REPORT

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DEVELOPMENT OF BIOMOLECULES LABELLED WITH ^{177}Lu FOR CANCER THERAPY

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Abstract

This research project will be focus in the development of methodology for labelling biomolecules with ^{177}Lu, quality control procedures to determine radiochemical purity and purification procedures to produce labelled molecules with high specific activity and with GMP grade to be applied in clinical trials. We have particular interest in work with BBN peptide derivatives to be applied in therapeutical procedures in prostate cancer. Studies with BBN(7-14)NH_2 conjugates radiolabelled with different radionuclides have demonstrated receptor-mediated trapping of these radiotracers by GRP-receptor-expressing cancer cells. Investigation into the synthesis and characterization of M^{3+}-radiolabeled BBN or GRP analogues have also been reported by several groups. We intend to develop technology for the production of ^{177}Lu in the research reactor IAE-R1 at IPEN and its radiochemical processing. After a very good experience of IPEN on working with somatostatine analogue labelled with ^{177}Lu, we intend to study labelling conditions to produce BBN derivatives labelled with this radioisotope and applied to therapy of prostate cancer. We also intend to perform in vivo and vitro studies to determine the specificity of the labelled peptide by receptor tumour cells. IPEN has a good relation with local Nuclear Medicine Society that indicates the Hospitals that will participate on the clinical protocols.

1. INTRODUCTION

Isotope production capability in IPEN

The Nuclear Energy Research Institute (IPEN-CNEN) was the first Institution in Brazil to produce radioisotopes and radiopharmaceuticals.

The IEA-R1 nuclear reactor at IPEN (Babcock & Wilcox Co), a swimming pool reactor, operates since 1957, with ^{235}U enriched 20%, maximum termal neutron flux of 5.10^{13}n/cm^2.s (best position). The reactor was recently reformulated to operate at 3.5 MW, 64 hours a week continously.

Nowadays, the IPEN reactor produces ^{153}Sm and ^{131}I applied in radiopharmaceuticals production. The IPEN nuclear reactor has no potency for the production of therapeutic radionuclides with high specific activity. The alternative is to import radionuclides like ^{90}Y and ^{177}Lu. IPEN also imports fission ^{99}Mo, applied in the production of ^{99}Mo-^{99m}Tc generators.

The IPEN produces in the Cyclone 30 cyclotron (IBA, Belgium) ^{123}I, ^{67}Ga, ^{201}Tl and ^{18}F (Table 1). The principal characteristics of the Cyclone 30 are: Particle: acelerated H^-, extracted H^+; energy 15-30 MeV; current 350μA; 2 beams and carbon foil extraction.

Table 1. Radionuclide production at IPEN cyclotron

Radionuclide	Activity (mCi)	production/ week
^{67}Ga	1200-1500	1
^{201}Tl	350-400	1
^{123}I	900-1000	1
^{18}F	6000	8

The local production of some radioisotopes represents an important economy on radioisotopes importation (Table 2).

Table 2. Anual economy with local production of radioisotopes

Product	Activity (mCi in calibration time)	Anual Economy (US$)
^{131}I (60% of demand)	25000	480,000
^{201}Tl (100% of demand)	350-400	330,000
^{67}Ga (100% of demand)	1200-1500	971,000
	TOTAL	**1,781,000**

Radiopharmaceutical production at IPEN

The Radiopharmacy Center (CR) of IPEN produces radiopharmaceuticals that are distributed to 260 Nuclear Medicine services in the country (Tables 3-6).

Table 3. Radiopharmaceuticals produced at IPEN

PRODUCT	**ACTIVITY (mCi) in 2005**
[^{131}I]NaI – solution	1,233,413
[^{131}I]NaI – capsules	293,815
^{67}Ga – citrate	57,697
^{201}Tl – chloride	15,948
[^{123}I] NaI - solution	2,659
^{32}P – phosphoric acid	1,830
^{32}P – sodium phosphate	712
^{51}Cr – chromate and chloride	696 and 1,300
^{35}S – sodium sulphate	648

In 1981 IPEN started the production and distribution of ^{99}Mo-^{99m}Tc generators using high activity fission ^{99}Mo. Nowadays, about 270 generators are produced every week, varying from 250 to 2000 mCi with a total acitivity distributed at calibration time of 420,000 mCi. About 50% of the total generators belong to 1250 and 2000 mCi (Table 4).

Table 4. ^{99}Mo-^{99m}Tc Generators produced at IPEN

mCi	250	500	750	1000	1250	1500	2000
GBq	9.25	18.50	27.75	37.00	46.25	55.50	74.0
N^{o} of generators produced/week	6	28	37	48	29	65	51

Lyophilized kits for labelling with technetium-99m are also produced at IPEN (Table 5) and are very important for the application of this radioisotope in different diagnostics procedures. Each kit contains 5 lyophilized vials.

Table 5. Lyophilized kits for labelling with ^{99m}Tc produced at IPEN in 2005

Product	Number of kits (2005)
MDP	11467
DTPA	3956
DMSA	2323
PYP	1626
ECD	1972
EC	410
Sn Colloidal	1145
DISIDA	498
DEXTRAN 70 /500	791
HSA	146
GHA	20
FYT	1926
MAA	2297

Table 6 describes the labeled molecules applied in diagnostic and therapeutic procedures distributed by IPEN.

Table 6. Labeled molecules produced at IPEN

Product	
[^{18}F]FDG	6105 doses*
[^{131}I] MIBG	14001 mCi
[^{123}I] MIBG	1586 mCi
[^{131}I] Lipiodol	1575 mCi
[^{131}I] Hippuran	242 mCi
[^{131}I] HSA	4 mCi
[^{51}Cr] HSA	4 mCi
[^{51}Cr] EDTA	560 mCi
[^{153}Sm] EDTMP	336 doses**
[^{111}In]DTPA-Octreotide	378 mCi
[^{153}Sm] HA	1950 mCi
[^{90}Y] Coloidal Citrate	505 mCi

*** one dose of [^{18}F]FDG = 20 mCi; ** one dose of [^{153}Sm] EDTMP = 100 mCi**

The application of therapeutic radiopharmaceutical in Brazil

The Radiopharmacy Center of IPEN produces, since 1961, 131-iodine as sodium iodide solution applied in thyroid diagnostic and therapeutical procedures. The increasing application of 131-iodine in therapeutic doses lead to the development of the capsules of ^{131}I, that facilitated and make more security the administration of high doses of this radioisotope. The use of ^{131}I-capsules increase every year reaching 293,815 mCi in 2005

and about 400,000 mCi in 2006.

The IPEN produces ^{131}I-MIBG for therapeutical applications, in the treatment of feochromocitoma and neuroblastoma, ^{131}I-Lipiodol for hepatoma therapy and ^{153}Sm-EDTMP, applied in bone pain palliation resulted from cancer metastasis.
Hydroxiapatate (HA) labeled with ^{153}Sm, has been applied in treatment of rheumatoid artritis. ^{90}Y-Coloidal citrate was also applied in radiosinovectomy but with good results in large joints (like knees) due to the high beta energy particles. The ^{90}Y-Coloidal citrate distributed nowadays by IPEN are imported from Nordion**.** The local production is in study.

Unfortunately, the production and distribution of radiopharmaceuticals for therapy application, with exception of 131-iodine, is not significant, especially if compared with the radiopharmaceuticals applied in diagnostic procedures.

The development of new compounds may change this view, especially when considering the radiopharmaceuticals based on biomolecules, like peptides and monoclonal antibodies, labeled with beta emmiters radionuclides and specifically directed against tumor cells. ^{177}Lu-DOTA-octreotate is an example of this kind of radiopharmaceutical that can contribute to increase the therapeutical application of radiopharmaceuticals in Brazil.

Products in Development

Two radiopharmaceuticals for therapeutical application were recently developed at IPEN and are in clinical trials: ^{177}Lu-DOTA-octreotate and ^{90}Y-Hidroxiapatate.

The development of ^{177}Lu-DOTA-octreotate started with an IAEA-CRP ("Comparative Evaluation of Therapeutic Radiopharmaceuticals") concluded in 2005. In this CRP we studied the labeling and quality control procedures and evaluated the specificity and affinity of the labeled compound for Somatostatin-receptors tumour cells, using *in vitro* and *in vivo* procedures.

The labeling procedure are performed using DOTA-Tyr^3-octreotate (Pichem, Austria or Anaspec, EUA, GMP grade) and $^{177}LuCl_3$ (IDB, Holand, specific activity of 820 GBq/mg or 22.16 Ci/mg) in a molar peptide to radionuclide ratio of 2.1 (~26.99 MBq/μg or 0.73 mCi/μg of peptide), in 0.4M sodium acetate buffer pH 4.5 for 30 minutes at 90^{o}C. Gentisic acid was used as stabilizer and DTPA was added in order to scavenge uncomplexed Lutetium. Radiochemical purity > 98% was obtained with very good stability (> 48 hours). The sterile and apirogenic labeled peptide was distributed in final concentration of about 740 MBq/mL (20 mCi/mL) and the labelling procedures are being performed using 200 - 500 mCi of $^{177}LuCl_3$.

The clinical trials with the ^{177}Lu-DOTA-octreotate started in March, 2006 and IPEN distributed until November 2006 about 2.0 Ci of the labeled compound in therapeutical

doses varying from 50 to 200 mCi and some diagnostic doses (5 mCi). Two hospitals are participating from this clinical protocol: Cancer Hospital – A.C. Camargo and Albert Einstein Hospital in São Paulo. The IPEN will start the routinary production and comercialization of this product in the next year, after the conclusion of the clinical protocol.

Another product that is in the clinical protocol is the ^{90}Y-Hidroxiapatate. This radiopharmaceutical presents some advantages in preparation when compared to the ^{90}Y-Coloidal citrate, particularly related to particle size and stability.

The Nuclear Physicians in Brazil have great interest for Anti-CD-20 antibody labeled with beta emiters applied in therapy of non-Hodking lymphoma. We studied the labeling of Anti-CD-20 antibody (Rituximab, Mathbera) with iodine-131 with good results of labeling yield and stability of the final product. However, the labeled antibody, when produced with specific activity of 80mCi/mg, undergos loss of immunoreactivity. The therapeutical usefulness of the low specific activity antibody (0.8 mCi/mg), apparently with high immunoreactivity as previously described [1] has to be evaluated.

The IPEN also participates of a IAEA-CRP ("Development of generator technologies for therapeutic radionuclides") with the objective of developing radiotherapeutical generator such as ^{188}W-^{188}Re and ^{90}Sr-^{90}Y. Our aim at this CRP is to produce in house ^{188}Re and ^{90}Y generators label molecules with these radioisotopes and distribute them to the clinics.

Some radiopharmaceuticals for diagnostic application based on biomolecules like somatostatin derivatives for labeling with technetium-99m (HYNIC-octreotide and HYNIC-octreotate) are being studied as well as the labeling of Anexin V (an apoptose marker) with ^{99m}Tc using different methods. The labeling of Arg-Gly-Asp (RGD) sequence peptide and a bombesin analog with ^{99m}Tc are being developed using the tricarbonil method, as part of a IAEA-CRP in course ("Development of ^{99m}Tc based small biomolecules using novel ^{99m}Tc cores").

Lyophilized kits of Ciprofloxacin, Metoxiisobutilisonitrila (MIBI) and Glucarate, for labeling with technetium-99m have been developed.

The participation of clinical groups on clinical protocols

The IPEN works with the colaboration of many clinical Hospitals and institutions to develop clinical protocols of new products. The IPEN and the Brazilian Society of Nuclear Biology and Medicine and Molecular Imaging (SBBMN) are stablishing now the rules for clinical protocols with the radiopharmaceuticals developed at IPEN. The Society will indicate the Clinical Institutions with competence and infrastructure to develop the clinical protocols.

2. THE OBJECTIVES AND PORPOSAL FOR THIS CRP

Labeling of bombesin analogs with ^{177}Lu

Radiolabeled small receptor-avid peptides have attracted considerable interest because of their wide applicability in the development of target-specific radiopharmaceuticals. Regulatory peptide receptors are overexpressed in numerous human cancers. These receptors have been used as molecular targets by which radiolabeled peptides can localize cancers *in vivo* and, more recently, to treat cancers with peptide receptor radiation therapy (PRRT) [2,3].

Gastrin-releasing peptide (GRP) receptors have been shown to be expressed with high densities on several types of cancer cells including prostate, breast, small cell lung, and pancreas cancer. Bombesin (BBN) has been known to bind to GRP receptors with high affinity and specificity [2].

BBN is a 14-amino-acide peptide present in amphibian tissues, whereas GRP, its human counterpart, consists of 27 amino acids. Like many of the regulatory peptides, GRP elicits a broad spectrum of biological responses including secretion of adrenal, pituitary, and gasrointestinal (GI) hormones as well as gastric acid secretion. It acts primarily in the central and enteric nervous systems, where it regulates several processes including satiety, thermoregulation, circadian rhythm, smooth muscle contraction and immune function [4,5].

BBN and GRP mediate their actions through membrane-bound, G protein coupled receptors (GPCR), characterized by 7 transmembrane domais, which cluster to form the ligand-binding pocket. There are 4 known subtypes of BBN-related peptide receptors including GRP-R (BB2, BRS-2), NMB-R (neuromedin B receptor, BB1, BRS-1), the orphan receptor bb3-R (BRS-3), and the amphibian receptor bb4-R, although cognate ligands for the last two have yet to be described for mammals [4].

The GRP receptor is becoming an increasingly attractive target for development of new radiolabeled peptides with diagnostic and therapeutic potential [6]. Significante progress has been made over the past few years in developing effective strategies to produce radiolabeled BBN analogs that specifically target GRP-receptor-expressing cancer cells, focused on developing radiometallated BBN analogs in which radiometal chelates are linked to the BBN(7-14)NH_2 sequence that serves as the highly specific GRP-recepto-binding motif. This sequence was selected since the BBN derivatives containing this sequence have been shown to bind in an agonist manner to the GRP receptors [2].

Studies with BBN(7-14)NH_2 conjugates radiolabeled with different radionuclides have demonstrated receptor-mediated trapping of these radiotracers by GRP-receptor-expressing cancer cells [2]. Investigation into the synthesis and characterization of M^{3+}-radiolabeled BBN or GRP analogues have also been reported by several groups, as described in Table 7.

Table 7: Representative BBN analogues labeled with different radionuclides

BBN analogue	Radio.	Results	Ref.
CHX-B-DTPA-8-Aoc-BBN(7-14)	^{111}In	-high binding to adenoviral vector AdCMVGRPr infected SKOV3.ipl ovarian cancer cells (~42.7%) - high degree of internalization and retention of the conjugate (60% at 15 min, 58% at 2 h)	[7] Rogers et al. (1999)
(DTPA-Pro^{1},Tyr^{4})BN (agonist) (DTPA-Tyr^{5},D-Phe^{6})BN(5-13)NHEt (antagonist)	^{111}In	- each of the two conjugates expressed high affinity for the GRP receptor in 7315b rat pituitary tumor cell membranes; -(DTPA-Pro^{1},Tyr^{4})BN demonstrate *in vitro* internalization inherent to agonistic binding also showed higher uptake in the tumor.	[8] Breeman net al. (1999)
(DTPA-Pro^{1},Tyr^{4})BN (DOTA-Pro^{1},Tyr^{4})BN (DTPA-ε-Lys^{3},Tyr^{4})BN (DOTA-ε-Lys^{3},Tyr^{4})BN	^{111}In	- The radiolabeled agonists bound specifically to the GRP receptor and were internalized in a temperature-dependent manner (CA20948 rat pancreatic tumor cells); -^{111}In-(DOTA-Pro^{1},Tyr^{4})BN showed a higher uptake of radioactivity in GRP receptor-positive tissues as well as higher target-to-blood ratios; -^{111}In-(DTPA-Pro^{1},Tyr^{4})BN was easier to handle and is more practical to use	[9] Breeman net al. (2002)
DOTA-X-BBN(7-14)NH_2 analogs, X = 0 carbon, β-Ala, 5-Ava, 8-Aoc or 11-Aun spacer moieties	^{111}In	- high binding affinities for GRP receptors in human PC-3 androgen independent prostate cancer cells *in vitro* (i.e. IC_{50} values ranging from 0.6 to 2.4 nM) included those with 3, 5, or 8-carbon spacer moieties; - analogs where X = 5-Ava and 8-Aoc exhibit high specific localization in the pancreas, a normal GRP receptor expresssing tissue, and efficient clearance from the blood primarily via the renal-urinary pathway. -^{111}In-DOTA-8-Aoc-BBN(7-14)NH_2 demonstrate *in vivo* uptake in human prostate PC-3 xenografed flank tumors (3.63 ± 1.11 %ID/g at 1 h p.i.)	[2] Hoffman et al (2003)
DOTA-8-Aoc-BBN(7-14)NH_2 (BBN-8)	^{177}Lu	-pre-clinical evaluation exhibits an IC_{50} of 0.5 ± 0.1nM in GRP recepor-expressing PC-3 tumor cells; - receptor-mediated, tumor targeting of the PC-3 xenografted SCID (severe combined immunodeficiency) mice resulted in tumor uptake and retention values of 4.22±1.09, 3.03±0.91, and	[10] Smith et al. (2003)

		1.54±1.14 %ID/g at 1, 4, and 24h, respectively.	
S_4-5-Ava-BBN(7-14)NH_2	^{105}Rh Rhodium	- IC_{50} value of 4.76±0.79 nM on *Swiss* 3T3 fibroblasts. - GRP receptor specific uptake in normal pancreas was found to be 2.25±1.02 %ID/organ	[11] Hoffman et al. (1997)
DTPA-DTyr^6,β-Ala^{11},Thi^{13} BBN(6-14) (BZH1) DOTA-DTyr^6,β-Ala^{11},Thi^{13} BBN(6-14) (BZH2) (Pan-BBN compounds)	^{177}Lu ^{90}Y ^{111}In	-both are internalized rapidly (10-20% in 30 min) by PC-3 or AR42J cells reaching 30-40% by 6 h. -blood clearance of both is very fast with 0.015 %ID/g remaining in the blood at 4 h. -^{90}Y-BZH2 and ^{177}Lu- BZH2 may already be good candidates for targeted radiotherapy in patients. -lower affinity for GRP-R and NMB-R recepors compared with Lu-AMBA.	[12] Zhang et al. (2004)
DOTA-G-4-aminobenzoyl-BBN(7-14)NH_2 (AMBA)	^{177}Lu	-^{177}Lu-AMBA binds specifically to GRP-R and NMB-R-bearing human tumor tissues with no binding affinity or low binding affinity to bb3 receptor. -excretion primarily renal; -great tumor retention (24 hs) than BBN-8; -kidneys uptake is ~ 50% lower than that of the SM receptor-targeted peptides and is not reduced further with coadministration of L-lysine; -the efflux after 2h is markedly reduced for ^{177}Lu-AMBA versus the BBN and Pan-BBN ligands. Very little radioactivity escapes from cells once targeted with ^{177}Lu-AMBA confirming the unique qualities of the molecule. -a phase I single-dose clinical trial with ^{177}Lu-AMBA is in progress (Bracco Imaging, started in October 2005 in hormone refractory prostate cancer) for imaging and systemic radiotherapy .	[13] Lantry et al. 2006

(Tyr4)BN
pGlu-Gln-Arg-Tyr-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2
Q – W – A –V – G – H – L –M - NH_2
(DTPA-Pro^1,Tyr^4)BN or (DOTA-Pro1,Tyr^4)BN
DTPA/DOTA-Pro-Gln-Arg-Tyr-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2
(DTPA-ε-Lys^3,Tyr^4)BN or (DOTA-ε-Lys^3,Tyr^4)BN
pGlu-Gln-(DTPA/DOTA-ε-Lys)Tyr-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2
DOTA-8-Aoc-BBN(7-14)NH_2
DOTA-8-aminooctanoic acid- Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2
DOTA-G-4-aminobenzoyl- BBN(7-14)NH_2
DOTA-glycyl-4-aminobenzoic acid- Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2

Further progress in developing GRP receptor targeted radiopharmaceuticals will

require additional efforts in understanding the structurally sensitive mechanisms involved in the binding of these derivatives to GRP/BBN receptors, the subsequent residualization of the radiotracer in GRP receptor expressing cancer cells, and finally, efficient clearance of non-residualizing radiolabeled peptide fron non-target tissues [6].

Pharmacologic side effects of the BN receptors agonists have to be considered. Because of the localization of NMB-R and bb3-R in normal human tissues is virtually unexplored, one cannot yet exclude the appearance of unwanted side effects related to yet unknown physiologic BBN targets [4].

However, acute toxicity tests of ^{177}Lu-AMBA have been performed in mouse, rat and monkey sufficient to allow clinical trials to start in cancer patients. No unexpected toxic or pharmacologic responses were noted, even at the highest blocking doses of 10 mg/kg in mice which is about 10 000 times the doses given to man [4].

Combined radiotherapy/chemotherapy continues to hold some promise as a treatment modality for human cancers and has been demonstrated in animals using ^{177}Lu-BBN analogue. In the recent Johnson et al´s paper, it is reported that GRP-receptor-targeted radiotherapy in combination with traditional chemotherapy results in 30% increase in mean survival as compared to targeted radiotherapy or chemoterapy administered as single-agent therapies [14].

Specific Objectives

This research project will be focus in the development of methodology for labelling biomolecules with ^{177}Lu, quality control procedures to determine radiochemical purity and purification procedures to produce labeled molecules with high specific activity and with GMP grade to be applied in clinical trials. We have particular interest in work with BBN peptide derivatives to be applied in therapeutical procedures in prostate cancer.

3. MATERIAL AND METHODS

- **Development of technology for the production of ^{177}Lu in the research reactor IAE-R1 at IPEN and its radiochemical processing**

^{177}Lu can be produced by two different routes: irradiation of natural or enriched Lu_2O_3 target, as also by irradiation of Yb target (Yb_2O_3) followed by radiochemical separation of ^{177}Lu from Yb isotopes. The two production routes lead to the product with different specific activities. Although the specific activity obtained in (n,γ) activation is usually low, it could be further enhanced considerably by using Lu target enriched in ^{176}Lu, by carrying out irradiation in a high flux reactor, as well as optimizing the duration of irradiation. The activation of ^{176}Yb and subsequent β^- decay gives no carrier added ^{177}Lu [16].

Our studies will include:

- Irradiation of targets containing Lu to measure the production yield and specific activity of ^{177}Lu and possible contaminants (direct reaction);
- Irradiation of targets containing Yb to measure the production yield and specific activity of ^{177}Lu and possible contaminants (indirect reaction);
- Development of a chemical separation method in order to separate ^{177}Lu and Yb;
- Establishment of quality control methods for ^{177}Lu: radiochemical, radionuclidic and chemical purities;
- Definition of the production route of ^{177}Lu;
- Establishment of the production capacity of ^{177}Lu at IPEN.

- **Development of ^{177}Lu based primary cancer specific radiopharmaceuticals: bombesin analogs for prostate cancer therapy including:**
  - Selection of the BBN analogue
  - Study of labeling conditions (pH, molar peptide:radionuclide ratio, reaction time and temperature);
  - Study of the *in vitro* stability of the labeled compound (in saline and plasma);
  - Stablishment of a protocol to determine radiochemical purity of the preparations (ITLC-SG, SepPack C18 cartdrige, HPLC);
  - Development of purification procedure (SepPack C18 cartdrige) in order to produce high specific activity radiopharmaceutical.

^{177}Lu is a radiolanthanide element that is ideally suited for radiotherapeutic applications due to its attractive physical characteristics and ready availability. ^{177}Lu has a 6.71 d half life and emits a medium energy β^- particle ($E\beta^-_{max}$ = 0.497 MeV). Furthermore, the presence of a low-abundance 208 keV gamma photon allows for *ex vivo* evaluation of the *in vivo* targeting efficacy of the administered ^{177}Lu biomolecular targeting agent. ^{177}Lu has a maximum particle range of about 2 mm, making it a more favorable radionuclide for radiotherapeutic application in smaller tumors [10].

Trivalent metallic radioisotopes, such as the radiolanthanides, are particularly attractive for use in diagnostic and/or therapeutic procedures in nuclear medicine. The radiolanthanides exist primarily in an oxidation state of 3+ and can be stabilized by hard donor atoms such as nitrogen or oxygen. The mechanism of ligand coordination is more often ionic, rather than covalent, for these trivalent isotopes, and therefore, multidentate, macrocyclic, polyamino-carboxylate ligand frameworks such as DTPA or DOTA (DTPA = diethylenetriaminepentaacetic acid; DOTA = 1,4,7,10-tetraazacyclododecane-N,N′,N′′,N′′′-tetraacetic acid) are often utilized to produce kinetically inert, *in vivo* stable conjugates [15].

Radiolabeling strategies for the conjugated peptide include heating (80 – 100^{o}C) for short periods of time (10 - 60 minutes) at pH 4.5 – 6.0 in order to obtains radiochemical yields ≥ 95% [2,6,10,13].

- *In vivo* and *in vitro* assays with the labeled peptide
  - Biodistribution studies in laboratory animals to predict pharmakocinetic behaviour, *in vivo* stability and tumor affinity (biodistribution in normal *Swiss* mice and *Nude* mice with tumor to determine the %I.D./g of tissue or tumor) and scintigraphic images of the tumor model animals;
  - Internalization assay in tumor cells (AR42J or PC-30 cells) - *in vitro* assay.

- Clinical protocol

The experimental conditions developed will be extended to the production plant in order to produce the labeled molecule under GMP conditions to be applied in clinical trials.The Radiopharmacy Center of IPEN will establish clinical protocols with Nuclear Medicine Center and Hospitals that are reference in cancer therapy in Brazil, in accordance and with the colaboration of the Brazilian Nuclear Medicine Society.

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