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Targeted Radionuclide Therapy using Peptide-Based Radiopharmaceuticals

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Abstract

The emergence of targeted radionuclide therapy has been one of the latest breakthroughs in cancer radiotherapy, with somatostatin analogs being the priorities. Some of the successfully used radiolabelled somatostatin analogs in cancer diagnosis and treatment include 68Da-DOTATOC and 177Lu-DOTATOC. On the other hand, the approval of everolimus by FDA for use in cancer treatment have been an important breakthrough as the medication has so far recorded positive reviews. This study sought to explore the combined use of everolimus and 177Lu-DOTATOC for the treatment of neuroendocrine tumours in a clinical study involving 9 patients. Peptide-based radiopharmaceuticals comprise of four parts that include a radionuclide, a targeting peptide, a bifunctional chelating agent and a linker moiety. the chelating agents used for labelling somatostatin analogs with radionuclides are based on DOTA. The most commonly used products of DOTA modifications are DOTATOC and DOTATATE, and they are produced through peptide conjugation of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid. For DOTATOC synthesis, the process involves conjugating 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid with Tyr3-octreotide to form DOTA0-Tyr3-octreotide (DOTATOC) Both DOTATOC and DOTATATE have the ability to bind to somatostatin receptors, and thus they are important vehicles of targeted radionuclide therapy after their conjugation with suitable radioisotopes. Some of the examples of radiolabelled DOTATOC compounds that are of significance to this paper are 177Lu-DOTATOC and 68Ga-DOTATOC. However, unlike 177Lu-DOTATATE which has been studied significantly in relation to targeted radionuclide therapy for its importance in the treatment of neuroendocrine tumors, 177Lu-DOTATOC has not been as widely studied. In this study, the combination of everolimus with 177Lu-DOTATOC as a treatment regimen was investigated for the efficacy, immediate and long-term complications, and the pharmacological characteristics when used for treating metastatic and/or inoperable neuroendocrine tumours. The subjects received daily everolimus 10 mg and four cycles of (100mCi) 177Lu-DOTATOC in order to determine the antitumor effects of the regimen. This study sought to explore the combined use of everolimus and 177Lu-DOTATOC for the treatment of neuroendocrine tumours in a clinical study involving 9 patients. In this study, the combination of everolimus with 177Lu-DOTATOC as a treatment regimen was investigated for the efficacy, synthesis using a new fluidic module and the radicals formed after radiodecay might be detected by this combination using Electron Paramagnetic Resonance EPR. The regimen was found to be efficacious as indicated by improvement of patients Karnofsky scores from 66 percent to 88.5 percent, a long progression-free survival of 33.5 months, overall objective response rate of 59.3 percent, and 34.5 months' duration of disease control. In addition the ccombined therapy produces minimal adverse events as well as safe biodistribution after administration. However, the synergistic effect of both drugs on Sulphur radical production should be further investigated by EPR using different radionuclides as well as external beam irradiation. Preparation of the radiopharmaceuticals and quality control were instrumental in the overall study outcomes.

Targeted Radionuclide Therapy using Peptide-Based Radiopharmaceuticals.

1.0. Chapter one: Background.

1.1. Introduction.

Traditionally, radiotherapy has been utilized for cancer treatment to suppress and kill the cancerous cells. The techniques used during treatment depend on several factors including the size of the affected tissue, site of the tumor plus the stage of its development. There are several treatment techniques available for patients depending on different factors.

1.1.1. Helical tomotherapy technique.

This technique involves delivering intensity modulated rotational therapy treatments with a CT gantry's continuously rotating fan beam. During treatment, a high-speed pneumatic binary multi-leaf collimator modulates the CT gantry's rotating fan beam by moving via the gantry ore to allow numerous dose is painting beamlets to be delivered (Jeraj *et al.,* 2004). As of 2016, the only clinically available helical slice-based radiotherapy system is the TomoTherapy®. This system provides high standards of dose delivery while at the same time avoiding destruction of the neighboring normal tissue structures. Helical tomotherapy differs from the conventional treatment units in that it has an onboard imaging capability (MVCT) and a dedicated intensity modulated radiation therapy system (IMRT) (OSL, 2011)

The distinctive field characteristics of a helical tomotherapy system are dependent on the design goals, with differences occurring in such areas as the treatment/imaging operation modes, collimators' shielding, presence/absence of a flattening filter, and the sizes of fan beam delivery. The helical tomotherapy CT-ring gantry platform has a "CTrue" detector that allows clear-cut monitoring of avoidance areas as well as targeting of the tumor volume. Helical tomotherapy can be used to treat several types of cancers because of its long range of coverage that allows targeting a single organ, a system or even the whole body (OSL, 2011).

1.1.2. 4D Radiotherapy technique.

This technique takes into consideration the size of the tumor, in addition to changes happening along a specific timeline – introducing the fourth dimension. In this technique, the initial step involves the acquisition of the image and it is followed by image-guided therapy to respond to various changes in tumor geometry. During the process, the dose delivered and the treatment set-up may be changed to adapt to various changes seen during the treatment. 4d radiotherapy allows both the treatment and the imaging of the target region to be performed simultaneously to avoid movement of the patient that may produce artifacts as well as make it difficult to delineate, localize and identify the lesion. The fourth dimension in the 4D radiotherapy technique is introduced prospectively or retrospectively by the use of motion tracking or respiratory gating techniques (OSL, 2011).

1.1.3. Intensity Modulated Radiotherapy (IMRT).

This is the radiotherapy technique that allows the technician to vary the size, intensity and the shape of the radiation ray to fit the location, shape and size of targeted tumor. This technique delivers radiation to the target region in a more precise manner than compared to the surrounding normal; tissues. It applies computer-controlled radiation deposition, normal tissue avoidance, and inverse planning to create an upper edge over the conventional trial-and-error approaches (Teh, Woo and Butler, 1999; Radiology Info, 2016). The reason for the wide range of applications of IMRT in radiation therapy is the ability to construct multiple avoidance structures and targets in order to treat different tumors concurrently regardless of their size differences and avoidance structures. The overall side effects of IMRT are lower compared to standard radiotherapy treatment as it gives minimal doses to tissues surrounding the target region (OSL, 2011).

1.1.4. Image-guided radiotherapy (IGRT).

Image-guided radiotherapy involves the use of such technologies as PET scans, MRI's and/or CT scans to aid in the therapy process. The imaging process generates both 2D and 3D images of the target region to generate coordinates to be followed during the treatment plan (Gupta

and Narayan, 2012). This process increases the precision as well as the accuracy of delivering the treatment. Image-guided radiotherapy also allows the oncologist to verify the consistency of the planned as well as the actual treatment geometry and adjustments to daily changes in order to improve the dose delivery. However, image-guided radiotherapy is limited by incremental irradiation caused by the imaging and the resource-intensive nature of the technique (OSL, 2011)..

1.1.5. Stereotactic radiotherapy (SBRT and SABR).

Stereotactic radiotherapy involves focusing and concentrating the radiation doses in order to deliver them within 1-5 fractions. The technique differs from conventional radiotherapies in that it reduces the dose fractions from 20-35 to 1-5. In SBRT (Stereotactic body radiotherapy), 3-5 fractions are delivered to organs lying outside of the brain, including liver, spine, lungs and pancreas. On the other hand, stereotactic ablative radiotherapy (SABR) delivers 1-5 fractions to such organs as the liver, lungs, bones and the brain (OSL, 2011)

1.1.5. Stereotactic radiosurgery/therapy.

This technique involves giving a single dose of radiotherapy from various angles with a precise targeting of the tumor. This technique is a type of image-guided radiotherapy that is applied on small tumors with well-defined edges. It gives a high-dose irradiation without affecting the untargeted area, the normal tissues. Some of the brain tumors treated using this technique include haemangioblastomas, acoustic neuromas, pituitary adenomas spinal cord tumors and haemangioblastomas (OSL, 2011).

1.2. Targeted radionuclide therapy.

1.2.1. General.

So far, radiotherapy techniques have proved important in treating as well as prolonging the patients' lives depending on the type of cancer in the question. However, the success of these techniques is limited by their lack of specificity as the anti-cancer agents, or cytotoxic technologies do no distinguish between the cancerous regions and the normal tissues. Most of the traditionally

used radiotherapy techniques apply a non-discriminatory destruction of the cells exhibiting uncontrolled growth without any degree of selection leading to the destruction of the healthy cells. Unlike external radiotherapy which damages cells' DNA with the aim of killing those with uncontrolled growth, targeted radionuclide therapy offers a systemic treatment by delivering toxic levels of radiolabelled molecules to the target sites for a highly selective destruction of the site. Radionuclide therapy acts the same way as the chemotherapy by targeting specific cells, but it is more advanced in that radionuclides also kill tumor cells lacking tumor-specific receptors and thus it has an ability for the direct as well as a bystander effect that ultimately kills the tumor cells. The biological effect of targeted radionuclide therapy results from energy absorption of radiation emitted by the radionuclide. Targeted radiotherapy involves the utilization of three particulate particles which are capable of irradiating tissue volumes with subcellular, cellular and multicellular dimensions (Kirwan, Constable, Murdoch and Khaw, 2003). These particles include Auger electrons, alpha particles, and beta particles.

1.2.2. Auger electrons and Auger-electron-emitting radionuclides.

Auger Electrons are particles released by some elements in a phenomenon referred to as the Auger effect. In this phenomenon, an atom emits an electron after filling an inner-shell vacancy resulting in energy release. Some of the current available or prospective Auger electron emitters include Indium-111 (In-111), Iodine-125, Iodine-123, and Bromine-77. These radionuclides can be used alongside targeting vehicles to localize sub-cellar radiations near the cellular DNA leading to an effective and a specific killing of the tumor cells. Using auger-emitting radionuclide therapeutics, highly tailored targeted radiotherapeutics could be engineered to fit the specific needs of a cancer patient (Kirwan, Constable, Murdoch and Khaw, 2003).

1.2.3. Beta particles and beta-emitting radionuclides.

Beta particles are fast-moving electrons emitted by nucleus during radioactive decay. Some of the currently approved beta-emitting radionuclides used in radiotherapy include yttrium-90 and iodine-131 (for non-Hodgkin's lymphoma treatment), strontium-89-chloride and samarium-153-

EDTMP (for bone metastases). Other potential beta-emitting radionuclides include rhodium-105, gold-199, copper-67, rhenium-186, and lutetium-177, amongst others. The major advantage of better particles is that they have minimal tissue penetration. These particles are emitted at high speed, but they become rapidly attenuated by biological tissues after striking it. As a result, when administered as a radiopharmaceutical it does not affect the surrounding tissues as it cannot travel beyond specific range within a biological structure. An additional protection of the untargeted tissue is also achieved by radioimmunotargeted (Kirwan, Constable, Murdoch and Khaw, 2003).

1.2.4. Alpha particles and alpha-emitting radionuclides.

Alpha particles comprise of two protons as well as two neutrons, and they are identical to helium atom's nucleus. Alpha-emitting radionuclides emit particles of only a few cell diameters in tissue. One of the advantages of alpha particles is that they have a high linear energy transfer that makes them more biologically effective as compared to the conventional radiotherapy techniques (Sartor, Maalouf, Hauck, and Macklis, 2012). In this vein, a few alpha particles are capable of human cancer cells, and it can happen in hypoxic conditions. Some of the available radionuclides that emit alpha particles include radium-223, astatine-211, and bismuth-2013. Alpha are preferred for radiotherapy for their ability to deliver lethal radiations in a localized version, within a range of 50-90 μm diameter. This aspect allows the emitter to target a specific tissue that is cancerous without destroying the healthy tissues nearby. Alpha particles offer a therapeutic benefit by breaking the DNA double strand and thus breaking the cell cycle (National Research Council and Institute of Medicine, 2007). Besides, these particles also cause chromosomal instability to the nearby cells leading to a bystander effect observed in radiotherapy (Sofou, 2008; Pouget *et al.*, 2015)

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1.3. Iodine-131 and thyroid cancer treatment.

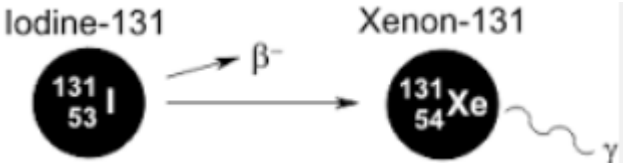


Fig 1: Iodine-131 radioactive decay path.

Iodine-131 is highly radioactive and has a half-life of 8.02 days, and when used in small doses it is used in cancer treatment. When iodine-131 is taken, it crosses the gastrointestinal wall, and it gets concentrated in the thyroid gland where it disintegrates to form Xneon-131 with the release of gamma radiations and beta particles. On the global scale, the use of radioactive iodine in differentiated thyroid cancer treatment has been the most common and the oldest targeted radiotherapy. The aim of the use of Iodine-131 in differentiated thyroid cancer treatment is to destroy cancer cells in order to ablate the remnant thyroid tissue in order to optimize follow-up conditions, and reduce cancer recurrence rate (Smith, 2015). The significance of radioactive iodine treatment in targeted radiotherapy is derived from the ability of both the follicular and the papillary cancers to express sodium iodide supporter for radioactive iodine uptake by cancer cells (Ersahin, Doddamane and Cheng, 2011). Low doses of radioactive iodine have high levels of efficacies as well as high safety profiles making it among the most acceptable thyroid cancer management modality across the world. Furthermore, the disintegration of the respective radionuclides results in additional cytotoxic effects on the target lymphoma cells.

1.3.1. Neuroblastoma/neuroendocrine tumors and I-131-metaiodobenylguanidine.

Since the 1980s, treatment of neuroendocrine tumors have been treated using I-131-metaiodobenylguanidine because of its high efficacy in treating chromaffin (paraganglioma, pheochromocytoma, and neuroblastoma). I-131-metaiodobenylguanidine uptake happens in a similar version to noradrenaline and increases after catecholamine excretion or adrenergic innervation. Stage III and IV patients with neuroblastoma are difficult to manage via chemotherapy

and surgery and most cases resort to the administration of I-131-metaiodobenylguanidine to control tumor growth as well as relief symptoms. A management plan for neuroblastoma using I-131-metaiodobenylguanidine involves taking the patient through series of studies including tissue biopsies, MRI/CT studies, ultrasonography, I-123-MIBG scintigraphy and FDG-PET/CT support the commencement of the I-131-metaiodobenylguanidine therapy. Moreover, recommendations point that I-131-metaiodobenylguanidine should last for longer than one hour in order to avoid metaiodobenylguanidine side effects. I-131-metaiodobenylguanidine may also be used for the treatment of such other tumors as paraganglioma, pheochromocytoma, medullary thyroid cancer, and carcinoid tumors. These tumors have a response rate of 30-75%, indicating high efficacies (Ersahin, Doddamane and Cheng, 2011).

1.4. Targeted radionuclide therapy and lymphoma treatment.

In the 2000s, two major targeted radionuclide therapeutic agents were introduced for lymphoma treatment to reduce the number of deaths resulting from low-grade lymphoma that is difficult to treat with chemotherapy techniques. These agents include I-131 tositumomab and Y-90 ibritumomab tiuxetan and they have been demonstrated to yield 50-80 percent response rates. I-131 tositumomab has an IgG_{2a} murine anti-CD20 antibody (tositumomab), while Y-90 ibritumomab tiuxetan has murine IgG_1 anti-CD20 antibody (ibritumomab), the difference between the two agents is their differential linkage to the radionuclide. By targeting CD20 antigens, these agents deliver the respective radionuclides mature B-lymphocytes, pre-B lymphocytes, and B-cell non-Hodgkin's lymphoma, and thus it ends up inducing apoptosis, antibody-dependent cytotoxicity, and complement-dependent cytotoxicity after the formation of the antibody-antigen immune complex (Ersahin, Doddamane and Cheng, 2011).

1.5. Yttrium-90 and liver tumors treatment.

Such metastatic tumors as pancreatic carcinoma, colorectal carcinoma, neuroendocrine tumors and breast cancers also occur in the liver after metastases leading to a fatal pathological burden. However, reduction of the burden is achieved through traditional therapies with an

additional administration of Y-90 microspheres for radioembolization. Radioembolization of the liver cancers with Y-20 microspheres generate between 27 and 100 percent response rates in clinical treatments (Ersahin, Doddamane and Cheng, 2011).

1.6. Palliation of metastatic bone pain.

During advanced stages of cancers, bone pain reduces the quality of life of the cancer patient to a significant extent. However, the administration of radiopharmaceuticals can palliate pain from metastatic processes. Some of the approved metastatic pain palliation radiopharmaceuticals include Re-186-etidronate, Sm-153-lexidronam and Sr-89-chloride, and their administrations result in high concentrations in bones leading to effective pain management (Ersahin, Doddamane and Cheng, 2011).

1.7. Advantages and disadvantages of targeted radionuclide therapy.

One of the advantages of targeted radionuclide therapy is that Auger electron, an alpha particle, and beta particle emitters are effective therapeutic particles as they can localize the delivery of cytotoxic ionizing radiation (Gnanasegaram, Kapse and Buscombe, 2005). By linking the emitters to biological agents, localized treatment can be achieved because of the high affinity of some elements for some organs and organ systems. As a result, the therapeutic capability of these agents provides a localized killing of specific tissues and cells. Another advantage of the application of targeted radionuclides in cancer therapy is their large-scale availability. Once a specific radionuclide is approved for use as radiopharmaceuticals, it becomes subject to large-scale production in the lab making it highly available for a wide scale application. Another advantage of targeted radionuclide therapy is high specificity and selectivity for the target cell types. Targeted radionuclides are linked to such biological components as antibodies that are specific for certain receptors expressed on cancer cells (National Research Council, 2007). As a result, when introduced into the body they become attached to the target cells where radionuclides decay to emit beta particles, alpha particles or Auger electrons that kill the antibody-associated cancer cells. The mechanism leads to a selective killing of the tumor.

On the other hand, this therapeutic technique carries several significant disadvantages that limit its application in treating human cancers. One of the disadvantages of targeted radionuclide therapy is the shortage of radionuclides. In this case, iodine-124, zirconium-89, astantine-89, bromine-77 and copper-67 are short of supply because of their high requirements of high-energy/complexity accelerators for production and this limits their availabilities comparative to the small cyclotrons available in PET centers. This limitation of radionuclide supply also limits the advancement of research and development in radiobiology and radiochemistry. In the list provided above, only yttrium-90 is available for clinical use, but its availability is severely low. Another disadvantage of targeted radionuclide therapy is resistance. By being a biologically determined process, targeted radionuclide therapy is limited by resistance because some tumors might lack receptor subtype leading to the inability to offer effective treatment (Dash, Knapp, and Pillai, 2013). For example, a tumor may exhibit a variant subtype of somatostatin receptors leading to resistance to somatostatin-active radionuclides. Another limitation is a mutation that can lead to resistance as well. For example, mutation of somatostatin genes will result in loss of efficacy of somatostatin-targeted radionuclides.

1.8. Application of radionuclide targeted therapy on solid and hematological malignancies.

The type of radionuclide targeted therapy applied on a specific type of cancer is dependent on the type of malignancies in the question. As a result, hematological malignancies require a different type of targeted radionuclide therapy from the one used for the solid tumors. In hematological malignancies, targeted radionuclide therapy is supported by three major factors. One of the reasons for the effectiveness of targeted radionuclide therapy is the expression of specific surface antigens by most cancer cell lines. These antigens are absent from other tissues in the organism and thus making targeted therapies possible. Another reason for the effectiveness of this approach is the rich availability of the high-quality antibodies against antigens expressed by hematological tissues (Gudkov, Shilyagina, Vodeneev and Zvyagin, 2015). Moreover, the effectiveness of this approach is also made possible by the high sensitivity of lymphomas and

leukemias to ionizing radiation. In addition, the effectiveness of the targeted radionuclide therapy is also increased by the availability of bone marrow transplantation technologies that allow for the replenishment of the hematological stem cells after the treatment of hematological malignancies with high dose radionuclides. Some of the target antigens in targeted radionuclide treatment of hematological malignancies include CD45, CD66, CD33, CD5, CD25, and the most commonly targeted CD20. On the other hand, Y-90 and I-131 have the greatest potentials for applications as radionuclides in targeted radionuclide therapy. Moreover, some of the common hematological tumors treated using targeted radionuclide therapy include T-cell leukemias, chronic lymphocytic leukemia, and Hodgkin's lymphoma.

However, unlike the treatment of hematological tumors with targeted radionuclides that is highly efficacious, the treatment of solid tumors has low efficacies and thus challenging the therapy. This challenge is presented by the inability of the ionizing particles to penetrate the tumor body leading to their localization in the periphery as well as low doses in the tumor parenchyma. In targeted radionuclide therapy of solid tumors, the cells lying to the surface of the tumor body have the same structure and function and as a result, their destruction does not always result in the complete destruction of the tumor (Gudkov, Shilyagina, Vodeneev and Zvyagin, 2015). Besides, the conditions inside of the tumor are hypoxic and do not permit the formation of reactive oxygen species that increase the damaging potential of the therapeutic agent. However, this problem can be addressed by the use of multi-step pre-targeted radionuclide therapy, which enhances exposure to tumor radiation and therapeutic selectivity.

Some of the successful applications of targeted radionuclide therapy of solid tumors include colorectal carcinoma, solid neuroendocrine malignancies, castration-resistant prostate cancer, metastasizing melanoma, pancreatic tumor and stage-IV melanoma, amongst others. The treatment of colorectal carcinoma involves the use of I-131-conjugated anti-CEA antibodies, and it produces up to 68 months median survival time. The application of anti-PSMA antigen antibodies with Lu-177 radionuclides offer a successful treatment of castration-resistant prostate cancer produces a

successful therapy with a median survival time of 10 months, while the application of anti-NG2 with Bi-213 produces a long-lasting effect in stage IV melanoma treatment (Gudkov, Shilyagina, Vodeneev and Zvyagin, 2015). For the metastatic melanoma, the survival time increases by nine months after administration of anti-NG2 antibodies conjugated to Bi-213 radionuclide, but the application of DOTATE in conjugation with Lu-177 produces a complication-free stable disease course in 46 percent cases.

1.9. Clinically important radionuclides in cancer therapy (Peptide-based radiopharmaceuticals).

Cancer therapy by the use of radionuclides involves the use of a variety of radionuclides labeled with monoclonal antibodies for specific tumors. At the basic level, this technique requires the monoclonal antibody to bind to the tumor-specific antigens in order to distinguish cancer cells from the normal cells and increase the therapeutic value of the radionuclides. After the first description of radio-immunotherapy by Korngold and Pressman in 1953, numerous radiopharmaceuticals have been developed by advanced techniques in genetic engineering and chelating techniques (Yeong, Cheng and Ng, 2014).

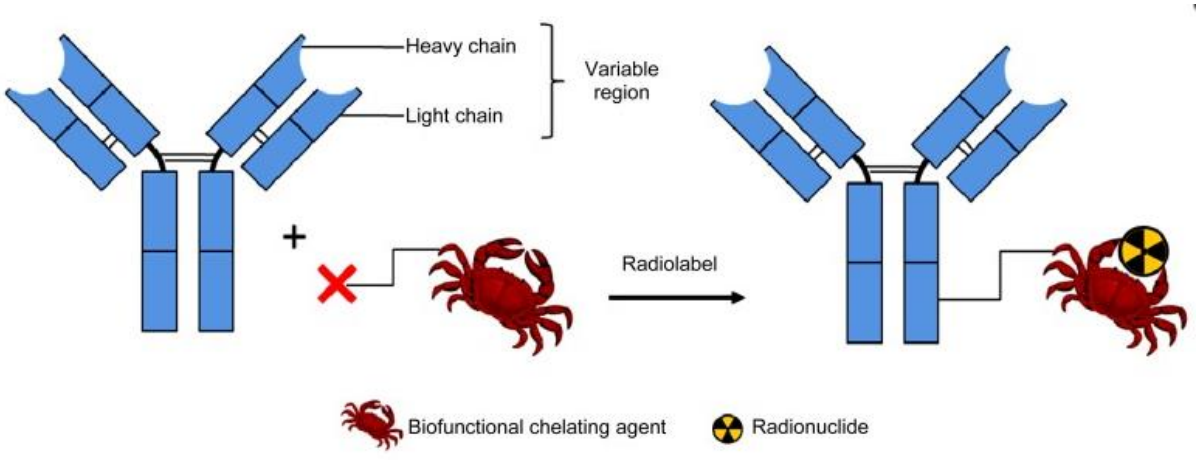


Fig 2: Conjugation of a monoclonal antibody with a radionuclide through a biofunctional chelating agent.

1.10. The composition of peptide-based radiopharmaceuticals.

Peptide-based radiopharmaceuticals comprise of four parts that include a radionuclide, a targeting peptide, a bifunctional chelating agent and a linker moiety (Yeong, Cheng and Ng, 2014).

1.10.1. Radionuclides as therapeutics.

Radionuclides used in cancer treatment release energy in the form of beta particles, Auger electrons or alpha particles to cause the destruction of cancer cells and result in improvement of the patient's condition. The radionuclides applied for the purposes of treating cancer depend on several factors, including, the nuclear emission properties, mode of radioactive decay, physical half-life, radionuclide production route, pharmacological features of the resultant radio-conjugate, radiation type and its energy, and the stability of the resultant daughter nuclides. Most of the cancer-destroying radionuclides have a physical half-life of between 10 hours and 10 days allowing them to deposit a large radiation dose. They also emit high LET radiation near the target cancer tissue and their daughter nuclides are stable and long-lived to increase the therapeutic effect of the radionuclide (Yeong, Cheng and Ng, 2014).

1.10.2. Targeting peptides.

A targeting peptide is delivery vehicle that takes a radionuclide to a specific antigen expressed by tumors. Most targeting peptides are regulatory peptides that can be synthesized by solid-phase peptide synthesis. These peptides can also be modified further to incorporate prosthetic groups, spacer moieties and chelating agents. In addition, these peptides are also chemically stable meaning that they can undergo radiolabeling processes without recording significant changes. Their small sizes also allow for a rapid clearance in order to facilitate diffusion of radiopharmaceuticals to the tumor as well as its excretion to avoid residual effects (Yeong, Cheng and Ng, 2014). However, most of these peptides are subject to biodegradation by various peptidases present in different biological systems. As a result, bio-engineered monoclonal antibodies form the other preferred option.

1.10.3. Bifunctional chelating agents and bioconjugation.

Bifunctional chelating agents provide a conjugation bridge between the targeting peptide and the radionuclide in order to prevent a direct contact between the two components that would minimize the binding affinity of the biopharmaceutical for the targeted receptor. The choice of a bifunctional chelating agent depends on the oxidation state of the metallic radionuclide in the question, but in the end it should allow for coupling with the peptide as well as a stable complexation of the radiometal. In addition, the resultant radionuclide-chelate complex should be biologically inert in order to avoid competition of binding between the target receptor and the endogenous plasma proteins. There are three chelating systems that may be utilized by bifunctional chelating agents (Yeong, Cheng and Ng, 2014). One of them is the acyclic chelating system, which forms a complex between a radiometal and an open-chain polyaminopolycarboxylates under mild conditions for temperature-sensitive biomolecules and under fast metal-binding kinetics for short-lived radiometals. Twoof the most common example of acyclic chelators for radiopharmaceutical applications include diethylene triamine pentaacetic acid (DTPA) and ethylene diamine tetraacetic acid (EDTA).

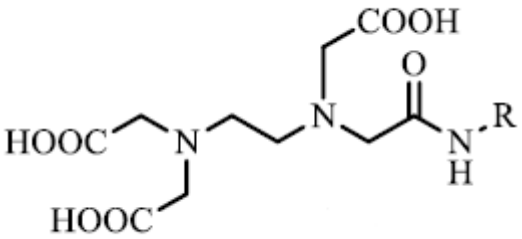


Fig 3: EDTA – a bifunctional chelator for radionuclide labeling with ^{111}In, ^{90}Y, ^{68}Ga and ^{89}Zr. In this figure, R is representative of a conjugated biomolecule.

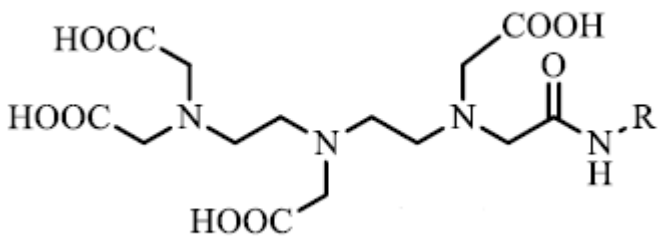


Fig 4: DTPA – a bifunctional chelator for radionuclide labeling with ^{111}In, ^{90}Y, ^{68}Ga and ^{89}Zr. In this figure, R is representative of a conjugated biomolecule.

Another chelating system utilized by bifunctional chelating agent is the macroacyclic system (Yeong, Cheng and Ng, 2014). The product of the macroacyclic chelatng systems are kinetically more inert and thermodynamically more stable than acyclic chelating systems their radiolabelling conditions are harsher. Someo fthe common mcroacyclic chelating agents include triaza, cyclen-type and cyclam-type tetraaza chelators. For example, DOTA (cyclen-type 1,4,7,10,-tetraazacyclododecane-1,4,7,10-tetraacetic acid), which is ubiquitous for +3-charged radionuclides.

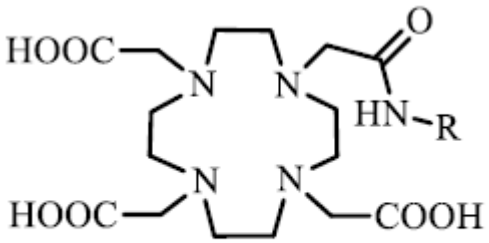


Fig 5: DOTA - an example of macrocyclic bifunctional chelator. It labels a peptide with a +2/+3 charge radionuclides.

The conjugation of bifunctional chelating agents with a peptide can also be achieved by conjugating to a peptide sequence through the side chain functionalities of the amino acids or the primary N-terminal amine. Some of the conjugation strategies use to achieve bioconjugation in this case include untilizing azides, maleimides, N-hydroxysuccinimide esters and isothiocyanates (Yeong, Cheng and Ng, 2014). Of all these examples, the N-hydroxysuccinimide esters are the most commonly used especially after their activation to form a primary amine of the amine or lysine group at the peptide sequence's N-terminus.

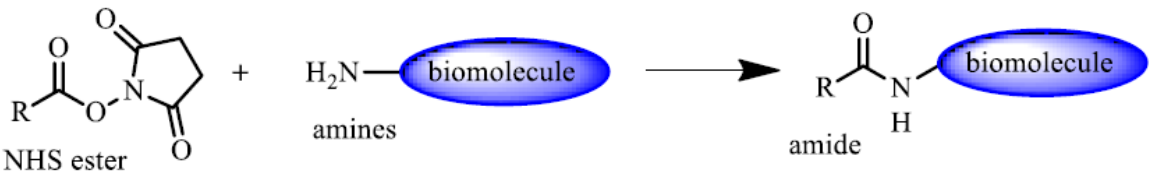


Fig 6: An activated N-hydroxysuccinimide ester.

1.10.4. Linkers.

A linker is a radiopharmaceutical component that has two orthogonal sites of conjugation for connection between the targeting peptide and the radionuclide-chelate complex. It serves as a spacer between the radionuclide and the targeting peptide in order to prevent binding interference between the radionuclide and the targeting peptide, and sustain a high binding affinity between the targeted receptor and the peptide. If the linker is electrically charged, hydrophilic or lipophilic, it modifies the pharmacokinetics of the conjugate to influence its clearance from the body, excretion or uptake by the tumors. Additionally, a linker provides an avenue to the creation of a bifunctional radioconjugate by providing an additional functionality. One of the commonly used linkers is beta-alanine (Yeong, Cheng and Ng, 2014).

1.11. Clinically available radiophharmaceuticals.

1.11.1. ^{90}Y-clivatumuzumab tetraxetan (PAM4).

PAM4 is a relatively new monoclonal antibody with a high reactivity with pancreatic cancer as well as precursor lesions. However, it does not exist in the normal tissues, and its reactivity with non-pancreatic non-pancreatic cancer is relatively low. Humanization and conjugation of PAM4 with DOTA - 1,4,7,10-tetraazacyclododecane-N,N′,N″,N‴-tetraacetic acid - and labelling with yttrium-90 produces a product with 5 mm radiation path of gamma rays. This product (^{90}Y-clivatumuzumab tetraxetan) is suitable for the treatment of bulky tumors (Picozzi *et al*., 2015). As a result, it creates a good agent for the treatment as well as the detection of pancreatic cancer. This radionuclide can be administered as a single dose although a higher efficacy is achievable through fractionated doses. The use of ^{90}Y-clivatumuzumab tetraxetan in combination with 200 mg/m^2 gemcidabine doses result in median survival of 7.7 months among pancreatic cancer patients with stage III and IV disease. However, the survival period may be extended by repeating the therapeutic cycles, but it produces myeloid suppression as a major side effect.

1.11.2. ^{131}I-metuximab.

^{131}I-metuximab is a murine monoclonal antibody Hab18 F(ab')2 for antigen Hab18G/CD147 that is associated with HCC cells. The high specificity of ^{131}I-metuximab to hepatocellular carcinoma allows for its concentration in the liver and as a result delivering the therapeutic radiation from the Iodine-131 to cancer cells (Ma and Wang, 2015). Besides the cytotoxic nature of ^{131}I-metuximab through irradiation, murine monoclonal antibody Hab18 F(ab')2 is also cytotoxic and it also a metastasis inhibiting effect on the hepatocellular carcinoma cells as it blocks prevents the formation of matrix metaloproteinases in fibroblast cells (Chen *et al.,* 2006). The survival rate recorded during clinical trials of Licartin® treatment have demonstrated that survival rate of hepatocellular carcinoma patients at the 21st month of treatment is up to 44 percent. This aspect represents Licartin® as an important candidate in targeted radionuclide treatment of hepatocellular carcinoma.

1.11.3. Pemtumomab.

Pretumomab is a ^{90}Y-conjugated monoclonal antibody that is derived from mouse and that is specific for MUC1 mucin's glycoform. In such tumors cells of the lung, ovarian, breast and gastric origin, the MUC1 mucin is over-expressed. Treatment of ovarian cancer with pretumomab has been in clinical trials to increase the survival by at least eight years among 67 percent of patients receiving the treatment (Yeong, Cheng and Ng, 2014). It has also been seen as a feasible treatment option for gastric cancers. However, it produces several immunological side effects as a result of human anti-mouse antibody leading to such conditions as myalagia, arthralgia and rashes.

1.11.4. ^{131}I-chTNT-1/B.

^{131}I-chTNT-1/B is a product of genetic engineered and it is a chimeric human/mouse monoclonal antibody that binds the DNA-histone H1 complex. DNA-histone H1 complex is an intracellular antigen expressed by solid tumors' necrotic core. Since necrotic cells have highly permeable cell membranes, administration of ^{131}I-chTNT-1/B results to its subsequent inward transfer across the cell membrane as well as the karyolema to bind the DNA-histone H1 complex

(Yeong, Cheng and Ng, 2014). This event results in the delivery of radiation energy in order to destroy the adjacent tumor cells and treat any cancer as the target antigen is universally expressed by almost all types of tumors. Some of the common types of cancers that have been tried using ^{131}I-chTNT-1/B include colorectal carcinoma, hepatocellular carcinoma, anaplastic astrocytoma, and brain glioblastoma.

1.11.5. ^{131}I-Lym-1.

^{131}I-Lym-1 is a specific radiolabelled monoclonal antibody that is specific to human leukocyte antigen-DR10 that is specifically expressed by B-lymphocytes. As a result, it specifically targets non-Hodgkin lymphoma cases for treatment. The higher proportion of HLA-DR10 protein that is expressed by non-Hodgkin's lymphoma in comparison to the normal blood cells allows ^{131}I-Lym-1 to be used lymphoma cancer treatment without destroying the health B-cells and thus ^{131}I-Lym-1 therapeutic approaches do not reduce the normal production of antibodies by B-cells (Yeong, Cheng and Ng, 2014). Clinical trials on ^{131}I-Lym-1 have indicated more benefits than then conventional therapies applied in cancer treatment, but produces a major decline in the blood cell counts.

1.11.6. Epratuzumab.

Epratuzumab comprises of anti-CD22 monoclonal antibodies labelled with yttrium-90 and it acts against B-cell surface antigens. At the basic level, Epratuzumab is applied in the therapeutic redress of several diseases characterized by abnormal B-cells, for example, chronic lymphocytic leukemia and non-Hodgkin's lymphoma (Yeong, Cheng and Ng, 2014)

. When introduced in the body, Epratuzumab binds to B-cells expressing CD22 in order to deliver beta particles from radioactive disintegration of yttrium-90. Clinical trials results of Epratuzumab have shown promising outcomes as the radiopharmaceutical has been shown to be of grade-1 toxicity, but with an aggressive anti-tumor activity against non-Hodgkin's lymphomas at doses ≥240 mg/m^2.

1.11.7. Labetuzumab.

Labetuzumab is an anti-CEA monoclonal antibody that is conjugated with iodine-131 or yttrium-90. Carcinoembryonic antigen (CEA) is present in at least 90 percent of colorectal cancer cells, making it a significant target in targeted radioimaging as well as targeted radionuclide therapy. Some of the cancer types that can be treated using Labetuzumab include pancreatic and colorectal cancers because of their high expressions of carcinoembryonic antigen. The treatment of patients with liver metastasis of colorectal cancer have been demonstrated in clinical trials to have 51.3 percent survival rates after five years of the treatment, placing Labetuzumab as significant treatment option for this cancer type. However, the treatment is associated with a transient suppression of the myeloid tissue (Yeong, Cheng and Ng, 2014).

1.11.8. ^{131}I-L19.

^{131}I-L19 is a radiolabelled monoclonal antibody comprising of L19 and iodine-131. It binds fibronectin on the surface of cells of such tumors as melanoma/carcinoma of the head/neck, hematological cancers and non-small cell lung cancer (Yeong, Cheng and Ng, 2014). This agent has been shown to have significant therapeutic importance in Hodgkin's lymphoma treatment.

1.12. Somatostatin and somatostatin receptors.

1.12.1. Biosynthesis.

The normal biosynthesis of somatostatin happens in several sites, including, the nervous system (central and peripheral nervous system), gastrointestinal tract, kidney, retina, immune cells and placenta. However, the hypothalamus forms the principle biosynthetic site for somatostatin

production (Fernstrom, ND). The process is activated the high cystosolic calcium concentration and membrane depolarization in neurons and peripheral secreting cells. The biosynthesis happens in two stages, where the initial stage involves the production of somatostatin peptide by ribosomal mechanisms. This stage is followed by post-translational cleavage of the peptide into smaller subsets, namely somatostatin-14 and somatostatin-28, by the action of trypsin-like proteolytic enzymes.

After synthesis, the release of SST is influence by several chemicals, including nutrients, neurotransmitters, neuropeptides, cytokines, hormones and growth factors (Bronstein-Sitton, 2006) Some of the examples of hormones that stimulate the release of somatostatin include corticotrophin-release hormone, neurotensin and growth hormone-releasing hormone. On the other hand, some of the substances that inhibit the release of somatostatin include leptin, cytokines, opiates and gamma-aminobutiric acid (GABA) (Ivell and Richter, 1983).

1.12.2. Somatostatin receptors.

There are three classifications of somatostatin receptors, including the rhodopsin-like family, the GABA-like family and the glucagon-receptor-like family that belong to the G-protein coupled receptor family to with all somatostatin receptors belong. A good understanding of the structure and function of somatostatin receptors can be achieved by exploring the G-protein coupled receptors to which they belong. G-protein coupled receptors are composed of seven transmembrane alpha helices that are linked to each other by six loops with extracellular ligand-binding domains as well as an intracellular signal transduction domain (Bronstein-Sitton, 2006). When a ligand binds the eternal receptors, a series of events ensue leading to signal transmission to the intracellular domain and a subsequent inhibition of the activity of adenyl cyclase, causing a decrease in the intracellular levels of cAMP, and calcium channels' activity. Further subsequent events in this cascade include tyrosine phosphatases activation and the ultimate upregulation of antimitosis. The initiation of this cascade also induces several other signal transduction pathways, including, Src, Erk ½, MAP kinase, Na+ -H+ exchangers, protein kinase and p38 mitogen activation. In the end all these

pathways result in such changes as vascular contractility, ion/nutrient absorption, lowered intestinal motility, neurotransmission modulation, reduced cellular proliferation, and inhibition of exocrine/endocrine functions, amongst others (Bronstein-Sitton, 2006).

1.12.3. Subtypes of somatostatin receptors.

Currently, there are six known subtypes of somatostatin receptors and they include ssr1, ssr2A, ssr2B, sstr3, sstr4 and sstr5. These receptors have a fair distribution across all body organs including kidney, pancreas, nervous system and the gastrointestinal tract. All the six ssrs have a structural semblance with G-protein coupled receptors but they have functional differences due to such variations as carboxyl terminal and amino sequences (Bronstein-Sitton, 2006). These variations result in the differential ligand affinities as well as specificities to ligands, in addition to the differential transmission of intracellular signals after activation. In human subjects, the six receptor subtypes are also present in the above mentioned organs in addition to several others. For example, sstr1, sstr2B, sstr4, sstr5, and sstr3 can also be found in the bronchial gland, sstr2B and sstr5 in parotid gland, and sstr1, sstr3, and sstr4 in the parathyroid gland.

1.12.4. Mechanism of action of somatostatin receptors.

The mechanism of action of somatostatin receptors is related to that of G-protein coupled receptors. In general the G-protein coupled receptors have three basic formations, namely heterodimeric units, homodimeric, and monomeric units occurring on the cell membrane. After activation, G-protein coupled receptors react either by remaining unaffected, dissociating into monomers, or dimerizing. However, different G-protein coupling receptors are expressed differently to cause differential ligand binding, receptor trafficking, and receptor desensitization (Bronstein-Sitton, 2006). Somatostatin receptors also act in a similar version where after activation by an extracellular ligand binding they dissociate into monomeric units. This event is followed by an intracellular transmission of signals and the subsequent phosphorylation of the intracellular region in a GPCR kinase-dependent event to activate the recruitment as well a binding of beta-arrestin-1/2 to the receptor. The last three events in this cascade include the receptor-ligand complex

endocytosis, dissociation of ligand from the receptor and the desensitization of the receptor, respectively, and the happen before the recycling of the receptors back to the membrane. This mechanism of action is exhibited in all of the three G-protein dependent signaling pathways after their activations by various somatostatin subtypes. In the protein phosphatases pathways, the binding of any of the ligand to any of the receptor subtypes activates such protein phosphatases as tyrosine phosphatases, calcium-dependent phosphatases, and threonine phosphatases. However, the binding of ligands to somatostatin receptors result in the inhibition of cAMP production and adenylate cyclase pathways (Bronstein-Sitton, 2006).

1.13. Radiolabelled somatostatin analogues.

1.13.1. Octreotide, lanreotide and vaprotide.

Octreotide has a long cyclic structure and its chemical properties are similar to those of somatostatin making it a somatostatin agonist. It has higher inhibitory potency than somatostatin in relation to insulin, glucagon and growth hormone, but it acts similarly to somatostatin in several biological processes including inhibition of hormone release, and suppression of response to hormone amongst others (Battershill and Clissold, 1989).

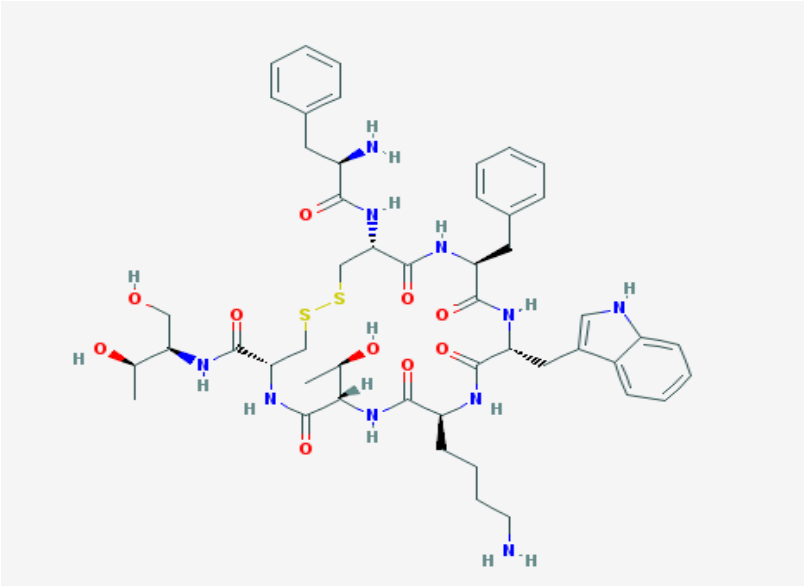


Fig 7: octreotide molecule.

Octreotide has been clinically tried in tumor treatments where it has been shown to over resolutions for such conditions as thyrotrophinomas and carcinoid syndrome (Battershill and Clissold, 1989). One of the radionuclides conjugated to octreotide to produce a radiolabelled somatostatin analogue for radionuclide targeted therapies is indium-111. This conjugation produces 111In-diethylenetriaminepenta-acetic acid (DTPA)0,octreotide, which emits Auger electrons and gamma-rays after administration in patients with metastatic tumors (Kaltsas, Papadogias, Makras and Grossman, 2005). Lanreotide is a somatostatin analogue with similar properties as octreotide as well as long-acting pharmacological properties. Lanreotide can be treated with indium-111 and yttrium-90 to produce two radiolabelled somatostatin analogues, namely 111In-DOTA-lanreotide and 90Y-DOTA-lanreotide, respectively. 111In-DOTA-lanreotide derives its tumor therapeutic effects from the inherent anti-tumor effects of lanreotide as well as the emission of Auger electrons and gamma-rays from after radioactive decay of indium-111. One the other hand, 90Y-DOTA-lanreotide acts in the same way, but its yttrium-90 emits beta particles (Kaltsas, Papadogias, Makras and Grossman, 2005).

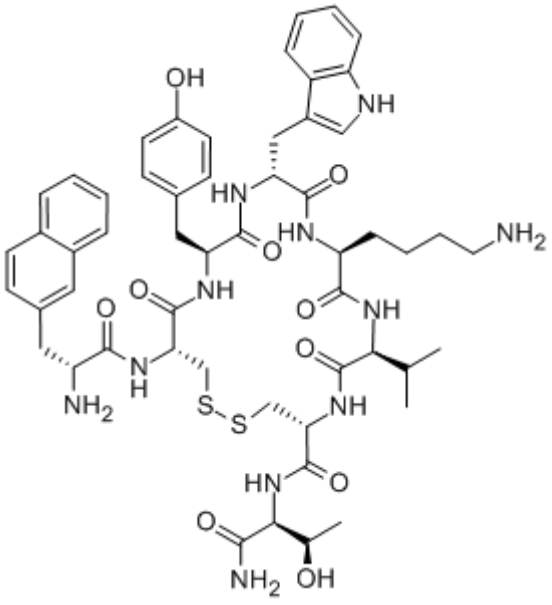


Fig 9: lanreotide molecule.

Vapreotide is marketed as Octastatin® and its structure is written as (D) Phe-cys-Tyr-(D) Trp-Lys-Val-Cys-Trp-NH2. Of the three major somatostatin analogues discussed above, octreotide is the most frequently used one and it is used in endocrine therapies, refractory pain and adjuvant

treatment. In oncology, octreotide is used in diagnosis as well as the treatment of neuroendocrine tumors because of its high affinity for sstr2A, which are highly expressed in those tumors (Kaltsas, Papadogias, Makras and Grossman, 2005). Other effective somatostatin analogues include MK363-301, MK 678, depreotide, and demotate.

1.13.2. Somatostatin analogues and anti-tumor effects.

Both in vivo and ex vivo studies have demonstrated the anti-tumor activity of various somatostatin analogues. Studies on the effects of octreotide on tumors conducted in the 1980s proved 20 percent partial response rate and 50 percent stable disease response rate. In the 1990s, six studies on the effects of octreotide on advanced neuroendocrine tumors resulted in stable disease among 15%-86% of the patients. Similar results were also observed in studies conducted to investigate the effects of lanreotide on tumor progression. Such supporting evidence as the one highlighted above has been the basis for the administration of Somatostatin analogues to stop tumor progression (Sideris, Dube and Rinke, 2012). However, the common practice is to use these compounds in conjunction with other treatments in order to increase the overall antiproliferative activities. It is also the rationale employed for their labelling with radionuclides to deliver targeted radionuclide therapy against different somatostatin-receptor-positive neuroendocrine tumors. Apart from the anti-proliferative treatment of neuroendocrine tumors, somatostatin analogues also provide relief of such symptoms as diarrhoea, wheezing and flushing, which are highly associated with neuroendocrine tumors. They two analogues act in a similar way as the natural somatostatin molecules in that after binding to somatostatin receptors, they initiate cascades of events that lead to direct or indirect anti-proliferative effect (Sideris, Dube and Rinke, 2012). Some of the effects of these analogues that are also present after the natural secretion of somatostatin include inhibition of cell signalling, protein synthesis and hormonal secretion, and increased apoptosis, as well as decreased cellular proliferation.

1.13.3. Somatostatin receptor expression in pathology.

Several pathological conditions have been associated with differential expression of somatostatin receptors. Some of these pathologies include tumors, neuroendocrine dysfunctions and

Alzheimer's disease (Note: Later in this paper, focus will be directed on tumors to explore how different cancers express these receptors). In Alzheimer's disease (AD) patients, there is a characteristic reduction of somatostatin distribution in the brain as well as the cerebrospinal fluid. The cortical areas of the AD brain also exhibit marked subtype-selective alterations of sstr distribution as a pathological characteristic of Alzheimer's disease. The characteristic depletion of somatostatin receptors in the AD brain is argued to be the consequence of over 70 percent reduction in the somatostatin receptor-immunoreactive neurons that have been established in the frontal cortex of the AD brain. In addition, postmortem studies on AD brains have indicated Alzheimer's disease also causes about 50 percent loss in the density of somatostatin receptors. Specific subtypes of somatostatin receptors are also affected by Alzheimer's disorder in a variety of ways. For example, Alzheimer's disease causes the reduction of the radioligand binding affinity of several somatostatin receptor subtypes, including sstr5, sstr2 and sstr3 in the temporal and the frontal cortex of the DA brain without affecting other somatostatin receptor subtypes (Burgos-Ramos *et al.,* 2010).

1.13.5. Somatostatin-receptor-positive neuroendocrine tumors.

One of the common characteristics of neuroendocrine tumors is that they show overexpression of somatostatin receptors. Some of these tumors include gastrinoma, insulinoma, small cell lung cancer, glucagonoma, VIPoma, and phaeochromocytoma. Around 80 percent of all of the above named neuroendocrine tumors exhibit the expression of somatostatin receptors on their cellular membranes making them important targets in somatostatin-based radiopharmaceuticals. The yearly incidence rate of gastroenteropancreatic tumors is about 1.2-3.0/100,000, but the risk of development in an individual increases by 460-720 percent within 30 years of one's life. Under the WHO classification system based on histological features gastroenteropancreatic and neuroendocrine tumors are classified into four classes, namely mixed exocrine-endocrine carcinomas, poorly-differentiated neuroendocrine carcinomas, well-differentiated neuroendocrine carcinomas and well-differentiated neuroendocrine tumors (Appetecchia and Baldelli, 2010).

However, most of these tumors do not produce detectable hormones, and thus they remain undiagnosed until their advanced stages of progression when such symptoms mass effects and metastases happen. Patients with nonmetastatic well-differentiated neuroendocrine carcinomas have been observed to have a survival rate of 60-100 percent over five years, but those with distant metastasis have a survival rate of 29 percent over a similar period. Different neuroendocrine tumors produce varying amounts of each of the five subtypes of somatostatin receptors. On average, 68, 86, 46, 93 and 57 percent of all neuroendocrine tumors express sstr1, sstr2, sstr3, sstr4 and sstr5 on their cells, respectively, and this shows a significant importance of somatostatin receptors on their diagnoses. However, 33 100, 33, 100 and 67 percent of all insulinoma cases express sstr1, sstr2, sstr3, sstr4, and sstr5 on their cells, respectively, while 33, 50, 17, 83, and 50 percent of all gastrinoma cases express sstr1, sstr2, sstr3, sstr4, and sstr5 on their cells, respectively. In addition, 67, 100, 67, 67, and 67 percent of all cases of glucagonoma express sstr1, sstr2, sstr3, sstr4, and sstr5 on their cells, respectively, but all cases of VIPoma express sstr1, sstr2, sstr3, sstr4, and sstr5 on their cells (Appetecchia and Baldelli, 2010).

1.14.0. Radiolabelled somatostatin analogs and radio-imaging.

Since neuroendocrine tumors express more somatostatin receptors than the normal tissue, somatostatin analogs have been seen as important molecules in for localizing the tumor by the radio-imaging protocol. Among all the five major subtypes of somatostatin receptors, sstr2 is the most overexpressed receptor in neuroendocrine tumors. Somatostatin imaging is mainly applied for four major reasons, including the determination of the SSTR status in relation to disease treatment, accurately stage the disease, follow up on the disease, and restage the disease (Navalkissoor and Gnanasegaran, 2015). Octreotide was the first somatostatin analog to be used in single photon emission tomography (SPET) somatostatin receptor analog imaging in the late 1980s. Octreotide exhibits a high affinity for sstr5 and sstr2, but a lesser affinity for sstr3 making it a popular fit for imaging neuroendocrine tumors. Due to this affinity for sstr2 and sstr5, a radiolabelled octreotide complex with indium-111 was developed. This complex has 68-hours long half-life and when used

for imaging an allowance of 24-48 hours is necessary to ensure hepatobiliary and renal clearance in order to reduce the background activity (Navalkissoor and Gnanasegaran, 2015).

1.14. 1. DOTA (1,4,7,10-tetra-azacyclo-dodecane-1,4,7,10-tetraacetic acid).

Most of the chelating agents used for labeling somatostatin analogs with radionuclides are based on DOTA. DOTA is organic, with a chemical formula $(CH_2CH_2NCH_2CO_2H)_4$, and its IUPAC name is 1,4,7,10-tetr-aazacyclo-dodecane-1,4,7,10-tetraacetic acid and a tetraaza ring. DOTA is a derivative of cyclen in a process that modifies the secondary amine groups by exchanging the N-H centers with the $N\text{-}CH_2CO_2H$ groups to form an aminopolycarboxylic acid, which has a high affinity for trivalent and divalent cations and thus making it a popular chelator for divalent and trivalent cations (Brechbiel, 2007). When interacting with divalent and trivalent metallic cations, DOTA forms a polydentate ligand where in the case of lanthanides it acts as an octadentate ligand allowing it to bind the metal via four carboxylate and four amine groups.

In cancer therapies, DOTA is used as a chelating agent for yttrium-90 when developing such radiopharmaceuticals as 90yttrium tacatuzumab tetraxetan and 90yttrium clivatuzumab tetrxetan.

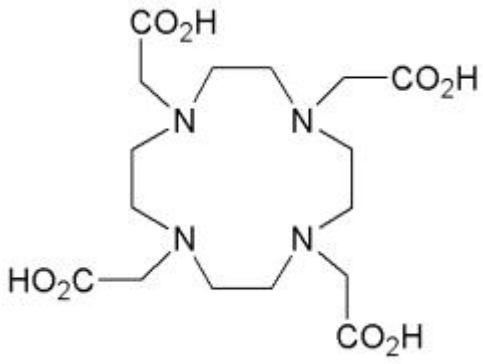


Fig 10: Structure of DOTA.

1.14.1.0. The structure of DOTA.

1.14.1.1. Modification of DOTA structure.

The most commonly used products of DOTA modifications are DOTATOC and DOTATATE, and they are produced through peptide conjugation of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid. For DOTATOC synthesis, the process involves conjugating 1,4,7,10-

tetraazacyclododecane-1,4,7,10-tetraacetic acid with Tyr3-octreotide to form DOTA0-Tyr3-octreotide (DOTATOC) (Moi, Meares and DeNardo, 1988)..

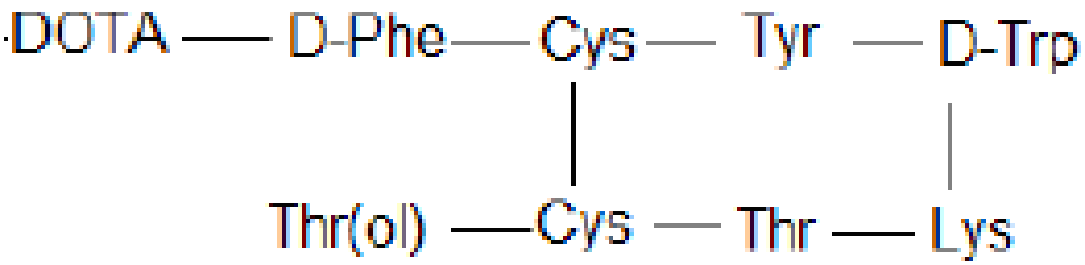


Fig 11: Structure of DOTATOC.

On the other hand, the production of DOTATATE involves a process of conjugating 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid with Tyr3-octreotate to form DOTA0-Tyr3-octreotate.

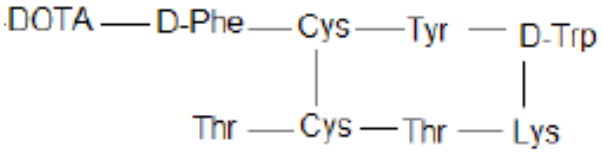


Fig 12: Structure of DOTATATE.

Both DOTATOC and DOTATATE have the ability to bind to somatostatin receptors, and thus they are important vehicles of targeted radionuclide therapy after their conjugation with suitable radioisotopes (Edreira, Melendez-Alafort and Mather, 2002). Some of the examples of radiolabeled DOTATOC compounds that are of significance to this paper are 177Lu-DOTATOC and 68Ga-DOTATOC. 177-Lu-DOTATOC is produced by radiolabelling DOTATOC with lutetium-177(Breeman, 2012). Lutetium-177 has a half-life of about 6.7 days, and it emits radio energy in the forms of beta particles and gamma rays, where 81 percent of the emissions are comprised of beta emission while the rest is gamma rays. However, unlike 177Lu-DOTATATE which has been studied significantly in relation to targeted radionuclide therapy for its importance in the treatment of neuroendocrine tumors, 177Lu-DOTATOC has not been as widely studied because it is only seen as a weaker alternative to the use of yttrium-90-laballed somatostatin analogs (Fraser, 2013).The achievement of the maximal specific activity of lutetium-177 is dependent on various factors. For

example, the presence of such impurities as Lu-176 and Lu-175 in Lu-177 produced from enriched Lu-176 causes a reduction of the peak achievable specific activity to 0.15 GBq Lu-177 for every nanomole of DOTA-peptide. In this example, higher specific activities of Lu-177 may be achieved by eliminating metallic impurities during radiolabelling as well as DOTA-conjugated peptide synthesis (Breeman, 2012). On the other hand, 68Ga-DOTATOC is produced by labeling DOTATOC with Ga-68 to form a radiolabeled somatostatin analog. Gallium-68's half-life is 68 minutes, and it emits high amounts of positron. This low half-life ensures the generation of high-quality radio images while minimizing radio exposure to patients or medical staff. Besides, it is generated from 68Ge/68Ga generator that has a considerably long shelf-life courtesy of its 270.95-days half-life (Velikyan, 2015). Preparation of Gallium-68 involves heating a mixture of 68Ga3+ solution and a small amount of DOTATOC in order to produce a radiolabelled complex. In this process, acid/base buffering is necessary in order to deprotonate the DOTATOC and maintain a PH of 5 (Bauwens *et al*., 2010).

1.15. mTOR drugs and Everolimus.

Mammalian target of rapamycin – mTOR- drugs target protein kinases responsible for cellular growth, survival, and proliferation. mTOR is organized into multi-protein complexes, the most important ones being mTOR complex 2 and mTOR complex 1. However, the general structure of the mTOR protein comprises of a 289-kDa serine-threonine kinase belonging to phosphoinositide 3-kinase family (Laplante and Sabatini, 2009; Ballou and Lin, 2008).

1.15.1. mTOR complex 1 (mTORC1).

mTORC1 comprises of five substructures, namely, proline-rich AKT substrate 40kdDa, a regulatory-associated protein of mTOR, DEP-domain-containing mTOR-interacting protein, mTOR, and mammalian lethal with Sec13 protein 8. The function of mTOR, DEP-domain-containing mTOR-interacting protein is unknown, but regulatory-associated protein of mTOR regulates the formation of the complex as well as the recruitment of mTOR substrates. Moreover, proline-rich AKT substrate 40kdDa inhibits the substrate binding to mTORC1, but after the

activation of mTORC1, DEP-domain-containing mTOR-interacting protein interacts with proline-rich AKT substrate 40kdDa to improve the level of substrate interaction with mTORC1 (Populo, Lopes and Soares, 2012).

1.15.2. mTOR complex 2 (mTORC2).

mTORC2 has six protein components that include DEP-domain-containing mTOR-interacting protein, rapamycin-insensitive companion of mTOR, mROR, protein observed with Rctor-1, mammalian stress-activated protein kinase, and mammalian lethal with Sec13 protein 8. MTORC1 and mTORC2 have distinctive roles in the mTOR pathway to influence different biological processes differently (Populo, Lopes and Soares, 2012).

1.15.3. mTOR pathway.

The mTORC1 is majorly involved in the regulation of metabolism and cell growth by upregulating such anabolic processes as lipid biosynthesis and protein biosynthesis, and downregulating catabolic processes. In the mTORC1 reacts to such environmental/nutritional stimuli as cellular stress, amino acids, energy levels and growth factors to bring about the phosphorylation of enzymatic substrates in order to activate or enhance the anabolic mentioned above processes and limit some catabolic processes as well. One of the potent stimulators of the activity of the mTORC1 activity is the GTP-bound small GTPase Rheb. GTP-bound small GTPase Rheb depends on TSC1/2 for negative regulation while the upstream regulation is dependent on Akt and TSC1/2. MTOR1 may also become activated due to the amino acid signal prompting it to be translocated to the lysosomal surface where Rheb sets off its activation (Populo, Lopes and Soares, 2012). In this process, there are coordinated actions of several complexes, including Regulator, GATOR1/2, Rag GTPases, and v-ATPase. On the other hand mTORC2's involvement in the mTOR signaling pathway includes promoting processes involved in cellular survival. This involvement includes Akt activation, activation of PKC-alpha, control of ion transport, and SGK1 phosphorylation (Populo, Lopes and Soares, 2012).

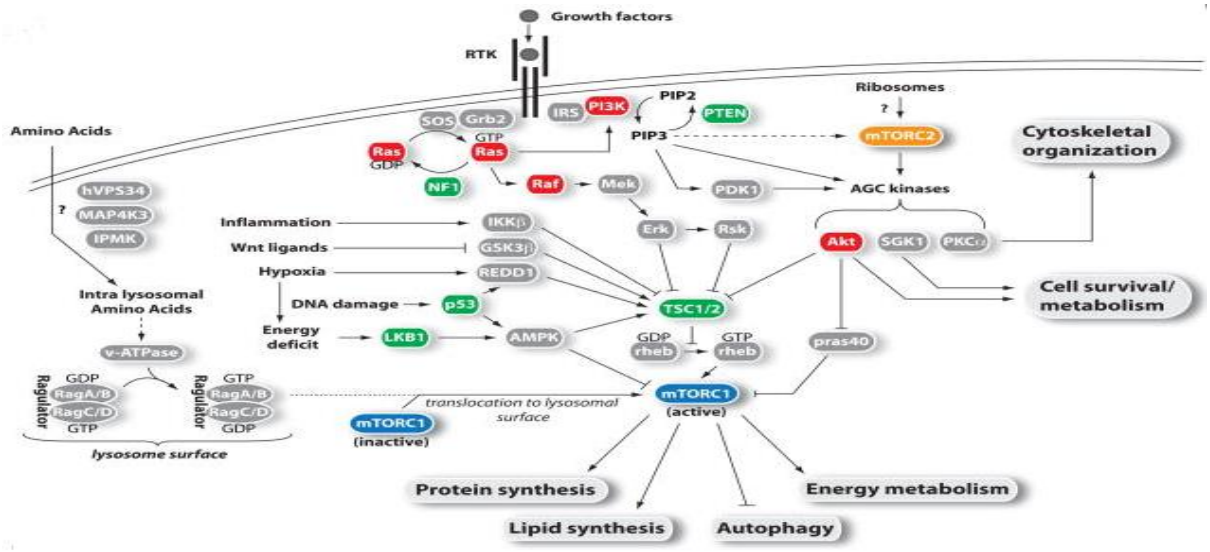


Fig 13: mTOR signaling pathway at a glance, showing multiple factors that lead to the activation of mTORC1as well as the downstream biosynthetic processes related to the activation of mTORC1.

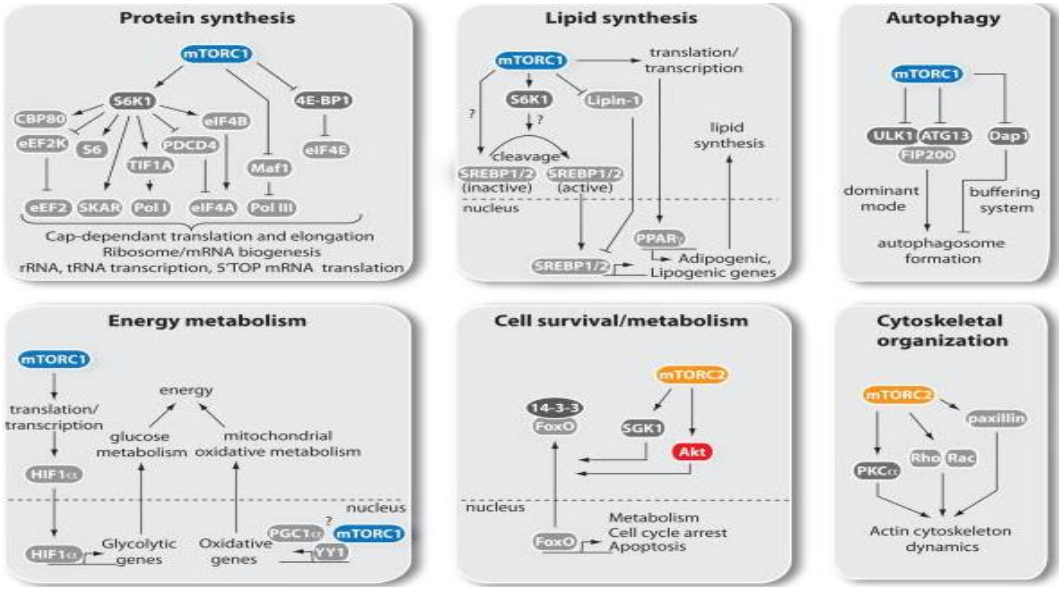


Fig14: the major six biological processes are occurring downstream after activation of both mTORC1 and mTORC2. In this paper, there is a significant focus on the impact of the combination of 177Lu-DOTATOC therapy with everolimus and mTOR drug in patients with neuroendocrine tumors.

1.15.4. mTOR drugs, mTOR kinase inhibitors, and everolimus.

Any compound targeting the same catalytic site of mTOR as ATP can inhibit the kinase-dependent processes of both mTORC1 and mTORC2. Almost all non-rapalog mTOR inhibitors were designed for the inhibition of class-I PI3Ks in order to limit their regulatory activity on

mTOR. However, rapalogs are specific in their inhibitory activities in that they only target mTORC1. Drugs used as mTOR inhibitors are generally the derivatives of rapamycin, and they include Sirolimus and everolimus (Populo, Lopes and Soares, 2012). Rapamycin's mechanism of action includes the formation of a complex with immunophilin FKBP12 and the subsequent binding of the complex exclusively to mTORC1. This event blocks the recruitment of substrates to the mTOR kinase domain leading to the inhibition of pathways related to enzymatic activities of mTORC1.

All the derivatives of rapamycin act in a similar version with regard to inhibition of mTORC1 leading to their therapeutic effects (Shimobayashi and Hall, 2014). Everolimus inhibits mTOR leading to downregulation of such cellular processes as cell motility, protein transcription and synthesis, cell proliferation, cell survival, and cell growth. During cancer development and progression, these processes are usually upregulated and rapid leading to uncontrolled cellular growth that is pathological. As a result, administration of everolimus in individuals suffering from cancerous growths benefits them by limiting such growth to relief or manage the associated symptoms (Ducker, 2013). When administered at sub-nanomolar concentrations, everolimus inhibits up to 50 percent growth, reduces the VEGF/HIF-1α expression, and inhibits human umbilical vein endothelial cells proliferation. These activities are popular targets for antitumor therapies and have allowed for the approval of everolimus by FDA after a spectrum of clinical trials. Nevertheless, its use is associated with several adverse side effects including a headache, fever, diarrhea, edema, stomatitis, abdominal pain, nausea, edema, fatigue, and rash (Houghton, 2010).

1.16. Electron Paramagnetic Resonance

Radiopharmaceuticals are used to carry unstable radionuclides close to target cells. The decay of the radionuclide causes the formation of radicals and, hopefully, to cancer cells death. However, little or no studies are present in literature investigating the radiopharmaceuticals irradiated by the radionuclide decay. In particular, after the decay, the radiopharmaceuticals are

irradiated and could decompose giving radicals. To the best of our knowledge, no information is present in literature on this topic. Nevertheless, since radiopharmaceuticals are close to receptor and thus, to the cell wall, the characterization of the radicals formed could shed light on mechanism of cell radical damage. In fact, after the nuclide decay, most of radicals are formed in the most abundant medium (water) leading to highly reactive species but also very short lived. Instead, radicals stemming from the drug skeleton, could be of intermediate stability and thus they could have sufficient time to diffuse and interact with cell components. For this reason, we decided to study the effect of irradiation on radiopharmaceuticals studied during this thesis. The first attempt was made by including 111-Indium with DOTATOC molecule. Since radicals are short lived at ambient temperature, the solutions were frozen and kept at liquid nitrogen temperature up to a complete decay of 111-Indium (i.e. the emitted radiation was negligible with respect to natural environmental radiation). However, since samples have been kept at low temperature for one month, the radicals observed were mainly peroxyl radicals.

1.15. Aims/objectives of the study.

The over-all objective of the study is to explore the targeted radionuclide therapy using peptide-based radiopharmaceuticals. In this objective, the study seeks to explore the pre-clinical as well as the possible clinical applications peptide-based radiopharmaceuticals in relation to targeted tumor therapy with reference to such perspectives as the patient's quality of life, overall survival, and tumor response. However, one of the specific objectives of the study is to determine the efficacy of a neuroendocrine tumor therapy involving the intravenous introduction of 177Lu-DOTATOC to patients in multiple cycles in combination with the mTOR drug (everolimus) regimen. Another specific objective of the study is to determine both the immediate and the long-term complications of inoperable and/or metastatic neuroendocrine tumors after taking the patient through a course of targeted radionuclide therapy using intravenous radiolabeled 177Lu-DOTATOC in combination with the mTOR drug (everolimus). In addition, the paper also has an objective to determine the electron paramagnetic resonance properties exhibited by radiopharmaceuticals used

during the combined therapy. An additional specific objective of this study is to make an evaluation of the affinity of somatostatin receptors with regard to 177Lu-DOTATOC as well as the pharmacodynamics/pharmacokinetic characteristics of this treatment regimen approach (Fani, Maecke and Okarvi, 2012).

1.17. The significance of the study.

This study is significant because, at its completion, it will generate significant and useful information for various applications. One of the significance of this study is that it will reveal new applications of 177Lu-DOTATOC as a radiopharmaceutical for targeted radionuclide therapy. By carrying out a complete phase-I trial for 177Lu-DOTATOC, vast information will be availed to help determine the suitability of this somatostatin analog as a potential agent for the treatment of neuroendocrine tumors. Another significance of this study is that it will generate important information with regard to the combined therapy of mTOR drug (everolimus) and 177Lu-DOTATOC for patients with inoperable and/or metastatic neuroendocrine tumors. Since both the two agents have significant anti-tumor activities, this study will reveal whether their combined use for the same purpose carries any synergistic effect (Fani, Maecke and Okarvi, 2012).

Another significance of this study is that it would open new windows of prospects of the regimen therapy approach. For example, if the study proves that the regimen to be highly efficacious and with limited adverse effects it would be possible to carry out further studies in relation to its clinical use for the treatment of neuroendocrine tumors. Ultimately, a better approach to the treatment would be accomplished. However, if the regimen fails to meet the threshold of the phase-I clinical trial, an opportunity to explore improvements would arise. In this case, the research community could explore such aspects as the preparation of the radioisotope, as well as the complexation process, to make improvements and reduce impurities before taking the refined radiopharmaceutical through another study with the aim of re-exploring its applicability as an efficacious and safe radiopharmaceutical agent for targeted radionuclide therapy for neuroendocrine tumors (Zukotynski, Jadvvar, Capala, and Fahey, 2016).

1.18. The hypothesis of the study.

A pre-requisite for consideration of this study is to demonstrate the presence of somatostatin receptors on the tumor. Using immunohistochemistry as well as radioiodinated analogs of somatostatin, both in vivo and in-vitro, somatostatin receptors were indicated to be highly expressed in neuroendocrine tumors. The therapy hypothesis was that receptor binding peptides using a bifunctional chelating agent (BFCA) labeled with positron emitter (Ga-68) could visualize receptor-expressing tissues as an imaging technique. Therefore if labeled with beta emitters (Lu-177) these peptides have the potential to irradiate somatostatin receptor expressing tissues, for example, the neuroendocrine tumor cells.

1.19. Summary of the experimental design.

This study involves synthesizing and investigating the performance of labeled peptide-based radiopharmaceuticals for nine patients with histologically confirmed neuroendocrine tumors. Each of the subjects would receive four cycles of (100mCi) 177Lu-DOTATOC in addition to everolimus for therapy. Additionally, the study is a phase-I trial. During the study, 68Ga-DOTATOC imaging would be performed at least three times. The first 68Ga-DOTATOC imaging would be performed before the commencement of the study while at least one image would be taken during and at the end of the treatment, respectively. The image results would be used to make recommendations of the dosage of everolimus and 177Lu-DOTATOC to be used in the phase-II study. Additionally, radiopharmaceuticals quality control would be done for every synthesis of radiolabelled peptides.

1.20. Assumptions and limitations of the study.

The primary presumption of this study was that all the nine cases did not have any other somatostatin-expressing pathological condition apart from neuroendocrine tumors. In this case, the study's assumption was that all subjects undergoing the trial did not have a pathological condition expressing somatostatin receptors otherwise it would lead to interferences leading to radio imaging of irrelevant artifacts not related to neuroendocrine tumors. In events of occurrence and subsequent resolution of a somatostatin receptor-positive pathology, the study would be limited by a false-

positive result. Another assumption of the study is that all the recruited subjects did not receive any other form of treatment apart from the one indicated in this project. In this assumption, the researcher understood that accurate results regarding the treatment of the subjects with everolimus and177Lu-DOTATOC could not be achieved if additional therapies were introduced before or during the study. For example, administration of any other anti-tumor therapies during the course would increase recovery from the disease leading to an inaccurate measurement of the effects of the therapy indicated in this research. Another assumption is that age, gender, genetics and other biological factors did not influence the natural progression of metastatic neuroendocrine tumors and as a result, they were of little significance with regard to the typical course of the therapeutic effects of everolimus in combination with 177Lu-DOTATOC. As a result, these factors would cause little deviations of the normal therapeutic effects of this new regimen approach.

1.21. Statement of the problem.

Somatostatin analogs have been established to produce high-quality images when they are complexed with radionuclide tracers. In particular, when somatostatin analog octreotide is radiolabelled with 68-Ga, it produces relatively high quality images for somatostatin scintigraphy. Besides, the substitution of the radionuclide extends the importance of somatostatin analog octreotide to the application as a therapeutic agent for neuroendocrine tumors (NETS). One of the alternative radionuclides that are applicable as a therapeutic agent for targeted radionuclide therapy of neuroendocrine tumors is Lu-177. Most studies of the importance of Lu-177 as an important targeted radionuclide therapeutic agent have majorly placed emphasis on the application of Lu-177 as a component of DOTATE and not as a component of DOTATOC. As a result, a research break exists in determining the importance of Lu-177 linked DOTATOC in relation to the treatment of metastatic neuroendocrine tumors.

In the same vein, mTOR drugs have been demonstrated to exhibit anti-tumor effects, with rapamycin (everolimus) being the first drug of this family to get FDA authorization for use in clinical management of tumors. mTOR drugs (everolimus) are used in conjunction with

radiolabelled somatostatin analogs. However, there exist a gap in determining where the use of Lu-177 radiolabelled DOTATOC in combination with mTOR (everolimus) drugs could produce similar results as to the use Lu-177 radiolabelled DOTATOC alone. Besides, the immediate, as well as the long-term complications of targeted radionuclide therapy for patients with inoperable and/or metastatic neuroendocrine tumors after treatment with everolimus and the intravenously administered 177Lu-DOTATOC in multiple cycles, have not been studied, and most oncology practitioners cannot foretell such events. Moreover, the efficacy of this treatment regimen has not been studied through logical approaches, for example, clinical trials. Furthermore, by being a new approach to the treatment of neuroendocrine tumors, there is a need for evaluating the receptors affinity as well as its pharmacodynamics/pharmacokinetic characteristics.

2.0. Chapter two: Chemistry of radionuclides.

2.1. Coordination chemistry.

2.1.1. Historical background of coordination chemistry.

Coordination chemistry is not as old as such other branches of chemistry as organic chemistry and inorganic chemistry, which date back to several centuries. Through the available literature, the date of discovery of the first coordination compound is difficult to determine, but Prussian blue is probably the first compound to have landed in the records. Prussian blue ($KCN \cdot Fe(CN)_2 \cdot Fe(CN)_3$ was first developed by Diesbach in the early 18^{th} century. However, the discovery of hexaamminecobalt (III) chloride in 1798 by Tassaert marked the clearest date for the beginning of coordination chemistry. After the discovery of hexaamminecobalt (III) chloride in 1798, considerable interest in coordination chemistry emerged as a result of the unique characteristics shown by this chemical. Tassaert particularly conducted a series of experiments whose findings could not be explained using the then available chemical theory (Widiarini, 2016).

These experiments aimed to establish how seemingly stable compounds could combine to form a more stable compound. Some of the other early contributors to the early coordination chemistry include Jons Jacobe Berzelius, Dmitri Mendeleyev, and Alfred Werner.

Jons Jacobe Berzelius's (1779-1848) work led to the development of the two-component theory as well as the discovery of three elements, namely silicon, thorium, and selenium. On the other hand, Dmitri Mendeleyev (1834-1907) contributed to the world of coordination chemistry by constructing the period table to classify different elements based on their chemical and electronic characteristics after vast analytical thoughts. However, the enormous contribution was made by Alfred Werner (1866-1919) who brought about the link between organic and inorganic chemistry. In his coordination theory, presented in 1892, Alfred Werner suggested that singly occurring atoms or ions form the central positions where some other atoms, compounds, molecules or ions are arranged in simple geometric orders (Widiarini, 2016). In this proposal, Werner stated that a pattern has several atoms grouped around a second valence (coordination number) and thus explaining the

reason for the existence of some compounds in two forms rather than three. This theory was sufficient in the explanation of several amminecobalt (III) chlorides in their reactions with silver ions to form equal ratios of solid silver chloride and therefore also explaining their lack of reactivity with acids. In his explanation, Werner observed that in these compounds, chloride was tightly bound to chloride ions through chloro ligand or loosely bound to chloride counter ion. Werner set the stage for the modern coordination chemistry by proposing that in any coordination complex, the peripheral ions, molecules, and atoms (L) are bound to the central atoms (M). Coordination complexes include compounds from both organic and inorganic origins, and they revolve around such elements of the periodic table as the lanthanoids, Group 12 elements, and transition elements. On the other hand, the ligands are classified into six groups depending on the number of donor atoms they contain. Most of the common ligands are of amine origin, but polydentate ligands are also common. Polydentate ligands are generally referred to as chelating agents, and some of the common chelating agents available in coordination chemistry include EDTA, DOTA, and DOTATATE, amongst others (Lundberg, 2006). The emergence, as well as the advancement of coordination chemistry, has resulted in the development and characterization of coordination compounds that are discussed in the following section of this chapter.

2.1.2. Coordination compounds.

Coordination compounds results from chemical reactions involving elements (of three categories, namely group 12 elements, lanthanides and transition elements) and Lewis bases. They are a group of compounds in which the metallic central atom is surrounded nonmetal groups or atoms referred to as ligands that are joined to the central atom by chemical bonds. Some of the naturally occurring coordination compounds include enzymes, pigments, dyes, chlorophyll, hemoglobin, and vitamins. In this case, the central metallic component is referred to as a Lewis acid, but the surrounding groups (ligands) are referred to as the Lewis Bases, and the number of ligand donor atoms describe the coordination number of the coordination compound in the question (Charlesworth, 2004).

2.1.3. The coordinate/dative bond.

Through the contribution of Alfred Werner, the nature of existence of coordination compounds and secondary valence was partly explained. So far, there are three basic theories that describe coordinate bonds and they include the crystal field theory, the molecular orbital theory as well as the valence bond theory. The valence theory was developed by Linus Pauling, and it explains the structure as well as the magnetic characteristics of metal complexes, which by extension also describe the chemical as well as the physical properties of coordination compounds. It seeks to explain the interaction of dissociated atoms with regard to the formation of chemical bonds in a molecule. According to the valence theory, every ligand contributes electrons resulting in the formation of a coordinate covalent bond. When an appropriate combination of the atomic orbitals of the metals is blended, they give a novel set of orbitals referred to as hybrid orbitals. If the case is of six-coordinated systems, there is involvement of such hybrid orbitals as the s, p_v, p_x, d_z^2, d_x^2-$_y^2$ and p_z atomic orbitals (Widiarini, 2016). From this combination, the resulting six d^2sp^3 or sp^3d^2 hybrid orbitals are pointed towards the corners of an octahedron. A good illustration of this concept can be seen in $[CoF_6]^3$ where the d orbitals have the same principal energy level compared to p and s orbitals. In this case, if the complex formed involves $nsnp^3nd^2$, it is referred to as an outer-orbital complex because of its use of outer d orbitals to form the complex. However, $[Co(NH_3)_6]^{3+}$ involves d orbitals of significantly lower energy level compared to p and s orbitals. As a result, a complex of $(n-1)d^2nsnp^3$ type is referred to as an inner-orbital complex because of its use of inner d orbitals.

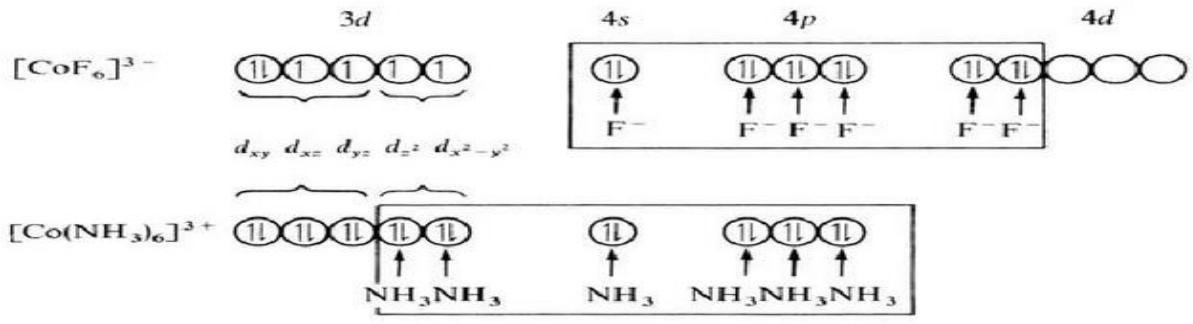


Fig 16: representations of $[Co(NH_3)_6]^{3+}$ and $[CoF_6]^3$ based on the valence theory.

On the other hand, the crystal field theory describes coordination compounds by describing the transition metal compounds behavior. It explains the influence of static electric field of anions on the degeneracies of the states of electron orbitals. It postulates that the positive cations of metals and the negatively charged anions of ligands attract each other leading to the interaction observed between any transitional metal and ligand (Widiarini, 2016). Unlike the valence bond theory which describes these compounds on the basis of covalent bonding, the crystal field theory explains the coordination compounds on the basis of ionic bonding models, where it explains the metal-ligand bonds as the products of the attraction between the negatively charged ligands and the positive metal ions. Additionally, under the crystal field theory, it is more difficult to visualize the 3D formations of complexes compared to the valence bonding theory. However, one of the most successful uses of the crystal field theory is the description of the colors of coordination compounds, particularly the transition metal compounds involving coordinate bonding. In this case, the transitional metal complexes involve small energy gaps between nonequivalent d orbitals and thus it is possible to excite the electrons in the lower energy levels to the higher energy levels by the visible light spectrum. As a result, these complexes appear colored. Moreover, the molecular orbital theory combines both the ionic and the covalent character of chemical bonds to describe chemical as well physical properties of coordination compounds. Molecular orbital theory first determines the positions of atomic nuclei before defining the orbitals surrounding the nuclei (Widiarini, 2016).

2.1.4. Nomenclature of coordination compounds.

Prior to Werner's contribution to coordination chemistry, through coordination theory, it was difficult to develop a comprehensive nomenclature for these compounds. However, the coordination theory paved the way for the description of the coordination compounds either as salts or nonionic complexes, which later made it possible to name these elements through a systematic scheme (Widiarini, 2016). The original systematic coordination compounds nomenclature system involved the giving salts two-word names and one-word names for nonionic compounds. For example, $[PtCl_2(NH_3)_2]$ was referred to as dichloroammine-platino while $[Co(NH_3)_6]Cl_3$ was referred to as

hexamminecobalti chloride, to show their nonionic and salt natures, respectively. Under this system, such suffies as –a, -i, -e, and –o, were used to designate such respective oxidation states of metals as +1, +3, +4, and +2. This system has so far been replaced by the Stock System, which uses Roman numerals and parentheses in order to indicate the oxidation states of the metals. Through this system, hexamminecobalti chloride ($[Co(NH_3)_6]Cl_3$) is named as hexaminecobalt(III) chloride, while dichloramine-platino ($[PtCl_2(NH_3)_2]$) is named as diamminedichloro-platinum(II) (Widiarini, 2016). The use of this system as recommended by the International Union of Pure and Applied Chemistry is outlined in the following guidelines.

1. When listing ions, the cation should be named first before naming the anion – just like when naming any other salt.
2. For nonionic or molecular complexes, one name is used.
3. When naming ligand, the positive ligands end in –ium while the negative counterparts end in 0-.

   For example, $NH_2NH_3^+$ (hydrazinium) and CH_3COO (Acetato).

   Rule three applies to all neutral ligands, with exceptions to such molecules as water, carbon monoxide, nitric oxide, and ammonia. For example, Caronyl (CO), Nitrosyl (NO), ammine (NH_3) and aquo (H_2O)
4. Ligands are mentioned in alphabetical order. For example, ammonium tetrabromo(ethylene-diamine)chromate(III) (NH4[CrBr4($NH_2CH_2CH_2NH_2$)]).
5. Numerical prefixes (di-, tri-, tetra-, etc) may be used before such simple expressions as oxalate, nitro, and bromo. On the other hand, such prefixes as tris-, bis-, and tetrakis-, amongst others, are used before the names of the complexes such as trialkylphosphine and ethylenediamine.

   For example, potassium trioxalatoaluminate(III) ($K_3[Al(C_2O_4)_3]$).
6. When terminating names, anionic complexes end with –ate or –ic while cationic as well as neutral complexes end with the name of the metal.

For example, calcium hexacyanoferrate (II) ($Ca_2[Fe(CN)_6]$) and bis(dimethylglyoimato) nickel (II) ($[Ni(DMG)_2]$.

7. When designating the oxidation state of the central atom, parenthesized Roman numeral are used. A minus sign is used (before the Roman numeral) in the case of a negative oxidation state while 0 is used if the charge is zero.

   For example. Potassium tetracyanonickelate(0) for $K_4[Ni(CN)4]$ and sodium tetracarbonylcobaltate(-I) for $Na[Co(CO)_4]$ (Widiarini, 2016).

8. When indicating the bridging groups, the Greek letter μ is repeated before different kinds bridging groups' names.

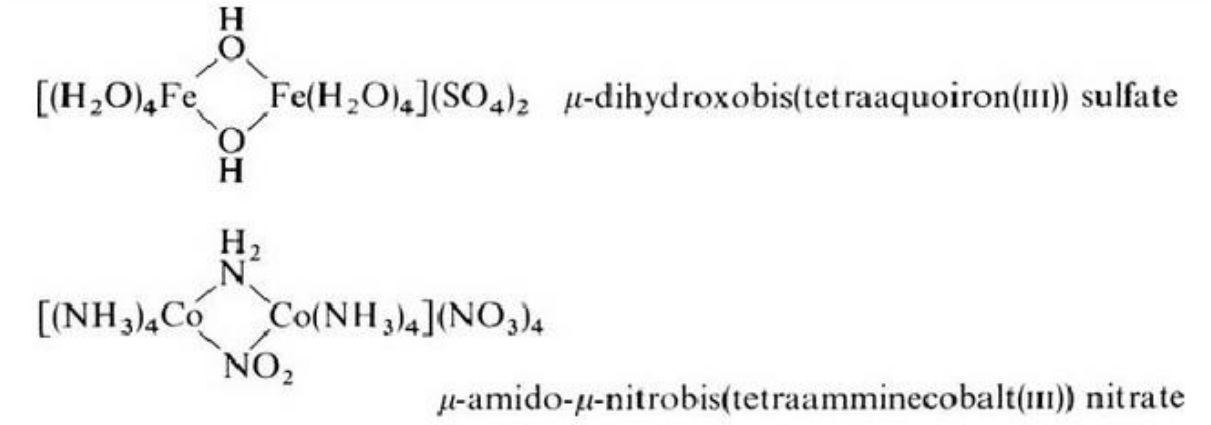


Fig 17: Writing different kinds bridging groups' names.

9. Specific italicized symbols are used to designate different points of attachment of ligands, which are placed in after the group's name.

   For example, ammonium hexathiocyanato-*N*-chromate(III).

10. When indicating geometric isomerism, the term *cis* is used to designate adjacent positions lying 90̊ apart while *trans* is used to designate opposite positions lying 180̊ apart.

11. When indicating optical isomerism, a (+) is used to a planar rotation to the right while a (-) is used to indicate a rotation to the left (Widiarini, 2016).

2.1.5. Structures of a coordination compound.

In a basic explanation, the structure of coordination compounds includes a central atom that is surrounded by ions/molecules referred to as ligands. This structure can be understood by exploring geometry, structural isomerism and optical isomerism of coordination compounds. Coordination complexes exist in several structures depending on the metal in the question. For example, cobalt(III) complexes have an octahedral structure, beryllium complexes for tetrahedral structures, while silver complexes are usually linear. These geometrical structures of coordination complexes, in addition to several other forms, are a product of electronic interactions among different orbitals (Widiarini, 2016). On the other hand, the general structure of these complexes are fixed, but they can be changed through reactions to form stable isomers.

Some of the common forms of isomerism observed in coordination compounds include structural isomerism and stereoisomerism. Structural isomerism results if different chemical bonds exist within the same complex, In this case, there are four isomeric forms that include linkage isomerism, coordination isomerism, hydrate isomerism, and ionization isomerism. Linkage isomerism exist when the complex has ambidentate ligands that are capable of binding at least one place, and NO2 exemplifies it. On the other hand, coordination isomerism exists when complex ions form both the positive and negative ions, and there is a difference of distribution of ligands in the cations and the anions. In addition, ionization isomerism occurs in coordination complexes of the same composition that give different ions when dissolved in water. This form of isomerism usually happens because of the capability of the complex's counter ion to act as a ligand as well. Moreover, hydrate isomers occur if coordination complexes have the same composition but different numbers of solvent ligand molecules and counter ions comprised in the crystal lattice (Widiarini, 2016).

On the other hand, coordination compounds exhibit characteristic facial-meridional isomerism and cis-trans isomerism. *Cis-trans* isomerism is absent in metal complexes whose coordination numbers are 2 or 3, in addition to metal complexes with tetrahedral formations. In these complexes, there are adjacent coordination positions. However, octahedral and squire planar complexes exhibit *cis-trans* isomerism characteristically. On the other hand, optical isomerism involves a phenomenon where the coordination complex is not superimposable with its mirror image. These isomers are optically active in that they cause opposite direction rotations of planes of polarized light (Widiarini, 2016).

2.1.6. Synthesis of coordination compounds.

Preparation and synthesis of coordination compounds are based on several types of reactions. Some of these reactions include substitution reactions, thermal dissociation, photochemical reactions, catalysis, and oxidation-reduction, amongst others. These reactions and related techniques are summarized in in the following subsections. Substitution reactions may involve aqueous solutions, non-aqueous solvents or may occur in the absence of any solvents. In substitution reactions involving aqueous solutions, the method involves mixing a metal salt solution with a coordinating agent in order to form a complex (Widiarini, 2016). Most substitution reactions involving coordinating agents and metal salt solutions are fairly fast, but some are slow, requiring modification of experimental conditions to increase the reaction rate. Substitution reactions involving entering ligands are the easiest to use for the preparation of coordination complexes. In such reactions, the coordinating agent may be added in excess to drive the reaction equilibrium towards the completely substituted complex. This reaction may be the basis for the preparation of [Cu(NH3)4]SO4 complex as illustrated in the following equation.

$$\underset{\text{blue}}{[Cu(H_2O)_4]^{2+}} + 4NH_3 \rightarrow \underset{\text{dark blue}}{[Cu(NH_3)_4]^{2+}} + 4H_2O$$

Fig 18: The formation of [Cu(NH3)4]SO4 complex after mixing copper hydroxide with excess ammonia.

In this reaction, an aqueous solution of copper sulfate is mixed with excess ammonia in order replace the coordinated water with ammonia. This interaction is indicated by the change of color from light blue to dark blue. Complexes are also prepared using non-aqueous solutions primarily in reactions where the ligand is insoluble in water or if the metal ion has a large affinity for water. Some of the common cations that exhibit high affinity for water, and that can form strong metal-oxygen bonds, include chromium(III), iron(III) and aluminium(III). If basic ligands are added to aqueous solutions containing any of these metal ions results in the formation of gelatinous hydroxide precipitates instead of the respective ligand-containing complexes (Widiarini, 2016). These gelatinous hydroxide precipitates contain intact metal-oxygen bonds and broken oxygen-hydrogen bonds. When these gelatinous hydroxide precipitates are hydrated, the metal ions behave like protonic acids. An example of such a reaction is shown in the following equation.

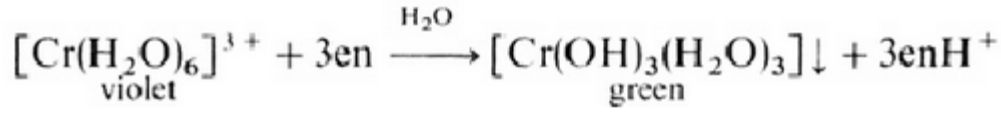


Fig 19: The formation of protonic acid-like metal ions.

If a non-aqueous solvent and an anhydrous chromium salt are used the reaction results in the formation of the required complex as demonstrated in the following equation.

Fig 20: The formation of a complex from a non-aqueous solvent and salt.

Among the most widely used non-aqueous solvents is dimethylformamide, and it makes it possible to make preparations of *cis*-$[CrCl_2(en)_2]Cl$ as illustrated in the following equation (Widiarini, 2016)

. A non-aqueous solvent may also be required for use in reactions involving ligands that insoluble in water, by adding the ligand in a water-immiscible solvent before adding this non-aqueous solution to concentrated aqueous solution of the central metal ion. A complex of $[Fe(bipy)_3]Cl_2$ as illustrated in the following equation.

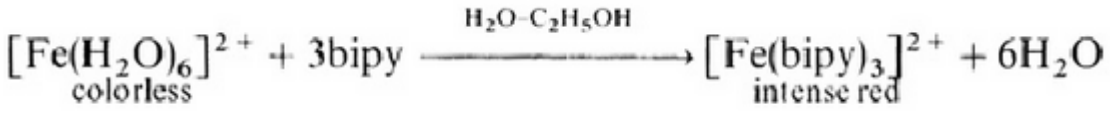


Fig 21: The formation of a $[Fe(bipy)_3]Cl_2$ complex.

Coordination compounds may also be prepared without involving any solvent in a substitution reaction involving an anhydrous salt and a liquid ligand. For example, most ammine complexes involve the addition of a metal salt to liquid ammonia before evaporating the mixture to dryness, which takes place at room temperature as ammonia's boiling point is -33℃ (Widiarini, 2016). The reaction results in the formation of a dry residue – a pure metal ammine. For example, when preparing $[Ni(NH_3)_6]Cl_2$ Nickel chloride is mixed with liquid ammonia and the mixture allowed to evaporate to dryness to form the required complex.

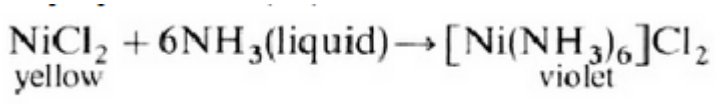


Fig 22: the preparation of $[Ni(NH_3)_6]Cl_2$ complex.

Another reaction exploited when preparing coordination compounds is the thermal dissociation, which is a substitution reaction involving solid state reactants. In this case, if the temperature of the solid complexes is elevated, there is a loss of volatile coordinated ligands followed by a takeover of their coordination sphere by complex's anions. When the temperature involved in the reaction is elevated, there is the liberation of coordinated water from the crystallized aquaamminemmetal complexes leading to the formation of a new product (Widiarini, 2016)

. Such a reaction as this is the basis for the preparation of halogenoamminemetal compounds as demonstrated in the following equation.

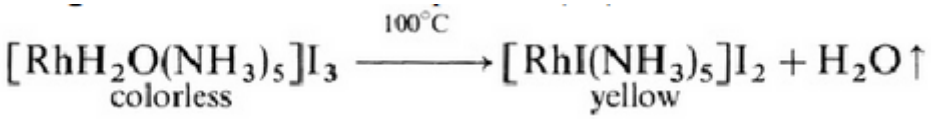


Fig 23: The preparation of halogenoamminemetal complexes.

A similar process is also used to prepare acidoamminemmetal complexes by liberating ammonia, and ammines from metal amines (Widiarini, 2016). For example, when preparing trans-$[PtA_2X_2]$ $[Pt(NH_3)_4]Cl_2$ is heated at about 250℃ leading to the liberation of ammonia gas as a byproduct as shown below.

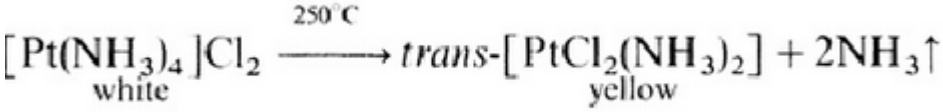


Fig 24: the preparation of trans-$[PtA_2X_2]$.

Coordination compounds are also involved in photochemical reactions, which is often exploited in the preparation of coordination complexes. In this reaction, specific wavelengths induce a reaction by imparting energy to a coordination compound. For example, exposure of light to stable Nitro results in the formation of an unstable nitrito complex, indicating the importance of photochemical reaction in the synthesis of metal ammine complexes as shown in the following equation.

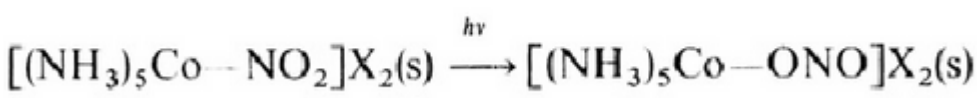


Fig 25: The preparation of metal ammine complexes by photochemical reactions.

For metal oxalates, exposure to the light results in the liberation of carbon dioxide and the metal in a lower state of oxidation (Widiarini, 2016)

. For example, the synthesis of coordinatively unsaturated platinum involves a similar reaction as the one described in this section, and it has been illustrated in the following equation.

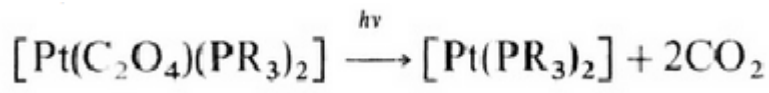


Fig 26: The preparation of coordinatively unsaturated platinum complexes.

When metal carbonyls are irradiated with ultraviolet light wavelengths, there is a loss of carbon (I) oxide leading to the replacement of carbon monoxide as illustrated in the following equation (Widiarini, 2016).

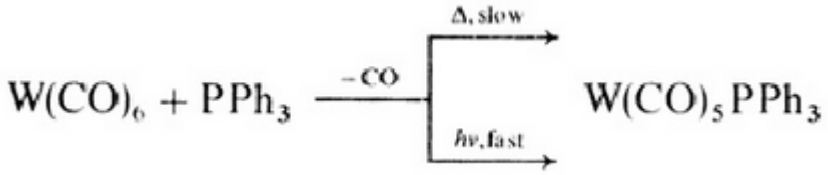


Fig27: The effects of UV radiation on metal carbonyls.

Coordination complexes are also involved in oxidation-reduction reactions, and this reaction type may be exploited during the preparation of some complexes. For example, the oxidation-reduction reaction is used in the preparation of cobalt (III) complexes with the starting material being a cobalt (II) salt. Simple cobalt salts have an oxidation state of 2, but when complexed, the stable cobalt salts gain an oxidation state of 3 after becoming coordinated with specific ligands (Widiarini, 2016). The best pathway to the preparation of cobalt(III) complexes is through cobalt(II) complexes because of their more readily reactions compared to reactions starting with cobalt(III) complexes. In this case, the reaction path involves a cobalt (II) salt and a ligand to form a cobalt(II) complex, which is further oxidized to form a corresponding Cobalt(III) complex. This process is illustrated in the following equations.

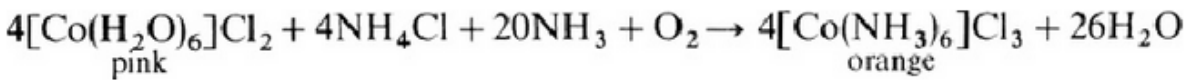


Fig 28: The formation of cobalt (II) complex.

$$\underset{\text{pink}}{[Co(H_2O)_6]Cl_2} + 6NH_3 \rightarrow \underset{\text{rose}}{[Co(NH_3)_6]Cl_2} + 6H_2O$$

$$4\underset{\text{rose}}{[Co(NH_3)_6]Cl_2} + 4NH_4Cl + O_2 \rightarrow 4\underset{\text{orange}}{[Co(NH_3)_6]Cl_3} + 4NH_3 + 2H_2O$$

Fig 29: Oxidation of cobalt (II) complex to form a cobalt (III) complex.

Often, air is used as the oxidation agent when preparing cobalt (III) complexes, but other oxidizing agents may also be employed. However, only a few of the available oxidizing agents are suitable for the preparation of cobalt(III) complexes. For example, although potassium dichromate and potassium permanganate are capable of oxidizing cobalt(II) to form cobalt(III) their reactions result in the formation of undesirable mixtures and thus making them unsuitable for use when preparing pure complexes (Widiarini, 2016). Some of the convenient oxidizing agents used in the preparation of such complexes include hydrogen peroxide, oxygen, and PbO_2. However, when PbO_2 is used, an insoluble residue, which is ultimately removed by filtration, is formed.

Reduction reactions are also used in the preparation of coordination complexes, but this approach is less common compared to oxidation reactions. However, reduction of the central metal to form a complex is limited by the formation of a reactive complex that must be kept in an oxygen- and moisture-free atmosphere (Widiarini, 2016). Nevertheless, by applying special precautions, it is still possible to prepare complexes with low oxidation state central metals. Such a reaction as the one described here may be illustrated by the following equation.

$$\underset{\text{yellow}}{[Fe(CO)_5]} + 4KOH \rightarrow K_2\underset{\text{colorless}}{[Fe(CO)_4]} + K_2CO_3 + 2H_2O$$

Fig 30: The preparation of complexes with low oxidation state central metals.

Some reactions involved in the preparation of coordination complexes require the use of catalysts in order to increase the reaction rates. In this case, there exist two types of catalysis used in these reactions, and they include heterogeneous catalysis and homogeneous catalysis. Homogeneous catalysis takes place if the reactants and the catalyst are in the same phase while heterogeneous catalysis takes place if the reactants and the catalysts have different phases. These

types of catalysis are used in a different instance. For example, heterogeneous catalysis is applied when preparing $[Co(H_3)_6]Cl_3$ (Widiarini, 2016). When preparing $[Co(H_3)_6]Cl_3$ a mixture of excess ammonia, ammonium chloride, and aqueous cobalt(II) chloride is exposed to the air for oxidation. This step is followed by addition of excess hydrochloric acid as an acidifying agent to form $[Co(NH_3)_5Cl)Cl_2$ and a small amount of $[Co(H_3)_6]Cl_3$. However, with the addition of charcoal as a catalyst the product is almost exclusively $[Co(H_3)_6]Cl_3$. In the latter reaction, the oxidation happens via a bridged intermediate. The ultimate product of this reaction is shown in the following equation.

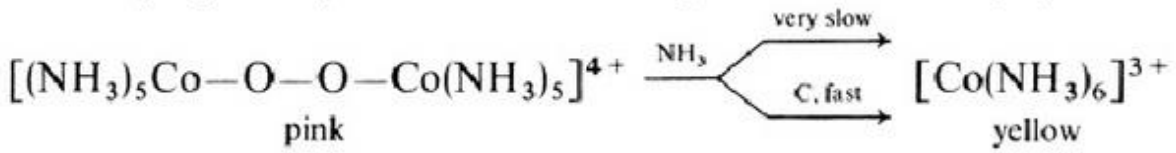


Fig 31: The catalytic effects obtained when preparing $[Co(H_3)_6]Cl_3$.

On the other hand, some platinum(IV) require homogeneous catalysis during their preparations to raise the reaction rates. For example, when preparing platinum (IV) complexes platinum (II) is used as a catalyst. In this case, the preparation of trans-$[PtCl(SCN)(NH_3)_4]^{2+}$ requires platinum (II) catalysis as shown in then the following equation.

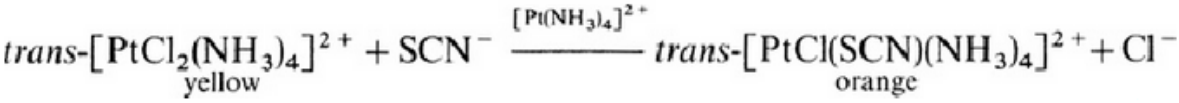


Fig 32: Platinum (II) catalysis.

Coordination compounds are also involved in substitution reactions in the absence of metal-ligand bond cleavage, where the formation of the complexes does not necessitate the breakage of any metal-ligand bond. For example, when preparing $[CoOH_2(NH_3)_5]^{3+}$ from $[CoCO_3(NH_3)_5]^{+}$ there is a breakage of carbon-oxygen bond to form carbon dioxide without interfering with the metal-oxygen bond (Widiarini, 2016). This reaction is illustrated in the following equation.

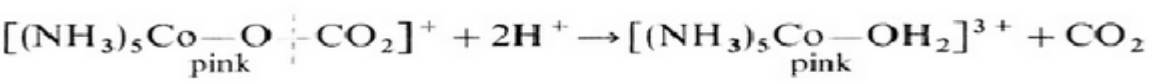


Fig 33: the formation of complexes without the breakage of the metal-oxygen bond.

Some reactions of coordination compounds favor the preparation of *cis-trans* isomers through mixing or stereospecific synthesis. Preparation of *cis-trans* by mixing isomers results in a heterogeneous mixture that requires a subsequent separation. However, this method is not widely used as the alternative stereospecific synthesis results in the production of a single product efficiently. This method exploits the *trans*-effect, for example in platinum (II) complexes. *Trans*-effect is the phenomenon that causes the replacement of certain groups of the ligands in the square plane. The *trans*-effect is exploited in the preparation of most platinum(IV) complexes as trans isomers (Widiarini, 2016).

2.1.7. Stability and stability constants of coordination complexes.

The thermodynamic and kinetic properties metallic ions in the coordination compounds are dependent the number as well as the nature of the ligands surrounding it. The two factors are the primary determinants of the stability of coordination complexes, but it can also be influenced by three other factors, including the size and charge of the metal ion, the crystal field effects and the class of the central metal. With regard to the size and the charge of the central metal ion, stability of the complex is influenced by electrostatic attraction and therefore small sized metal ions with a large charge of metal ion produces significantly stable complexes. In this case, the stability of the complexes increases with the increase in the charge-to-radius ratio of reference metal ion (Widiarini, 2016).

The stability of the complexes is also influenced by the crystal field stabilization energy. In this case, the crystal field effects influence the order of stability of complexes involving the first-row transition metals. Then again, with regard to the class of the central metal class a, and class b metals exhibit significant differences in the stability of their respective metal complexes. Class a comprises of highly electropositive metals – including Ca, Al, Na, Ti, Fe and lanthanides – while class b metals include metals that are less electropositive metals that include Rh, Pb, Hg, Pt, and Pd. Complexes of class a metals are more stable than those formed by class b metals, and they involve ligands with such donor atoms as O, F, or N. On the other hand, the stability of class b metals is a

product of the electron density transfer to the ligand from the metal by sigma-bonding as well as the covalent involvement of metal-ligand bonds. In a complex, ligands make significant contributions to the stability of the complex and it is influenced by four factors, including base strength, chelate effect, steric strain, and chelate ring size. With regard to the base strength, ligands tend to form stable complexes with the class a metal if there is a significant ligand base strength in relation to H^+. On the other hand, the chelate effect influences the stability of the complex in a version characterized by greater stability of a metal chelate when compared to an analogous nonchelated metallic complex (Widiarini, 2016). This influence is demonstrated by the higher stability of $[Ni(en)_3]^{2+}$ relative to $[Ni(NH_3)_6]^{2+}$. By extension, extensive chelation results in the formation of a stable system, and this is exemplified by the significant stability of hexidentate EDTA complexes. On the other hand, the size of the chelate ring, saturated ligands form stable metal chelates when they involve five-membered chelate rings. However, unsaturated ligands result in stable metal chelates especially if they involve six-membered rings. Moreover, large bulky ligands results in the formation of less stable metallic complexes compared to the analogous smaller ligands because of steric factors – steric strain. For instance, $(CH_3)_2NCH_2CH_2N\text{-}(CH_3)_2$ form less stable complexes compared to $H_2NCH_2CH_2NH_2$ due to their differential steric strains. Differences in the steric strains of metal chelates are largely attributed to the metal complex's stereochemistry and ligand's geometry (Widiarini, 2016).

2.1.8. Applications of a coordination compound.

Coordination compounds have a wide variety of applications in industries, pharmaceuticals, and medicine. Among the most exploited and relevant use (in relation to this paper) are medicine and pharmaceuticals. Some of the complexes that have found a wide range of applications with regard to medicine and pharmaceuticals include platinum complexes, arsenic complexes, ruthenium, gallium, bismuth and silver salts. So far there are several cis-platinum compounds used as anticancer agents, with the classic example being cisplatin (cis-$[PtCl_2(NH_3)_2]$). Cis-$[PtCl_2(NH_3)_2]$ was discovered in the 1970s, and it has been demonstrated to treat several cancers, including small

cell lung cancer, ovarian cancer, gestational trophoblastic tumors and germ cell cancers. Besides, this agent is used as a palliation agent for esophageal, head, neck, cervical and bladder cancers. Some other platinum compounds have also been taken through clinical trials as novel anticancer agents (Farrell, 1990). One of such platinum-based anticancer agents is cis-[$PtCl_2(NH_3)$(2-methylpyridine] and it is designed to avoid resistance to cisplatin by circumventing thiol-mediated drug resistance pathways. Another platinum-based anticancer agent that is under clinical investigation is satraplatin (cis-[Ptl(I)(NH3)(cha)]). Other non-platinum metal complexes have also been studied with regard to cancer treatment and have shown similar significances as effective anticancer agents.

Most of the ruthenium compounds studied have shown significant potentials, even though none of them have made it to the clinical applications. For example, ruthenium ammine complexes have been demonstrated to exhibit significant activity, but their low solubility in water have been their chief limiting factor to their testing. The significance of ruthenium complexes with regard to their anticancer activities is attributed to their rich DNA chemistry (Farrell, 1990). When used as a treatment agent for acute promomyelocytic leukemia, arsenic trioxide kills cancer cells by affecting several intracellular pathways of transduction. Another coordination complex used in medicine as a pharmaceutical is Gallium Nitrate, which has been approved as a treatment agent for malignancy-related hypercalcemia. In this case, gallium ions reduce the activity of osteoclasts and thus stopping bone loss as well as the associated high levels of calcium ions in the blood.

Coordination compounds are also used in other realms of pharmaceuticals like antibiotics. For example, bismuth is also used as a component of antiulcer drugs. Bismuth compounds have astringent and anti-acid properties that are exploited in the treatment of different gastrointestinal disorders. They also have bactericidal effects on Helicobacter pylori, and thus when bismuth citrate is combined ranitidine it forms an effective regimen for the management of Helicobacter pylori-related peptic ulcers (Farrell, 1990). Another coordination complex available for use as a treatment agent for bacterial infection is silver. Silver in the form of silver nitrate is used in the treatment of

severe burns as an antibacterial agent in a polymeric material that releases silver ions and thus preventing the infection of the burns with bacteria. Other coordinate complexes that have found applications in the world of medicine include lanthanum carbonate, cold complexes, vanadium complexes, lithium carbonate, titanium complexes and manganese complexes, amongst others (Farrell, 1990).

2.1.9. The role of coordination chemistry in the development of peptide-based radiopharmaceuticals.

2.1.9.1. Peptide-based radiopharmaceuticals.

Peptide-based radiopharmaceuticals comprise of three components that include a chelating agent, a radionuclide, and a peptide. Some of the commonly used chelating agents in the development of radiopharmaceuticals include DOTA, DPTA, and DOTATE, amongst others, while some of the commonly available radionuclides used in cancer therapies include iodine-131, samarium-153, strontium-89, yttrium-90, ruthenium-106, palladium-103, cobalt-60, caesium-137 and iridium-192 (Delgado, 1995). On the other hand, one of the commonly used peptides, in this case, is somatostatin and its analogs. The development of peptide-based radiopharmaceuticals is highly dependent on coordination chemistry. In the following sections, the significance of coordination chemistry in the development of anti-cancer peptide-based radiopharmaceuticals is discussed.

2.1.9.2. Synthesis of peptide-based radiopharmaceuticals (characteristics and challenges).

In human bodies, peptides help with the regulation of cellular processes both in the normal and tumor cells. In modern medicine, the development of the radiolabeled peptide analogs has paved the way for the localization as well as the treatment of tumors in vivo. So far the development of radiolabeled peptides of desired affinity properties has been developed through the combination of techniques resulting from advances in coordination chemistry, bioconjugates, solid phase peptide synthesis, organic chemistry, and phage display techniques. Apart from peptides, other biological carriers available for use in therapies include proteins, antibodies, and small

molecules. However, peptides have a significant advantage over all of them because of their high receptor binding affinity, low molecular weights, and their ease of synthesis (Delgado, 1995). They also have good tumor penetration characteristics, favorable pharmacokinetics, and can be modified using bio-conjugation through simple methodologies. Also, their therapeutic value is also enhanced by their high efficacies when applied in low doses and as a result, they cause a shorter spectrum of side effects in comparison to most of the conventional drugs available in the market. The current rising popularity of peptide-based radiopharmaceuticals can be attributed partly to these advantages.

With peptide-based radiopharmaceuticals, several strategies are available for the enhancement of radiolabeled peptides' bioavailability. Some of these strategies are the introduction of D-amino acids to the biological active sequence as well as the shortening of the sequence of the natural molecule (Delgado, 1995). For example, radiolabeled RGD peptide may be enhanced by multimerization to improve its affinity for the binding alpha-v-beta-3 receptor. Other pharmacokinetic modifications that may be applied in order to enhance the bioavailability of peptide-based radiopharmaceuticals include PGylation, glycosylation, and the introduction of charged amino acids.

Some of the most frequently used elements for the preparation of peptide-based radiopharmaceuticals include radioactive halogens and metals. When labeled with radioactive elements, peptides allow for the targeting of specific molecules for radiotherapy and molecular imaging. Since most metallic radionuclides are capable of forming stable complexes with chelators, it is possible to label peptides with a variety of radionuclides in order to meet specific purposes. Some of the common labeling protocols include the use of bifunctional chelating agents, direct/indirect labeling using prosthetic groups or covalent labeling. Bifunctional chelating agents contain two different moieties that include a functional group for covalent attachment to peptide and a radio-metal complexing chelating unit. On the other hand, a prosthetic group comprises of bifunctional agents with functional groups for the attachment to the peptide by covalent bonding and a suitable fluorination or radioiodination sites (Delgado, 1995).

2.1.10. Coordination chemistry and chelating agents.

2.1.10.1. Coordination chemistry and macrocyclic/polycyclic chelators

Macrocyclic chelators can be synthetic or natural, and they comprise of donor atoms in they cyclic backbones. The donor atoms may also be incorporated in the substituents attached to the macrocyclic backbones. Macrocyclic chelating agents have not less than three donor atoms and at least nine atoms in their rings. The formation of metal complexes with macrocyclic chelating agents happens through coordinate bonds. In general, macrocyclic chelators coordinate strongly with metallic ions whose size have the best match for the cavity of the ring resulting from complexation, and thus forming the best complementary pair (Jamous, Haberkorn, and Mier, 2013). Through this complementarity, the largest bond energies present when all the donor atoms have been utilized fully. As a result, the macrocyclic agent would have a peak selectivity size which determines the size of the metal that best fits the ring cavity leading to the macrocyclic effect – the phenomenon in which small metal ions would fall through the cavity, while the large ones would be too large to be accommodated (Delgado, 1995). The macrocyclic effect as a behavior is determined by investigating the stability constants ratios of the macrocyclic ligand complexes to the analogous acyclic ligands of the same binding sites sets, in a given solvent, in a given cation and at a given temperature (Jamous, Haberkorn, and Mier, 2013). The macrocyclic effect of these cyclic polyaminopolycarboxylic ligands is the basis for the radiometallation of peptides with such radiometals as Cu_{2+}, Y_{3+}, Lu_{3+}, $In_{3+,}$ and Ga_{3+} in order to improve the radiopharmaceuticals' pharmacokinetics. One of the common macrocyclic chelating agents used in complexation of radiometals is DOTA.

The synthesis of DOTA involves a reaction of chloroacetic acid with cyclen 50 under alkaline conditions, in water. DOTA complexes have significantly high stabilities making them suitable chelators for different therapeutic and diagnostic applications (Jamous, Haberkorn, and Mier, 2013). Additionally, DOTA is also a favorable bifunctional chelating agent for use when preparing most of the therapeutic lanthanide radiopharmaceuticals, besides the preparation of stable

complexes with trivalent and divalent radionuclides, for example, $_{64/67}$Cu, $_{86/90}$Y, and $_{67/68}$Ga. The coordination chemistry of DOTA allows for the formation of several species of DOTA-based bifunctional chelators after attaching biomolecules to the DOTA-unit (Delgado, 1995). Such new species of DOTA-based bifunctional chelators formed after the attachment of the biomolecules to DOTA-unit included DOTA-derivatives, active DOTA esters, and protected DOTA forms. DOTA-active esters are prepared by the activation of at least one carboxylic groups, and they are used for the optimization of DOTA-conjugated biomolecules synthesis. Some of such DOTA-conjugated biomolecules include the active ester DOTA-NHS and the DOTA-phenolic active esters (Jamous, Haberkorn, and Mier, 2013). Coordination chemistry also plays an important role when synthesizing Chelator-peptide conjugates. One of the commonly used methods of synthesizing Chelator-peptide conjugates is post-conjugation. This strategy involves first synthesizing protected peptide on a solid phase and the subsequent conjugation of a bifunctional chelating agent to the resin bound peptide (Delgado, 1995).

2.1.10.2. Development of iodine-labeled peptide radiopharmaceuticals.

Peptides used in cancer treatment may be lined with radioiodine via conjugation or electrophilic substitution. In this case, such side chains of the peptide as histidine or tyrosine provide the possibility of high-efficiency electrophilic radioiodine aromatic substitution under mild conditions. This is the direct approach to linking peptides with radioiodine. On the other hand, conjugation of the peptide with radioiodine is used when direct labeling is impossible. In this approach, a radioiodinated prosthetic group is utilized for the conjugation with such functional groups of the peptide as amine, thiol, and aminooxy (Jamous, Haberkorn, and Mier, 2013). Another group of peptide-based radiopharmaceuticals used in cancer treatment and that utilizes coordination chemistry during its development is fluorine-labeled peptide radiopharmaceuticals. When labeling peptides with radiofluorine, direct labeling by nucleophilic substitution is impossible because the reaction requires the elevated temperature to induce radiofluorination and thus destroying the peptidic biomolecules. As a result, the conjugation of fluorine to peptides remains as the only

remaining option where 18-fluorine-labeled prosthetic groups are used to bind to such functional groups as an alkyne, hydrazine, azide groups, amine, and aminooxy.

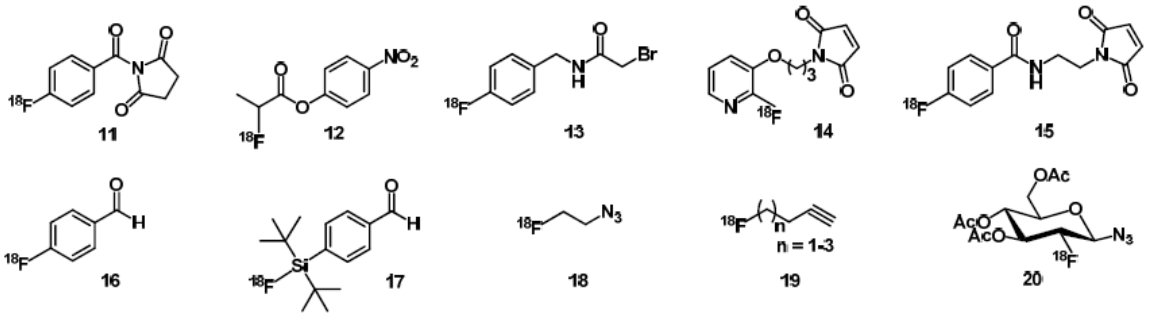


Fig 34: chemical structures of some of the prosthetic groups used in peptide fluorination.

Most 18-Fluorine labeled radiopharmaceuticals have high lipophilicity and as a result, they have a low tumor and unspecific liver uptake (Jamous, Haberkorn, and Mier, 2013). Moreover, one of the most widely used radiolabeled peptide radiopharmaceuticals is ^{99m}Tc. ^{99m}Tc is frequently used for diagnostic applications, and it offers rich labeling chemistry, and it has ideal nuclear physical properties. ^{99m}Tc-complexes used in most radiopharmaceuticals have an oxidation state of +V, and they are prepared from chemically inert generator whose oxidation state is +VII. The preparation involves reduction of the generator using such reducing agents as Zinc, phosphines, $SnCl_2$ and $Na_2S_2O_4$ in the presence of a suitable ligand. Generally, the preparation of peptide-based radiopharmaceuticals using ^{99m}Tc applies the post-conjugation labeling strategy where a bifunctional chelating agent is first attached to the peptide by covalent bonds. This step is then followed by the reduction of $^{99m}TcO_4^-$ with Sn(II) before being completed by the chelating agent. In the end, this scheme results in the formation of technetium complexes whose structure and oxidation states depend on ligands, ligands and the reducing agent (Jamous, Haberkorn, and Mier, 2013). If the tetradentate bifunctional chelating agent used in this case is based on mercaptoacetyltriglycine, N_3S or N_2S_2, and the ligand is tetraamine, the resultant complexes are octahedral. The advantage of using N_4 cores is that it allows for the formation of hydrophilic Tc-complex without the structural influence of isomerism. Another component used when coupling technetium to peptides is hydrazinonicotinic acid (HYNIC), which acts either as a monodentate or a

bidentate ligand. In both cases, the complete coordination of the core of the [Tc]-HYNIC is achieved only after the addition of such acids as nicotinic acid, tricine or EDDA. ^{99m}Tc is may also be linked to peptides by combining organometallic [^{99m}Tc(CO)3] with the core through the ^{99m}Tc carbonyl approach.

2.1.10.3. The role of coordination chemistry of radiometals used in peptide-based radiopharmaceuticals.

Some of the commonly used radionuclides in the development of peptide-based radiopharmaceuticals include Cu, Y, Ga, In, and Zr. Copper(II) radio-ion is the most common of radioactive copper ions present in aqueous solutions. It has been found to have a high plasticity of coordination geometry making it suitable for complexation with multiple chelating agents. Copper(II) radiation's coordination number ranges from 4 through 6, with geometries of octahedral, trigonal bypyramidal, square planar and square pyramidal. AS a result, the square-planar geometry is exploited to design tetradentate chelating agents that complement the high affinity of this geometry where the common donor set comprise two charge-neutralizing anionic oxo, thiolato or amido sites combined with imino or amino nitrogen (Wadas, Wong, Weisman and Anderson, 2012). The coordination numbers of copper(III) radioions are also the driving force behind the use of cyclic hexadentate chelators where they allow the Cu(II)-EDTA to interact with Cu(II) ions along one O-Cu-O axis in a tetragonally-distorted N_2O_4 octahedron. Coordination number five of copper(II) radioions is also exploited to develop five-coordinate Cu(II) complexes, for example, bispidine (3,7-diazabicyclo[3.3.1]nonane).

Coordination chemistry is also credited for the formation more inert Cu(II) complexes in 14-membered N_2S_2 macrocycle compared to other ring sizes. In this complexes, and thus allowing carboxymethyl arms to be appended and complexed with 64Cu(II) and C(II). Another aspect that indicates the role of coordination chemistry in the development of peptide-based radiopharmaceuticals from Cu(II) ions is the radio-copper chelation potential (Wadas, Wong, Weisman and Anderson, 2012). In this regard, some chelating agents form more stable complexes with radio-copper regardless of the size of the ring. For example, dioxocyclan has been observed to

have highly stable complexes because of the influence of their distorted square-planar coordination geometry.

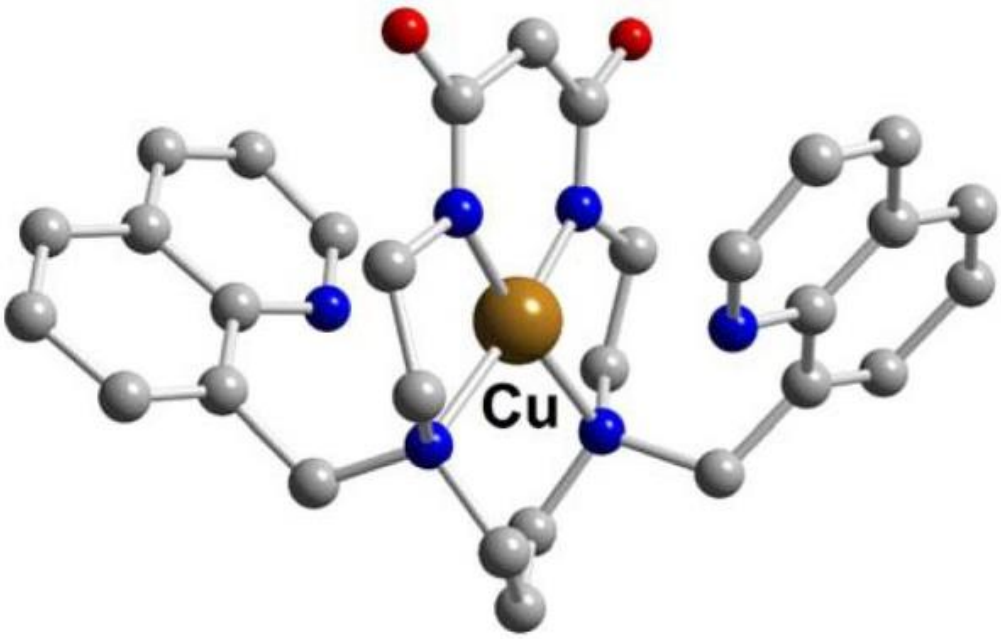


Fig 35: A distorted square-planar coordination geometry.

The coordination chemistry of Gallium (III) also influences the development of peptide-based radiopharmaceuticals in several ways. In the aqueous state, the most prevalent oxidation state of gallium is +3 (Wadas, Wong, Weisman and Anderson, 2012). The ion has a coordination number of 4-6 and an ionic radius of 47-62 pm, and a *pKa* of 2.6 when hydrated. Accordingly, it has a very strong affinity for hydroxide ions and therefore at high pH it tends to demetallate from the complex to form gallate anion ($Ga(OH)_4^-$. The coordination properties of gallium (III) ions are responsible for the interaction of the metal with tetrahydrate ligands and hexadentate ligands in peptide-based radiopharmaceuticals involving radio-gallium. In tetradentate ligands, such iminodiacetic acid derivatives as tetradentate o-hydroxybenzyl provide NO3 donor set in order to complete the distorted octahedral coordination around two cis-coordinated water molecules and Ga(III) in their centers. Additionally, the small size of Ga(III) as well as its low coordination number causes the formation of a distorted square pyramidal GaCl complex after a reaction between GaCl and a bis (aminothiolate) N_2S_2. With regard to hexadentate ligands, a reaction between N2O2S2 donor sites

and N,N'-ethylene-di-L-cysteine (an acyclic hexadentate chelator)results to the formation of a stable complex if Ga(III) is added to the mixture (Wadas, Wong, Weisman and Anderson, 2012). This complex has a distorted octahedral structure that has two carboxylate O's in *trans*-arrangement.

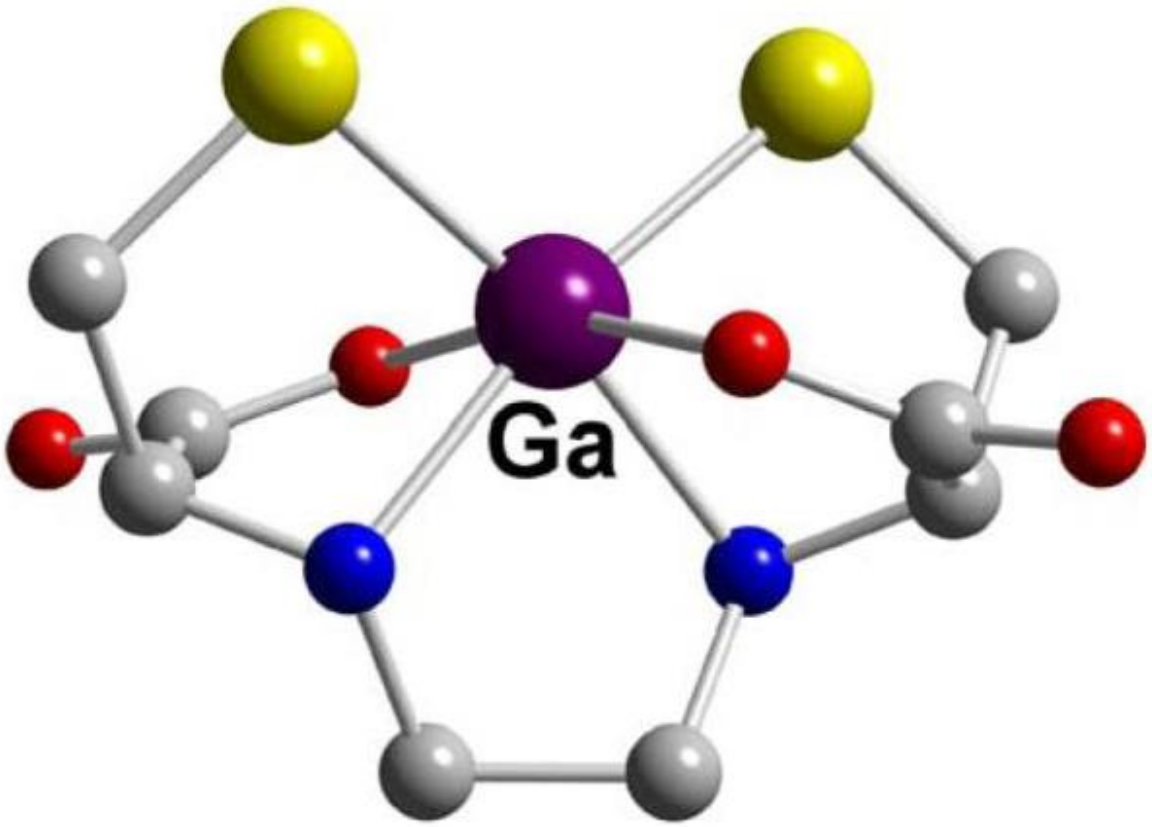


Fig 36: A very stable complex of Gallium (III) and N,N'-ethylene-di-L-cysteine.

Coordination Chemistry of Gallium (III) is also the cause of high stability of several other complexes from such chelates as 1,4,7-triazacylononane (NOTA), and DOTA. NOTA, in addition to it relatives, forms highly stable complexes with Ga(III) as a result of the formation of coordinate bonds as demonstrated by the envelopment of the cation by distorted octahedral N_3O_3 (Wadas, Wong, Weisman and Anderson, 2012). This complex is significantly inactive, and thus it can survive acidic conditions for extended periods.

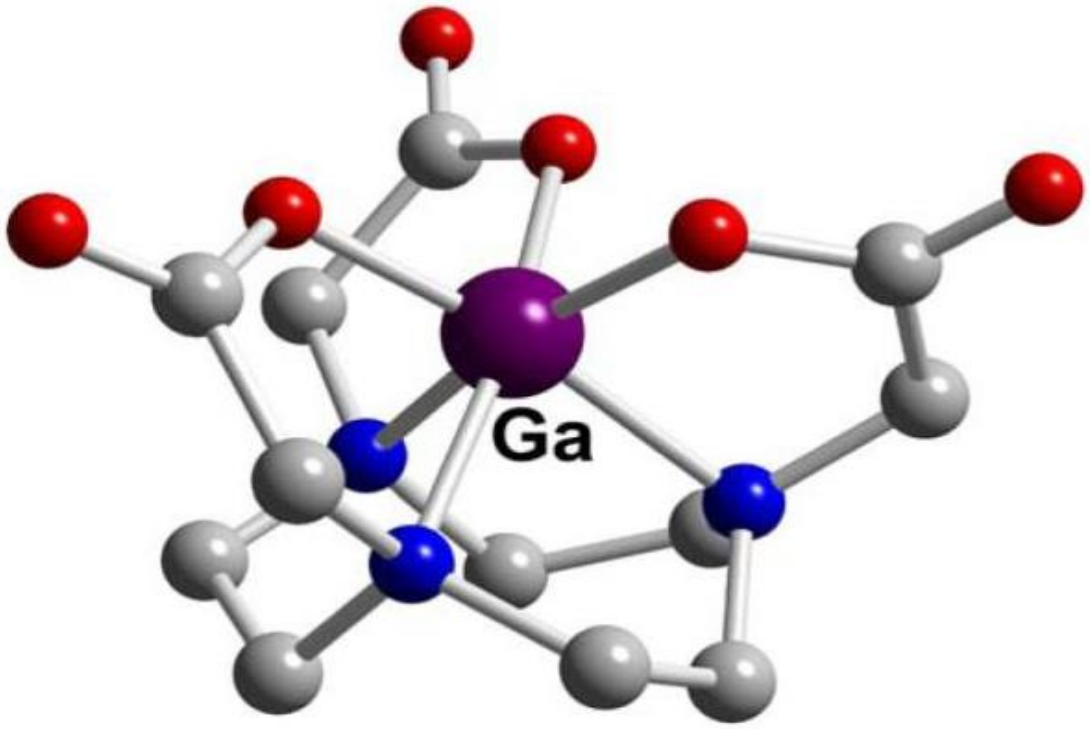


Fig 37: stable Ga-NOTA complex.

On the other hand, the 6-coordination sphere of Ga(III) is saturated by octadentate (DOTA) to form complexes of two different structures, namely di- and monoprotonated structures that are similar to the distorted octahedron coordination of four macrocyclic N's and two cis-carboxylates (Wadas, Wong, Weisman and Anderson, 2012).

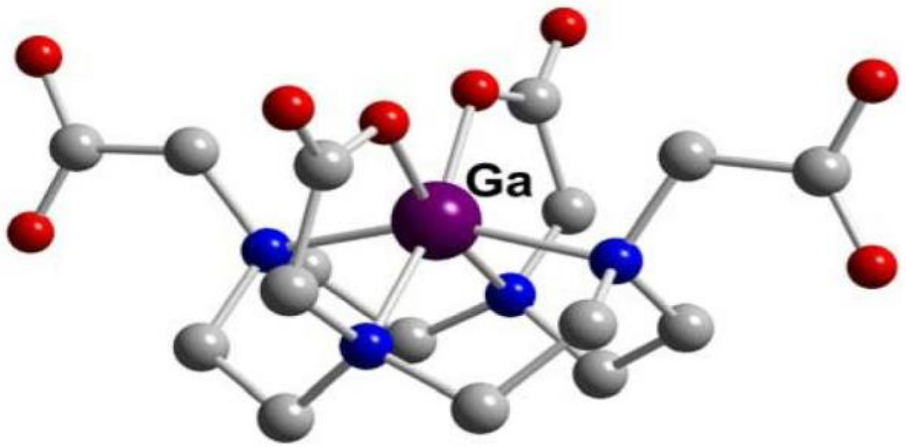


Fig 38: Ga-DOTA complex.

Another radiometal whose coordination chemistry influences the development of peptide-based radiopharmaceuticals is indium (III). Indium is like gallium in that its stable oxidation state is

+3. However, its coordination number is 4-8, and its atomic radius is 62-92 pm. Coordination chemistry of indium also influences the development of peptide-based radiopharmaceuticals through its interaction with tetradentate and hexadentate chelating agents to influence the stability of the entire radiopharmaceutical Wadas, Wong, Weisman and Anderson, 2012). One of the tetradentate chelating agents complex with Indium(III) is InCl-bis(aminothiolate). This structure is 5-coordinate, and it interacts with an axial chloride in a near square pyramidal version. This complex is stable in aqueous acetonitrile solutions.

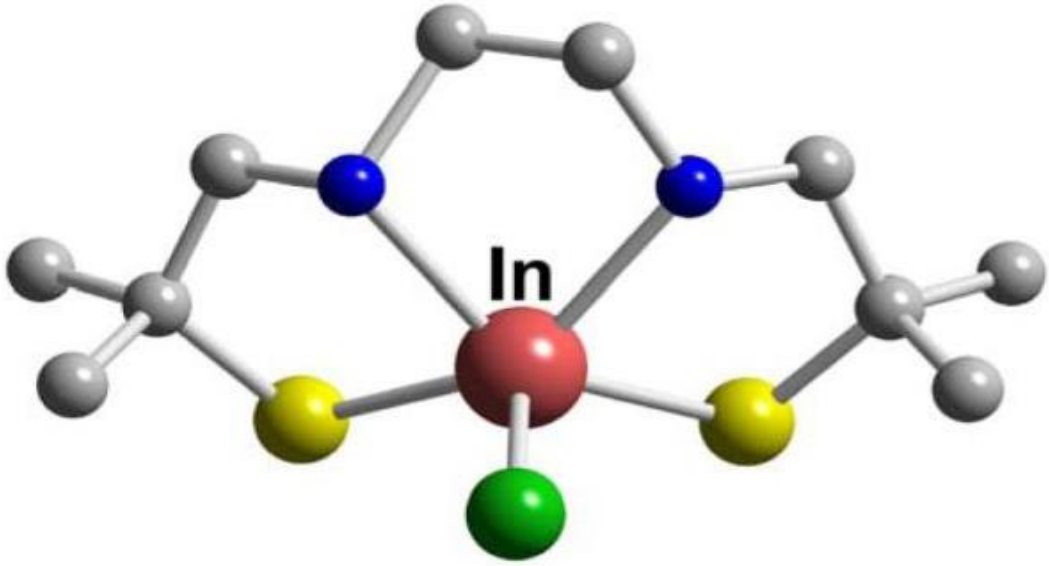


Fig 39: InCl-bis complex.

On the other hand, the interaction of indium with hexadentate, heptadentate and octadentate chelators results in the formation of a distorted octahedral Indium(III) complex that has carboxylate donors at its axial sites. For example, when EDTA or DTPA interact with indium the product is a thermodynamically stable structure. In this case, interactions with EDTA results in the formation of 7-coordinate In-EDTA that includes an hexadentate chelator and has a pentagonal bipyramidal geometry. On the other hand, the In-DTPA complex has about 7- and full 8-coordination by the chelator, and it has a distorted pentagonal bipyramidal as well as square antiprismatic geometries, respectively. Another chelator whose complexation with indium influences the structure and stability of peptide-based radiopharmaceuticals is DOTA Wadas, Wong, Weisman and Anderson, 2012)

. One of the derivatives of DOTA that has been studied in relation to the stability of peptide-based radiopharmaceuticals is DTA-AA, which has a twisted square antiprismatic geometry. Other coordination features present in this complex include the O_4 and N_4 coordination planes.

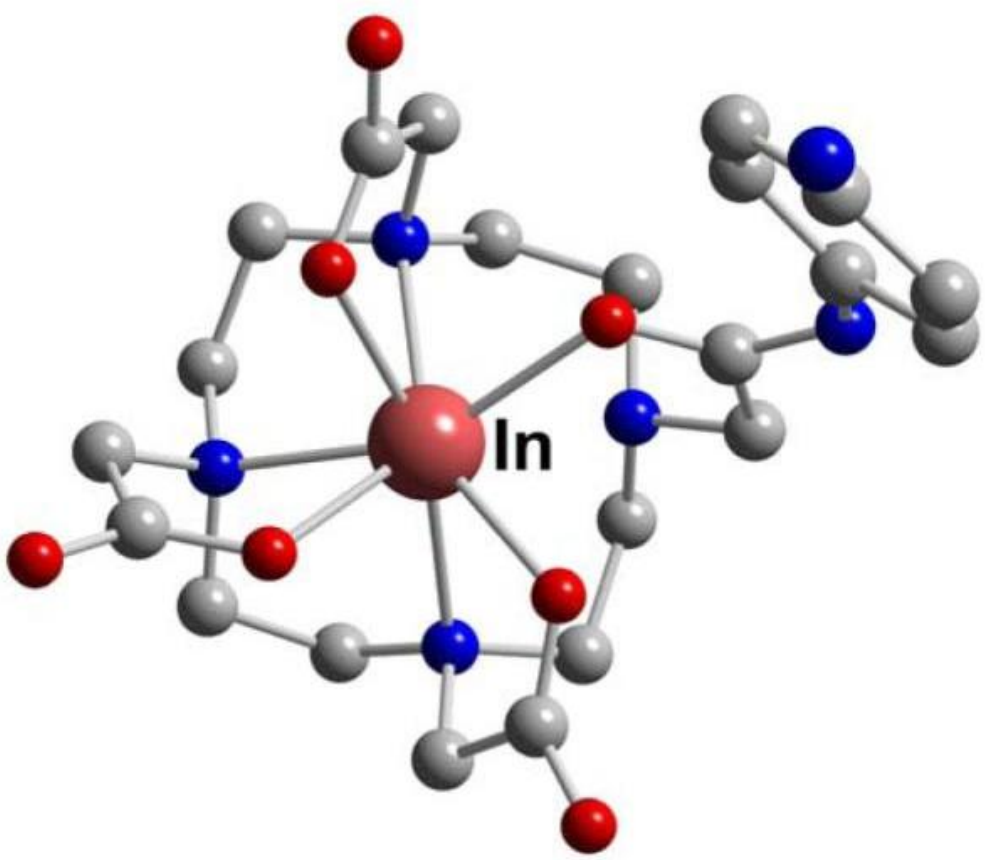


Fig 40: In-DOTA-AA complex.

With regard to yttrium(III) ions, some of the extensively studied chelating agents include DTPA, EDTA, Tris(carbamoylmethyl) derivative (NOTAM) DOTA and DOTATOC, amongst others. Both DPTA and EDTA have significantly stable complexes with Y(III), where YF2-EDTA exists as a dodecahedron while Y-DPTA exists as a 9-coordinate comprising of a monocapped anti-prismatic geometry (Wadas, Wong, Weisman and Anderson, 2012). In both YF2-EDTA and Y-DPTA, the 9-coordinate has a distorted tricapped trigonal prismatic geometry in which a coordinated solvent molecule and octadentate chelator exists.

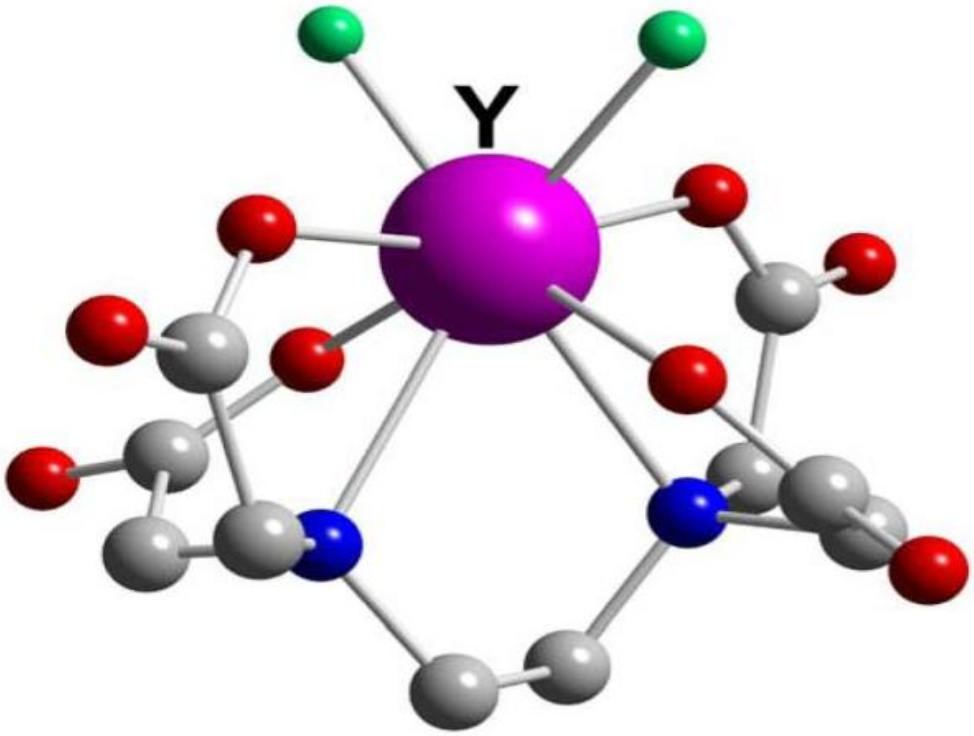


Fig 41: YF2-EDTA complex.

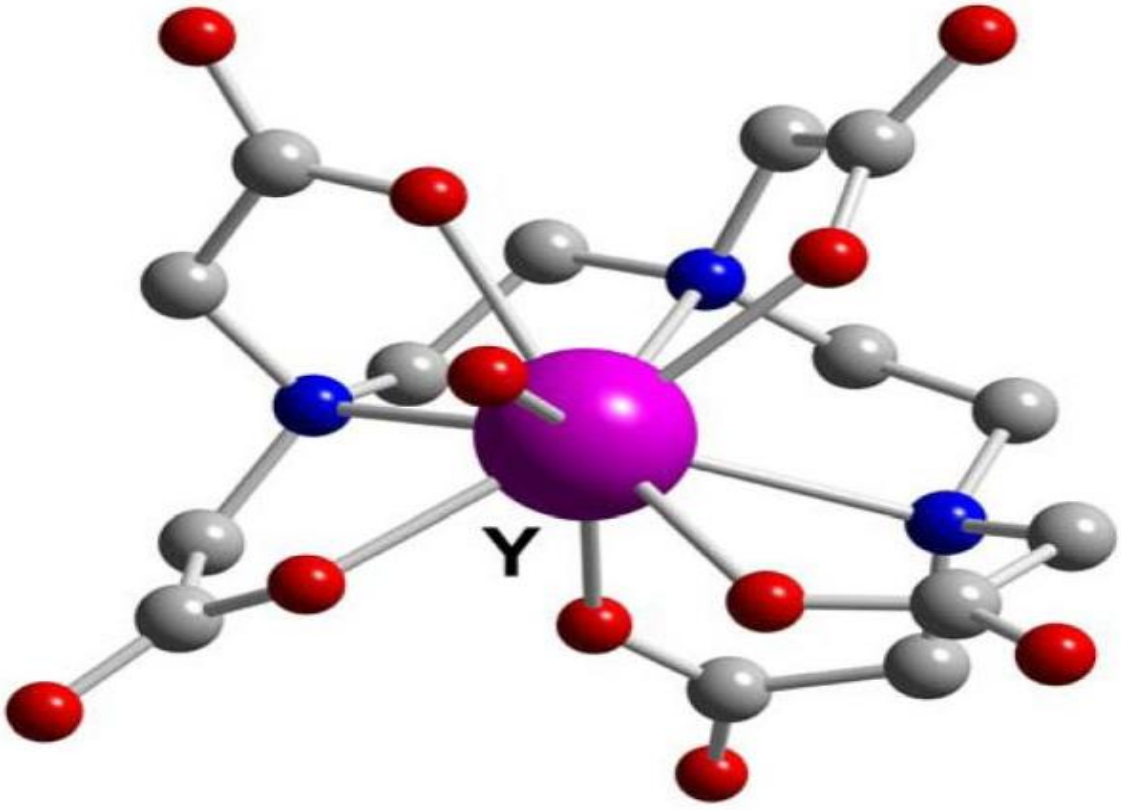


Fig 42: Y-DPTA complex.

Also, the coordination chemistry of zirconium has been seen to influence the stability of complexes used in peptide-based radiopharmaceuticals. Zirconium ion is highly positively charged, and it has a small radius of 59-89 pm for coordination number 4-9. It also has an extreme hardness leading to high stability constants of DPTA and EDTA complexes (Wadas, Wong, Weisman and Anderson, 2012). The complex of Zr and EDTA forms a dodecahedral geometry while the complex of Zr-DPTA complex forms a full envelopment of Zr(IV).

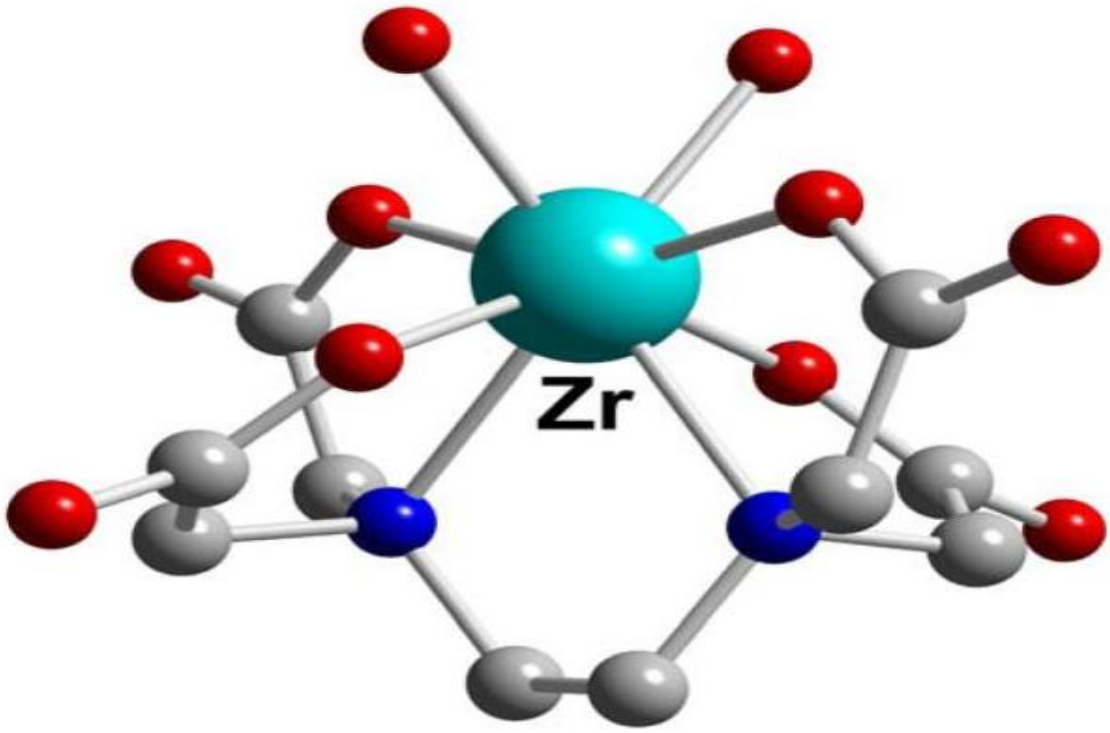


Fig 43: Zr-EDTA complex.

2.1.11. The concept of matched-pair radiometals.

In some cases, some radionuclides have both the imaging and the therapeutic values. For example, some iodine radioisotopes are useful for imaging as well as therapy, which makes them ideal matched-pairs. Usually, the concept of matched-pair emerges from the ability of some radiometals to emit both particulate forms of energy (alpha and beta particles) and gamma radiations, which allow for their use both as therapeutic and imaging agents (theranostic agent) (Giblin, Veerendra, and Smith, 2005). In basic terms, matched-pair radiometals are radionuclides with value for imaging as well as therapy, and thus they can be utilized for molecular imaging as well as therapeutic agents. Coordination chemistry of radiometals also extends the concept of

matched pairs further as different radionuclides may be used together for imaging as well as therapy after linking them to suitable ligands to form complexes. In this case, radionuclides any two radionuclides of a single element that may not be applied as theranostic agents may be coordinated through a bifunctional chelator and tethering them to the biologically active molecule for targeting in order to deliver a theranostic performance.

The application of matched pair radiometals in theranostics may be demonstrated by several examples. One of the examples of the application of this concept in theranostics is the use of ^{99m}Tc/^{188}Re (Ricardo, Kumar and Wiebe, 2015). In this matched pair strategy, ^{99m}Tc provides diagnostic information by Single Photon Emission Computed Tomography (SPECT), which demonstrate the availability of the receptors on the primary as well as the metastatic tissue, while ^{188}Re is administered as a therapeutic agent for diagnosed problem. Such a concept as the one indicated in this approach; the target receptor guides the administration of the treatment agent. In addition, the diagnostic radiopharmaceutical allows for the pre-screening of receptor-positive patients before the administration of the therapy in order to characterize such aspects as the receptor density, drug pharmacokinetics as well as the patient dosimetry and ultimately reduce or eliminate the unsuccessful radiotherapeutic regimens. In this case, the therapeutic value of Re-188 is contributed by its attractive physical characteristics (Gamma emission =155keV, half-life = 16.94 hours and maximum beta particles release = 2.12MeV) and its widespread available for use. Other radioisotopes of rhenium that are available for radiotherapy include Re-186, which emits gamma photon of 137KeV, beta emission of 1.07MeV and a half-life of 3.7 days. However, Re-186 has a lower specific activity compared to Re-188, and this limits its usefulness as a radiotherapy agent. Another example of the successful application of the matched pair concept is the use of In-111 and Y-90 (Ricardo, Kumar and Wiebe, 2015). In this case, 111In-Octreotide is administered in the body as a somatostatin tracer to localize the somatostatin receptors expressed by neuroendocrine tumors in order to guide the treatment of the target tumor with Y-90 radiopharmaceuticals. Elements of

theranostic significance have been explored widely in the recent years from 2000, and the value of the theranostic matched pairs is outlined in the following paragraph.

The concept of matched pair is aimed at using a radiotherapeutic as well as an imaging analog of similar pharmacokinetic and biodistribution characteristics in order to allow for the prior estimation of dosimetry as a tool for sound clinical decisions (International Atomic Energy Agency, 2009). In the history of radiotherapy practices, the use of matched pairs have been rare, but the approach has been gaining popularity since 2000 due to the need to predict outcomes of different therapies as well as avoid irrelevant as well as expensive treatments. These have been the reasons behind the concerted development of matched pair radiotherapy treatment techniques. However, the matched pair concept is limited by several factors. For example, although In-111 have in history been used, as an imaging surrogate, along with Y-90, the former does not offer effective quantitative prediction because the two metals do not have the same coordination chemistry. On the other hand, the use of Tc along with Re-186/188 is also limited by the low imageable emissions of Re-186/188, which limits the ability of technetium images to determine the dosimetry of Re isotope (International Atomic Energy Agency, 2009).

2.2. Gallium-68 chemistry and generator.

2.2.1. Gallium-68 for Positron emission tomography.

One of the dominant diagnostic imaging methods in nuclear medicine is positron emission tomography, and its popularity is due to the ability to quantify the lesions' tracer uptake as well as its high sensitivity. Some of the commonly used tracers include ^{13}N, ^{18}F, ^{15}O and ^{11}C. Among these positron emitters, ^{18}F is commonly used, and it replaces hydrogen atom because of similarity of its van der Waal's radius in comparison with ^{1}H. On the other hand, such emitters as ^{13}N, ^{15}O and ^{11}C are commonly used when labeling most biological molecules, a function that is supported by their biological properties (Maecke and Andre, N.D). By being biological, these emitters do not change the chemical structure of the target molecule and thus representing an ideal positron emitter.

However, these radioisotopes are short-lived and as a result, they are used in imaging practices supporting short half-lives, for example, on-site cyclotron.

Another method of producing positron emitters is through a generator. Some of the radionuclides produced from generators include ^{62}C, ^{82}Rb and ^{68}Ga, and their respective generators include $^{62}Zn/^{62}Cu$, $^{82}Sr/^{82}Rb$ and $^{68}Ge/^{68}Ga$. The primary advantage of generators over other positron emitters is that they allow clinical studies to be performed without necessitating the use of onsite cyclotron. Besides, they may also provide radioactive probes and radionuclides for use at any time on demand whenever cyclotron beam time is unavailable (Maecke and Andre, N.D). Among these generators, Gallium-68 is the most important positron emitter used in nuclear medicine for positron emitting tomography.

2.2.2. Gallium-68.

The physical half-life of gallium-68 is 67.71 minutes, and it makes it compatible with the pharmacokinetics characteristics of such low molecular weight radiopharmaceuticals as peptides, antibody fragments, oligonucleotides, and aptamers. 89 percent of gallium-68 decay happens through positron emission, while the rest 11 percent happens by electron capture. On average, the average positron energy emitted by gallium-68 in every disintegration is 740 KeV. The production of gallium-68 involves the use of a generator system in which an organic or an inorganic matrix immobilizes the parent radionuclide germanium-68 (Maecke and Andre, N.D). The parent radionuclide, germanium-68, has a half-life of 270.95 days and thus it is used to produce long-lived as well as cost-effective generator systems that allow for its manufacture as well as the shipment. Then again, there is sufficient difference in chemical properties between Ge^{4+} and Ga^{3+}, which allows for the employment of methods of separation. In addition, Ga^{3+} has a well-established coordination chemistry and thus it is possible to develop robust agents with high resistance to in vivo $^{68}Ga^{3+}$ trans-chelation. The generator system used in this case also results in the development of reconstitutable and labeled freeze-dried kits that can be used as soon as it is required. Some of the recently published studies have reiterated the significance of $^{68}Ge/^{68}Ga$ generators as useful

agents in imaging, by demonstrating the importance of ^{68}Ga labeled somatostatin analogs that have been found to be more specific compared to [^{18}F]fluorodeoxygucose (FDG). When selecting the best agent for use in clinical PET imaging, some of the parameters considered include in vivo behavior, production methods, and dosimetry, amongst others (Maecke and Andre, N.D).

2.2.3. Early ^{68}Ge/^{68}Ga generator developments.

The early attempts to make routine generator based on liquid-liquid extraction chemistry were impossible. However, the earliest ^{68}Ge/^{68}Ga generator systems used columns for separation of Ga3+ from Ge3+ with 0.005 N EDTA solution and absorbed on zirconium oxides or alumina in order to provide neutral Ga-EDTA eluates. In other generator systems, antimony oxide (Sb2O5) was used to retain 68Ge while oxalate solutions were used to elute 68Ga. In this case, ion exchange resins with the dilute hydrofluoric acid solution were used as an eluent to allow for high-purity separations. In these generators, 68-GaCl4 was eluted in strong hydrochloric acid after adsorption of Ge4+ complex with phenolic groups in 1,2,3-trihydroxybenzene-formaldehyde resin, which is resistant to radiation-related dissociation (Roesch and Riss, 2010).

The electron configuration of gallium is $3d_{10}4s_24p_1$. Based on the small size and the high charge density of three positive charges, Ga3+ cation fits well to such five-membered chelate rings as glycine-like chelators and ethylene-1,2-diamine, and it forms stable complexes with such hard donors as amino and oxy functional groups (Roesch and Riss, 2010). In addition, the main coordination number of gallium is six, which corresponds to distorted octahedral geometry which is exhibited by the most stable of gallium complexes with chelating agents. However, sufficient stability of gallium complexes is also possible for lower coordination numbers where stability is enhanced by interactions between the metal core and the sulfhydryl donor. This case is possible due to the electronic interactions between the empty d-shell of sulfur and the complete d-shell of gallium. Extraordinarily stable complexes between Ga3+ and soft thiophenol donors is also possible. In moderately acidic-basic pH values, Ga3+ is susceptible to aqueous hydrolysis leading to the formation of stable complexes.

2.2.4. Gallium-68 generator.

68Ge/68Ga generator is almost an ideal generator strategy because of the long half-life (270.95 days) of Ge-68 (parent radionuclide) and the short half-life (67.71 minutes) of the resultant radionuclide (Ga-68) (Roesch and Riss, 2010). The (p, 2n) reaction on gallium targets is the preferred production route of Ge-68, and it requires the use of ion exchange chromatography as a perfect separation system that prevents the break-through between the parent radionuclide and the soluble daughter (Maecke and Andre, N.D). The early 68Ga/68Ge generator systems used Al_2O_3 and ZrO_2 as the matrices in which Ge-68 is fixed on the column while Ga-68 is extracted using EDTA solution.

2.2.4. Characteristics of Ge-68/Ga-68 generator systems.

As established in the above sub-section, the primary advantage of Ga-68 is that it has a high positron emission fraction and a half-life of 67.71 minutes, which results in high radioactivity levels as well as high-quality PET images without exposing the patients or the personnel to high doses of irradiation. Additionally, this radionuclide also requires short scanning time, which makes it possible to stage repetitive examinations. Modern generator systems exploit the stable oxidation state (Ga3+) to obtain ionic forms that can be aligned with the chemistry characteristics of the subsequent labeling steps (Maecke and Andre, N.D). This oxidation state allows for the production of stable complexes with ligands capable of fitting the Ga^{3+} coordination sphere through the six coordination sites.

As a basic principle, a generator contains both the parent and the daughter radionuclide mixture in the state of equilibrium. As a result, a simple commercial generator is a chromatographic column located within a shielding container. In $^{68}Ge/^{68}Ga$ system, a high energy cyclotron is used to produce 68Ge from Ga-69 (Maecke and Andre, N.D). The resulting Ge-68 is immobilized on the matrix-filled column where spontaneous decay results in the formation of Ga-68 that is subsequently eluted using a suitable eluent. Additionally, radioactive decay of Ga-68 results in the

formation of stable Zn^{2+} and as a result, the three elements, namely Ge, Ga and Zn accumulate in the generator and the can be traced in the eluate (Roesch and Riss, 2010).

Generally, when exploring the performance of a generator, seven parameters are considered, and they include 68-Ge breakthrough, chemical separation specificity, eluent type, elution profile, eluate sterility/pyrogenecity, and the chemical stability and radiation resistance of the column material. Additionally, most generator systems use acidic eluent in order to allow for further direct chemistry as provided by cationic Ga^{3+}. On the other hand, there are several types of eluents used in elution of Ga-68 from column matrices. When performing 68-Ga elution, various eluents, including, acids, buffering agents or bases may be used (Maecke and Andre, N.D). In the case of N-methylglucamine functional groups containing organic resins, it is possible to use such eluents as EDTA, citrate, HCl and NaOH depending on the subsequent application or chemical processes. As a general rule, the modern 68Ge/68Ga generators are required to have a robust performance as well as high levels of reproducibility (Roesch and Riss, 2010).

2.2.5. Organic and inorganic matrices used in 68Ge/68Ga generators.

Reactions between Ge4+ and phenolic groups results in the formation of stable complexes. Based on this property, it has been possible to use pyrogallol-formaldehyde polymer resins for eluting $^{68}Ga^{3+}$ in the form of $^{68}GaCl$, where small Dowex anion exchange column is used for purification (Maecke and Andre, N.D). Another organic matrix used in $^{68}Ge/^{68}Ga$ generators is N-methylglucamine, and it employs 0.1 M trisodium citrate as the elution agent. Then again, the most commonly used inorganic matrices used in $^{68}Ge/^{68}Ga$ generators include TiO2, ZrO, Sb2O5, Al2O3, and SnO2. Inorganic matrices offer efficient separation, but in the case of Al2O3 or ZrO2 the elution of 68Ga3+ and as 68Ga(EDTA) is time-consuming as well as tedious. On the other hand, a TiO2-based generator is currently available, and it provides 68Ga3+ in 0.1M HCl. In both the organic and inorganic matrices systems, the major shortcoming is the 68Ge breakthrough and thus a matrix that supports low 68Ge breakthrough is considered as the best option (Maecke and Andre, N.D).

2.2.6. Chemistry of gallium.

Gallium is a member of group thirteen elements in the periodic table and when dissolved in water its most stable oxidation state is +3. Its +3 oxidation state also exists in the presence of high concentration of Cl ions. However, this oxidation state is unstable and subject to a further oxidation to +3. In addition, a high stability of Ga+3 in aqueous solution is only possible in low PH (acidic conditions) (Maecke and Andre, N.D). In this case, if the pH of the solution is 3-7 Ga+3 can hydrolyze to form insoluble trihydroxide when its concentration is raised to levels above nanomoles. However, stabilizing agents may be used to prevent this precipitation reaction and reduce the formation of $[Ga(OH)_4]^-$ ions.

In aqueous solutions, Ga exists as Ga3+ ions and its coordination chemistry is similar to that of high spin Fe^{3+} ions in terms of their charges, ionic radii as well as the major coordination numbers (number six) (Roesch and Riss, 2010). For Ga3+ to be used as a suitable radiopharmaceutical, it has to exhibit kinetic stability for clinical applications and a high thermodynamic stability towards hydrolysis under physiological pH conditions. Additionally, another requirement of Ga3+ as a suitable radiopharmaceutical is that its chelate should not undergo exchange with transferrin, which has high affinity for Ga3+ due to two binding sites present in this protein. On Ga3+ ion coordination chemistry, gallium-68 is a hard Lewis acid that forms thermodynamically stable complexes when reacted with hard Lewis bases. These hard Lewis bases are ligands that are rich in oxygen and nitrogen donor atoms, which form good chelating agents for Gallium-68 (Maecke and Andre, N.D).

Coordination chemistry of Gallium-68 makes it possible for the formation of complexes with polydentate ligands, particularly open chain, and cyclic structures. Most of the ligands used in the formation of stable complexes with Ga3+ are hexadentate, and their coordination numbers are four and five. Most of these chelating agents are bifunctional because they allow for a covalent coupling to at least one targeting vector besides binding the metal cation. A perfect bifunctional chelating agent for Ga3+ needs to meet two basic requirements, including the ability to chelate the

radiometal sufficiently and rapidly and the ability to form kinetically stable chelate that can resist demetallation under the conditions of pH range 4-8 as well as the presence of such serum cations as Mg^{2+}, Zn^{2+} and Ca^{2+} (Wadas, Wong, Weisman and Anderson 2012). One of the common ligands used for complexation with Ga3+ is desferrioxamine-B (DFO). DFO has three hydroxamate groups for coordination of metal ions. As a bifunctional chelator, DFO gives high radiochemical yield labeling with $^{67/68}Ga^{3+}$. In nanomolar concentrations, DFO does not act as a good gallium chelating agent, but it is affected by different incubation conditions. On the other hand, DFO also has coupling ability to biomolecules via the –NH2 group as well as the succinyl spacer. On the stability of Ga3+ complexes, highly stable complexes are rapidly cleared from the liver while lowly stable complexes are retained in the liver due to ion exchanges with transferrin. In this case, the most stable of complexes of gallium and chelating agents include those formed from tetradentate and pentadentate ligands. Complexation of tetradentate tripodal ligand and Ga3+ results in the formation of neutral species that are lipophilic and that can pass the blood-brain barrier as well as that exhibits a high heart-to-blood ratio. As a result, this chelate retains its tetrahedral geometry even in the presence of water. The stability of 68Ga-labelled peptides is also increased by the conjugation of NS3 chelating agents to phenylalanine. Other tripodal polycarboxylic acid chelating agents used increase the stability of 68Ga-labelled peptides are based on TAME structure, including DPTA (Wadas, Wong, Weisman and Anderson 2012). On the other hand, chelation of Ga3+ with macrocyclic chelating agents, including triaza ligands, exhibit high selectivity in terms of conformation and size. In this regard, complexation of triazamacrocyclic ligands with Ga3+ results in the formation of highly thermodynamically stable complexes because of the good fit of the metallic cation in the cyclic cavity of the chelating agent. In this case, then chelator encapsulates the metal ion efficiently and thus keeping the ion away from competitors, for example, blood transferrin.

With regard to tetradentate ligands, such a derivative of tetradentate 0-hydroxybenzyl derivatives as iminodiacetic acid provides an NO3 donor set that as a result completes the

octahedral coordination around the central Ga3+ in addition to two *cis*-coordinated water molecules. In this case, the highest stability constant is exhibited by p-OMe derivatives while the lowest stability constant is shown by *p*-NO_2 derivatives. Moreover, Hexadentate ligands, especially such an acyclic hexadentate chelating agents as N,N'-ethylene-di-L-cysteine with N2O2S2 donor sites, also form stable complexes with Ga (III) (Wadas, Wong, Weisman and Anderson 2012). The complex forms a distorted octahedron structure containing two carboxylate O's *trans* arrangement. Another hexadentate ligand that forms a stable complex with Ga3+ is N,N'-bisethylenediamine-N,N'-diacetic acid and this is attributed to the presence of N2O4 donor set in this aminophenolate chelator. Moreover, another hexadentate ligand that forms a stable complex with gallium is NOTA, and it has been demonstrated through X-ray to envelop the cation through distorted octahedral formation due to N3O3. On the other hand, octadenate DOTA saturates the 6-coordination sphere of Ga(III) through a distorted octahedral coordination containing four macrocyclic N,s and two cis-carboxylates. However, the stability of this complex may be increased by conjugating targeting moieties to DOTA through its free carboxymethyl arm. For example, linking DOTA to a mitochondrion-targeting triphenylphosphonium moiety results in the formation of DO3A-TPP, which when complexed with gallium results in the retention of 6-coordinate pseudo-octahedral geometry. However, the chemical structure of DOTA makes it unsuitable for complexing with gallium and consequently a poor bifunctional chelator for this metal. For example, DOTA has a large cavity size and as a result, its complex with 68Ga is of low thermodynamic stability, besides being non-selective to metal ions for coordination. In addition, it has slower complexation kinetics and thus creating a need for longer reaction times and elevated temperatures for the complexation reactions to happen (Wadas, Wong, Weisman and Anderson 2012).

When designing 68Ga radiopharmaceuticals, three complex chemistry requirements are to be considered in order to fit the Chemistry of Gallium. One of the requirements is that all chelators used for complexation purpose need to be a targeting moiety itself. Alternatively, it has to have a functional group to allow for the conjugation of the respective complex to a specific targeting

moiety. In this case, the specific targeting moiety is a complex ligand that is referred to as a bifunctional chelating agent. Another requirement is that the resultant Ga-ligand complex needs to have a high thermodynamic stability. In this case, a high thermodynamic stability is exhibited by high equilibrium constants, which is the ratio of chemical activities of the complexed cation to the free cation in the solution. An additional requirement is that the corresponding complex of gallium and ligand needs to exhibit high levels of kinetic inertness under conditions of physiology. In this case, the Ga3+ rate constants between the unbound and the bound Ga3+ in the complex need to be reasonable low. In this case, the displacement of bound Ga3+ in the blood is also influenced by core-exchange to other cations (for example Mg2+) in the blood (Roesch and Riss, 2010).

2.2.7. Formation of the complex with Ga^{3+}.

At moderate temperature, When Ga3+ is exposed to such chelating agents as NOTA, DPTA, and EDTA, amongst others, a rapid reaction takes place where a respective complex is formed. In this reaction, the stable octahedral Ga3+-chelate complexes are formed with these multidentate chelating agents (Roesch and Riss, 2010). Moreover, at moderate temperature twelve-membered macrocycles react with gallium ions to lowly stable complexes that are nonetheless stabilized by the application of sufficient activation energy by heating. Formation of complexes of $68Ga^{3+}$ and ligands happens under acidic conditions of pH 2.8 to 3.8 with such buffers as citrate, acetate as well as 4-(2-hydroxyethyl)-1-piperanineethanesulfonic acid being used to prevent the hydrolysis of Ga^{3+} to $Ga(OH)_3$. The immediate complex resulting from a reaction between Ga3+ and a ligand forms a semi-stable complex but more stable complex may be obtained through trans-chelation at higher pH values (Roesch and Riss, 2010).

2.3. Lu-177 chemistry and physical characteristics.

2.3.1. Lutetium atom and element.

The atomic number of lutetium is 71. When in dry conditions it is resistant to corrosion, it moist air encourages its corrosion. It is a member of the lanthanide series, as the last element in the

series. Lutetium was discovered independently by three scientists, namely, Baron Carl Auer Von Welsbach (mineralogist), Georges Urbain (scientist), and Charles James (chemist). Initially, this element was thought to be exclusively composed of ytterbium as at the time of its discovery it was proposed to be an impurity of mineral ytterbia (Chemicool, 2016). However, it was later to be erudite that mineral ytterbia comprised two different compounds, namely lutetium oxide and ytterbium oxide. Lutetium is considerably a rare element in the earth's crust, and it is therefore referred to as a rare earth element even though it is more abundant compared to silver. The discovery of Lutetium-177 by the three personalities lauded the previous discoveries and the subsequent analyses of other rare earth metals. Such other rare metals whose discoveries and analyses were echoed by the discovery, as well as the analysis of Lutetium-177, include lanthanum, terbium, and erbium, which discovered by Carl Gustaf Monsander. However, the first successful isolation of lutetium was made in 1906/7 by Charles James through a bromate fractional crystallization process that he patented. His technique was employed for isolating lutetium as well as other rare earth metals until the 1940s when ion exchange techniques were discovered for the same purpose (Chemicool, 2016).

2.3.2. Physical characteristics of lutetium-177.

Lutetium is a rare metal whose physical appearance is silver to white. Its atom has 71 electrons and a configuration of $[Xe]4f^{1}45d^{1}6s^{2}$. The atom participates in chemical reactions by shedding its two outermost electrons as well as the single 5d-electron, unlike all other lanthanides that exclusively involve f-shell electrons during chemical reactions. Another interesting physical characteristic is that lutetium has the smallest atomic size compared to other lanthanide elements. The smaller size of lutetium is attributed to the lanthanide contraction. Lanthanide contraction is the phenomenon by which the members of the lanthanide series (lanthanum to lutetium) have smaller ionic radii compared to those with atomic numbers 72 and above. It is a product of poor nuclear charge shielding by 4f electrons, making the 6s electrons be drawn closer to the nucleus, which results in the contraction of the atomic radius (Bains, 2014). The shielding effect is the phenomenon

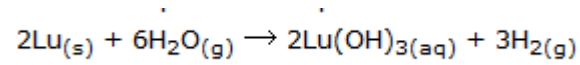
by which the inner-shell electrons block the effects of the nuclear charge on the outer-shell electrons. Poor shielding effect describes the events where the positively charged nucleus attracts the electrons to a greater extent and thus resulting in reduced atomic radii with the increase in the atomic number. The largest shielding is offered by s orbital while the least shielding is provided by f orbitals. In between the two orbitals exists p and d orbitals where p provides a greater shielding compared to d orbitals.

The Lanthanide Contraction affects all members of the lanthanide series where their atomic radii decrease with the increase in the atomic numbers. Apart from lutetium, other members of the lanthanide series to which the Lanthanide Contraction applies include Holmium, Dysprosium, Europium, Terbium, Thulium, Samarium, Gadolinium, Neodymium, Cerium, Ytterbium, Praseodymium, and Promethium (Bains, 2014). The ultimate effects of the Lanthanide Contraction are the increased density, melting point and hardness of these elements, with lutetium being the hardest, the densest, and with the highest melting point, amongst the lanthanides. Another physical characteristic of Lutetium and its isotopes is that when it is exposed to the air is that it tarnishes and that if exposed to air at 150 ºC it burns to the oxide. Lutetium and its isotopes also form compounds with other elements and ions to exist as a trivalent ion (Lu^{3+}) with most of its salts being colorless.

The melting point of lutetium is 1663 ºC while its boiling point is 3402 ºC at one atmosphere. It is density is 9.84 g per cubic centimeter. Additionally, its electronegativity based on Pauling Scale is 1.0 while its electropositivity on the same scale is 3.0. In addition, this element has a specific heat capacity of 0.154J/g ºC[7], a heat of fusion of 106.3J/g, thermal conductivity of 16.4(W/m)/K, 27 ºC[8], and its heat of vaporization is 2034.1J/g (Bains, 2014). Lutetium exists in 35 isotopes, with the most stable isotopes being Lu-175 and Lu-176, whose abundance is above 97.41 percent of the total lutetium. On the other hand, Lu-177 also forms one of the most important isotopes because of its application in radiation therapy for different tumors. It is the most stable of all lutetium isotopes, and it has a half-life of 160.4 days.

2.3.3. Chemical reactions involving Lutetium-177.

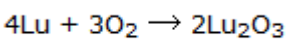
Lutetium-177 reacts slowly with other chemical substances, with the rates of reactions increasing with increased temperatures. In most cases, the most stable ionic form of Lu-177 is trivalent (with three positive charges (3+)). When exposed to cold water, lutetium, which is electropositive, reacts slowly but the reaction is more rapid in hot water, and it results in the formation of two moles of lutetium hydroxide and three moles of hydrogen gas for every two moles of lutetium and six moles of water consumed in the reaction as demonstrated in the following equation (International Database of Educational Objects, 2016).



$$2Lu_{(s)} + 6H_2O_{(g)} \rightarrow 2Lu(OH)_{3(aq)} + 3H_{2(g)}$$

Fig 44: the reaction between Lu and water.

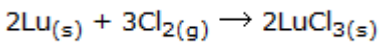
A reaction between lutetium-177 and air results in the formation of lutetium (III) oxide when the metal tarnishes slowly in the air, to burn (International Database of Educational Objects, 2016). The following equation represents this reaction.



$$4Lu + 3O_2 \rightarrow 2Lu_2O_3$$

Fig 45: The reaction between Lu and oxygen.

In addition, lutetium may also reacts with group-7 elements to form lutetium (III) salts. For example, the reaction between lutetium and chloride results in the formation of lutetium (III) chloride as depicted in the following equation (International Database of Educational Objects, 2016).



$$2Lu_{(s)} + 3Cl_{2(g)} \rightarrow 2LuCl_{3(s)}$$

Fig 46: The reaction between Lu and chlorine.

Similar reactions also happen with other halogens, including iodine, fluorine, and bromine as depicted in the following equations (International Database of Educational Objects, 2016).

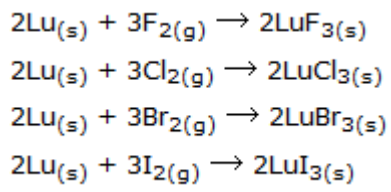


Fig 47: reaction between Lu and halides.

Like most other metallic elements, lutetium-177 also reacts rapidly with acids, where it dissolves as soon as it lands on dilute sulphuric acid resulting in the formation of colorless aqueous solution of Lu(III) ions and hydrogen gas. This reaction is presented in the following equation. Similar reactions also happen when lutetium-177 is exposed to such other inorganic acids as hydrochloric acid and nitric acid (Banerjee, Pillai and Knapp, 2015).

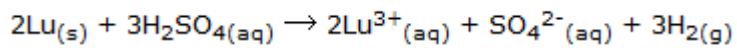


Fig 48: The reaction between Lu and sulfuric acid.

2.3.4. Radioactive decay of Lu-177.

The radioactive decay of Lu-177 results in the emission of two major radio particles, namely gamma radiation (γ-photon) and beta-particles. Of the beta particles emitted during radioactive decay of Lu-177, 498 keV accounts for 79.3 percent, 380 keV accounts for 9.1 percent while 176 keV accounts for 12.2 percent of all beta-emissions by abundance (Banerjee, Pillai and Knapp, 2015). These events are depicted in the following scheme.

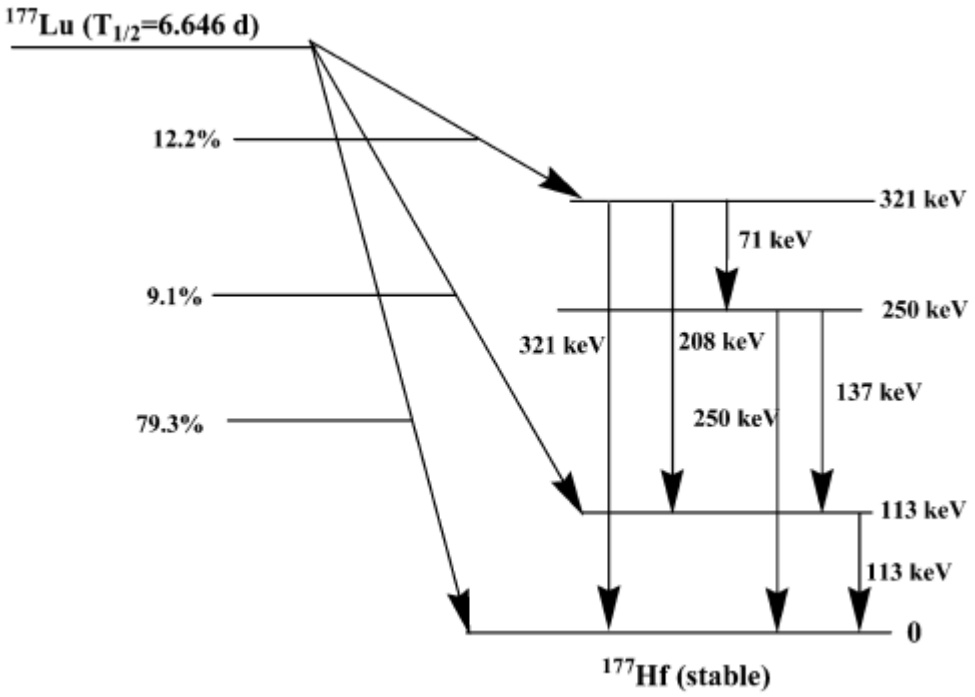


Fig 49: a schematic representation of the major Lu-177 Beta/gamma emissions.

Using a high-purity germanium detector (HPGe detector) for the assessment of the gamma-ray spectrum of Lu-177, it has been observed that the photon peaks of 208 and 112keV obtained during radioactive decay can be used for the single-photon emission computed tomography (SPECT) (Banerjee, Pillai and Knapp, 2015). As a result, the scintigraphic images obtained from this radioactive decay offer good dimensions for the evaluation of pharmacokinetics, excretion and targeting behaviors of radiopharmaceuticals labeled with Lu-177. Apart from a favorable half-life (6.647 days) and low-energy beta particles' emission, other characteristics that make Lu-177 a good therapeutic agent include high multi-Curie activity levels as well as its capability of producing high specific activity. The major advantage offered by a long half-life of Lu-177 is that it allows for transportation from the source to the hospital setting where therapy is performed on cancer patients. Additionally, the radioactive decay of Lu-177 results in the emission of 208 KeV (11 percent) and gamma photons (E = 113 (6.4 percent)), which can be used for radio imaging and thus this radionuclide has a significant theranostic value in cancer therapy.

2.3.4. Production of Lu-177.

Lu-177 can be produced through three approaches, namely, charged particle acceleration, neutron irradiation in a nuclear reactor and a cyclotron. The production of Lu-177 by charged particle acceleration route yields lower radioactivity through an expensive process making it impractical. On the other hand, the preparation of Lu-177 in a nuclear reactor involves the neutron activation by the indirect activation of ^{176}Yb before allowing its decay to Lu-177 or direct activation of enriched Lu-176 (Banerjee, Pillai and Knapp, 2015).

The employment of the accelerator route for the production of Lu-177 is limited. However, there are several techniques available for the production of Lu-177 by a cyclotron. For example, the stacked-foil activation technique may be used in the generation of carrier-free Lu isotopes by deuteron-inducing reactions on the natural Yb. Under this technique, a deuteron energy of 12 MeV have been reported to give a maximum cross-section of 217 mb for Lu-177 production. Most of the cyclotron techniques used in the production of Lu-177 results in the production of other lutetium radionuclides (for example, Lu-174, Lu-173, Lu-172, Lu-170, and Lu-171) but the coproduction of these radionuclides may be avoided by using enriched Yb-176. However, the use of Yb-176 results in the introduction of Yb impurities in the resultant Lu-177, which necessitates the employment of efficient methods of separation in order to make this route useful. Another example of an efficient production technique using cyclotron LU-177 production method is through deuteron-induced nuclear reactions. This method requires an energy of 11 MeV in order to avoid the production of ^{177m}Lu. However, this method is set back by the production of low-activity Lu-177, which is about one-tenth of Lu-177 produced by indirect route in a nuclear reactor. This aspect makes it impossible to produce Lu-177 commercially as cyclotron has been viewed as an uneconomical way of producing Lu-177 whose activity is of a significant therapeutic value (Banerjee, Pillai and Knapp, 2015). On the other hand, Lu-177 may also be produced by nuclear reactors by direct or indirect route, which is independent routes.

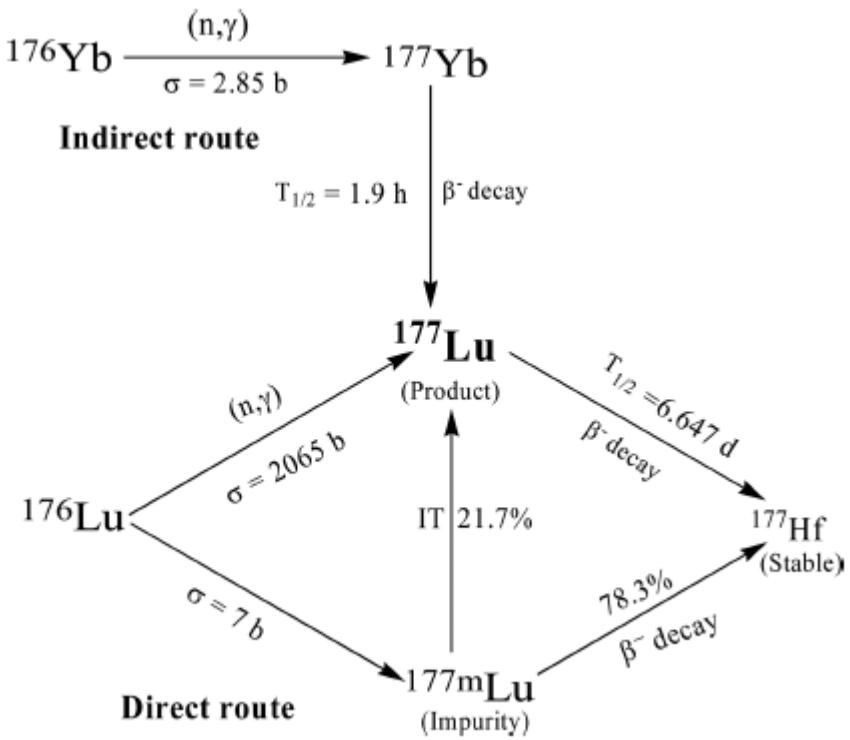


Fig 50: production of Lu-177 by direct and indirect routes in nuclear reactors.

The indirect production of Lu-177 in nuclear reactors involves first producing the short-lived Yb-177 from enriched Yb-176 by neutron capture before allowing radioactive decay to provide no carrier added Lu-177 that is finally isolated by the appropriate radiochemical process. This process is depicted in the following equation (Banerjee, Pillai and Knapp, 2015).

$$^{176}\mathrm{Yb}(\mathrm{n},\gamma)^{177}\mathrm{Yb} \xrightarrow{\beta^- \text{decay}} {}^{177}\mathrm{Lu}$$

Fig 51: Indirect production of Lu-177.

One of the major advantages of production of Lu-177 through this route is that its product is no carrier added Lu-177 and the absence of ^{177m}Lu in the final product. However, this route is challenged by the lack of an adequate method of separating Lu-177 from irradiated Yb_2O_3 as both have significant lanthanide similarity, but the use of such methods as column chromatography and solvent extraction, as well as their combinations, offer efficient methods of separation. On the other hand, the production of Lu-177 through the direct route involves Lu-176 neutron capture through ^{176}Lu(n,γ)^{177}Lu nuclear reaction in order to provide Lu-177. Direct production of Lu-177 by the nuclear reactor results in high specific activity as well as production yields as ^{176}Lu thermal neutron

capture's cross-section is 2065 barn while its epithermal neutron capture is 1087 barn. There are two major pluses associated with the direct production route of Lu-177 in this case. One of the advantages is that it results in high specific values (above 70 Ci per milligram. Another advantage of this route is that Lu-177 activity levels can be achieved by irradiating highly enriched targets in medium-high flux reactors. Yet, this method results in the production of the long-lived ^{177m}Lu, which has activity levels that are dependent on neutron influx and the duration of irradiation. As a result, the production of Lu-177 at high neutron flux and for a long duration results in the coproduction with ^{177m}Lu whose half-life is 160.5 days (Banerjee, Pillai and Knapp, 2015).

2.3.4. Complexation of Lu-177 with bifunctional chelators.

Lu-177 is capable of forming complexes with several chelating agents. At the basic level, a good bifunctional chelating agent has an active functional group at the end of the molecule and a chelating moiety at one of its termini. Some of the functional groups that allow for the conjugation with versatile activated groups include –COOH, -NH2 and any other pendant moieties. During complexation of Lu-177 with bifunctional chelators, the pharmacokinetic characteristic of the conjugate may be improved by incorporating a linker moiety between the targeting vector and the chelator. These linkers are usually polypeptide linkers, or polyethylene glycol, and can change the biodistribution and the pharmacokinetics characteristics by influencing the charge as well as the hydrophilicity of the resultant form intended for use as a therapeutic agent (Banerjee, Pillai and Knapp, 2015).

When in aqueous conditions, the most stable oxidation state of Lu is +3, and thus it forms complexes with such hard donor atoms as N, O, and F^-. The coordination number of Lu in +3 oxidation state is 8 or 9 and as a result, it forms stable complexes with such ligands as polyaminopolycarboxylic as well as acyclic ligands whose numbers of donor atoms are 8 or 9. For example, DOTA (a macrocyclic ligand) forms a complex of high kinetic inertness and thermodynamic stability with Lu-177 (Banerjee, Pillai and Knapp, 2015). It also forms a complex with DPTA, but the product is of lower thermodynamic stability as well as kinetic inertness

compared to that of DOTA. So far, there is not available data on the structure of the product of complexation between Lu and macrocyclic ligands, but it is assumed to exhibit similar characteristics to the general lanthanide behavior. In this case, the complex of DOTA with Lu-177 is assumed to demonstrate a capped square antiprism geometry with the basal plane being occupied by macrocycle's amine nitrogens. Additionally, the capped plane is assumed to be occupied by four carboxylic residues' carboxylate oxygen atoms while a water molecule occupies the capping position (Banerjee, Pillai and Knapp, 2015). This structure is shown in the representative structure presented below.

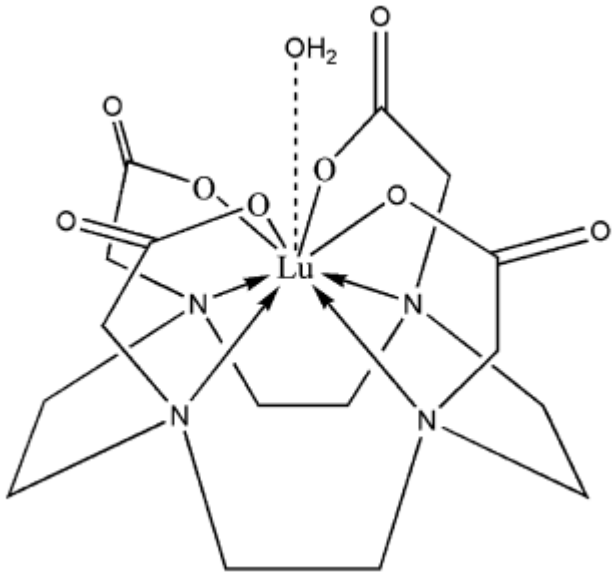


Fig 52: Lu-DOTA complex.

If this complex is exposed to aqueous conditions, the orientation of the macrocyclic ring and the carboxylic side arms results in the formation of two coordination isomers whose twist or torsion angle differ at the capped O_4 squares and the basal N_4. In this case, a twist angle of 29° describes a twisted square of antiprismatic coordination geometry while torsion angle of 39° describes a square antiprismatic structure, but the interconversion of these coordination isomers is possible by ring inversion or arm rotation (Banerjee, Pillai and Knapp, 2015).

2.4. **Safety considerations of handling Gallium and lutetium radioisotopes**.

By being radioactive substances, both Ga-68 and Lu-177 pose a significant danger to the users and handlers both in the research and the medical settings. Ga-68 emits a positron, while Lu-

177 emits beta particles, both of which are ionizing radiations. The maximum permissible whole body occupational exposure to ionizing radiations for adults is five rem/yr TEDE. However, any level of exposure to radiation is risky and as a result, all persons handling or working with such radioactive substances as Ga-68 and Lu-177 need to be exposed to as low as reasonably achievable and thus making it a necessity to take precautions at different levels (University of Pittsburgh, 2001). There are two major levels of precautionary measures to be observed in order to limit exposure to ionizing radiations emitted by both Ga-68 and Lu-177, and they include the external radiation protection and the internal radiation protection. The external radiation protection is based on three basic principles that include distance, time and shielding. The radiation dose to which the worker is exposed is influenced by the length of time on a direct proportion scale, where the longtime of exposure translates to a higher radiation dose.

On the other hand, the distance between the source of radiation and the worker influence the radiation dose on an indirect proportion scale. In this case, the radiation dose received by an individual is indirectly proportional to the squared distance of separation. Moreover, shielding has a tendency of attenuating the radiation and thus the acceptable levels of radiation may be achieved by shielding a person with a thick substance. In this case, a thin paper shit is sufficiently enough to attenuate alpha particles while beta particles are sufficiently attenuated by 0.15 cm thick sheets of aluminium. Moreover, gamma rays are sufficiently attenuated by substances composed of high atomic numbers as well as high density – for example, such materials as steel, lead, concrete and brick could reduce gamma radiations to acceptable levels (University of Pittsburgh, 2001).

On the other hand, the internal level of protection considers hazards resulting from the entry of radionuclides to the body by ingestion, injection, inhalation or direct absorption across the skin. Exposure to radionuclides at this level results in more complex outcomes because of such factors as the individual's mode of metabolism, mode of entry of the radionuclide and the physical as well as the chemical characteristics of the radionuclide material. Besides, once the radionuclide has gained entry into the body irradiation happens continuously until its complete elimination/decay. This

aspect means that the tissue is affected by all the ionizing energy. In order to avoid internal introduction of Ga-68 and Lu-177 in the body, the most important precautions include the containment of their sources as well as the avoidance of contamination of the personnel, equipment, and the working surfaces (University of Pittsburgh, 2001). Furthermore, precautionary measures are necessary as the working precautions when working with radionuclides in order to limit unprotected contact with them as well as offer enough convenience to evacuations in case of emergencies pertaining any of the two radionuclides. One of the general safety considerations is that the laboratory staff need to wear the protective garment, for example, lab coats, and gloves, for effective protection of their skin as well as clothes. Another general consideration is the separation as well as the isolation of the radioactive and the non-radioactive work by designating as well as labeling different areas of the lab. Other general considerations include the similar procedures to those applied in the general chemistry laboratories. On the other hand, emergency precautions are there to take care of the safety needs arising from any spill of radioactive materials, and they are aimed at improving communication, human traffic and ultimately minimizing effects of the spill (University of Pittsburgh, 2001).

3.0. Chapter Three: Literature Review.

3.1. Introduction.

Metastatic neuroendocrine tumors, just like other tumors, are difficult to treat because of the uncontrolled cellular growth that they exhibit. This chapter seeks to review research on the treatment of neuroendocrine tumors as conducted by different scholars in order to develop the theoretical foundation for the current study. The chapter is organized to include eight subsections that include treatment of neuroendocrine tumors, the importance of peptide receptor radionuclide therapy, methods of peptide receptor radionuclide therapy, radiolabelled 177Lu-DOTATOC therapy, and everolimus therapy for neuroendocrine tumors. Other subsections include somatostatin receptor affinity in somatostatin-based radionuclide therapy, the success of radiolabelled

somatostatin analogs in targeted radionuclide therapy and combination of mTOR drug (everolimus) with targeted radionuclide therapy for neuroendocrine tumors.

3.2. Treatment of neuroendocrine tumors.

In a review paper published in by Mathew Kulke on the Journal of Gastrointestinal Cancer Research in 2008, the author sought to explore whether there exists a standard treatment for neuroendocrine tumors. In this paper, Kulke (2008) stated that neuroendocrine tumors present significant paradox because it is not only indolent but also difficult to treat. The author observes that the treatment of malignant neuroendocrine tumor has been undergoing a significantly rapid evolution, with such new methodologies as targeted therapies emerging to support the traditional methods of treatment. However, despite the presence of new options to the treatment of these malignancies there still no standard recommendation for the best treatment option for malignant neuroendocrine tumors. In this view, the author thinks that oncology practitioners are yet to agree on the standard treatment approach to these tumors.

Neuroendocrine tumors do not have a standard treatment method because of their complex characteristics as the described by Kulke (2008). For example, these tumors are known to express high levels of vascular endothelial growth factor (VEGF) as well as its receptor and thus allowing researchers to develop better therapies over the cytotoxic therapies traditionally used on this disease. In this case, such a medication as bevacizumab has been developed to inhibit tyrosine kinases and block tumor progression. Another drug targeted against VEGF is mTOR inhibitors, which targets the downstream functions of tyrosine kinases. Other anti-tumor agents developed against neuroendocrine tumors include PI3 and IGFR1-R inhibitors, and their responses have been seen to be positive. The author also observes that traditional therapies, like somatostatin analogs, are also of significant value in neuroendocrine treatment as results to the improvement of the condition by controlling tumor growth as well as controlling the symptoms, with the emergence of radiolabelled somatostatin analogs presenting new hope in somatostatin-based anti-tumor therapy. Moreover, Kulke (2008) also observed that cytotoxic regimens are also important as they have also

shown significant importance in the management of neuroendocrine tumor progression and presentation of clinical symptoms. By presenting these options, Kulke (2008) also presents the daunting task involving the classification of each of the options in order of importance to present the best treatment option for the disease. This paper was a review, and thus author's biases influenced the conclusion. Besides, most of the treatment options examined by the author were still under clinical investigations meaning that early conclusions may be overtaken by new developments after the completion of these clinical studies (Kulke, 2008). Another study related to the treatment of neuroendocrine tumors was presented by Harring, Nguyen, Goss, and O'Mahony (2011) in a review paper.

Harring *et al*. (2011) were a comprehensive review paper that explored the treatment of metastases of the liver in patients with neuroendocrine tumors. The author states that 946%-90% of neuroendocrine tumor patients also have neuroendocrine liver metastases at the time of diagnosis. The author establishes a set of therapeutic approaches available for a patient with neuroendocrine liver metastasis, each of which has special attributes that give it an edge over the others. For surgical interventions, the author observes that it produces superior outcomes as compared to the non-operative therapies. In this case, resection produces superior long-term outcomes as compared to complete surgical extirpation. Another treatment offered to individuals with neuroendocrine liver metastases is the liver-directed therapies where the introduction of chemotherapy creates hypoxic conditions to limit the progression of tumors in the liver. For example, radiofrequency ablation destroys the tumors using intense, destructive heat to provide symptomatic relief for the patient. Another liver-directed therapy presented by the author is hepatic artery embolization/chemoembolization, and its success rate is dependent on the level of liver involvement where patients with over 50 percent liver involvement do not record notable improvements. The authors also present selective internal radiation therapy and hepatic artery radioembolization where yttrium-90 radioactive microspheres are used to deliver selective internal radiation therapy leading to improvement of survival to above 20 months after the treatment (Harring *et al.,* 2011).

On the other hand, the non-surgical therapies presented by Harring et al. (2011) include biotherapy, targeted radiotherapy, and chemotherapy. For biotherapy, Harring *et al.* (2011) outline various somatostatin analogs that produce encouraging results on patients. These analogues include autogel, lanreotide, octreotide long-acting repeatable and octreotide, which produce tumor and symptomatic response at the rates of 64.4%, 46.6%, 6.8% and 57.4%, and 67.5%, 63.0%, 77.3% and 74.2%, respectively. Harring *et al*. (2011) also present interferon-alpha as another biotherapy approach that may be employed to address the problem of neuroendocrine liver metastases. On chemotherapy approaches, Harring *et al*. (2011) presented several drugs that have shown different efficacies in the treatment of neuroendocrine liver metastases. Some of the drugs presented by the author include platinum-based regimens, temozolomide, capecitabine, nitrosurea streptozocin, and oxaliplatin-based regimens, and they result in different survival rates and periods. Other treatments presented by Harring *et al*. (2011) in this write-up target such biological pathways as the microRNA-Regulated pathways, mTOR pathway as well as the vascular endothelial growth factors (VEGF) pathway. However, Harring *et al*. (2011) presented a review paper where evidence was based on secondary data. As a result, any limitations of the primary research could be carried on to Harring et al. (2011)'s work without giving enough options for controlling the reference experiments. Besides, by being a review paper, the quality Harring *et al.* (2011)'s work was subject to influence by their research experience and bias. A similar paper was published by Alonso-Gordoa *et al*. (2015) that was published in Rare Cancers and Therapy Journal in 2015.

In this paper, Alonso-Gordoa *et al*. (2015) sought to present an overview of pancreatic neuroendocrine tumors' (pNETs) sequential treatment. According to Alonso-Gordoa *et al*. (2015), the treatment of pNETs depend on such factors as tumor characteristics, concomitant medication, comorbidities, somatostatin research findings, tumor-related symptoms, clinical course /stage of the tumor, and rate of tumor progression, amongst others. Like Harring et al. (2011) and Sulke (2008), Alonso-Gordoa *et al*. (2015) recognized the current treatments of neuroendocrine tumors to include mTOR inhibitors, cytotoxic drugs, and STZ-based chemotherapy, amongst others. Besides Alonso-

Gordoa *et al*. (2015) observed that series of clinical trials are taking place to explore the best combinations of therapeutic approaches to offer better efficacies in the treatment of neuroendocrine tumors. About the current advancements in pancreatic tumor treatment, Alonso-Gordoa *et al*. (2015) observed that new treatment approaches are being developed to target VEGF pathway and DNA methylation. The authors also introduced other treatment methods, for example alkylating agents, endothelial growth factor receptor inhibitors, vascular endothelial growth factor receptor plus the fibroblast growth factor receptor dual inhibitors, antiangiogenics, and peptide receptor radionuclide therapy. On peptide receptor radionuclide therapy, Alonso-Gordoa *et al*. (2015) identified the most important radiolabeled somatostatin analogs as 177Lu-DOTATATE, 90Y-DOTATOC, and 111In-DOTATOC as they have shown promising results in advanced-stage clinical trials. For antiangiogenic, Alonso-Gordoa *et al*. (2015) identified bevacizumab (anti-VEGF monoclonal antibody), and tyrosine kinase inhibitors as the most successful therapies because they have made it to the advanced phases of clinical trials.

Moreover, for vascular endothelial growth factor receptor and fibroblast growth factor receptor dual inhibitors, Alonso-Gordoa *et al*. (2015) presented brivanib as the first-line medication and sorafenib as the second-line treatment after showing high efficacy levels in clinical trials. In addition, Alonso-Gordoa *et al*. (2015) presented such endothelial growth factor receptor inhibitors as sulfatinib, SNX-5422 mesylate (plus everolimus), small interfering RNA (TKM-080301), dovitinb, and gefinib, amongst others, as the likely successful medications as they have shown positive results in the early phases of their clinical trials. Moreover, Alonso-Gordoa *et al*. (2015) also presented some alkylating agents that may offer effective treatment of pancreatic neuroendocrine tumors. Some of the medications presented in this case include temozolomide, dacarbazine, and capecitabine, and the authors also highlight some of their unpleasant side effects. Like Harring et al. (2011) and Sulke (2008), the paper by Alonso-Gordoa *et al*. (2015) was also a review paper and thus its importance in research is limited by the use of secondary data as the basis for conclusions. Besides, the experience and personal biases of Alonso-Gordoa *et al*. were also of

significant importance to the paper development (Alonso-Gordoa *et al.,* 2015). Nevertheless, one of the primary research paper related to the treatment of neuroendocrine tumors was developed by Kim et al. who published the results on BMC Cancer.

Kim et al. (2010) sought to establish the biological characteristics as well as the treatment outcomes of metastatic/recurrent neuroendocrine tumors. In this study, Kim et al. (2010) made a retrospective analysis of patients with metastatic/recurrent neuroendocrine tumors to study their biology, treatment patterns as well as treatment outcomes. Kim et al. (2010) also found out that for the cases analyzed, liver comprises the primary metastatic site and that some of the treatment options availed to them included radiofrequency ablation, TACE, metastasectomy, chemotherapy, interferon, and somatostatin analogs. In the end, Kim et al. (2010) stated that treatment of neuroendocrine tumors would produce the best results after making enough considerations of the tumor characteristics in order to the device the treatment approach for individuals. However, this paper did not examine how different treatment options work to address the problem to stop tumor growth as well as to manage the symptomatic characteristics of the disease (Kim *et al*., 2010). An additional primary research about this problem was developed by Raymodn *et al.* (2011) who published the results in the New England Journal of Medicine.

In this research Raymodn *et al.* (2011) sought to explore the use of sunitinib malate as a treatment regimen for pancreatic neuroendocrine tumors. Raymodn *et al.* (2011) stated that preclinical models and phase ½ trials have demonstrated the ability of multi-targeted tyrosine kinase inhibitor sunitinib to have antitumor activity against pancreatic neuroendocrine tumors. In order to assess the significance of this antitumor activity, Raymodn *et al.* (2011) conducted a randomized, double-blind placebo-controlled trial of sunitinib on subjects recruited from different countries. In the end, the study showed that sunitinib treatment with 37.5 mg daily dose results in increased overall survival and improvement of progression-free survival of the subjects. This study was a controlled one and thus the results were dependable for making a logic conclusion about sunitinib malate's efficacies in pancreatic neuroendocrine tumor treatment. Besides, it was multinational,

allowing for more extended progression of results to cover people from different geographical locations of the globe. However, the research was made on pancreatic neuroendocrine tumors and thus it could not be used as a representative study for all neuroendocrine tumors. All these studies were summed up by Diez, Teule and Salazar (2012) in their paper that explored the diagnosis as well as the treatment of gastrointestinal neuroendocrine tumors.

In this paper that was published in Annals of Gastroenterology, Diez, Teule and Salazar (2012) stated that gastroenteropancreatic neuroendocrine tumors could be sporadic or familial, and that successful diagnosis and treatment of these conditions are a product of an interdisciplinary approach involving numerous specialties. However, the summary of the treatment strategies used to treat these conditions effectively included radiological intervention, surgery, somatostatin analogs, everolimus, sunitinib, and cytotoxic chemotherapies. In this study, Diez, Teule and Salazar (2012) observed that peptide receptor radionuclide therapy should be used in the events where disease progresses beyond the ability to manage it using the strategies mentioned above. However, this paper was a review that utilized secondary research strategies and experience of the authors to come up with a conclusion. As a result, it is limited by the author's biases (Diez, Teule and Salazar, 2012).

3.3. The importance of peptide receptor radionuclide therapy.

Several studies have demonstrated the importance of peptide receptor radionuclide therapy in ensuring better health for individuals after its administration. One of such studies was presented by Delpassand *et al*. (2012) in a paper published in the Journal of Theranostics. In this paper, Delpassand *et al*. (2012) sought to explore the long-term survival as well as the toxicity profile in patients suffering from progressive neuroendocrine tumors after peptide receptor radionuclide therapy with 111In-pentreotide. In order to establish the benefits of the therapy to the recipients, the authors used F-18 FDG PET-CT scan for prognosis in order to explore these benefits. In this paper, Delpassand *et al*. (2012) stated that the standard chemotherapy approaches the treatment of neuroendocrine tumors are lowly effective and that they that they have numerous adverse effects on the recipient leading to low survival rates as well as several effects of their high toxicity profiles.

Also, the authors observed that the introduction of peptide receptor radionuclide therapy in the 1990s provided new hopes for successfully battling neuroendocrine tumors. In this case, the authors referred to the radiolabelled somatostatin analogs as an efficacious treatment method for patients with inoperable and/or metastatic neuroendocrine tumors with elevated expression of somatostatin receptors (Delpassand *et al*., 2012).

Delpassand *et al*. (2012) found out that the therapy does not cause significant acute toxicity meaning that the recipients could not develop toxic reactions to the therapy immediately after the therapy. In addition, the authors found out that this therapy causes grade-I renal toxicity on 6.1 percent of the recipients, and liver toxicity in 18.4 percent of the recipients. Besides, the study found out that grade II and grade III hematological toxicity occurs in 7.6 percent of the patients. Moreover, the study reported an average of 18.9 months survival after the last administration of the regimen. Besides, the regimen was found to produce 85 percent patients with stable disease, 7.5 percent with partial response and only 7.5 percent with progressive disease and this was indicative of the therapy as a highly efficacious strategy. In the end, the study established that 111In-pentreotide therapy is an important approach that increases the survival time of the individual to up to 45 months after the last cycle administration. However, it highlighted the therapy like the one that is toxic to such organs and tissues as the liver, the hematological tissue and the kidney, which is the source of its contraindications (Delpassand *et al*., 2012). This study was primary, and it utilized one of the popular somatostatin analogs for the investigations. Its results could be extrapolated to cover similar analogs of the somatostatin nature, for example, DOTATOC and DOTATATE. As a result, it presented important insights about the significance of applying radiolabelled somatostatin analogs in the treatment of neuroendocrine tumors. However, the study was limited by the fact that it was a nonrandomized clinical trial. Besides, the study was also negatively affected by the use of a small population to explore the survival advantages of neuroendocrine tumors about the radiolabelled somatostatin treatment. A similar study was published by Vinjamuri *et al*. (2013) in the British Journal of Cancer.

In this study, Vinjamuri *et al*. (2013) sought to explore the response, toxicity and survival aspects related to the treatment of patients with progressive metastatic neuroendocrine tumors using 90Y-DOTATATE and 90Y-DOTATOC. Vinjamuri *et al*. (2013) stated that treatment of patients with metastatic neuroendocrine tumors with peptide receptor radionuclide therapy had gained popularly not only among patients but also among oncologists, but the overall survival advantage of the approach remains unclear. Out of the 57 patients put through this therapy by Vinjamuri *et al*. (2013), the radiological response was achieved in 71.5 percent, with progressive disease cases achieving 18 months overall survival. In the same vein, cases of positive response and stable disease achieved overall survivals of 51 and 56 months, respectively. In the end, the study established that peptide receptor radionuclide therapy with 90Y-DOTATATE and 90Y-DOTATOC results in increased overall survival advantage for the recipients in comparison with biochemical therapeutic approaches. This study supported Delpassand *et al*. (2012) findings with regard to the overall survival of the patients, but it did not explore the importance of the toxicity profiles of the therapies and thus created a requirement for further investigations about the subject.

A better understanding of the importance of peptide receptor radionuclide therapy was made by Filice et al. (2012) in a paper published in the Journal of Nuclear Medicine.

In this paper, by Filice et al. (2012) sought to explore the role played by peptide receptor radionuclide therapy (90Y-DOTATOC and 177Lu-DOTATOC) in patients suffering from neuroendocrine lung tumors. The researchers administered 90Y-DOTATOC to five patients, 177Lu-DOTATOC to two patients and a combination of the two analogs to six patients in order to establish their roles in cancer treatment. In the end, 7 out of 13 patients treated with this regimen recorded partial response, 3 out of 13 patients recorded progression disease, and 3 out of 13 recorded stable disease. Besides, for combination therapy, 67 percent of the recipients recorded partial response while 3 percent of the recipients recorded stable disease. Moreover, the treatment with 90Y-DOTATOC resulted in 40 percent partial response and 60 percent progression disease. However, 50 percent of patients treated with 177Lu-DOTATOC recorded partial response while the other 50

percent had a stable disease. In the end, the study established that pulmonary neuroendocrine tumors could benefit from the use of peptide receptor radionuclide therapy using either 90Y-DOTATOC or 177Lu-analogues alone, with combination therapy yielding superiors results. This study demonstrated the specific importance of 177Lu-DOTATOC in the treatment of neuroendocrine tumors. However, the research was limited by the small number of the subjects that limited the generalizability of the results. As a result, it created a need for further studies on a larger sample for the confirmation of the results (Filice *et al*., 2012). The importance of peptide receptor radionuclide therapy in the treatment of neuroendocrine tumors was further explored by Praasad, Brenner and Modlin who published a review in the European Journal of Nuclear Medicine and Molecular Imaging.

Praasad, Brenner, and Modlin (2013) sought to present the perspective of the clinician regarding the smartness of peptide receptor radionuclide therapy when treating neuroendocrine tumors (with a special interest in the salvage settings). In this review, Praasad, Brenner and Modlin (2013) observed that emergence and advancement of new technologies in the biological perception of the disease have resulted in increased confusion in the managerial protocols because, for example, therapeutic drugs target not only the tumor cells but also the healthy cells leading to toxicity. Praasad, Brenner and Modlin (2013) observed that one of the importance of the peptide receptor radionuclide therapy over the generalized treatment is that it allows for the reduction of therapy toxicity by targeting only the tumor cells without affecting the normal tissues laying close to the tumor. Another importance of peptide receptor radionuclide therapy, according to Praasad, Brenner and Modlin (2013), is that it allows for specific targeting of a tumor when dealing with a complex and heterogeneous disease. However, they also observed that peptide receptor radionuclide therapy has a major shortcoming, which is toxicity, as evidence in different studies assessing toxicity profiles of such somatostatin analogs as DOTATOC and DOTATE. They observed that major toxicities of peptide receptor radionuclide therapy are observed within the first few months of treatment and that if some resultant somatostatin receptor positive lesions are left untreated they

may progress within 6-14.3 months of the last treatment cycle. However, this paper only made a review of the issues of the therapy without showing how empirical evidence was achieved (Prasad, Brenner and Modlin, 2013). Nevertheless, an empirical approach was employed by Wedinger et al. in 2011 when they published a similar paper in the World Journal of Nuclear Medicine.

In this paper, Wedinger *et al*. (2011) sought to explore the importance of peptide nuclides in improving the quality in patients. The authors stated that although peptide receptor radionuclide therapy has been popularized as a significant treatment strategy for patients with somatostatin receptor-expressing tumors little evidence is available to show the importance of the approach in terms of the improvement of the patient's quality of life. In this research, Wedinger *et al.* in 2011 administered cycles of 90Y-DOTALAN and/or 90Y-DOTATOC to patients before following them up until death to establish their response in terms of improvement of the quality of life through such indicators as the general symptoms, the karnosfsky score and pain intensity. For 90Y-DOTATOC therapy, all patients recorded stable disease while for 90Y-DOTALAN, one patient recorded a stable disease and three recorded progressive disease. Moreover, even with treatment with 90Y-DOTALAN or 90Y-DOTATOC 9 out of 13 patients died after the PRRT. In addition, the therapy achieved pain relief in 3 out of 3 patients, karnfsky score, general well-being, weight and appetite improved significantly for all individuals with the stable disease. The overall finding of this study was that PRRT offers a successful treatment options patients with somatostatin receptor positive tumors because of the marked improvement of their quality of life after the treatment as well as the presence of transient side-effects. However, Wedinger *et al.* (2011) used a very small sample of 13 patients and thus limiting the generalizability of the conclusion until further investigations confirm these results.

A similar paper was developed by Romer et al. (2014), where they compared the use of 177Lu-DOTATOC and 90Y-DOTATOC in somatostatin-based radionuclide therapy for neuroendocrine tumors. In this study, Romer et al. (2014) hoped to establish benefits and harms of the two approaches. To accomplish this goal, the authors used a comparative cohort study targeting

patients with advanced neuroendocrine tumors for treatment with 177Lu-DOTATOC or 90Y-DOTATOC to such endpoints as disease progression or permanent adverse events. After 1,804 cycles of 90Y-DOTATOC in 910 patients and 259 cycles of 177Lu-DOTATOC treatment, the median survival was established to be 45.5 months and 35.9 months, respectively. These results were comparable. However, for the subgroups with extra-hepatic and solitary tumors, or low tumor uptake, the median survival of patients treated with 177Lu-DOTATOc was higher compared to 90Y-DOTATOC. In the end, the only advantage identified with regard to the use of 177Lu-DOTATOC instead of 90Y-DOTATOC was its lower hemotoxic nature (Romer et al., 2014).

3.4. Techniques in peptide receptor radionuclide therapy.

Over time, several techniques of administering peptide receptor radionuclide therapy have been described. In a paper published in Best Practice & Research Clinical Gastroenterology in 2005, Teunissen *et al*. described peptide receptor radionuclide therapy as a new treatment modality for patients with neuroendocrine tumors where they presented radionuclides and somatostatin analogs applied. In this review article, Teunissen *et al*. (2005) indicated that the most commonly used radionuclides for targeted radiotherapy included lutetium (177Lu), indium (111In) and yttrium (90Y) and cited that they have been taken through several clinical trials in the past. Besides, Teunissen *et al*. (2005) also indicated that somatostatin analogs labeled with these radionuclides differ in their affinities for various subtypes of somatostatin receptors. Teunissen *et al*. (2005) also observed that somatostatin analogs with high affinities for somatostatin receptor-2 are of high therapeutic values because of their inherent affinities for neuroendocrine tumors that allows them to deliver the radionuclides to the tumor much rapid and without affecting tissues that do not express this receptor subtype. The paper also emphasized the higher affinity for sstr2 that is exhibited by analog DOTATATE in comparison with DOTATOC and stated that DOTATATE has nine times higher affinity for this receptor subtype in comparison with DOTATOC.

On the other hand, Teunissen *et al*. (2005) highlighted the major somatostatin analogs used for peptide receptor radionuclide therapy to include DOTATOC, DOTATATE, and DPTA.

Moreover, the study also highlighted some of the somatostatin analogs that have been subject to clinical studies to include [111In-DPTA] octreotide, [90Y-DOTA] lanreotide, 90Y-DOTATOC, 90Y-DOTATATE, and 177Lu-DOTATATE. For all these clinical trials, Teunissen *et al*. (2005) found out that there were varied levels of toxicity with hematological toxicity presenting in the form of low white blood cell counts, lowered hemoglobin levels and lowered platelet counts. In addition, other forms of toxicities presented in these studies include hepatotoxicity and renal toxicity. In the end, Teunissen *et al*. (2005) observed that advancements in peptide receptor radionuclide therapy have led to promising trials with several radiolabelled somatostatin analogs, leading to an opportunity for targeting tumors efficiently. This paper was a review of various works, and it was based on secondary data to develop a conclusion. As a result, it is limited by the likely incorporation of the authors' biases during the review. A similar paper was developed by Jong and Krenning in 2002, and it was published in the Journal of Nuclear Medicine.

In this paper, Jong and Krenning (2002) sought to explore new peptide receptor radionuclide therapy advances in a review. Jong and Krenning (2002) observed that multicenter preclinical and clinical studies had established the usefulness of radiolabelled somatostatin analogs in treating somatostatin receptor positive tumors, but one of the major concerns is the high uptake of these analogs by the kidney leading to renal toxicity. One of the advances observed over years of clinical trials is the usefulness of DOTA as a chelator, where it has gained the importance of universal chelator that can be used to form complexes with such radioisotopes as 65Cu, 68Ga, 111In, 86Y, and 67Ga. Another advance made in this area is the increased tumor responses to 90Y-DOTATOC, and 177Lu-DOATATE, which according to Jong and Krenning (2002) can vary up to 100 percent cure depending on the size of the tumor. In this paper, the early results of clinical trials of radiolabelled somatostatin analogs are presented to have recorded varying levels of successes depending on the analog somatostatin type and the radionuclide employed in technique. However, the paper was limited by being a review of various clinical trial reports as well as being old since its publication. Since it was published in the early years of the 20th century, it does not include

advancements made after 2001. Another paper that highlights a method of administering peptide receptor radionuclide therapy was presented by Jong *et al*. in 2005 and it was published in the Journal of nuclear medicine.

In this paper, Jong *et al*. (2005) explored the peptide receptor radionuclide therapy by combining 90Y- and 177Lu- labeled somatostatin analogs. Jong *et al*. (2005) observed that the use of radiolabelled somatostatin analogs is promising due to their likely use in administering therapies for somatostatin receptor-expressing tumors. The added that application of 90Y- and 177Lu- labeled somatostatin analogs offers an advantage of delivering higher amounts of beta particles because both particles are beta-emitters of different capacities. In the end, the 90Y- and 177Lu- labeled somatostatin analogs' radiotherapeutic effects were shown in rats in varying intensities. However, the overall effect of combined therapy of 90Y- and 177Lu- labeled somatostatin analogs was more significant as compared to those of the two radiolabelled therapeutic somatostatin analog when used alone. In the end, Jong *et al*. (2005) established that combination of two radiolabelled somatostatin analogs in a therapeutic modality generates superior results to the application of a single radiolabelled somatostatin analog. The paper produced insightful information regarding the development of combined radiolabelled somatostatin receptor analogs by presenting the therapeutic advantage of that approach. However, it used animal models (rats) for the study and thus limiting the projections of results and conclusions to cover humans. As a result, it presented a need for further research on humans through clinical trials. A similar research to Jong *et al*.'s was made by Bison *et al*. in 2015 and the report was published in EJNMMI Research Journal.

In this paper, Bison *et al*. (2015) sought to explore methods of optimizing combined peptide receptor radionuclide therapy and temozolomide in mice (after multimodality molecular imaging studies). Bison *et al*. (2015) observed that both temozolomide and somatostatin receptor radionuclide therapy by the use of 177Lu-DOTATATE have individually resulted in significant success in the treatment of somatostatin receptor-expressing neuroendocrine tumors, and that additive results could be achieved if both the two agents are used in a combination (Bison *et al.,*

2015). As a result, they set a test model using murine to study the tumor characteristics as well as the therapeutic responses after the administration of peptide receptor radionuclide therapy in combination with temozolomide in the model murine. This method was found to cause enhanced tumor perfusion as well a reduction in the tumor size, in addition to an increased uptake of somatostatin analogs due to temozolomide administration. Moreover, the study found out that a complete response to 177Lu-DOTATATE was possible at day fourteen of temozolomide administration. In the end, Bison *et al*. (2015) concluded that the use of temozolomide in peptide receptor radionuclide therapy results in enhanced uptake of somatostatin analogs leading to higher therapeutic efficacies. However, the study was carried out on murine models and thus limiting the extrapolation of results to cover human subjects and as a result, further studies on human subjects are necessary to confirm this conclusion in a clinically relevant way.

3.5. Immediate and long-term side effects of peptide receptor radionuclide therapy.

Traditional, surface radiotherapy, is associated with several adverse outcomes presenting immediately or sometime after completing the cycle. Similarly, peptide receptor radionuclide therapy also results in some adverse outcomes that present immediately or sometimes after the last cycle. Some researchers have taken an interest in this area as adverse effects of the therapy decrease the overall importance of a regimen. One of the scholarly work conducted with the aim of exploring the therapy side effects of the peptide receptor radionuclide therapy was conducted by Pach *et al*. who published a report in the Radiotherapy and Oncology Journal in 2012.

In this study, Pach *et al*. (2012) sought to explore the levels of effectiveness of multiple cycles of peptide receptor radionuclide therapy when administered to individuals with malignant neuroendocrine tumors, in addition to the resultant side effects of the therapy. Pach *et al*. (2012) 16 out of 89 patients were treated on a repeated cycle, of which one was subjected to peptide receptor radionuclide therapy as neoadjuvant therapy. The analysis of the side effects of the treatment showed that the use of PRRT as a neoadjuvant therapy resulted in a reduction of the tumor sizes. In addition, stabilization of the disease was achieved in a period of 6-18 months, but death occurred in

ten individuals who received repeated PRRT cycles. However, Pach *et al*. (2012) observed that repeated PRRT cycles do not cause significant toxicities to the hematological functions as well as the kidney because toxic changes were transient. Nevertheless, the paper was based on results of a small sample that is unrepresentative. Besides, sample collection was not randomized and thus results and conclusions were of little generalizability. A similar study was conducted in Germany by Horsch *et al*. in 2016 and the report was published in the European Journal of Cancer.

In this paper, Horsch *et al*. (2016) wished to determine the effectiveness as well as the side-effects of PRRT for neuroendocrine neoplasms among patients in Germany. Horsch *et al*. (2016) followed 450 patients for 24.4 months (averagely), where 54 percent of them were treated with Lu-177, 17 percent with Y-90, and 29 percent with both nuclides. Ultimately, Horsch *et al*. (2016) conducted overall and progression-free survival determination using univariate log-rank test COX models and Kaplan-Meier curves, where the median overall survival of the patients was found to be 59 months. However, patients treated with Lu-177 recorded longer survival as compared to those treated with Y-90, with survival rates of patients having grade II and grade III neuroendocrine neoplasms being lower than those with grade-I neuroendocrine neoplasms. From the study, 0.2-1.5 percent cases of adverse events of kidney and bone marrow function higher than grade III were recorded. In the end, the paper showed that peptide receptor radionuclide therapy results in low-grade adverse events. However, the research was conducted on a localized population in Germany and result may not be generalized to cover a wide global region as different regions may be subject to differential local influences (Horsch *et al*., 2016).

3.6. Receptor affinity and pharmacokinetics/pharmacodynamics of radiolabelled somatostatin analogs.

Different somatostatin analogs have distinctive receptor affinities as pharmacodynamics characteristics. A study published in Anticancer Research Journal by Laznicek, laznickova, and maecke in 2012 revealed some of the characteristic features of radiolabelled somatostatin analogs with regard to receptor affinity and bio-distribution. In this study, Laznicek, laznickova, and maecke

(2012) explored how different radiolabelled somatostatin analogs are taken up by somatostatin-rich tissues in relation to the affinity to somatostatin receptor subtype2. Laznicek, laznickova, and maecke (2012) administered six derivatives of 111In-labelled octreotide and octreotide to adrenals and pancreas in vivo before measuring the associated organ radioactivity 24 and 48 hours after administration in order to correlate the results with the affinity to somatostatin subtype2 determined in vitro – IC-50 values. Adrenal uptake of radiolabeled analogs was determined by establishing the exponential dependence best fit against IC-50 while linear dependence was used for correlation regarding pancreas. In the end, Laznicek, laznickova and maecke (2012) established that adrenal and pancreas radioactivity correlates with in vitro determined receptor affinities for specific subtypes of somatostatin. However, the study used rat models to come up with the conclusion and thus it cannot be applied for clinical purpose until further investigations involving clinical work. In another paper published by Harris (1994), the author offers as a comprehensive exploration of the pharmacokinetics and pharmacodynamics effects of somatostatin and somatostatin analogs.

In this paper, Harris (1994) described the structures of the two somatostatin molecules, namely somatostatin 14 and somatostatin 28, as well as their biological distributions in the body. In this case, Harris (1994) observed that the gut lumen, the endocrine cells, the visceral autonomic nervous system and the pancreas form the primary localization sites of somatostatin. Regarding actions, Harris (1994) observed that somatostatin is an inhibitory molecule that blocks endocrine and exocrine secretion. The natural somatostatin has a short half-life making its effects short lived as well as insufficient in controlling unspecific growth that characterizes tumors. As a result, for tumor treatment somatostatin analogs are used to overcome the problem of shortened half-life. For example, octreotide is one of the analogs of somatostatin, and it has a half-life of 113 minutes as opposed to 2-3 minutes that natural somatostatin takes to reduce by half its original quantity. According to Harris (1994), after administration of octreotide in the body, a 50 percent reduction in growth hormone secretion is recorded. One of the pharmacokinetics characteristics of octreotide is that its oral administration is characterized by low rates of

gastrointestinal absorption leading to lowered bioavailability. However, administration by intravenous or subcutaneous injection results in similar bioavailability and a peak serum concentration within 30 minutes of administration. The increase in the concentration of octreotide in the serum after administration increases linearly and its plasma clearance in persons with renal impairment is 50 percent lower than those without renal malformations. The endocrine effects of octreotide are similar to those produced by natural somatostatin molecules. Harris (1994) concluded by presenting similarities between octreotide as a representative somatostatin analog and the natural somatostatin. Ultimately, Harris (1994) provided a resourceful overview of the pharmacodynamics and pharmacokinetics characteristic of octreotide with regard to its application as a pharmaceutical. However, the paper was a review, and it depended on secondary data to make conclusions.

Another study by Lesche *et al*. (2009) sought to establish the differences between octreotide and pasreotide with regard to somatostatin receptor internalization as well as trafficking in vitro. In this paper, Lesche *et al*. (2009) observed pasreotide has a high binding affinity to such receptors subtypes as sstr1, sstr2, sstr5 and sstr3, unlike octreotide that has a high affinity for sstr2 only. However, results showed that octreotide has a higher potency for causing internalization as well as signaling of sstr2 in human embryonic renal cells. Additionally, octreotide-mediated activation of the receptor was shown to cause events leading to the internalization of beta-arrestin-2 and sstr2 into the same endocytic vesicles while receptor activation by SOM230-mediated receptor led to the formation of lowly stable complexes that disintegrated at the plasma membrane. However, both octreotide and lanreotide caused rapid sstr3 down-regulation. In the end, Lesche *et al*. (2009) found out that octreotide and somatostatin modulate receptor trafficking distinct ways as compared to pasreotide.

3.7. Everolimus therapy for neuroendocrine tumors.

Everolimus is one of the most recent breakthroughs in neuroendocrine tumor therapy as it stops tumor progression by targeting the downstream reactions of the mTORC1 pathway. As a result, numerous studies have been made to ascertain the importance of this breakthrough. One of

such studies was conducted by Yao et al. and the report was published in the New England Journal of Medicine in 2011. In this study, Yao *et al*. (2011) sought to explore the use of Everolimus for advanced pancreatic neuroendocrine tumors. In this study, Yao *et al*. (2011) stated that pancreatic neuroendocrine tumors are difficult to treat as most of the cases they are diagnosed lately. Besides about 65 percent of the cases diagnosed in the late stage present with the inoperable or metastatic disease, making surgical strategies infeasible. As a result, oncologist result to the recommendation of chemotherapeutic agents for the management of this disorder, where streptozocin is prescribed for use by patients.

However, according to Yao *et al*. (2011) Everolimus is a promising agent that has shown high antitumor efficacies in several phase-2 clinical trials. Yao *et al*. (2011) stated that everolimus treats pancreatic neuroendocrine tumors by suppressing cell proliferation, growth, and angiogenesis, by inhibiting mTOR. In their study, Yao *et al*. (2011) made a phase 3 study on everolimus to evaluate it in a perspective and a randomized way where 410 patients with low-grade, advanced or intermediate-grade pancreatic neuroendocrine tumors received everolimus at a daily dose of 10 mg (207 patients) or placebo (203 patients). In addition to this medication, each group also got high-profile supportive care for the condition. In the end, Yao *et al*. (2011) investigated the survival of the two groups to establish the effectiveness of the therapy, where those who received everolimus recorded 11.0 months median survival while those who received placebo recorded a median survival of 4.6 months. Besides, at the eighteenth month after the treatment, only 34 percent of those treated with everolimus were alive, compared to only 5 percent that had received placebo treatment. In addition, drug-related adverse effects were higher among those treated with everolimus while it was lower among those treated with placebo, at the rate of 34 percent and 9 percent, respectively. Additionally, the some of the commonest adverse events recorded by those treated with everolimus included anemia, hyperglycemia, diarrhea, fatigue, infections, rash, and stomatitis. Moreover, the study revealed a median exposure rate of 34 weeks for everolimus compared to 16 weeks for placebo.

As a conclusion, Yao *et al*. (2011) observed that everolimus therapy results in prolongation of progression-free survival of the recipients as compared to placebo treatment. Additionally, the study also concluded that use of everolimus results in numerous, severe adverse effects as compared to the administration of placebo. This study was generalizable as it was randomized and with a high number of participants. However, it was conducted on pancreatic neuroendocrine tumors making it difficult to extrapolate the findings to cover other classes of neuroendocrine tumors and thus further studies are necessary to establish where the treatment of other neuroendocrine tumors with this agent results in similar outcomes (Yao *et al*., 2011). Another study that explored the use of everolimus in the treatment of neuroendocrine tumors was conducted by Porta, Paglino and Mosca and reported in Frontiers in Oncology in 2014.

In this study, Porta, Paglino and Mosca (2014) sought to explore the cancer treatment by targeting PI3K/Akt/mTOR pathway. Porta, Paglino and Mosca stated that mTOR and PI3K are important pathways in determining the growth as well as the growth of cells both in physiology and pathology. In this review paper, Porta, Paglino and Mosca (2014) described the functions of mTOR in the normal cellular physiology as well as the likely targeting in the event of tumor development to offer treatment. While exploring the importance of everolimus in neuroendocrine tumors therapy Porta, Paglino and Mosca (2014) explained that rapamycin inhibitors act by inhibiting the mTOR pathway by forming an FKBP-12 to prevent the mTOR activity and as a result inhibiting three events, namely, angiogenesis, survival and cell cycle progression. With this idea in mind, Porta, Paglino and Mosca (2014) described everolimus phase-III trials that have resulted in noteworthy successes in the treatment of neuroendocrine tumors. Porta, Paglino and Mosca (2014) observed that the use of everolimus in combination with octreotide LAR in a phase-II trial had demonstrated a significant anti-tumor activity that resulted in a median in 15.7 months of progression-free state, 80 percent of stable disease, and 17 percent remission. In RADIANT-1 phase-II trial 115 participants were put on everolimus at a dosage of 10 mg per day while 45 patients have put on ten everolimus 10 mg per day and octreotide LAR at a dosage of 30 mg per 28 days. Porta, Paglino and

Mosca (2014) found a 9.6 percent response rates in individuals put on everolimus alone, and 4.4 percent response rate among those put on everolimus in combination with octreotide LAR.

In addition, the progression-free survival time for those who took everolimus alone was 9.7 months, but the progression-free survival of those put on both everolimus and octreotide LAR was 16.7 months. These results were confirmed in phase-2 and phase-3 (RADIANT-II and RADIANT-III, respectively) studies - randomized, placebo-controlled, multicentre, and internalized studies. Following the RADIANT-3 study, in 2011, everolimus was approved for the use as a therapeutic agent for neuroendocrine tumors, and the medication has ever since been in the market. This paper provided important theoretical backgrounds regarding mTOR as well as the mechanism of action of mTOR inhibitors with regard to neuroendocrine tumors. Besides it also confirmed the theory by presenting the clinical trial results of everolimus (Porta, Paglino and Mosca, 2014). However, the paper was a review and its quality highly dependent on the experience of the authors in this field as well as the quality of the primary data, which was out of their control. Besides, the paper was also subject to the biases of the authors, and these factors could affect the conclusions made by the authors. Another paper regarding the use of everolimus in the treatment of neuroendocrine tumors was presented by Neychev et al. (2015) in the Biomedical Journal (BMJ).

In this paper, Neychev et al. (2015) set a phase-II clinical trial to explore the effectiveness of mutation-targeted treatment using sunitinib or everolimus in cases of intermediate-grade or advanced-level pancreatic or gastrointestinal neuroendocrine tumors with or without cytoreductive surgery. Neychev et al. (2015) observed that several studies and works are in progress in order to find the optimal strategies for managing such inoperable or metastatic neuroendocrine tumors as pancreatic neuroendocrine tumors or gastrointestinal neuroendocrine tumors. Neychev et al. (2015) were a prospective study that designed an open-label phase-II trial to determine whether nutation-targeting treatment with everolimus or sunitinib could result in longer progression-free survival of patients with intermediate-grade or low-grade neuroendocrine tumors. The research design involved putting patients on sunitinib or everolimus and making a prospective study on them until disease

progression warranted switching to the other drug and consequently following the patient until unacceptable toxicity, consent to withdrawal or disease progression. The paper explored the importance of sunitinib and everolimus as important therapeutic agents for treating neuroendocrine tumors, in addition to the effect of the mutation on the progression of the disease during the treatment course. However, the paper was incomplete as it did not report the findings (Neychev *et al.*, 2015).

3.8. Other studies.

By everolimus being among the first mTOR drug to be approved for use in the treatment of neuroendocrine tumors, numerous studies have been conducted to explore different aspects of the drug. In a paper published in the Therapeutic Drug Monitoring Journal, Taber et al. (2015) explored the pharmacokinetics as well as the pharmacodynamics characteristics of everolimus. In this study, Taber et al. (2015) sought to make a comparative analysis of the pharmacokinetic and pharmacodynamics characteristics of everolimus with regard to different racial groups comprising of adult kidney transplant recipients. In this paper, Taber et al. (2015) stated that limited data exists with regard to both the pharmacodynamics and pharmacokinetic properties of everolimus with regard to two races, namely, Caucasians and African-Americans, and thus it is difficult to establish their differences. According to Taber et al. (2015), both races exhibit similar baseline demographics, immunologic risk, and immunosuppression. However, after experimental treatment with everolimus, the African-American group of recipients exhibited higher concentrations as compared to the Caucasian cohort. However, the overall outcome demonstrated everolimus as an effective way of preventing as well as causing an improvement in the graft function regardless of the race of the recipient. This study provided important insights of the pharmacokinetics and pharmacodynamics characteristics of everolimus in African-American as well as the Caucasian users, but it provided no information with regard to neuroendocrine tumor therapy. As a result, it necessitated for further studies on the pharmacodynamics and pharmacokinetic properties of everolimus with regard to the treatment of neuroendocrine tumors. Besides, no randomization was

made and thus making it difficult to generalize the conclusion. Another study on everolimus and octreotide was made by Tippeswamy *et al.* in 2015 and published in the Indian Journal of Cancer.

Tippeswamy *et al*. (2015) presented an Indian experience of the use of everolimus in combination with octreotide long-repeatable for treating advanced neuroendocrine tumors performed in tertiary cancer care setting. In this study, Tippeswamy *et al*. (2015) explained that the treatment of neuroendocrine tumors is challenged by their insensitivity to the conventional system chemotherapy. As a result, the successful management of this condition involves using three strategies, namely antiangiogenic therapy, rapamycin inhibition and somatostatin analogs. In this study, patients with prior exposure to chemotherapy were put on everolimus at a dose of 10 mg per day in addition to intramuscular injection of octreotide long-repeatable at a dose of 30 mg per 30 days for a period leading to unacceptable toxicity or disease progression. In the end, 69 percent of the recipients recorded clinical benefits – with 63 percent of them recording stable disease and 6 percent recording partial response – after administration of everolimus in combination with octreotide long-acting repeatable. The patients also showed high tolerance to this therapy as well and thus allowing Tippeswamy *et al*. (2015) to conclude that combination of everolimus and octreotide long-acting repeatable has is highly efficacious as well as safe for use in the treatment of neuroendocrine tumors. However, the paper did not explore the use of everolimus in combination with a radiolabelled octreotide molecule and thus being different from the current study (Tippeswamy *et al.*, 2015).

A similar study was conducted by Bajetta *et al*. who published the findings in the American Cancer Society Journal in 2014. In this study, Bajetta *et al*. (2014) sought to explore the efficacy of a combined regimen of everolimus and octreotide LAR when used in the first-line setting for neuroendocrine tumor patients. Bajetta *et al*. (2014) stated that suggestions from preclinical as well as clinical studies indicate that the concurrent use of everolimus and somatostatin analogues produces synergy in the treatment of neuroendocrine tumors and, as a result, they sought to assess the activity as well as the safety of the regimen with regard to the treatment of neuroendocrine tumors of lung

and gastroenteropancreatic origin. They used the 2-stage minimax design developed by Simon to set a phase-2 multicenter trial where the participants were treated with a daily dose of 10 mg everolimus, combined with a 28-day dose of 30 mg octreotide long-acting repeatable. As a result, Bajetta *et al*. (2014) found out that most adverse events were of grade 1 or 2, with one patient recording grade-4 adverse events (mucositis), but grade 3 adverse events included 1 case of skin rash, 4 cases of stomatitis and 11 cases of diarrhea. Additionally, the study resulted in 18 percent overall response rate and 92 percent clinical benefit. As a result, the study suggested the combined use of everolimus and octreotide long-acting repeatable as an effective regimen for first-line treatment of patients with neuroendocrine tumors. However, Bajetta *et al*. (2014) used a small sample of fifty patients that was unrepresentative. Besides, the study was also not randomized leading to the lack of generalizability of related conclusions. Combined therapy of neuroendocrine tumors using a somatostatin analog and everolimus was also explored by Claringbold and Turner (2015) with the aim of establishing the safe dose of this combined therapy.

In this study, Claringbold and Turner (2015) sought to establish the dose-limiting toxicity of combined use of 177Lu-octreotate and everolimus when used for the treatment of progressive gastro-entero-pancreatic neuroendocrine tumors. In this study, all the patients with unresectable, progressive GEP-NETs received 7 GBq 177Lu-octreotate at intervals of 8 weeks, while successive cohorts of 4, 9 and three patients received escalating doses of everolimus of 5, 7.5 and 10 mg for 24 weeks, respectively. This combined therapy resulted in 44 percent response rate, and the maximum allowable dose of everolimus was established to be 10 mg, at a point where reduced creatinine clearance, neutropenia, and thrombocytopenia showed up. In the end, the maximum tolerable dose of everolimus was found to be 7.5 mg daily (Claringbold and Turner, 2015). The only shortcoming of this study was the use of a small sample of 16 patients, which limited the generalizability of conclusions.

A different study that explored the importance of everolimus with regard to the treatment of neuroendocrine tumors was published by Liu, Marincola and Oberg who reported their findings in the Therapeutic Advances in Gastroenterology journal in 2013.

In this paper, Liu, Marincola and Oberg (2013) reviewed the latest studies with the aim of interpreting the use of everolimus as a treatment approach for patients with advanced pancreatic neuroendocrine tumors. Additionally, Liu, Marincola and Oberg (2013) stated that the traditionally available treatment option for pancreatic neuroendocrine tumors involves the use cytotoxic agents, most commonly streptozotocin in addition to doxorubicin or 5-fluorouracil. However, the authors also recognize the emergence of sunitinib (a tyrosine kinase inhibitor) and everolimus (an mTOR inhibitor) as the latest additions to the treatment options. Liu, Marincola and Oberg (2013) reviewed the results of three clinical studies, namely RADIANT-1, RADIANT-2 and RADIANT-3, and their importance with regard to the use of everolimus in treating pancreatic neuroendocrine tumors. Regarding RADIANT-1, the study established that the use of everolimus resulted in a median overall survival of 24.9 months, confirming the previous studies that had established high rates of disease stabilization with the use of everolimus. In addition, with regard to RADIANT-2 Liu, Marincola and Oberg (2013) found out that disease progression-free survival was higher with the use of everolimus as compared to the use of placebo, even though its use was associated with a wide range of outcomes. Moreover, regarding RADIANT-3, v Liu, Marincola and Oberg (2013) found several clinical benefits of using everolimus over placebo (including 11 months progression-free survival versus 4.6 months, 5 percent response rate versus 2 percent, and 73 percent stable disease versus 51 percent).

Another study that is related to the current study was conducted by Petrik et al. (2011) with the aim of radiolabeling peptides using a fully automated disposable cassette system in order to produced PET, SPECT, and therapeutic agents. In this study, Petrik et al. (2011) wished to radiolabel DOTA derivatives with 177Lu, 90Y, 68Ga and 11In, and meet both the radiation safety requirements as well as their pharmaceutical requirements. According to Petrik et al. (2011), the

system's major components include a syringe pump, a holder, a heater and radiation shielding, which is removable. This system uses an acetate buffer for 68Ga labeling and ascorbate buffer for 177Lu, 90Y, and 111In labeling. On the other hand, use of disposable cassettes and thin-layer chromatography prevented cross-contamination and radiochemical purity, respectively. According to Petrik et al. (2011), the system produced radiolabeled products of over 80 percent radiochemical purity and radiochemical yield. However, the method utilized in this system cannot be generalized to cover other somatostatin analogs as different such analogs have different chemical properties.

4.0. Chapter Four: Materials and Methods.

4.1. Study population.

The research proposal was presented to research ethics committee and upon approval, nine patients were selected randomly as phase I-II clinical trial participants. In this study, patients with neuroendocrine tumors who met the inclusion/exclusion criteria were included as study participants. These patients were diagnosed with inoperable and/or metastatic neuroendocrine tumors. During the study, 68Ga-DOTATOC was used to produce PET/CT images in order to establish the uptake of 177Lu-DOTATOC by neuroendocrine tumors. After histological confirmation of the neuroendocrine tumor cases, the treatment of the recruited patients was performed using intravenous 177Lu-DOTATOC and oral everolimus as outlined in the proceeding sections. All the nine patients were treated with 177Lu-DOTATOC in four cycles without any other anti-tumor agents except everolimus. However, other antitumor agents were allowable in cases of disease progression.

4.2. Inclusion and exclusion.

4.2.1. Inclusion criteria.

1. All those who participated in the study were required to have histologically confirmed neuroendocrine tumor, with a ki67 index of at most 20 percent.
2. They were also supposed to have measurable disease based on the RECIST (Response Evaluation in Solid Tumors) criteria.
3. Patients with advanced neuroendocrine tumors were also eligible for the study - they were supposed to exhibit progressive disease on the basis of Response Evaluation Criteria in Solid Tumors.
4. The study also required the participants to have their diagnostic PET/CT 68Ga-peptide images to demonstrate high levels of tumor uptake in order for the researcher to determine the impact of the treatment from commencement to the conclusion of the study.

5. All participants were also required to be FDG PET negative, with SUV being less than 2.5.
6. The study also required the participants to have a medically determined life expectancy of at least six months.
7. For female participants of childbearing age, it was a requirement for them to agree to the use of effective contraceptive methods (including, condoms, oral contraceptives, barrier controls, sterilization, and intrauterine devices) at the beginning of the study and for a period lasting for three months after the last cycle of 177Lu-DOTATOC and the last oral administration of everolimus. Besides, they were required to have a negative serum test for pregnancy within 14 days leading to the study commencement.
8. All the study participants were also required to exhibit healthy renal, hematological and liver function. In this case, the participants were required to have hemoglobin level of at least 9 g/dl, platelets count of at least 100 x 109/L, and total neutrophil count of 1.5 x 109/L. Besides, the participants were required to have a bilirubin level of at most 1.5 x upper normal limit, creatinine level of less than 2mg/dl, and alanine transaminase of less than 2.5 x upper normal limit (and less than 5 x upper normal limit for liver metastases cases).
9. The participants were also required to have ECOG performance status of less than 2.
10. All participants were also required to be willing as well as able to provide informed consent for participating in this study.
11. The study also allowed concomitant somatostatin analogs assumption (National Institutes of Health, 2016; Neychev *et al*., 2016).

4.2.2. Exclusion criteria.

Patients who had any of the following issues did not meet the inclusion criteria, and therefore, they were excluded from the study.

1. Those with Ki 67 index of more than 20 percent were ineligible for participation in this study.

2. The study also excluded those who had to participate in a different clinical trial involving an investigational agent within 30 days leading to the commencement of the current study.
3. Additionally, the study also excluded willing patients who had a history of allergic reactions due to the agents of similar chemical properties to 68Ga-DOTATOC, 177Lu-DOTATOC or/and everolimus.
4. The study also excluded patients who had been treated with therapeutic radiotherapy and chemotherapy within a period of four weeks before the commencement of the study. It also excluded patients who had undergone hormonal or biological therapy, or palliative radiotherapy within a period of two weeks leading to the commencement of the study.
5. The study also excluded patients with any uncontrolled intercurrent condition, for example, cardiac disorders (symptomatic congestive heart failure, cardiac arrhythmia, or unstable angina pectoris), active infectious disease, psychiatric instability or social disturbances that could have limited one's compliance with the requirements of this study.
6. This study also excluded all patients affected by adverse events of toxic effects of previous chemotherapies or any kind of radiation therapy.
7. In addition, the study also excluded neuroendocrine tumor patients with operable or benign conditions.
8. Those patients who had been treated with radiometabolic therapy and with absorbed dose to bone marrow more than 15 Gy and 25 Gy for kidney were also excluded from the study.
9. The study also excluded patients who exhibited uncontrolled hypertension – more than 150/100 mm Hg.
10. Pregnant or nursing mothers (patients) were also excluded from this study as it was postulated that everolimus and 177Lu-DOTATOC could cause unknown effects on the human fetus. In the same vein, it was recommended to exclude the nursing mothers as adverse effects of the treatment could extend to their children.

11. The study also excluded patients with pulmonary diseases of clinical importance, for example, asthma, or severe chronic obstructive pulmonary disease (COPD).
12. Patients with HIV infection and undergoing antiretroviral therapy were also excluded from the study because there was a possible pharmacokinetics interaction with 177Lu-DOTATOC or everolimus.
13. Subjects were also excluded from the study due to such conditions as malabsorption syndrome, the absence of upper gastrointestinal tract physical integrity or the inability to take medications orally (National Institutes of Health, 2016; Neychev *et al*., 2016).

4.3. Study design.

This study was a prospective study involving the intravenous administration of 177Lu-DOTATOC in four cycles in combination with daily oral everolimus. At the beginning of the study, histological confirmation of neuroendocrine tumors was performed to ensure that all the nine participants of the study had an inoperable or/and metastatic neuroendocrine tumor. All of the nine patients received four cycles of (100mCi) 177Lu-DOTATOC at intervals of 28 days. A daily 10mg dose of oral everolimus was added to the regimen for this period. For everolimus administration, the treatment continued until the withdrawal of consent, disease progression, interruption of the drug for at least three weeks, or progression of the disease. On the other hand, periodic PET/CT 68Ga-images were taken before, during and at the end of the treatment. Additionally, for every synthesis of radiolabelled peptides radiopharmaceutical quality control was done.

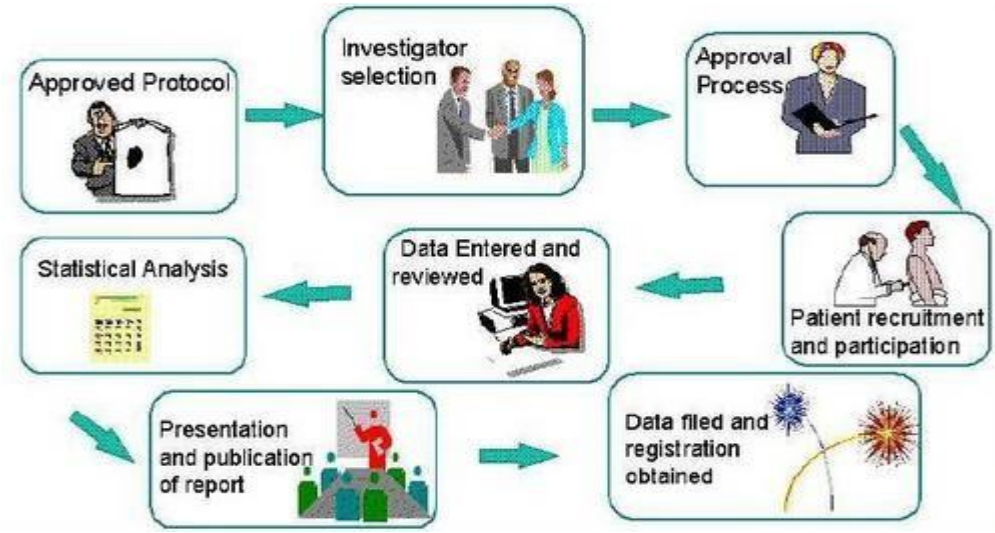


Fig 54: Clinical data management processes applied in this study.

4.4. Radiochemistry.

4.4.1. Preparation of 68Ga-DOTATOC.

68Ga-DOTATOC needed in this study was produced by following a guidelines described by Seemann, Waldron, Parker and Roesch (2016). All reagents used in this procedure were bought from Merck® and no further purification was made. The procedure also used Purite® water and a Millex® Millipore filter membrane for all filtering, while TLC-plates were used to monitor the reaction progress and KMnO4 was used for visualization. Also, silica gel 60 used to perform column chromatography while Avance III HD 400 was used for recording NMR spectra. Additionally, A meta-free Dionex ICS-5000 system that had an AS-50 autosampler, a quaternary pump, an automated fraction collector AFC-3000, and a UV/Vis detector was used to perform HPLC. The chelator synthesis was performed using a procedure outlined in Seemann *et al.,* (2016) while the radiolabelling stage of 68Ga-DOTATOC preparation followed the NaCl (Mueller) method.

In this method, 1ml 5.5M HCl and 10 ml of water was used for preconditioning the silica-based SCX cartridge in preparation for the elution step. A standard radio-thin layer chromatography (using ITLSC-SG strips) was used to determine the peptide-bound percentage while 0.1 percent

trifluoroacetic acid/50 percent acetonitrile formed the mobile phase (Schultz *et al*., 2012). The radio-High Performance Liquid Chromatography method was used to validate the procedure, and it operated under conditions outlined here.

Table 1: Conditions of operating Radio-High Performance Liquid Chromatography.

Feature	Condition
Multiwavelength Detector	Jasco MD 1510
HPLC pump	Jasco PU-1580
Quaternary gradient unit	Jasco LG-1580-04
Radio detector	Biostep IsoScan LC
Column	RP-18
Detector	Jasco MD 1510

1M sodium acetate was used to buffer the pH o the content to 4.5.

4.4.2. Automated and manual labeling of Ga-68 with DOTATOC.

4.4.2.1. Automated labelling of 68Ga-DOTATOC.

In this section, an automated process of 68Ga-radiolabelling was described with reference to the work of Aslani et al. (2014). According to Aslani et al. (2014), a complete radiolabelling is achieved in one preliminary step and two steps of the protocol. The preliminary step was to test the cassette for leaks by applying a pressure of 200 kPa to different sections of the cassette. When performing this test, a high purity nitrogen gas supply is attached to the cassette in order to deliver 200 kPa of pressure. The pressure and the synthesis modules are then driven to by Modular Lab Pharm Tracer®'s software in order to test the cassette using pressure. The software also develops a visual progress graph to display the loss of pressure. In this case, the cassette is considered to have passed the test if there is no loss of pressure below 100 kPa, but if the test fails the cassette is reattached before repeating the test Aslani et al., 2014.

After passing the preliminary test (the cassette pressure test), such pre-prepared reagents as ethanol, DOTATOC peptide, eluent and 0.9% saline were placed in their respective sections of the synthesis cassette before connecting the 68Ge/68Ga generator tube, and attaching waste bottle, product vial, and empty containers. The first step of the automated radiolabelling system as adopted from Aslani et al. (2014) is the synthesis phase, and it also is driven by Modular Lab Pharm Tracer®. This step forms the core of the entire automated radiolabelling process, and it takes 33 minutes to be completed. In this phase, a ten mL syringe that is used to drive all liquids. At the initial part of this phase, ethanol, and 0.9% saline are used to prime the SepPak cartridge, before eluting the generator with 0.05 M HCl (4 mL) and passing it through Strata-X-C ion exchange cartridge to trap the gallium-68 chloride. Of the resultant mixture, the excess 0.05 M HCl is released to the wastewater while Ga-68 chloride is released into a reaction vial of acetate buffer and DOTATOC peptide. This mixture is then exposed to heating at 95 °C for a duration of 400 seconds in order to link DOTATOC to gallium-68 chloride as well as evaporate acetone/HCL (the eluent). This step is followed by passing resultant content through SepPak cartridge to trap Ga-68 DOTATOC and release unbound DOTATOC and Ga-68 to the waste bottle. In the end, the elution of the trapped Ga-68 DOTATOC is accomplished using ethanol plus 0.9% saline Aslani *et al*., 2014.

On the other hand, the last step of the automated radiolabelling process as adopted from Aslani et al. (2014) is the Filter Pressure Test, and it is driven by Modular Lab Pharm Tracer ®. In this step, the operator removes the 022 μm filter as well as the needle from the vial containing the product in order to allow for the filter to be subjected to 200 kPa pressure. A successful filter pressure test is the one in which pressure remains above 100 kPa, and it indicates the sterility of the product. In case of pressure that is lower than 100 kPa, the product is considered unsterile, and the operator needs to draw the product up manually (Aslani *et al.,* 2014)

On the other hand, a variant method for radiolabelling DOTATOC with 68Ga was made available (as an alternative method) by adopting methodology described by Eppard, Perez-Malo and Rosch (2016). In this case, standards set to be followed involved heating a buffer with DOTATOC

to develop high yields by varying the temperature conditions between 95 and 100 °C for a duration of 10 to 20 minutes. DOTATOC used in this system was sourced from ABX Advanced Radiochemical Systems in order to prepare an aqueous stock solution (Eppard, Perez-Malo, and Rosch, 2016).

4.4.2.2. Manual synthesis of 68Ga-DOTATATOC.

In the current study, manual preparation of 68Ga-DOTATOC was conducted according to Romero et al. (2016), in a two-stage protocol. In this case, the first step of the protocol was the elution, purification as well as concentration of 68Ga. Concentration of the eluate used the fractionated method, where the fractions were collected in Eppendorf plastic tubes and were measured using a calibrator, on the 68Ga window. Simultaneously, the measurements were made using an installation with ionization chamber. The data shows that 80-92 percent of the eluted radioactivity was found in 2^{nd} fractions 1^{st} and third fraction was considered waste. This method leads to the concentration of the activity in a small volume, 1.5 mL, and thereby to the increasing of the radioactive concentration. In this case, 4 mL 0.05MHCl was used to elute ITG 68Ga generator. The second step was the radiolabeling step, which included the linkage of 68Ga to DOTATOC. The 68Ga so eluted in the previous step was added directly to a vessel containing DOTATOC solution, at PH of 3.5-4, and heated to 95 °C for five minutes before being purified using a solid-phase extraction cartridge Sep-Pak. The final product was then evaporated to get rid of ethanol, and mixed with normal saline before being passed through HPLC to determine its radiochemical purity and any possible peptide radiolysis damage. For quality control purpose, the product was subjected to radio-High Performance Liquid Chromatography to determine the radiochemical purity of the product.

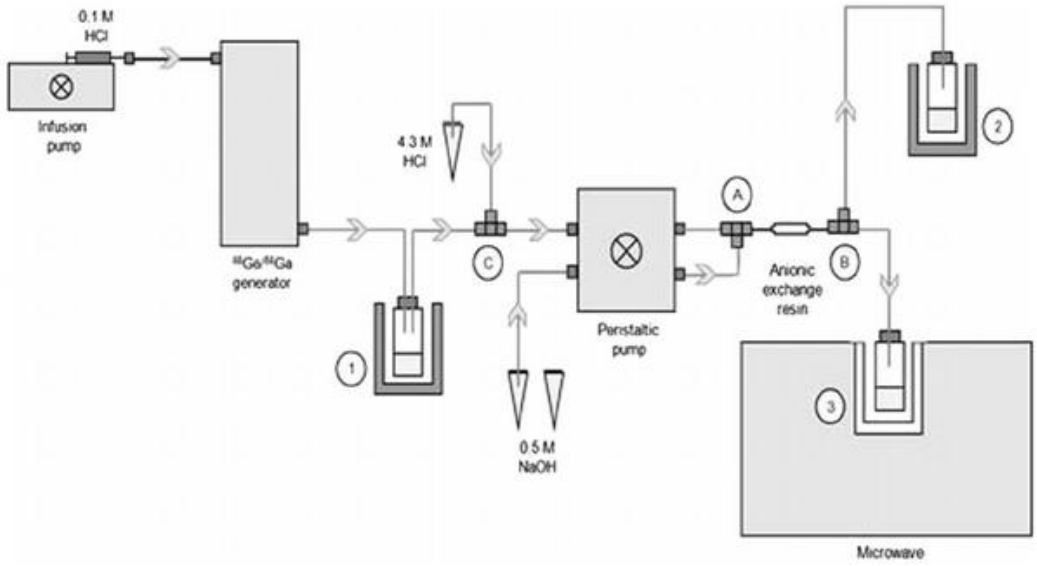


Fig 53: the manual preparation of 68Ga and 68Ga-DOTA-peptides.

4.4.3. Image acquisition and analysis.

In this study, 68Ga-DOTATOC PET/CT was used to localize primary neuroendocrine tumors as well as detect the associated metastatic disease and follow up the condition throughout the study period. For every imaging session, the research technician prepared the patients in three ways. In this case, the technician explained the imaging test to the patients through all means possible to the recipients' satisfaction. The patients were also required to stop cold octreotide therapy to avoid somatostatin blockade. They were also advised not to fast prior to imaging because taking meals does not have any negative implications on image acquisition (Virgolini *et al*., 2010). Before 68Ga-DOTATOC injection, the technician was required to be aware of the relevant history of the primary tumor and metastasis. The technologists were also required to be aware of the results of such imaging modalities like CT, plain radiography, MRI, and ultrasonography. 68Ga-DOTATOC was administered by an indwelling catheter in order to avoid extravasation. The activity ranges of 68Ga-DOTATOC administered in this study varied from 100 to 200 MBq in order to produce good quality images. Besides, 25 μg of 68Ga-DOTATOC was administered for all cases.

In this project, a dedicated PET/CT scanner was used to acquire data at the 60^{th} minute after 68Ga-DOTATOC injection. An iterative reconstruction algorithm was used to reconstruct the

images, and it included such corrections as attenuation correction, normalization, dead time, model-based scatter correction and decay correction (Virgolini *et al*., 2010). During analysis, the normal, as well as the abnormal accumulations of 68Ga-DOTATOC, were evaluated by a nuclear medicine physician. Any accumulation of 68Ga-DOTATOC in tissues not known to take up the tracer physiologically was considered pathological. However, findings with clearly demarcated tracer uptake were considered positive for malignant neuroendocrine tumors.

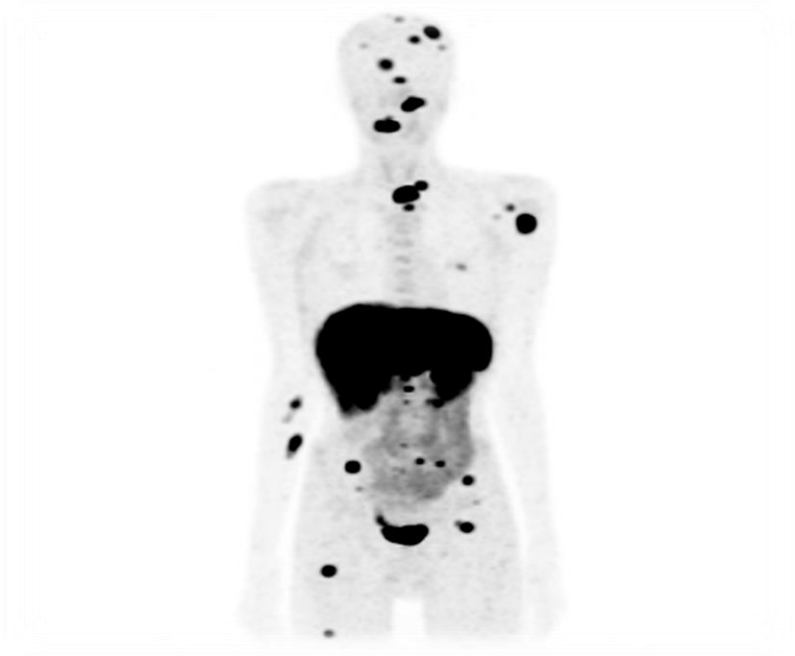


Fig 54: Sample one (PET) 68Ga-DOTATOC images.

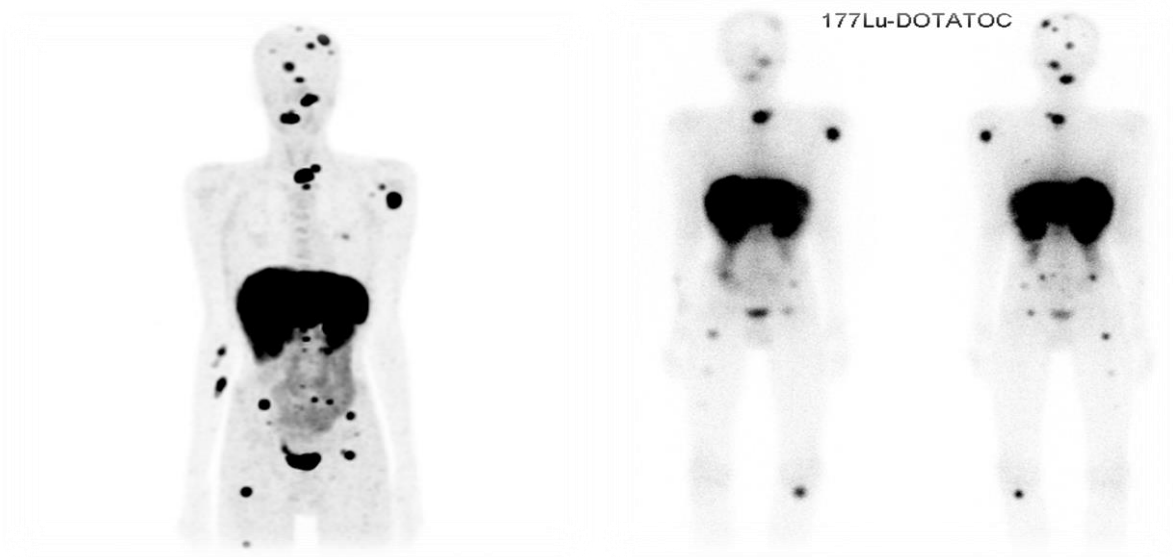


Fig 55: Sample two (PET) 68Ga-DOTATOC Pre-Therapy vs 177Lu-DOTATOC 1st Cycle.

4.4.4. Possible sources of errors in 68Ga-DOTATOC PET/CT scan.

In this study, seven possible sources of errors were identified and resolved as follows.

Table 2: Possible sources of errors in 68Ga-DOTATOC PET/CT scan.

A possible source of error.	Remedy
Skin/clothe contamination with urine.	Patients wore clean clothes and took a bath before the test.
Overexpression of somatostatin receptors in the body.	Clinical and laboratory investigations to rule out other somatostatin receptor expressing diseases.
Octreotide therapy.	Preclusion of patients under octreotide therapy.
High somatostatin receptor density.	Histological confirmation of malignancy.
Tumor differentiation/heterogeneous somatostatin receptor expression.	Histological confirmation of malignancy.
Variable uptake of the tracer.	Adequate timing before image acquisition.
Accumulation of radioactivity in some organs (spleen, pituitary, and kidneys).	Histological confirmation of neuroendocrine tumors.

(Virgolini *et al*., 2010).

4.5. 177Lu-DOTATOC.

Four cycles of (100 mCi) 177Lu-DOTATOC were administered at an interval of 28 days.

Production route of 177Lu

The direct route of production of 177Lu was utilized 176Lu was irradiated to promote neutron capture in order to produce 177Lu from 176Lu. This production route required an enriched target material in order to circumvent the problem presented by the limited natural abundance of 176Lu, which is just 2.6 percent. An example of enriched targets used in this production route is Lutetium oxides, and it is enriched in 176Lu up to 60-80 percent. However, this production method

is limited by the production of carrier-added 177Lu because of the presence of stable 175,176Lu isotopes in the target. Besides, high-flux reactors are required to obtain the maximum specific activity. An alternative 177Lu production method was also available, as the indirect production route. In this route, the production of 177Lu could have been achieved by beta decay of 177Yb after its production by 176Yb neutron capture in a reactor. In this route, enriched target material was needed in order to suppress the formation of two isotopes, namely, 169Yb and 175Yb, as well as increase the process effectivity. However, this alternative method was limited by the loss of the maximum activity of 177Lu during radiopharmaceutical production if ytterbium impurities are not separated (Pandey, Byrne, Jiang, Packard and DeGrado, 2014). The first step of production of 177Lu involved the irradiation of lutetium or ytterbium oxide targets in 2.05×10^{15} cm^2s^{-1} thermal neutron flux. In this case, irradiation of lutetium oxide results in a practical-to-theoretical production ratio of 1.66, but if ytterbium oxide is used the practical-to-theoretical ratio obtained is 0.79. In the end, the choice of the production route to be used is determined by the product-specific activity, presence of effective method of Lu/Yb separation, the available thermal neutron flux and the availability of isotopically enriched targets.

4.5.1. Production of 177Lu-DOTATOC

Dotatoc used in this study was purchased from ABX Advanced Biochemical Compounds. Its molecular mass is 1421.7 while its chemical structure is $C_{65}H_{92}N_{14}O_{18}S_2$ and it occurs as a white to off-white solid that is packaged in plastic vials.

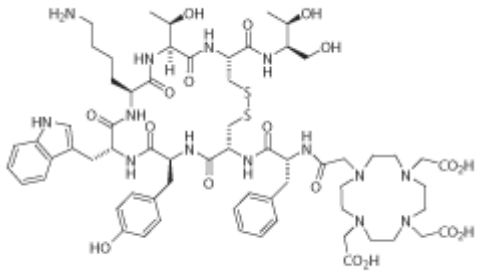


Fig 56: Dotatoc.

177$LuCl_3$ solution produced by direct route was purchased from IDB Company Holland DOTATOC stock solution was prepared by adding 115 µg of the ligand in pure water, the radiolabelling was achieved by adding DOTATOC solution to 3.7 GBq of 177$LuCl_3$ in a vial and buffering the pH using 0.4M sodium acetate buffer, before heating the content to 90 °C in a water bath for 30 minutes. During this process, the radiochemical purity of the mixture was checked using instant thin layer chromatography. 8ml of water was added to the mixture before passing it through C18 Sep-Pak column. In the end, the column was washed with 1ml of 0.9%NaCl and 0.5ml ethanol. When preparing radiolabeled 177Lu-DOTATOC, quality control test was achieved by checking the radiolabeled complex's radiochemical purity using instant thin layer chromatography and paper chromatography involved the use of 0.9% NaCl as the mobile phase and Whatman No. 3 paper (Yousefnia *et al*., 2015).

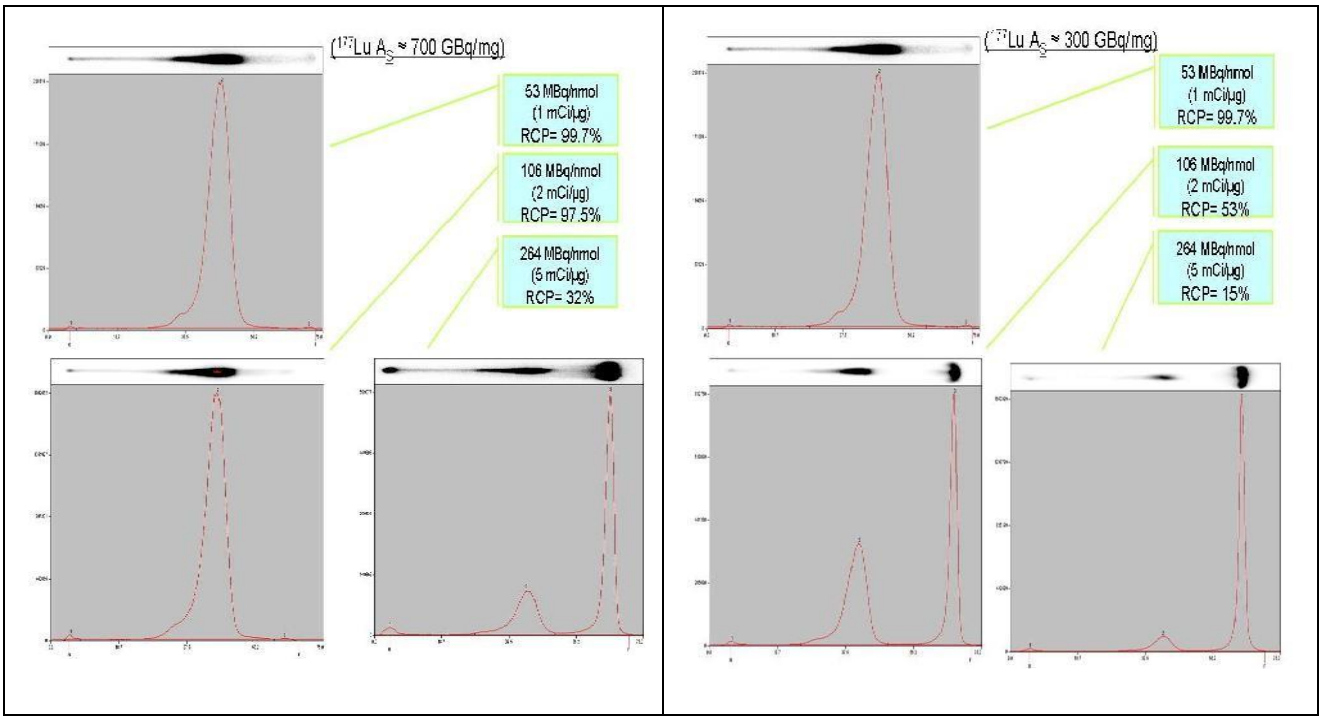


Fig 57: RCP of ^{177}Lu-DOTATOC radiolabeling at different A_S starting from "fresh" $^{177}LuCl_3$ sample (~700 GBq/mg) and 1-week old sample (~300 GBq/mg)

4.5.2. Summary of 177Lu-DOTATOC reaction.

When radiolabeling DOTATOC with 177$LuCl_3$, a DOTATOC stock solution (purchased from ABX Advanced Biochemical Systems) was prepared by dissolving 115 µg of DOTATOC in pure water. The DOTATOC solution was then added to 3.7 GBq of 177$LuCl_3$ in a vial before

adjusting the pH of the mixture using 0.4 M sodium acetate buffer (from ABX Advanced Biochemical Systems). The mixture was then put in 90 °C water bath for half an hour, and its radiochemical purity assessed using Instant thin layer chromatography. Then, eight mL of water was added to the reaction vial before passing the mixture via a C18 Sep-Pak column (preconditioned with ethanol (5 mL), water (10 mL) and air (10 mL), respectively). In the end, the column was washed with one mL of 0.9% NaCl and 0.5 mL ethanol. The labelling conditions applied in this study were informed by literature provided by the work of Yousefnia, Mousavi-Daramoroundi, Zolghadri, and Abbasi-Davani, (2015).

4.5.3. 177Lu-DOTATOC peptide receptor radionuclide therapy.

177Lu-DOTATOC was prepared onsite as outlined in the above subsection. In every cycle, 177Lu-DOTATOC was infused slowly by intravenous route over 15 minutes. In this case, nephroprotective agent was co-administrated with 177Lu-DOTATOC. For each of the four cycles of 177Lu-DOTATOC, the activity was 3.7 GBq while the maximum activity administered cumulatively was 14.8 GBq.

4.6. Everolimus.

The mode of action of everolimus involves the inhibition of mTOR leading to the down-regulation of intracellular serine-threonine kinase and therefore, affecting such processes as apoptosis, angiogenesis, cell proliferation, and cell growth. FDA has already approved the use of everolimus as a treatment agent for cases of advanced, progressive and inoperable neuroendocrine tumors. Some of the commonly observed side effects of everolimus use include rash, diarrhea, stomatitis, respiratory tract infections and fatigue. It is also associated with Grades 3 and four toxicities, including hyperglycemia and anemia. In this study, Everolimus was administered orally at 10 mg in one event every day, and the treatment was continued until the development of unacceptable toxicity, the progression of the disease, withdrawal of consent or drug interruption for at least three weeks. In cases of development of adverse events, doses were delayed or reduced in order to avoid unpleasant results (Baum et al., 2016).

4.7. Histological characteristics of neuroendocrine tumors.

In this project, patients recruited to undertake the treatment were assessed to confirm that their cases were of inoperable and/or metastatic neuroendocrine tumors. The grade and differentiation of neuroendocrine tumors in patients included in this study involved using histological concepts to describe their biologic aggressiveness. The WHO grading system was used to determine the number of mitoses in every ten high-power microscopic fields and their ki-67 indexes (Carcinoid, 2016). The histological confirmation of neuroendocrine tumors was performed according to concepts outlined here.

Table 3: The histological confirmation of neuroendocrine tumors.

Basis of histological classification of neuroendocrine tumors	Poorly differentiated neuroendocrine tumors – high-grade tumors, G3	Moderately differentiated neuroendocrine tumors – intermediate grade tumors, G2.	Well-differentiated neuroendocrine tumors – Low-grade tumors, G1
necrosis	Present	Information unavailable from medical literature	Absent
Ki-67 Index	Over 20 percent	3-20 percent	Less than 3 percent
Prognosis	Poor	Intermediate	Prolonged survival
Appearance	Cellular pleomorphism	Information unavailable from medical literature	A monomorphic population of small, round cells
Rate of mitosis	More than 20 mitoses per high-	2-20 mitoses per high-power microscopic fields	Less than two mitoses per high-

	power microscopic fields		power microscopic fields

4.8. EPR materials and methods.

We decided to perform an easier experimental setup, to avoid the long time needed for decay in the samples and safety precautions for radiations as well. In the new experimental setup, the samples (without radionuclide) were frozen at liquid nitrogen temperature and then irradiated by gamma radiation using 60-Co gamma source. The external irradiation should mimic the radiolytic processes occurring after the decay of radionuclide but avoiding safety issues due to the manipulation of the radionuclides. Moreover, the irradiation of frozen solution presents several advantages. In particular, the radicals formed are not free to move and thus to recombine. This opens the possibility to identify the primary radicals formed and to investigate radical reactions after annealing at higher temperatures.

All the samples were irradiated at 2 kGy with gamma rays (^{60}Co source, dose rate 0.14 kGy/h in October 2015) at liquid nitrogen temperature (77 K). EPR measurement and analysis were conducted following a standard method previously developed [D. Dondi, A. Buttafava, A. Zeffiro, R. Cherubini, V. De Nadal, S. Gerardi, A. Faucitano The Origin of the Radiobiological Damage in Cells Stored in Cryostatic Conditions Radiation Physics and Chemistry (2012) 81 (9) , pp. 1445-1450]. Spectra were recorded at 120 K with a Bruker EMX-10/12 spectrometer (Bruker BioSpin GmbH, Karlsruhe, Germany) operating in Xband, equipped with an ER4119HS cavity.

4.9. Efficacy and safety of the regimen.

In this research, 68Ga-DOTATOC was used to evaluate response to treatment as well as diagnose progression. Images were taken at the before the commencement of each 177Lu-DOTATOC cycle as well as at restaging, where restaging happened 16 weeks after the last 177Lu-DOTATOC cycle. Additionally, such hematology parameters as neutrophils count, leucocytes

lymphocytes, erythrocytes, platelets, and hemoglobin were assessed at the end of every 177Lu-DOTATOC cycle. In addition, other laboratory tests performed in relation to the regimen efficacy and safety included the liver function tests as well as the renal function tests (Baum *et al*., 2016).

4.10. Pharmaceutical quality in Peptide Receptors Radionuclides Therapies PRRT.

4.10.1. Media Fills.

The media fills were used to validate the aseptic preparations for 68Ga-DOTATOC and 177Lu-DOTATOC. In this media fill test was used for validating the radiopharmaceutical development method, testing the quality of the product, and detecting microbial contamination, besides evaluating the sterility of the method as well as the product of this study (Choudhary, 2014). A media fill involves performing an aseptic procedure of manufacturing using a microbiological growth medium instead of the drug solution in order to test the effectiveness of the aseptic procedures in preventing contamination in the course of drug production. It is simply used for validating the aseptic manufacturing procedure used in pharmaceutical development. The media fill evaluates the aseptic assembly/operation of equipment, operators, and technique to ensure they meet the minimum requirements of sterile pharmaceutical development (Choudhary, 2014).

In this study, the media fill was performed in two steps. The first step involved media fills design. In this step, the media fill was set to align with all the aseptic manipulations included in the course of the radiopharmaceutical production (Center for Drug Evaluation and Research, 2012). The simulation included the product container preparation as well as the assembly, the product container's transfer to the fill area, as well as all downstream steps till the final product was packaged. Then again, the incubation of finished product containers was performed to allow for growth of any contaminating microbes for any contaminated equipment. A broad range of aseptic manipulations was made to check the likely events during production, with controls being utilized in every case. In addition, the media fill reproduced all steps of aseptic manufacturing, including sampling as well as the final product dilution, and the researcher took an important role in media fill simulations (Center for Drug Evaluation and Research, 2012). This simulation process duplicated

the actual process of production, but the formation of the final product is followed by its incubation in a temperature-controlled incubator for 14 days with regular inspections after ever two days. In addition, all steps of the simulation were performed in the same location as the radiopharmaceutical production steps (Center for Drug Evaluation and Research, 2012).

The growth medium of choice in this media fill test was soybean casein digest medium. This medium was obtained from commercial vendors as Trypticase Soy Broth and its shipping, preparation, handling and handling procedures were carefully designed, recorded and adhered to so that to uphold the integrity as well as the stability of the media. The aseptic process used in this facility was qualified after the performance of three media fills that were doe on three separate days (Center for Drug Evaluation and Research, 2012). In addition, media fills were performed two other times during the course of the study after changes in components and equipment, which affected the aseptic process. Moreover, the media fill tests were performed by a previously trained operator who was well-trained in such techniques as disinfection, sterile materials handling and proper gowning. In order to qualify the vendor, three commercial lots of medium sourced from a single supplier were subjected to quality control tests to assess such aspects as sterility, growth promotion, pH and visual characteristics. The results of these quality control tests were reported in a certificate of analysis (Center for Drug Evaluation and Research, 2012).

4.10.2. Pyrogen tests.

In pharmaceutical preparation, pyrogen test is used for reducing febrile reactions related to the administration of the final product. In simple, during pyrogen test intravenous injection of rabbits with the product is accompanied by temperature monitoring to assess the development of fever. In the end, it helps with the prediction of corrosivity, and toxicity of the product, amongst other safety issues, associated with chemicals, medical devices, and new drugs. As a general rule, pyrogen tests are performed on animals before proceeding to administer the tested substance on humans (New England Anti-Vivisection Society, 2016). In this study, pyrogen test was performed according to the guidelines recommended by the U.S. Pharmacopeia (U.S. Pharmacopeia, Nd.).

During the pyrogen test, the needles, syringes, and glassware were rendered free from pyrogen by exposing them to high temperature (250 °C) for 30 minutes. Also, all solutions and diluents used in this study were treated to ensure their sterility as well as their pyrogen-free nature. Periodic control pyrogen tests were also performed on representative portions of the solutions and diluents in order to check their conditions. An accurate temperaturesensing device (thermometer) was used to check the rabbits' core temperature by inserting it into the rectum to a depth of 4.5-7.5 cm. The device had been calibrated to an accuracy of +/-0.1 °C. The rabbits used for pyrogen test were healthy and mature, and the environmental temperatures varied between 20 °C and 23 °C. The pyrogen test was performed in designated area under normal rearing conditions. During pyrogen testing, no food was given to the rabbits, but water access was allowed throughout the testing period. Six rabbits were selected for the test, where three received 68Ga-DOTATOC and others, 177Lu-DOTATOC, by injecting into the ear vein at 10 ml per Kg of body weight. Devices and equipment were also tested for any pyrogen, where rinsings and washings of their surfaces were administered into rabbits, before recording the temperature changes after every 30 minutes for two hours. The radiopharmaceuticals were declared free of pyrogens as all rabbits did not vary their respective body temperatures by more than 0.5°C (U.S. Pharmacopeia, Nd.).

Another test performed on the radiopharmaceuticals is a determination of the mutagenicity and carcinogenicity potentials of the two radiopharmaceuticals. Radiopharmaceuticals are postulated to cause several genetic effects due to irradiation that may result in cell mutations as well as the ultimate development of cancer. In this study, the AMES test was performed to screen both 177Lu and 68Ga for mutagenic potential, in addition to DNA and cell damage (New England Anti-Vivisection Society, 2016).

4.10.3. Sterility tests.

Sterility tests were conducted to validate the sterilization of radiopharmaceuticals/devices. In this case, the membrane filtration sterility test was used to ensure that all radiopharmaceuticals were sterile and safe for administration (Richter, 2008). In principle, the membrane sterility test was

supposed to collect microorganisms by filtering them using a 0.45 micron pore size filter, before segmenting it and transferring the segments to the appropriate media, namely soybean casein digest medium (SCDM) and fluid thioglycollate medium (FTM). A closed system of testing was employed to ensure full sterility testing of the products as well as the devices and avoid extrinsic contamination. In the end, both the devices and the radiopharmaceuticals were found to be sterile (Richter, 2008).

4.11. Statistical analysis.

In this project, simple random sampling was used to select nine participants. This method was observed to be an easy way of assembling the sample, and to be fair in terms of representation of patients with metastatic and/or inoperable neuroendocrine tumors as everyone has an equal odd of being selected.

4.11.1. Mean, standard deviation and median.

In this study, calculation of the mean was performed using the following formula.

Sample mean $(\bar{\mathbf{x}}) = (1/n)\sum_{i=1}^{n} a_i$

Where,

$\bar{\mathbf{x}}$ = the sample mean.

a = sample values.

n = total sample volume.

One the other hand, the stand deviation was calculated using the following formula:

$$Standard\ deviation\ (\boldsymbol{\sigma}) = \sqrt{\frac{\mathbf{1}}{\boldsymbol{N-1}}\sum_{i=1}^{N}(x_i - \bar{\mathbf{x}})^2}$$

Where,

σ (Sigma) = standard deviation.

N = the sample volume.

X = sample value.

$\bar{x}$ = sample mean.

N-1 = sample variance.

Median.

The median was determined using the following formula:

$$\textbf{Median} = L + \frac{(n/2) - B}{G} \times w$$

Where,

W = group width.

G = median group frequency.

B = the cumulative frequency of the groups preceding the median group.

N = the total number of values.

L = the class boundary (lower) of the median containing group (Darton College, 2016).

4.12. Ethical considerations.

4.12.1. Privacy, security, and confidentiality.

The research was aware of the importance of personal privacy and confidentiality and therefore, the subject patient's confidentiality were central tenets of the investigation. For that purpose, the researcher allowed patients to choose the amount of their personal information that they would reveal as well as the circumstances. This determination was allowed at the beginning of the project when the researcher was recruiting the participants. The researcher also distributed letters to the participants explaining the study as well as providing a way of contacting the research if a need arose among the participants. Another step taken to uphold confidentiality and privacy of the participants included discussing the allowable limits. In this case, the participants were informed about the limits of data use as well as the fate of PET/CT scans or any other data relate to the project – where no data would be used for any other purposes apart from the current research (Smith, 2003). An additional step was taken with regard to the practical security of confidential

records, and all records were records were locked in safe rooms in order to limit access as well as deny the unauthorized parties access to any identifying information. Also, the researcher also limited the use of the internet for data sharing to predetermined private email addresses to avoid possible data breaches and maintain the confidentiality of electronic information. Additionally, for any information shared among researchers anonymity was maintained to avoid breaching terms established by the consent. In this case, data was coded to hide identities of the research participants. Moreover, all federal and state laws regarding data security, privacy and confidentiality were taken into consideration in order to facilitate compliance. In cases of legally ambiguous areas, the researcher sought the assistance of a qualified legal expert (Smith, 2003).

4.12.2. Protection of subjects.

This study recognized the importance of subject protection as it involved administration of highly toxic agents when taken in high doses or if it accumulates in some organs. One of the considerations made was renal protection. The researcher was aware that the kidneys are highly susceptible to irradiation because of proximal tubular reabsorption of 177Lu-DOTATOC and the subsequent retention of the interstitial fluid. Besides, such other factors as diabetes mellitus or hypertension increase the risk of nephrotoxicity further. The researcher sought to protect the subjects by limiting the reabsorption of 177Lu-DOTATOC in order to reduce exposure of the renal tissue to irradiation. For that purpose, an amino acid protocol for renal protection was adopted to protect the subjects. In this case, a single-day protocol involving 1500 ml of nephroprotective L-arginine and L-lysine was applied, where it was administered 15 minutes before the commencement of the treatment and continued until 240 minutes after 177Lu-DOTATOC infusion. In this study, nephroprotective L-arginine and L-lysine were used to competitively inhibit the reabsorption of 177Lu-DOTATOC in the proximal tubules of the renal tissue and prevent irradiation of the tissue. The protocol ultimately protected the subjects from excessive renal irradiation during the study (Zaknum *et al.,* 2013). Besides, the subjects were protected from adverse outcomes of everolimus treatment through recommended daily dose of 10 mg. In addition, patients who developed

unacceptable side effects were allowed to either withdraw their consent to the investigational treatment or get lower doses to increase their treatment tolerance.

Another consideration made in order to protect the subject was the proper training of the research personnel. In this perspective, all members of the research team received relevant training regarding human subjects' protection in order to inform them their responsibilities and facilitate upholding protection of research participants throughout the project. As part of human subject protection plan in this research, there was sufficient minimization of the conflict of interest by clearly defining the primary as well as the secondary interests of the investigator, individual community members, the research partners as well as the participants undertaking the treatment. Additionally, with regard to data monitoring as an aspect of subject protection a smooth scheme of reporting adverse events as well as response to these events was developed to avoid extreme effects on the participants. Besides, the research project also outlined clear guidelines to be followed when halting the research so as to minimize risks of adverse effects of the regimen on the subjects (Ross *et al*., 2010).

4.12.3. Honesty.

Researcher considered honesty in research to be an important component of research integrity. As a result, honesty was upheld by avoiding all forms of financial as well as scientific misconduct. In addition, research participants were given free access to relevant research information in order to answer their questions fully. Also, misleading inferences of research were avoided by reporting research records in a prompt, accurate, objective and clear manner. Moreover, relevant information was also availed to the public as well as the participants appropriately (Ross *et al*., 2010).

4.12.4. Informed consent.

The researcher drafted an informed consent form that indicated both the benefits and risks associated with participation in this research in order to facilitate informed decisions from the subjects. In this case, four elements were integrated into the form to serve the purpose. One of the

elements considered when seeking the consent of the subjects was the comparison of the radioactivity of 177Lu-DOTATOC and 68Ga-DOTATOC with the maximum allowable radiations in nuclear medicine to inform the subjects of the safety levels associated with the two agents. In this regard, a list of adverse events likely to be observed after everolimus use was also included. Another element considered when obtaining consent from subjects in this research was the expression of quantitative nature with regard to the absolute risk of stochastic effects associated with the administration of both 177Lu-DOTATOC and 68Ga-DOTATOC. Also, the third element considered by the research was the expression of the extent of the uncertain events associated with the administration of both 177Lu-DOTATOC and 68Ga-DOTATOC as they do not have a substantially long history of use in nuclear medicine and cancer management. Moreover, the researcher also considered the element of understandability, where the consent form was created using understandable language for all participants to read and comprehend. In the end, a printed consent form was given to every participant to append their respective signatures after reading and agreeing to the terms and conditions outlined (Reiman, 2013).

4.13. Internal and external validity.

In this paper, internal validity was ensured through random selection of patients. However, external validity was ensured by limiting the elements of exclusion criteria (Godwin *et al*., 2003).

5.0. Chapter Five: Results and Discussion.

5.1. Introduction.

The study is still taking place and data on objective response rate, progression-free survival, duration of disease control, and overall survival is still being collected. As a result, the currently available data only covers death rate and the improvement of the quality of life of the subjects.

5.2. Clinical results.

5.2.1. Medical information and Clinical data.

In this study, the following medical information was collected as represented in the table 4

Table 4:

Symptoms	Frequency
Diarrhea	9
Skin flushing	9
Abdominal pain	7
Rectal pain	4
Jaundice	6
Unexplained weight loss	8
Headache	9

The clinical trial data management standards used in this study are summarized in the following scheme.

5.2.2. Types of neuroendocrine tumors and their respective frequencies.

In this study, the patients were diagnosed with either Gastroenterol intestinal neuroendocrine tumors or other forms of neuroendocrine tumors. All cases indicated hepatic metastases.

Table four: Frequency table for neuroendocrine tumors.

Type of gastrointestinal tumor	Gastroenterointestinal neuroendocrine tumors.	Pancreatic neuroendocrine tumors	Others (Thyroid gland tumors)	Liver metastases
Frequency	4	3	2	9

All the 9 patients underwent the first cycle of ^{177}Lu-DOTATOC treatment. None of them died during the course of the treatment and the research is still taking place. Besides, the quality of life of the nine patients improved to a significant extent as indicated by the rise in their respective Karnofsky performance scores, high survival rates, long progression-free survivals, high objective survival rates and long durations of disease control.

Table 6: Deaths and withdrawals from the study.

Total number of patients recruited for treatment with 177 Lu-DOTATOC in combination with everolimus	Total number of deaths recorded during treatment with ^{177}Lu-DOTATOC in combination with everolimus	The total number of complete response recorded before the fourth cycle of treatment with ^{177}LuDOTATOC in combination with everolimus.
9	0	0

5.2.3. Karnofsky performance statuses versus treatment cycles in ^{177}Lu-DOTATOC monotherapy.

Karnofsky performance status scale indicated an improvement of the disease conditions of the patients with the advancement of the treatment cycles. At the baseline, the mean Karnofsky performance status of the nine patients was 66. It rose to 69.5 in the second treatment cycle and 77.9 in the third treatment cycle. In the last cycle of the treatment, the mean Karnofsky performance status was 88.5. Overall, the elevation of the Karnofsky Performance Statuses of the patients was a clear indication of improvement in the quality of life of the nine patients.

Table 7: Karnofsky status scale.

Treatment cycle	Cycle one	Cycle two	Cycle three	Cycle four
Karnofsky performance status	66	69.5	77.9	88.5

5.2.3. Adverse events.

A combination of 177Lu-DOTATOC and everolimus in this therapy was well tolerated with minimal serious adverse events being reported. In total, 67 percent of patients (6 out of 9) reported 91 adverse events that included general disorders, events at the site of administration, and gastrointestinal disorders, amongst others, as shown this table.

Table 8: Adverse events.

Adverse event	Frequency	Number of patients with one episode of adverse event	Number of patients with two episodes of adverse event	Number of patients with three episode of adverse event	Number of patients with four episodes of adverse event	Number of patients with five episode of adverse event
Diarrhea	12	9	0	1	0	0
Fever/chills	8	8	0	0	0	0
Chest pain	13	5	0	0	2	0
Bloody nose	3	3	0	0	0	0
General discomfort	13	7	1	0	1	0
Loss of appetite	13	7	1	0	1	0

Feeling bloated	11	9	2	0	0	0
Labored breathing	5	3	1	0	0	0
Fatigue	9	5	2	0	0	0
Soreness at the site of injection	4	4	0	0	0	0

However, all adverse events recorded and that had a causal relationship with the administration of 177Lu-DOTATOC had mild to moderate intensity and they resolved without medical attention.

5.2.4. Hemoglobin level.

At the beginning of this study, 61 percent of the subjects recorded normal hemoglobin levels. However, 32 percent of the subjects had grade 1 anemia, while 7 percent had grade 2 or grade 3 anemia. After treatment with 177Lu-DOTATOC in combination with everolimus, 25 percent of subjects previously with normal hemoglobin levels at the baseline developed grade 1 anemia. On the other hand, 30 percent of the subjects with baseline grade 1 anemia developed grade 2 anemia. The study also established that the total red blood cells count reduced with the advancement of the treatment cycles with those who made it to fourth 177Lu-DOTATOC treatment cycle. In addition, serum liver enzymes levels were found to decline with the advancement of the treatment, with such enzymes as SGPT, SGOT, and SAP being the most affected, along with alkaline phosphatase. However, hemoglobin level, platelets count, neutrophils count, lymphocytes count and leucocytes count did not show significant fluctuation from the baseline records.

5.2.5. Renal function.

Standard clinical parameters of potassium ions, creatinine and blood urea nitrogen, were assessed for this study. However, they did not show fluctuation from the baseline in correlation with the cumulative administered radioactivity or the number of cycles administered on the patient.

However, the subtle dysfunctions of the renal proximal tubules were captured by determining the tubular extraction rate by the use of ^{99m}Tc-MAG-3 renography. In this case, the tubular extraction rate was seen remain constant throughout the four cycles of 177Lu-DOTATOC in combination with daily everolimus intake. There was a temporal relationship between the treatment cycles and the renal toxicities.

5.2.6. Biodistribution, and pharmacokinetics/pharmacodynamics of 177Lu-DOTATOC and everolimus.

In this study, administration of 177Lu-DOTATOC was done over a period of fifteen minutes in each of the treatment cycles. In all the four cycles, the maximum serum 177LuDOTATOC concentration was reached after 1.3-1.8 hours of administration to 0.94±0.25 ng per ml. However, the post-dose concentration of 177Lu-DOTATOC declined trendily for the following 24 hours. Additionally, at preparation 177Lu-DOTATOC was found to have a radiochemical purity of at least 99 percent. Besides, the complex remained stable throughout in human serum at 37 °C as well as at room temperature. In addition, 177Lu-DOTATOC was found to be highly concentrated in such organs as pancreas, gastrointestinal tract, thyroid gland, pituitary gland and the liver and this indicated its primary sites of concentration in neuroendocrine tumors to be related to such tumors as pancreatic neuroendocrine tumors, gastroenterointestinal neuroendocrine tumors, neuroendocrine tumors of such glands as the thyroid and pituitary gland. Besides, it also indicated high concentrations and radioactivity in such sites of neuroendocrine tumor metastases as the liver, and the renal tissue. In contrast, the oral administration of everolimus resulted in peak concentrations after 1-2 hours as tabulated below.

Table 9: Peak concentration times after everolimus administration.

Week	Week one	Week two	Week three	Week four
Weekly peak concentration time (averages)	1 hour	1.75 hours	1.5 hours	2 hours

In this case, patients who took the test before after taking breakfast exhibited longer peak concentration times as compared to those who did not take any food before taking the medication.

5.3. Radiochemistry results.

5.3.1. Production of radiopharmaceuticals.

In this study, the number of synthesis made for 68Ga-DOTATOC were 80, 60 automated synthesis cycles, and 20 manual synthesis cycles. On the other hand, 177Lu-DOTATOC was synthesized in 36 manual synthesis cycles.

5.4. EPR results.

In this study, 3 samples were considered: DOTATOC, everolimus and DOTATOC + everolimus. After the irradiation at low temperature, samples were transferred inside the EPR cavity and measured at 120 K. The identification of radicals was performed by gradually heating the samples inside the EPR cavity at different temperatures from 120 K to 300 K, and restoring the measurement temperature (120 K) before each spectrum acquisition. The relative amount of organic radicals was measured by double integration of the EPR spectra. In figure 58 are collected and stacked the spectrum obtained after heating at different temperature for the DOTATOC sample.

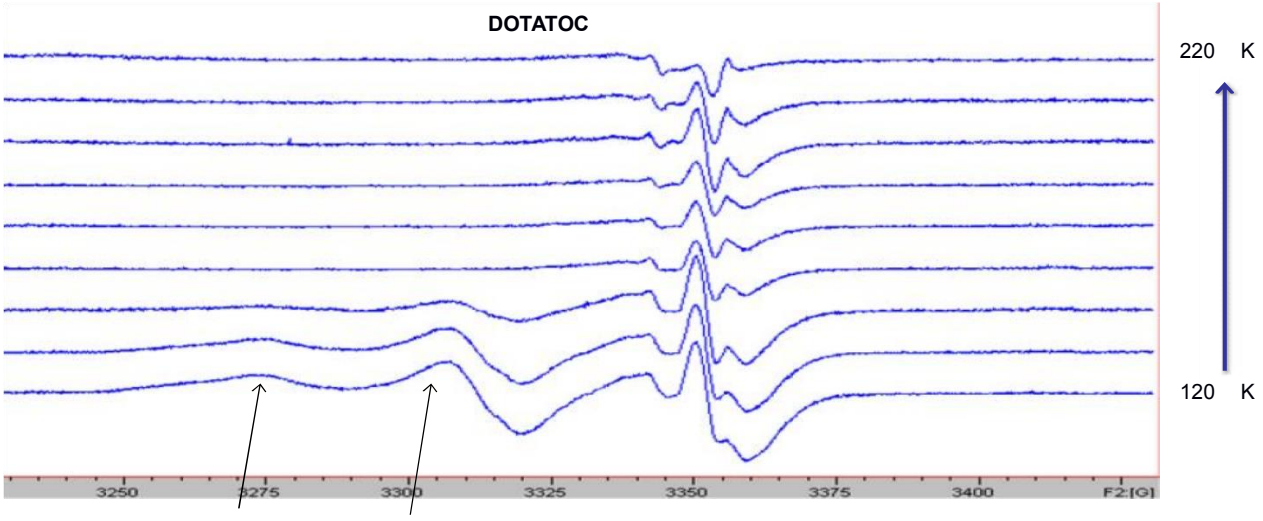


Fig 58: EPR spectra at different temperature for the DOTATOC sample.

Since the main component of the solution is water, it is not surprising to observe OH and OOH radicals (indicated by arrows in figure 58). Due to their high reactivity, an increase of temperature give rise to coupling and disappearance of radicals. At higher temperatures, almost no radicals are

detectable (the central part of the spectrum is dominated by radicals stemming from the quartz tube used).

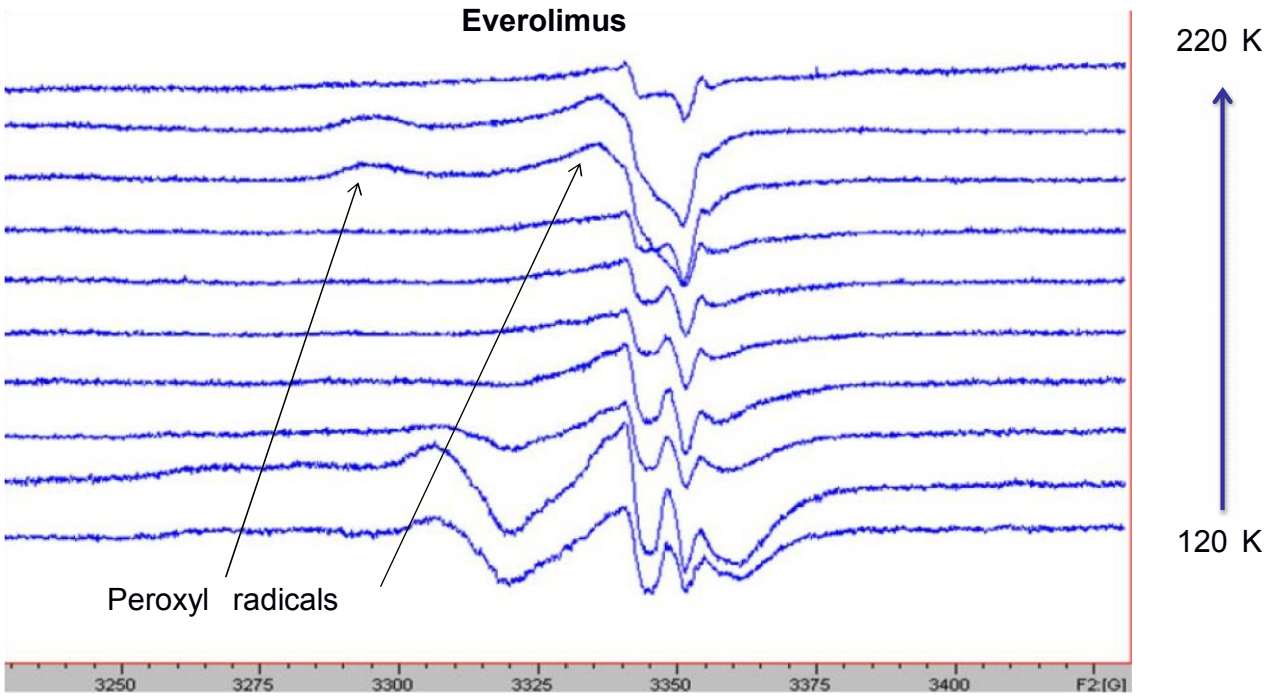


Fig 59: Everolimus submitted to a similar procedure.

5.4. Discussion.

5.4.1. Discussion of clinical results results.

The results show the combination therapy of 177Lu-DOTATOC and everolimus as a safe and efficacious regimen for the treatment of neuroendocrine tumors. At the baseline, 4 out of 9 patients were confirmed to have gastroenterointestinal neuroendocrine tumors. This number indicated that 44.4 percent of all cases were of gastroenterointestinal neuroendocrine tumors. On the other hand, pancreatic neuroendocrine tumors recorded in this study was 3, making 33.3 percent of all cases. Other types of neuroendocrine tumors recorded in this study were two in number, making 23.3 percent of the study sample. In the general public, the prevalence of neuroendocrine tumors presents a different picture. Lungs and bronchioles have among the highest risks of development of neuroendocrine tumors, accounting for up to 31.9 percent of all cases, according to Hauso *et al*., (2008). Hauso *et al*., (2008) also found out that gastroenterointestinal tract is also highly susceptible to neuroendocrine tumors with a prevalence rate of up to 46 percent where stomach, small

intestines, colon, rectum and appendix accounting for the highest cases. In addition, the pancreas also accounts a significantly large proportion of neuroendocrine tumor cases 41 percent of all neuroendocrine tumors. Other significantly important neuroendocrine tumors present to the general public include prostatic neuroendocrine tumors, neuroendocrine ovarian tumors, and breast neuroendocrine tumors, amongst others. The difference of neuroendocrine tumor prevalence indicated between the study population and the general population can be attributed to the concentration of the cases in hospitals where different individuals seek medical attention from varying motivations (Hauso *et al*., 2008).

Of the nine patients recruited to undergo combined therapy of 177Lu-DOTATOC and everolimus, none of them died as a result of adverse events and natural disease progression. Everolimus alone has been observed to result in such adverse events as diarrhea, fever, and general sickness, amongst others. On the other hand, lack of literature about the use of 177LuDOTATOC as a radiolabeled somatostatin analog for use in the treatment of neuroendocrine tumors also limited the availability of information with regard to the adverse events likely to cause death after treatment. Dosimetry determines the optimal number of treatment cycles for peptide receptor radionuclide therapy for different individuals. Since 177LuDOTATOC has not been studied adequately relative to other related radiolabeled somatostatin analogs, it was not possible to offer the ultimate protection to various organs at risk. Some of the organs at the primary risk of 177Lu-DOTATOC toxicity include the liver, kidneys and bone marrows. According to Bison *et al*. (2014), about 9 percent of patients treated with 90Y-DOTATOC usually develop severe permanent renal toxicity of grade 4. However, this level of toxicity is observed among patients treated for extended periods of 1-10 years. That notwithstanding, this level of renal toxicity is possible within a short time in cases where administration of the radiopharmaceutical is done in significantly high doses. Bison *et al*. (2014) also observed that treatments with radiopharmaceuticals also cause severe hematological toxicity in 13 percent of the treatment recipients, and it may be transient or progressive to leukemia or myelodysplastic syndrome. Such a toxicity level is enough to cause

further health constraints to the already ailing tumor patient leading to deterioration of the general health, and the likely death (Bison *et al.,* 2014).

In this study, the Karnofsky performance scale was used to determine the average performance statuses of the patients in relation to the treatment cycles. At the baseline, the average performance status of was 66, and gradual improvement was recorded after every treatment cycle. The Karnofsky performance status scale is used in the assessment of patient's functional status. High Karnofsky performance statuses indicate that the subject is capable of doing the basic things in life without assistance with 100 indicating that a person will demonstrate pre-disease performance. In this study, there was a significant improvement of Karnofsky performance statuses from 66 at the baseline to 88.5 at the fourth cycle. These improvements resulted from the use combined the use of 177LuDOTATOC and everolimus in the therapy. These findings are similar to the work of Fonseca et al. (2013) who found out that the prolonged use of everolimus in addition to octreotide longacting repeatable in cases of high-grade pancreatic neuroendocrine tumors results in the resolution of symptoms and the consequent improvement of the patient's Karnofsky performance status. Fonseca et al. (2013) also found a low baseline Karnofsky performance status of 70 percent similar to the current study which found an average of 66 percent. The gradual improvement of the patient's Karnofsky performance status in this study indicated that the use of 177Lu-DOTATOC in combination with everolimus in therapy is an effective treatment regimen that resolves the constitutional symptoms of neuroendocrine tumors (Fonseca *et al.,* 2013).

The current study indicated a synergistic interaction of everolimus and 177Lu-DOTATOC to increase the efficacy of the regimen. According to Lehar et al. (2009), drug interactions in disease management occurs when a combination of medications is used to bring about a synergistic effect. A combined use of therapeutic agents leads to an interaction that can overcome toxicity as well as reduce the side effects of high doses of a single dose. Such synergistic combinations result from counteraction to biological compensation, access to context-specific multi-target mechanisms and sparing doses on each compound. According to Tallarida (2001), drug synergism is achieved

through similar independent action, additivity, or isobologram. In the similar independent action of drugs, a combination of at least two agonist drugs the mechanism of action includes the activity of each of the drugs on different drug receptors to bring about concerted results without affecting each other's pathways. On the other hand, additivity of drugs results in an increased potency of the combination after the pairing the effects of each of the therapeutic agents. In this case, the final efficacy of a drug is equivalent to the combined effect of each of the therapeutic agents. In the end, the therapeutic result of the two agents is more concentrated than any of the two drugs used in the combination. Moreover, combination therapy involving two agents can result in synergy in isobologram where two agonist drugs result in additivity in addition to the effects of individual dose-response (Tallarida, 2001). The common effect of the three pathways to drug synergy is the increased progression-free survival the combination exhibits higher potency compared to any of its constituents. In this study, the combination of everolimus and 177Lu-DOTATOC resulted in synergy and ultimately gave a higher potency in comparison to any of the two agents.

This study established a high level of tolerance of 177Lu-DOTATOC in combination with everolimus, where only 67 percent of the subjects reported adverse events. Additionally, a total of 91 adverse events were reported, and they included soreness at the site of injection, fatigue, bloating, chest pain, diarrhea, bloody nose, loss of appetite, labored breathing, and general discomfort. Combined use of 177Lu-DOTATOC and everolimus for neuroendocrine tumor treatment may have resulted in suppression of some adverse events or encourage the development of others. Like demonstrated in the current study, the use of everolimus for cancer treatment results in mild to moderate adverse events. Many papers published lately also found that the use of everolimus in cancer treatment results in such mild to moderate adverse events as stomatitis, dyspnea, fatigue, and rash. For example, Aapro et al. (2014) published a paper exploring the management of oral everolimus adverse events in advanced cancer cases. According to Aapro et al. (2014), stomatitis is one of the common adverse events related to the use of everolimus in advanced cancer treatment, and it presents as inflammation as well as ulceration of the oral cavity's mucosal

lining. A cancer patient exhibits stomatitis as an adverse event complaints of gingival pain, mouth ulceration, glossodynia, glossitis, and lip ulceration. Aapro et al. (2014) added that up to 44 percent of patients receiving everolimus complain of stomatitis related health issues.

Another adverse event reported by Aapro et al. (2014) is rash. This condition presents as an acneiform dermatitis with blackheads, and it presents in 29-49 percent of all cases undergoing everolimus monotherapy. Aapro et al. (2014) also reported that the use of everolimus as a treatment modality for advanced cancer also results in such metabolic adverse events hyperlipidemia and hyperglycemia. In pancreatic neuroendocrine tumors, the incidence of hyperglycemia is 13 percent. On the other hand, cases of hyperlipidemia reported after everolimus treatment appear in 24-44 percent of the patients.

Another paper by Kaplan, Qazi and Wellen (2014) also reported similar adverse events that included such organs as the kidneys, lymph nodes, the skin, the reproductive system, lungs and the hematologic tissue. According to Kaplan, Qazi and Wellen (2014), some of the common dermatologic disorders after everolimus treatment include acne, oral ulcers, and rash. In addition, the metabolic disorders that result from everolimus treatment include dyslipidemia, hyperlipidemia, hypercholesterolemia and hyperglycemia, while adverse events related to the renal tissue include proteinuria and nephrotic syndrome. In general, the adverse events reported in the current study relate to such organs as kidneys, the pulmonary system, the skin, gastrointestinal tract and the cardiac tissue (Kaplan, Qazi and Wellen, 2014).

On the other hand, the use of 177Lu-DOTATOC as a monotherapy treatment modality for neuroendocrine tumors has not been extensively studied, and there is not widely available literature with regard to the side effects. However, the use of most other radiolabelled somatostatin analogs, for example, 90Y-DOTATOC and 177Lu-DOTATATE, amongst others, in the treatment of neuroendocrine tumors is widely documented to cause different forms of toxicities as side effects. For example, Kwekkeboom et al. (2008) reported some of the toxic events related to the use of 177Lu-DOTATATE in the treatment of both gastroenterointestinal neuroendocrine tumors and

pancreatic neuroendocrine tumors. In this paper, Kwekkeboom et al. (2008) reported the common adverse events to include liver toxicity and myelodysplastic syndrome. Some of the discomforts reported in relation to toxicity after the administration of radiopharmaceuticals included abdominal pain (10%), vomiting (10%), and nausea (25%). In addition, adverse events related to hematological toxicity were reported in 3.6-9.5 percent of all cases. These adverse events, together with temporary hair loss in 62 percent of the patients, were reported as acute or sub-acute events. Kwekkeboom et al. (2008) also reported several delayed toxicities, including renal insufficiency, hepatic failure, and myelodysplastic syndrome. In the current study, however, the long list of adverse events and side effects reported is attributable to the use of both everolimus and 177Lu-DOTATOC as a combined therapy. In this case, the interaction between everolimus and 177Lu-DOTATOC is likely to have subdued some adverse events while encouraging others. For example, the effect of everolimus on the liver is likely to have been raised by irradiation of the hepatic tissue by 177Lu-DOTATOC.

In all the four cycles, the administration of 177Lu-DOTATOC resulted in peak concentrations after 1.3-1.8 hours of administration to 0.94±0.25 ng per ml. On the other hand, the off-peak concentration was achieved after 24 hours of administration. Also, the study found the primary sites of 177Lu-DOTATOC concentrations in the subjects' bodies to include the Thyroid gland, the liver, pituitary gland, pancreas, and gastrointestinal tract, amongst others, depending on the primary tumor sites and the sites of metastases. The pharmacokinetics and pharmacodynamics characteristics of the 177Lu-DOTATOC are highly dependent on the affinity of DOTATOC for somatostatin receptors. In particular, the highest affinity of DOTATOC for somatostatin receptors is exhibited towards somatostatin receptors subtype-2. Currently, there is a limited library of information in relation to the biodistribution of 177Lu-DOTATOC in human subjects. However, one of the widely studied radiolabeled somatostatin analogs is 68GaDOTATOC, and it can be used to describe the occurrences observed in this study. One of the studies whose findings correlated to findings established in the current study was prepared by Naderi et al. (2016), and it explored the biodistribution of 68Ga-DOTATOC in rats and human subjects.

In this study, Naderi et al. (2016) demonstrated concerted uptake by such organs as adrenal glands and the pancreas. In brief, these tissues had the highest concentrations of somatostatin receptors explaining their high affinities for 68Ga-DOTATOC as demonstrated by Naderi et al. (2016). By extrapolation, it is also likely that the high uptake of 177Lu-DOTATOC by the thyroid gland, the liver, pituitary gland, pancreas, and gastrointestinal tract, amongst others, is as a result of their respective high concentrations of somatostatin receptors subtype-2. As a result, such a high concentration of this class of somatostatin receptors could explain the high affinity of 177Lu-DOTATOC for these organs in human subjects as demonstrated in this study. Besides, it could also relate 177Lu-DOTATOC to its primary sites of metabolism that include the one demonstrated in the current study. In another paper prepared by Huang (1997), the pharmacokinetic/pharmacodynamics characteristics of somatostatin correlate to those of 177Lu-DOTATOC as demonstrated in this study. In his paper, Huang (1997) observed that the highest concentrations of somatostatin-producing cells are found in the gut, pancreas, adrenals, thyroid gland, kidneys, placenta, and the prostate. Huang (1997) also indicated that neuroendocrine tumors of these organs also express somatostatin receptors leading and as a result explaining the high concentrations of 177Lu-DOTATOC in these tissues where they exert therapeutic effects on the said tumors (Huang, 1997). These characteristics are the favoring factors for the use of 177Lu-DOTATOC as a therapeutic agent for neuroendocrine tumors which express somatostatin receptors and as a result allowing metabolism of 177Lu-DOTATOC to bring about a therapeutic effect from both irradiation from Lu-177 and octreotide. According to Diao and Meibohm (2013), the biodistribution of somatostatin peptides involves both diffusion and connective extravasation while their elimination from the body organs is dependent on proteolytic degradation. Besides, the elimination of these peptides from the body systems is also influenced by glomerular filtration rate during excretion.

This paper found a peak concentration of everolimus after an average of 1-2 hours of oral administration. Like any other orally administered drugs, the oral administration of everolimus results in the movement of the drug across the drug across the gastrointestinal wall and followed by

its uptake by the tumor leading to the inhibition of mTOR. According to Houghton (2010), everolimus has been observed to cause inhibition of such mTORC1 dependent processes as elF4G an S6 phosphorylation. This effect is more concerted in the skin tissue than tumors (Houghton, 2010). The current study also established delayed achievement of the peak concentration of everolimus in subjects who had taken meals before its administration. According to Coppin (2010), the peak concentration of everolimus is delayed or affected by meals rich in fat and everolimus metabolites are excreted through the hepatic-biliary-fecal route. Coppin (2010) also reported that everolimus treats neuroendocrine tumors y limiting cell growth, cell survival, cell proliferation and angiogenesis by forming complexes with both mTORC1 and mTORC2 (Coppin, 2010)

This study found a significant improvement in the quality of life of patients treated with 177Lu-DOTATOC in combination with everolimus as indicated by long overall objective response rate, low mortality during the treatment, and long duration of disease control, amongst others. Besides, the average karnofsky performance status of the sample was raised from 66 percent to over 88 percent. In fact, the treatment eliminated the need for occasional assistance in patients as observed at the baseline to allow the subjects to exhibit normal activity as well as minimal signs and symptoms of neuroendocrine tumors. According to Peus, Newcomb and Hofer (2013), high quality of life is experienced by individuals with high karnofsky performance status and as a result healthy individuals who have 100 percent karnofsky performance statuses enjoy a higher quality of life compared to those with lower percentages and thus sick. Most studies involving the exploration of the treatment of the neuroendocrine tumors with somatostatin analogs have also reported improvement of the subject's quality of life. For example, a study by
Khan et al. (2011) established an improvement of the quality of life scores by at least 10 points after the treatment of subjects with 177Lu-DOTATATE. Besides, Khan et al. (2011) did not report any deterioration of the quality of life of patients who did not report severe neuroendocrine tumors' symptoms after the study. Another paper by Trub-Weidinger et al. (2011) reported that the

application of peptide receptor radionuclide therapy as a treatment modality for neuroendocrine tumors results in a significant improvement of the quality of life among the treatment recipients.

Discussion of EPR.

In this case, it is possible to define, in addition to OH and OOH radicals present at low T, the appearance of more stable peroxyl radicals. This is not surprising since the everolimus molecule presents many sites that might give rise to stable radicals after hydrogen abstractions. Some of the sites are evidenced by red dots in the figure below

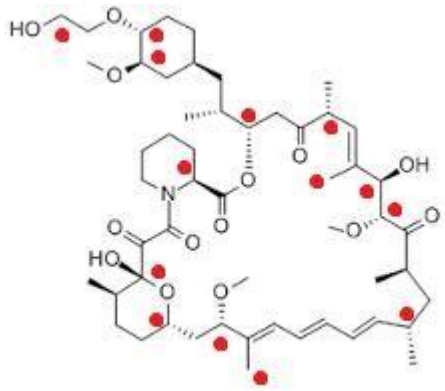


Fig 60: Stable radicals after hydrogen abstractions.

The everolimus molecule is thus more prone to give rise to many stabilized radicals with respect to DOTATOC in which a protein residue is present. The irradiation of a (frozen) solution of both DOTATOC and everolimus give rise to the result at a first glance which is similar to the sum of the two behaviors (see figure 61).

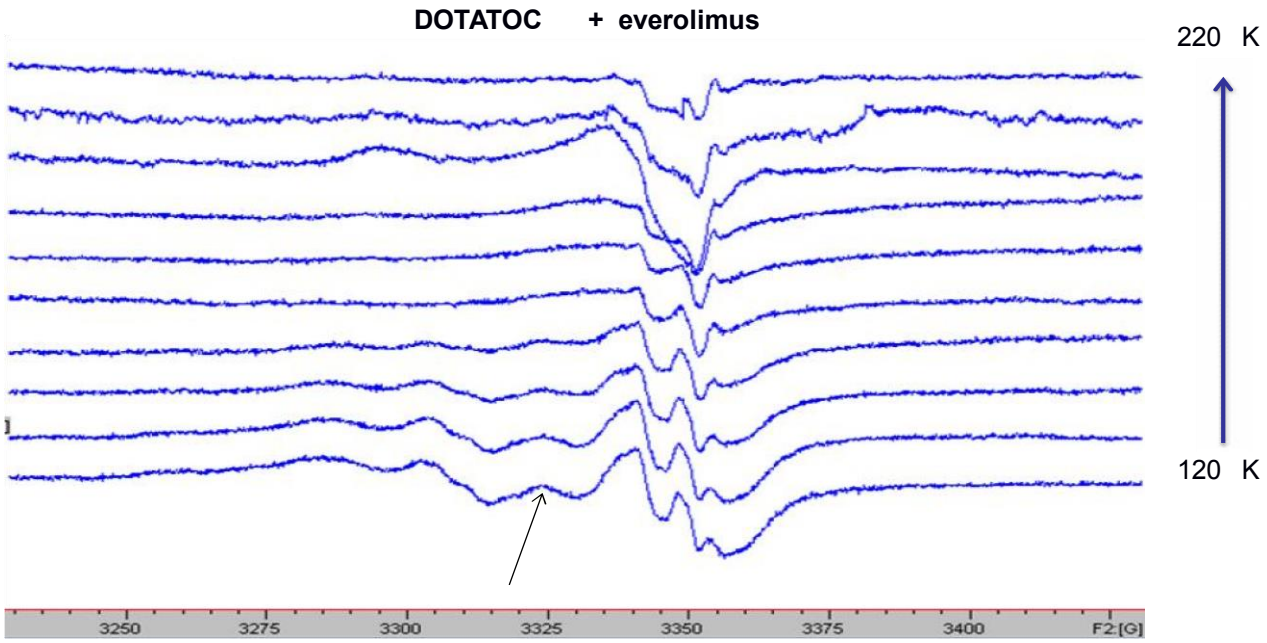


Fig 61: The irradiation of a (frozen) solution of both DOTATOC and everolimus.

However, after a more careful analysis, another specie was visible at low temperature, indicated in fig. 61 with an arrow. For position and shape of the signal, the specie is tentatively assigned to a sulfur radical. Since only DOTATOC possess sulfur (cysteine S-S bridge), we can conclude that the co presence of everolimus is responsible for the formation of radical. Everolimus could act as an efficient radical shuttle due to the possibility to form stabilized radicals and thus damage indirectly the DOTATOC. However, the probable synergistic effect of both drugs on sulfur radical production should be further investigated. If this phenomenon happens also in cells treated with both drugs this could explain the synergistic effect observed during the course of this thesis.

5.5. Discussion of radiochemistry.

5.5.1. Effect of chemical purity (metallic contamination).

As a general observation, the presence other chemical elements, for example, radioisotopes, affect the process of radiolabeling by decreasing the labeling efficiency of radiopharmaceuticals. In addition, they also increase the unnecessary burden for the patient as they increase the radiation dose that is not targeted to as specific condition or organ. The effectiveness of both 68Ga and 177Lu as radiopharmaceuticals is affected by chemical impurities in similar ways, as they reduce their radiopharmaceutical effectiveness besides increasing the untargeted radiation dose in the recipient

patient. For 177Lu, radiolabeling requires it to have a high specific activity as well as a low proportion of other metal impurities. Besides, the proportion of 177mLu isotope occurring along with 177Lu should also be low (Mikolajczak,
Bazaniak, Iller, 2005). After preparation, $^{177}LuCl_3$ has about 20 elemental impurities that may affect the radiolabeling efficiency because of the chemical competition challenge that they present to 177Lu during the radiolabeling process. These elemental impurities include Al, Cd, Ca, As, Cu, Cr, Pb, Si, Mn, Ni, Lu, and Ba. Others include Zn, Te, Mg, and B, amongst others, whose concentration range between μg per mL. It is hypothesized that elemental impurities of the present in the $^{177}LuCl_3$ solution are a result of the improper opening of the quartz ampoules during the solution preparation phase. On the other hand, the most important radioisotope impurity in $^{177}LuCl_3$ solution is 177mLu, which affects the radiolabeling process by increasing the untargeted radiation dose and as a result causing therapeutic contraindications (Mikolajczak, Bazaniak, Iller, 2005).

Like 177Lu, the properties of 68Ga are also influenced by impurities, where the higher the radionuclidic impurity proportion, the lower quality of the PET image. High levels of impurities also result in increased radiation exposure to the patient (Gawii and Chirmade, 2016). The gamma-ray spectrometry method, using HPGe detector, is used to determine the radionuclidic impurity level while a beta spectrometer is used to determine the presence of betaemitters present in 68Ga solution. On the other hand, then the presence of radiochemical impurities in the 68Ga solution affect the in-vivo localization of radiopharmaceuticals, by sometimes decreasing the target/non-target ratio due to the localization of the unintended organs. On the other hand, the presence of chemical impurities in radiopharmaceuticals results from the decomposition of active chemical ligand. Like in 177Lu, the presence of chemical impurities results in lower radiolabeling efficiency as a result of chemical competition (Gawii and Chirmade, 2016). Some of the most common metallic impurities present in 68Ga solution include Ti(IV), Zn(II), In(III), Al(III), Ti(IV) and the long-lived parent 68Ge(IV). Among all metallic impurities present in 68Ga solution, Fe(III) is the

strongest chemical competitor. Some of the commonly available methods of determining impurities present in radionuclide solutions and reduce their influence on the radiolabeling process include the use of well scintillation counters, dose calibrators, and radio-chromatogram scanners. On the other hand, the purity quality of 68Ga solutions may be improved using such methods as anion exchange chromatography, fractionation as well as cation exchange chromatography, which are used for pre-purification as well as pre-concentration (Velikyan, 2014).

5.5.2. Effect of PH and temperature.

Both pH and temperature are instrumental factors that influence the radiolabeling process. In 177Lu radiolabeling procedures, a rise in pH results in an increase in the radiolabeling yield with the maximum yield being attained at the pH range of 2.8-3.8 (Roesch and Riss, 2010). The influence of PH on the radiolabeling process is attributed to the coordination chemistry of both 177Lu and 68Ga, which influence their interactions with DOTATOC and DOTATOC, respectively. According to Xie, Tay, Zhang and Chen (2015), an increase in the number of hydroxyl ions in the reaction environment involving a bifunctional chelating agent and a metal results in the strengthening of their chemical bonds due to the dissociation of hydrogen ions (Xie, Tay, Zhang and Chen, 2015). This aspect explains why the increase in PH from acidic ranges to slightly alkaline PH ranges results in higher yields of complexes during radiolabeling processes of both 68Ga and 177Lu with DOTATOC and DOTATOC, respectively. On the other hand, temperature also has a similar influence on the radiolabeling process of both 177Lu and 68Ga, with DOTATOC and DOTATOC, respectively. In general, the kinetic effect of raising reaction temperature in coordination chemicals is that it allows the reactants to achieve the activation energy for the reaction to proceed towards a chemical equilibrium state (Xie, Tay, Zhang and
Chen, 2015).

5.5.3. Radiolabeling specific activity and its influencing factors.

The characterization of both the chemical and nuclear properties of 177Lu in relation to molecular and cellular biology paradigms has resulted in the concerted advancement of radionuclide therapy. Currently, use of 177Lu-labelled radiopharmaceuticals is currently under application in

numerous clinical applications. Although the targeted radionuclide therapy depends on the selection of the appropriate targeting mechanisms and radiopharmaceutical, the specific activity of 177Lu needed for a specific application depends on the target sites' number available for radiopharmaceutical targeting (Dash, Pillai and Knapp, 2015). For example, when the therapy is targeted at the treatment of trabecular bone 177Lu with high specific activity is not a requirement as the bone is a large-capacity site. As a result, if the neuroendocrine tumor in the question involves such organs as the bone and the hepatocellular tissue the application of a high specific activity 177Lu is irrelevant as high masses of low specific activity 177Lu can accomplish the same goal easily. Besides, medium specific activity 177Lu can also accomplish the same goal without many difficulties. On the other hand, if the target site is of low capacity it is logical to use high specific activity 177Lu because such sites present in low numbers, and this is the case for the application of somatostatin peptide receptor radionuclide therapy. In this case, the use of high specific activity 177Lu is necessitated by the expression of cognate somatostatin receptors on the surface of the tumor cell. The maximum specific activity of 177Lu is achieved when using carrier-free 177Lu. Theoretically, the carrier free 177Lu refers to the radiochemical containing 177Lu only as the radioisotope of lutetium, meaning that the isotopic composition comprises of 177Lu alone. However, it is difficult to have 100 percent carrier-free radioisotope as this is an idealistic requirement. However, it is possible to determine the isotopic purity to establish the amount of carrier present (Dash, Pillai and Knapp, 2015).

5.5.4. The quality of 177Lu to be used for radiolabeling.

In this study, quality management practices for 177Lu were aimed at producing 177Lu of refined attributes. One of the characteristics of 177Lu sought to be attained through quality control of 177Lu in the course of this study is to have 177Lu whose mean beta particles penetration range of 670 μm in order to deliver radiotherapeutic energy to the target tissues in order to destroy both the small and the metastatic neuroendocrine tumors without affecting the surrounding tissues (Bahrami-Samani, et al., 2012). As a result, such a characteristic is important to seek through

effective quality control in order to also offer personalized theranostics to the research subjects. Another characteristic of 177Lu sought to be achieved through quality control is the emission of moderate energy beta particles as well as low-energy gamma photons. In this case, the importance of quality control is to purify the chemical in order to ensure all other sources of beta particles and gamma photons are eliminated. As a result, the purified 177Lu has low radiation dose which is important for chemical handling when preparing and formulating Dotatoc-based radiopharmaceuticals, as well as their administration to subjects, in the course of the research (Bahrami-Samani et al., 2012).

The quality control practices applied in this research for 177Lu also sought to ensure the exclusive existence of 177Lu as Lu^{3+}. This property allows 177Lu to form nine coordination complexes, besides precluding all solution chemistry reduction-oxidation complications. By ensuring the intactness of this property through quality control, the researcher wished to assure of its usability in radiolabeling of such molecular carriers as the somatostatin analog used in this study (Bahrami-Samani et al., 2012). This property derives its importance from the ability to form metabolically resistant covalent bonds with bifunctional chelating agents. In addition, the quality control activities utilized in this study also aimed at ensuring the radiochemical purity of 177Lu to avoid having any other chemicals that may increase or lower the physical half-life of the radionuclide from 6.65 days. In this case, any impurities with less than 6.65 days half-lives would affect the research procedures that require extended durations to be accomplished. Besides, by ensuring the purity of the radiochemical, it is possible to calculate decay losses and use compensatory approaches to ensure that the therapeutic doses of 177Lu-Dotatoc are sustained to the point of administration in the research subjects. In the end, the quality control procedures of aim at ensuring the maintenance of the quality standards of $177LuCl_3$ (BahramiSamani et al., 2012).

After production, 177LuCl was taken through quality control process as described in a paper developed by Zolghadri, Yousefnia, Jalilian and Fasaeli (2015). In this case, irradiated Lu_2O_3 target was dissolved in 1.0 M HCl (200 µL) in order to produce $177LuCl_3$, which was then diluted with

clean water to a volume of 50 ml. For biologic purity, the solution was passed through a 0.22 μM filter before being sent to the radiolabeling stage. On the other hand, the radionuclidic purity of the solution was checked using an HPGe detector for four hours by gamma-ray spectroscopy based on 177Lu photons. On the other hand, ITLC was used to check the radiochemical purity of $^{177}LuCl_3$ where two solvent systems were used (Zolghadri, Yousefnia, Jaililian, and Fazaeli, 2015).

5.5.5. 177mLu contamination and waste management.

The direct route of production of 177Lu is the preferred route as it offers several advantages. One of the advantages of using this route is that it is simple to design and use. In this case, it is a lowly intricate method for targeting irradiation in the reactor, plus it has minimal requirements of design in processing facilities and reactor irradiation. Another advantage of the direct route is that it allows for the use of 176Lu2O3 as the target (Dash, Pillai, and Knapp, 2015). 176Lu2O3 is advantageous to use based on its high stability under irradiation conditions as well as its high compatibility with reactor irradiation. Another advantage of the direct route is its high flexibility that allows for the increase as well as the decrease in the levels of production depending on the target size. In addition, the method is also inexpensive, and it generates insignificant levels of radioactive waste. The method also offers an easy, fast as well as technically less demanding method of irradiated target processing, which is accomplished by dissolving the target in a mineral acid without complex requirements, or facilities. Additionally, the direct route of production of 177Lu also allows for the preparation of a receptor-specific therapeutic radiopharmaceutical (Dash, Pillai, and Knapp, 2015). With all these benefits compounding the significance of the direct production route, the process is not without disadvantages as depicted below.

One of the concerns raised in relation to the direct production route is that it requires the use of enriched 176Lu targets in order to augment 177Lu production yield and specific activity. This requirement is because of limited natural availability of 176Lu if the target is unenriched. Another disadvantage is that the direct production of 177Lu 740-1,110 GBq (20-30 Ci)/mg can be enough for peptide-receptor radionuclide therapy, but the decrease of SA with time makes the shelf-

life of the product be limited for peptide receptor radionuclide therapy as well as other uses requiring high SA. Another limitation is the difference in the theoretical specific activity of 177Lu compared to the practical one (Dash, Pillai, and Knapp, 2015). The practical, specific activity of 177Lu obtained via the direct production route is lower than the one obtained practically as a result of the presence of 175/176Lu atoms, which are nonradioactive. Another major disadvantage of the direct production route in 177Lu preparation is the co-production of 177mLu. The co-production of 177mLu poses a major challenge in radiation protection as well as waste disposal, and this is f significant importance to this study.

The use of 176Lu-enriched lutetium oxide is popularly adopted as a successful way of producing 177Lu with specific activities of above 740GBq (20 Ci)/mg. This specific activity level is perfect for application in radionuclide therapy. However, the method results in the coproduction of 177mLu, which has a half-life of 160.1 days. The amount of 177mLu in the endproduct is dependent on two factors, namely the time lapsed after the end of the radiation and the irradiation time. As a result, although this product has a long half-life as well as a low neutron absorption cross section, the product is of low specific activity, but of possible concern. In the end, the production of 177mLu poses three major concerns (Dash, Pillai, and Knapp, 2015). One of the concerns is the radiation dose because with continued use of 177Lu for the preparation of radiopharmaceuticals may result in the increased radiation dose. In this case, 177mLu impurities result in the compounding of doses regardless of the ratio of 177mLu to 177Lu present in the radiopharmaceutical. Another concern resulting from the presence of 177mLu is the laboratory waste. The radiolabelling process and treatment results 2-5 percent loss of 177Lu activity, while 28-90bkBq 177mLu is released. Then again, the permissible177mLu release limit is 10Bq/g, and as a result it is necessary to collect 177Lu wastes separately before transporting them to an authorized radioactive waste management facility where decay is allowed to happen without causing harm to the surroundings. In this case, a radioactive waste management facility is needed because of the long duration required for the complete decay of 177mLu, which has a half-life of 160.1 days.

Another concern raised by the presence of 177mLu after the direct production of 177Lu is the waste water. After administration of 177Lu-DOTATOC to a patient, approximately 80 percent of the dose is excreted via urine and feces. As a result, if these wastes are not handled precautionary there is a danger of the accumulation of 177mLu in the waste water holding tanks (Dash, Pillai, and Knapp, 2015). The accumulation of 177mLu in waste water could result in regulatory violations, for example, the European Radiation Safety Regulation permits a maximum of 50kBq/m3 of 177mLu for the municipal sewage. As a result, there should be an adequate evaluation of the water holding tanks in order to ensure the accumulation of 177mLu does not exceed the maximum allowable limit.

In general, 177mLu (0.01%-0.02%) is a radiation dose of little significance to the patients but the danger may be compounded by the handling as well as the disposal of residual 177mLu during research because the customary waste disposal practices are rarely adequate in circumventing the danger (Dash, Pillai, and Knapp, 2015). One of the considerations of radioactive waste management that is of importance is the segregation, transportation as well as the temporal storage of the waste. This consideration forms an important step in the effective management of radioactive waste, and it might involve segregating both the biological wastes and the chemical wastes resulting from radiopharmaceutical synthesis and use in this study. This step results in the isolation of the radioactive waste from the nonradioactive one at the research facility, during transportation, or temporal use in order to prevent their leakage to the surroundings as well as the associated harm. Currently, there are no universal standards for the temporal storage or transportation of radioactive medical wastes, but the general principle is to use lead or other blocking agents to avoid irradiation of the surroundings (Khan *et al*., 2010).

Another consideration worth noting in relation to waste management is dilution and dispersion of the radioactive waste. In the case of the low activity solid particle, the researcher may dispose of it as ordinary hospital waste if its activity is below 1.35 microcuries (Okot, N.d.). On the other hand, liquid radioactive waste of lower activity than the microacuries level may be disposed as a sewer as long as adequate water is used to flush the system after disposal. That

notwithstanding, it is important to consider the local regulations to avoid exceeding the maximum prescribed limits (Khan *et al*., 2010). The third consideration is delay and decay, because unlike the wastes produced via nuclear fuel cycle operations which pose long-term management problem, radioactive medical waste does not present a significant long-term waste disposal problem. As a result, the radioactive medical waste may be managed effectively by storing them in order to allow for their complete decay to eliminate harms. The fourth consideration that may be applied to manage radioactive wastes resulting from the use of 177Lu is the waste concentration and continence. Since 177mLu has a high level of activity besides its long half-life, this technique presents an effective method of its management in the course of the study. In this case, the effective management of the waste is achieved by collecting it in superbly designed containers before burying them in designated areas to allow for radioactive decay of the waste. Another consideration is incineration, where thermal energy is used to transform the waste into waste gasses and non-combustible residue/ash. This approach also allows for the sterilization of the waste mixture, energy recovery, and waste volume/mass reduction. However, the method poses a significant environmental concern as a result of gaseous products of waste combustion (Okot, N.d.).

The researcher also applied the basic principles of Pollution Prevention Act to minimize the buildup of radioactive wastes resulting from the preparation as well as the use of both 177LuDOTATOC and 68Ga-DOTATOC in this project. In source reduction, the researcher prevented the buildup of radionuclide wastes by using the standard methods of preparation and administration. In this case, the production of both 177Lu and 68Ga was optimized to avoid surplus production in order to avoid the accumulation of their waste (National Research Council (US), 2011). The second principle employed was recycling, where both 176Lu and 68Ge were reused till their exhaustion for the production of 177Lu and 68Ga, respectively. The third principle employed for this matter was waste treatment, where all excreta and radioactive waste related to the project was isolated and stored in the lead shielded containers to allow for the safe radioactive decay of the radionuclides in order to protect their leakage to the environment as well as the consequent

radiation exposure to the occupants. The fourth principle was the disposal, where the containers were transported to designated radioactive disposal point to be buried in order to allow for safe radioactive decay (National Research Council (US), 2011).

5.5.6. Operator dosimetric considerations.

As described in the entire paper, both 177Lu and 68Ga are radioactive, and one's exposure to these radionuclides may result in significant health implications as a result of irradiation. As a result, the operator is placed in a coincidental risk of exposure to these radiations in the course of the study (Loke et al., 2011). In order to explore the dosimetric considerations worth noting in this project, it is important to discuss the Medical Internal Radiation Dose Schema first. The Medical Internal Radiation Dose Schema is used for calculating radiation dose by referring to age- as well as sex-specific standard data for the human body and anatomy compositions. Of significant importance to the Medical Internal Radiation Dose Schema in relation to this study are organs mostly affected by radionuclides leading to disease, and these organs include kidney, bone and bone marrow (Loke et al., 2011).

For the red bone marrow, the absorption of as a low dose as 3 Gy results in the induction of one percent leukemia within ten years of exposure, with the probability of survival reducing to about 50 percent death rates if the dose increases to 4-6 Gy (Loke et al., 2011). In most studies, the toxicity of the red marrow in relation to radiation uptake it has been seen that the organ is dose-limiting. However, since the red bone marrow is affected by lower dose compared other organs it is important to for operators to note that it is more highly likely to be affected by 177Lu and 68Ga than other organs. On the other hand, exposure of the kidney to irradiation results in the development of radiation nephropathy. At first, the acute radiation nephropathy signs develop within 6-12 months of irradiation, but pathologic kidney emerges as mesangiolysis, atrophy, thrombotic microangiopathy and tubulointerstitial scarring. However, the kidney is more resistant to irradiation compared to the bone marrow, but chronic kidney pathology may develop at the doses of 23 Gy (Loke et al., 2011).

In this study, the justification of exposure to diagnostic 68Ga-PET was considered by weighing the benefit against the costs of radiation, which included effects of radiation. The primary detriment associated with exposure to 68Ga is the development of cancer, and this risk decreases with the advancement of age. Exposure to an effective dose of more than 100 mSv, there is 5 percent increase in the risk of cancer development for every Sv. However, a dose lower than 100 mSv also carry the risk of cancer development, but on a lower scale compared to doses above 100 mSv. In this case, 1 in every 1200 individuals exposed to dose lower than 100 mSv has a nominal risk of cancer development if he or she is 30-60 years of age. However, this risk is lower (1 in 3000) if the person is at least 70 years of age. Since an operator stands significant chances of exposure to radiation, it is important to pay several considerations for protection from irradiation and reduce dosimetric exposure (Australian Radiation Protection and Nuclear Safety Agency, 2008).

One of the considerations is the time spent around the radionuclide. As a general principle, the more the time spent near a specific radionuclide, the higher the radiation dose received. As a result, the operator would receive high 68Ga or 177Lu doses if he/she spends much time near them. A typical workplace involving nuclides may involve a characteristic dose rate that may be used to determine the radiation dose received per unit time. As a result, if the operator follows all safety precautions they may not receive more than the predetermined radiation doses during the research period. However, some operators may be complacent about their safety, which may result in such behaviors as telephone calls or breaks when operating near the source of radioactivity leading to a higher exposure to radiation and ultimately making them receive higher doses of radiation (Australian Radiation Protection and Nuclear Safety Agency, 2008).

The second consideration that needs to be observed by the operators with regard to 177Lu and 68Ga dosimetry is the distance from the source of radioactivity. Again, the dosimetric amount of radiation received by an operator is indirectly proportional to the distance between the source and the operator and therefore an operator working close to the source of radiation (177Lu and 68Ga) will receive a high radiation dose. By using this equation $(I_1(D_1)^2 = I_2(D_2)^2$, where D is the distance

from the source, while I is the radiation intensity at the source), it is possible to determine the radiation dose rate achieved at a specific distance from the source (Bolus, 2008). This equation may be employed when developing surveying the study work areas in order to establish how radiation exposure may be reduced by varying the distance from the source of radiation. Then third consideration to be made in order to reduce exposure to high dose of 177Lu or 68Ga is shielding. The type of shielding employed to counter radiations in the work area depends on the type of ionizing radiation in the question. In this case, 68Ga emits gamma radiation while 177Lu releases beta particles. As a result, the operators needed to pay consideration to the individual characteristics of both gamma rays and beta particles before selecting the shielding material to be applied. Since beta particles are highly penetrating, the operators need to select heavy blocking materials with lead and concrete being prioritized for covering the storage sites, wastes as well as the radiopharmaceutical (177Lu) in order to reduce exposure to high dose beta radiation. On the other hand, gamma radiation from 68Ga should also be blocked using lead to reduce radiation exposure to the operator (Bolus, 2008).

Another important safety consideration involved in this study as a way of reducing radiation exposure to operators was area surveys. In this case, the exposure rate measurements were made on a daily basis to assess the room and the environment in order to reduce background radiation levels in the room. Some of the areas monitored for radiation included stairwells, rooms, and hallways of the study location, and the aim was to ensure that exposure rate does not exceed 100 mrem/y or 2 mrem/hour. According to Thompson (2001), the important to conduct daily wipe tests in all areas within the laboratory in order to detect radioactive contamination early enough and eliminate background radiation that may harm not only the patient but also the operators involved in the study. Also, areas found to be contaminated with e 177Lu and/or 68Ga should be cleaned to make it free from background radiations that increase the radiation dose to which the operators are exposed (Thompson, 2001).

5.5.7.3. Quality assurance and documentation.

For effective quality control practices to be applied in this study, Zanzonico (2008) guidelines were followed as highlighted in this section. The first guideline was the electromechanical inspection of the research instruments for safety. In this case, safety features of such equipment that interface directly with patients as PET and gamma-camera were inspected on a regular basis by manual means. The inspections involved looking for frayed wires, loose electrical/mechanical connections, dents, sharp edges and any other physical damage. This process was carried out manually to allow for the determination of the safety issues associated with the equipment (Zanzonico, 2008).

A sound, compliant quality control program was used for documenting the QC procedures as well as recording the results of those procedures (Zanzonico, 2008). All quality control procedures (including tolerance ranges and corrective action) were described in written documents, under the supervision of the research supervisor who signed their authorization of use. Every quality control test performed was accompanied by a record of such data as the test performed, results of the test, date/time of the test, the identity of the device tested, reference sources, and test results. These records were archived in a chronologic order in an accessible location in order to help to track these QC results longitudinally. The records were maintained electronically, but printed copies were made to help retrieve the data in the case of any loss. The researcher utilized longer-lived surrogate radionuclides as reference sources for quality control tests of the research instrumentation, following commercially available standards provided that their activities be traceable to records of the National Institute of Standards & Technology in order to assure of the calibrated activity's accuracy (Zanzonico, 2008). The long-lived nature of the reference sources allowed for the use of a single standard throughout the research period, and this eliminated the need for the regular preparation of sources besides eliminating possible radioactive contamination as well as inaccuracies in activity dispensing. In addition, an up-todate inventory was maintained, besides checking for radioactivity leakage for all sealed sources.

Also, survey meters were used for monitoring exposure rates, as an important part of the radiation safety program of the study (Zanzonico, 2008). Geiger counters were used to check for radioactive contamination. The survey meters were taken through a daily battery check in order to confirm the battery supplied the acceptable range of voltage. They were also checked for radioactive contamination from a remote radioactivity-free environment, as well as for constancy of response by evaluating their response to a long-lived reference source. In addition, they were also calibrated by the use of long-lived reference sources. Gas calibrators were also tested for linearity, accuracy, and constancy, while quality control tests for the well counters involved photopeak energy window checks, and constancy, efficiency, and background checks, as well. Before using well counters to count samples containing 68Ga, the energy spectrum was always checked to ensure their proper peaking in relation to 68Ga radionuclide photopeak. Moreover, the QC tests of the gamma-camera included in the QC program included spatial linearity, spatial resolution, energy resolution as well as peaking, and uniformity tests. For PET scanners, daily blank scans were performed in order to allow for the overall assessment of the response of the detector (Zanzonico, 2008).

6.0. Chapter Six: Summary, Conclusions and Recommendations.

6.1. Summary.

For the later years of the second half of the 20th century, the discovery of somatostatin and somatostatin analogs elicited research of their use in cancer treatment. Besides, the development of methods of complexing these analogs with radionuclides allowed for their in targeted radiotherapy in the form of peptide receptor radionuclide therapy. On the other hand, the discovery of the mTOR pathway and mTOR inhibitors resulted in the development of everolimus for use as in the suppression of the cellular metabolic processes related to angiogenesis, cell growth, and cell proliferation, amongst others, which are key to the development of cancers. After the approval of everolimus by FDA, the agent has proved efficacious in the treatment of such neuroendocrine

tumors as the breast tumors, pancreatic neuroendocrine tumors, and gastreoenterointestinal neuroendocrine tumors, amongst others.

In this clinical trial, we tried to look in to the efficacy and safety of combined therapy using everolimus (mTOR inhibitor) and 177Lu-DOTATOC regimen in the patient with inoperable/ metastatic neuroendocrine tumor. The study also focus on the affinity, and pharmacodynamics / pharmacokinetics characteristics of the regimen. After approval of the study design from the research and ethical committee we enrolled 9 subjects who fulfilled the inclusion criteria; after explaining the study protocol and taking informed consent. All the nine subjects received 4 cycles of fixed dose of 100 mCi per-cycle of 177LuDOTATOC every 6-8 weeks. In between each cycle patient got 10mg of everolimus every day.

The patient initial assessment along with all the coexisting comorbid conditions were documented to limit the bias. The quality of life was measured using Karnofsky performance status. 24hour 177Lu-DOTATOC plasma concentration measured after each cycle to look for the any change in pharmacodynamics / pharmacokinetics of the radioisotope. Additionally, such hematology parameters as neutrophils count, leucocytes lymphocytes, erythrocytes, platelets, and hemoglobin were assessed at the end of every cycle. Reno protective regimen was used to minimize the renal radiation dose. For the response evolution 68Ga-DOTATOC PET/CT scan was used a tool in the beginning, mid and end of treatment. There was no death occur during and after the study period till now. Diarrhea, headache and skin flushing was most common side effect of the regime. No grade 4 toxicity observed during the trial with respect to hematology, hepatotoxicity or GI symptoms. On contrary the regimen reported 91 mild to moderate adverse events (Grade 2 and 3 toxicities) in 67 percent of the patients, and these included diarrhea, fever, chest pain, bloody nose, general discomfort, loss of appetite, bloating, fatigue, labored breathing and soreness at the site of injection. Besides, the regimen resulted in the development of anemia, but liver and kidney toxicities were minimal because of the co-administration of L-arginine/L-lysine during treatment. In

the end, the treatment was found to be safe and efficacious, and with favorable pharmacokinetics and pharmacodynamics characteristics.

In this study, 68Ga-DOTATOC involved both manual and automated processes, while for 177Lu-DOTATOC only manual synthesis was used. The study resulted in the improvement of the average Karnofsky Performance Status from 66 to 88.5 percent, and this was indicative of the therapeutic value of this combination with regard to the improvement of recipient's quality of life.

EPR technique showed the formation of radicals after the (external) irradiation of drugs used in this study. This could be a starting point for the study of radicals stemming from radiopharmaceuticals in order to better understand the mechanism of action of these drugs. We know that this is only a preliminary study and further experiments should be carried out for example with real (in vitro) cancer cells treated with radiopharmaceuticals. Unfortunately, due to different expertise (and expenses) needed to carry out the experiment with cells we were not able to follow the entire route; nevertheless, we laid the foundation for a collaboration among IEO, Maugeri and Pavia University.

6.2. Conclusions.

In conclusion, combined therapy of everolimus and 177Lu-DOTATOC for neuroendocrine tumors is an effective regimen that produces acceptable adverse events as well as safe biodistribution after administration. The therapeutic effect of this regimen results from the additive effects of each of the respective constituents where everolimus kills cancer cells by targeting mTOR in order to limit the basic cellular activities associated with the development of cancer. On the other hand, 177Lu-DOTATOC kills neuroendocrine tumor cells through the combined action of octreotide as a somatostatin analog and irradiation of cells by Lu-177. This treatment modality results in several benefits that include the improvement of the quality of life of the patients as measured by the Karnofsky Performance Scale where the treatment raises one's independence with personal activities. Besides, the regimen has a low profile of adverse events as only 67 percent of the patients recorded mild-to-moderate adverse events that were easily manageable. In addition, the automated

synthesis of 68Ga-DOTATOC was found to be superior to manual synthesis on the basis of product purity, labelling efficiency, and safety of the operator. In addition, quality control measures and radiopharmaceutical production variables (including radionuclide production and radiolabeling factors) played significant roles in the accomplishment of the goals of this study, but refinement of methods and related protocols could improve results. In fact, if the correlation between radicals and activity of radiopharmaceuticals are confirmed, then a new kind of drugs could be designed for these treatments.

6.3. Recommendations.

One of the recommendations is the additional research with regard to the preparation and dosimetry of 177Lu-DOTATOC. Currently, there exists a significant research gap in this area as there is limited literature of 177Lu-DOTATOC compared to other radiolabeled somatostatin analogs. As a result, it is difficult to develop measures to prevent excessive irradiation of specific subjects and thus leaving the only protective method for 177Lu-DOTATOC administration to be the co-administration of the radiopharmaceutical with L-arginine and L-lysine. In this case, intensive investigations are necessary to establish the 177Lu-DOTATOC dosimetry and avoid assignment of the radiopharmaceutical doses to subjects without prior knowledge of their respective safety thresholds. This research could improve the current understanding by reducing cases of toxicities associated with radioactivity.

Another recommendation is the for the researcher to record further data for analysis of such aspects as the overall survival, objective response rate, long-term safety concerns, and disease control, among other variables. In addition, it is recommendable to adapt the automated synthesis system for 68Ga-DOTATOC production because it offers such advantages as minimal radiation exposure to the operator, high purity/labelling efficiency, and low incident of failures. In EPR experiment, it shows probable synergistic effect of both drugs on sulfur radical production, however further investigation is needed.

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