and blood. LUTRIN is cleared relatively quickly from the blood and normal tissues but accumulates in tumors, providing the potential to selectively treat large tumors with reduced damage to adjacent normal tissues.

Lutrin is being developed for the possible treatment of a number of cancers. In July 1997, the compound entered Phase II trials for treatment of advanced refractory breast cancer. Lutetium texaphyrin has also been investigated as a photo-activated agent for the treatment of atherosclerotic plaque in coronary heart disease and for treatment of age-related macular degeneration (AMD). Lutetium-177 incorporated in the Lutrin framework may also be useful for the treatment of the above type of cancers.

10. SUMMARY

Lutetium-177 has emerged as a promising radionuclide for therapy, and the clinical efficacy of several ^{177}Lu -labeled targeted agents has been demonstrated. The favorable nuclear characteristics as well as the easy availability of high activity levels of high specific activity ^{177}Lu are the prime factors for the widespread interest in the clinical use of this radionuclide. The relatively long half-life of this radionuclide offers distinct logistical advantages, particularly for availability for use in countries with limited reactor facilities. In addition, the feasibility of large-scale production of ^{177}Lu from enriched ^{176}Lu with adequate specific activity and acceptable radionuclidic purity in medium flux research reactors constitutes yet another desirable feature. No carrier added ^{177}Lu can be obtained via the indirect route involving the irradiation of a ^{176}Yb -enriched Yb_2O_3 target followed by the separation of ^{177}Lu from the irradiated target. This route of production also eliminates the possibility of production of the long-lived isotope $^{177\text{m}}\text{Lu}$ as an impurity, thereby solving the problem of radioactive waste management. The minimum decay loss encountered during the distribution of ^{177}Lu to users remotely located from the site of production directly contributes to providing cost-effective supply of the radionuclide.

The chemistry of Lu^{3+} allows facile radiolabeling of different molecules either directly or through BFCAs such as DOTA. The complexes are generally formed in high radiochemical yields and exhibit good postpreparation stability. On identifying a suitable

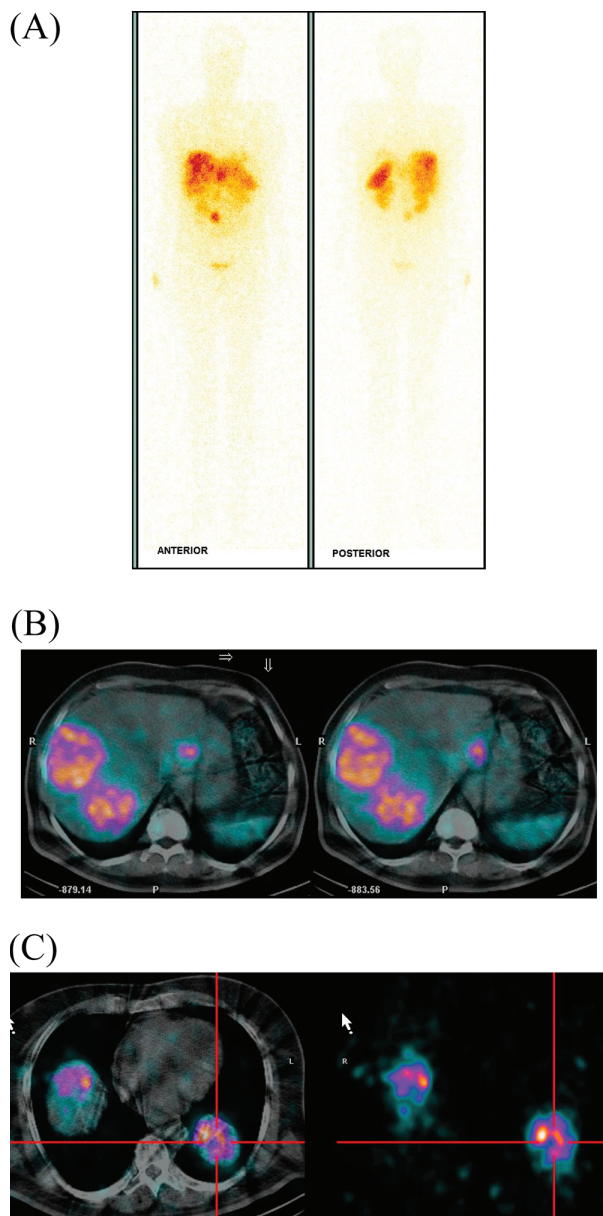


Figure 37. ^{177}Lu -DOTATATE images. (A) Whole body image with SPECT/CT of (B) liver and (C) lung lesions. Reprinted with permission from ref 290. Copyright 2011 Society of Nuclear Medicine India.

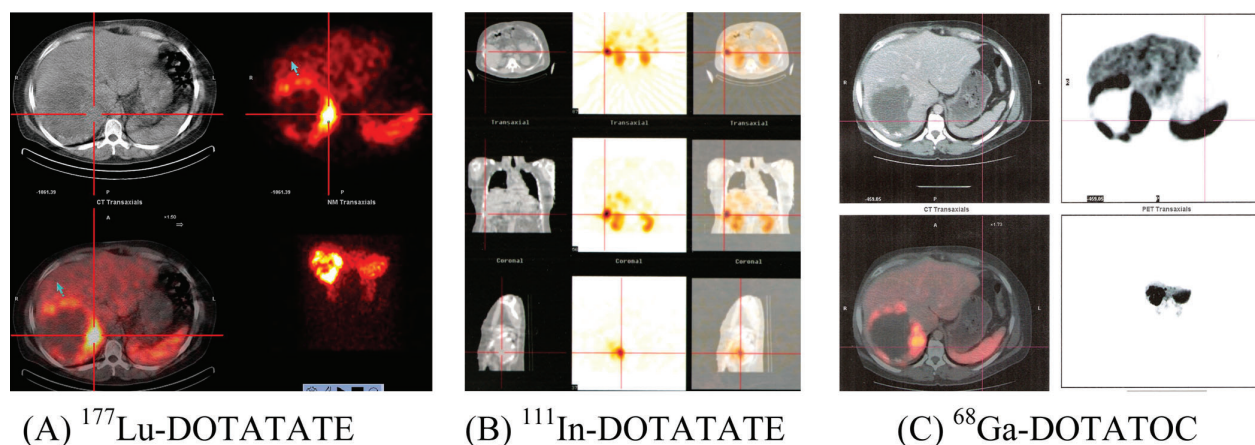


Figure 38. Comparison of (A) ^{177}Lu -DOTATATE SPECT-CT with ^{111}In -DOTATATE SPECT-CT and ^{68}Ga -DOTATATE PET-CT scans in the same patient. Reprinted with permission from ref 290. Copyright 2011 Society of Nuclear Medicine India.

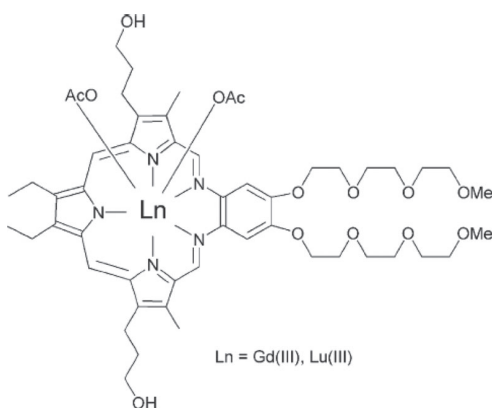


Figure 39. Structure of gadolinium and lutetium texaphyrin complexes.

chelating agent that will complex with ^{177}Lu at room temperature, the radiolabeling of carrier molecules which are heat sensitive will become facile and also result in simplifying the radiopharmacy practices.

The low soft tissue penetration of the ^{177}Lu beta emissions results in more efficient energy deposition in small-sized lesions of less than 3 mm diameter. At the same time, the low beta energy of ^{177}Lu ensures minimum radiation exposure to nontargeted healthy tissues during therapy. This property is particularly advantageous in designing bone pain palliation agents where accumulation of the ^{177}Lu -labeled agents on the bone surface minimizes any potential bone marrow damage. Similarly, in the case of ^{177}Lu -labeled agents intended for use in peptide receptor radionuclide therapy, the lower kidney dose delivered while using ^{177}Lu is considered a distinct advantage and hence often preferred over use of similar ^{90}Y -based peptides. From the point of view of radiation exposure, the low energy of the beta particles ensures a substantial reduction of the handling dose to the radiopharmacy staff performing the radiolabeling procedure.

This review presents an overview of the evolution of the use of the ^{177}Lu for therapy and describes the wide spectrum of applications that have been pursued in the development of ^{177}Lu -based radiopharmaceuticals for targeted radiotherapy. There is an ever-increasing possibility of identifying a varied range of molecular vectors such as newer peptides, monoclonal antibodies, and other cancer-seeking small molecules for the develop-

ment of ^{177}Lu -based therapeutic radiopharmaceuticals for targeted therapy. Therefore, the development and application of ^{177}Lu radiopharmaceuticals is expected to dramatically increase over the coming years. As envisioned by the authors, ^{177}Lu is indeed a gold mine for radiopharmaceutical development, and exploring its immense potential for therapeutic applications is still in the early stages.

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Notes

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sponsored multi-country research projects. She has more than 140 publications in peer-reviewed international journals and has coauthored three review articles, five book chapters, and four technical reports. She has published more than 30 peer-reviewed research papers on ^{177}Lu radiopharmaceuticals. She is an Editorial Board member of *Annals of Palliative Medicine* and *BioMed Research International*. She is a Fellow of the Indian College of Nuclear Medicine.



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F. F. (Russ) Knapp, Jr., Ph.D., completed his doctoral studies in biochemistry at the St. Louis University School of Medicine and was Head of the Nuclear Medicine Program in the Isotope Development Group at the Oak Ridge National Laboratory (ORNL) during the 1977–2012 period, until his retirement in 2012, and is now an Emeritus in the Medical Radioisotope Program at ORNL. He consults and

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ACKNOWLEDGMENTS

Dr. Madhava B. Mallia is acknowledged for his valuable technical support in the preparation of this manuscript. S.B. and M.R.A.P. thank Dr. Tapas Das and Dr. Sudipta Chakraborty who are researchers in lutetium radiopharmaceuticals chemistry in India as part of their Ph.D. program. The authors thank Dr. Guillermina Ferro-Flores, Departamento de Materiales Radiactivos, Instituto Nacional de Investigaciones Nucleares, Estado de México, Mexico, for her kind input. R.K. acknowledges ORNL for the data used in Figures 8–10.

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