Available online at www.sciencedirect.com
ScienceDirect
Nuclear Science and Techniques, Vol.18, No.2 (2007) 88–100
NUCLEAR SCIENCE AND TECHNIQUES

Peptides radiolabeled with Re-186/188 and Tc-99m as potential diagnostic and therapeutic agents

E. GARCÍA-GARAYOA[1] R. SCHIBLI[1, 2] P.A. SCHUBIGER[1, 2*]

([1] Center for Radiopharmaceutical Science of the ETH Zurich-Paul Scherrer Institut-University Hospital Zurich, CH-5232, Villigen PSI, Switzerland;

[2] Department of Chemistry and Applied Biosciences ETH Zurich, 8093 Zurich, Switzerland)

Abstract Small peptide-based compounds have attracted an enormous interest as carrier molecules to selectively deliver radionuclides to target tissues, sparing critical normal organs. When looking for "matched pairs" of radionuclides, suitable for radiolabeling of peptides for diagnosis and therapy, technetium and rhenium represent an almost ideal constellation. The important role of technetium-99m and Re-186/188 is based on the decay characteristics, suitable for tumor diagnosis and therapy. Tc-99m and Re-188 are readily available by either a ^{99}Mo/^{99m}Tc or the ^{188}W/^{188}Re radionuclide generator system. Furthermore, technetium and rhenium are chemically related and share structural as well as reactive similarities, which prompt an attractive "matched-pair" situation. This article shows an overview of ^{99m}Tc- and $^{186/188}$Re-radiolabeled peptides that have been tested for their potential use as imaging and therapeutic agents in oncological diseases.

Keywords Radiolabeled peptides, Imaging, Therapy, Oncology, Technetium-99m, Rhenium-186/188

CLC numbers Q516, R817

1 Introduction

^{99m}Tc is the most commonly used radioisotope for diagnostic applications in nuclear medicine because of its ideal physical properties (γ-emission energy = 140 keV, 89% abundance), optimal for diagnostic imaging, and its availability because of an inexpensive ^{99}Mo/^{99m}Tc generator.[1] At the same time, the 6 h half-life is ideal for most applications; this is especially valid for small peptides that clear rapidly from the blood pool and readily localize at the target tissue. The half-life is short enough to enable the administration of reasonably high doses without harming the patient, but still allowing good quality images. Rhenium-186 is a β-emitting radionuclide with good therapeutic decay characteristics. In addition to emitting moderate energy β^- particles at 1.07 and 0.933 MeV, it has a low-abundance γ emission at 137 keV (9%), which allows in vivo tracking of the radiolabeled biomolecules and dosimetry calculation. The 3.7-day half-life of ^{186}Re allows sufficient time for the synthesis and shipment of potential radiopharmaceuticals. The drawback of ^{186}Re for radioimmunotherapy or receptor mediated radiotherapy is the low specific activity resulting from the production method utilizing the (n,γ) reaction in a nuclear reactor. Therefore, several groups have recently put efforts into the production of ^{186}Re with high specific activity, for example, via a proton or neutron bombardment of enriched target materials.[2-5] ^{188}Re is an interesting radionuclide with favorable physical characteristics, which offers several advantages for potential therapeutic applications.[6] It is relatively cheap and can be obtained carrier free in a good yield from an in-house ^{188}W/^{188}Re generator. Moreover, its decay characteristics (high-energy β_{max} emission = 2.12 MeV; γ-emission of 155 keV, 15% abundance) and its relatively short half-life (17.0 h) allow high dose rates and repeated application, which

* Correspondig author. E-mail: august.schubiger@pharma.ethz.ch

Received date: 2006-12-20

renders ^{188}Re suitable for radiotherapy using peptides. The average penetration of the $^{186/188}$Re β^-particles circumscribe a region of high energy deposition to the tumor area and induce minimal damage to adjacent healthy organs.

2 Coordination chemistry of Tc and Re used for peptide labeling

Technetium and rhenium are members of the same transition metal group (group 7). They both possess a rich coordination chemistry. The elements share similar physico-chemical and chemical properties as a consequence of the lanthanide contraction. Thus, metal chelators applicable for technetium are usually also suitable for rhenium and vice versa. However the reaction conditions and particularly the radiolabeling conditions are often quiet different. Reduction of ^{99m}Tc-pertechnetate ($[^{99m}TcO_4]^-$) requires only a small amount of Sn^{2+}.[7] To reduce the corresponding $[^{186/188}ReO_4]^-$, the Sn^{2+} concentration must be about 2-3 orders of magnitude higher. This is a direct consequence of the lower redox-potential of rhenium as compared to technetium. In addition, the pH of the reaction solution has to be rather acidic to avoid the fast back-oxidation or hydrolysis of the reduced rhenium species and intermediates.[8] Reaction kinetics is also frequently reported to be slower in the case of rhenium. Consequently, labeling of biomolecules with rhenium generally implies the exposure of the biological entity to rather harsh reaction conditions for a prolonged time. In this respect peptides are much more tolerable than large proteins, such as, antibodies. Yet, the optimization of the radiolabeling conditions as well as the stability of radioconjugates with rhenium (and technetium) remain important and challenging for (radio) chemists.

Three approaches have been used for ^{99m}Tc/$^{186/188}$Re-labeling of peptides: (1) direct labeling; (2) the preformed chelate or prelabeling approach; and (3) the post-labeling approach.

The direct approach uses a reducing agent to open a disulfide bridge of a peptide or protein, thus forming two thiolates that coordinate with the metal center. This method is easy to perform, but there is little knowledge about the geometry of the coordination sphere and the donor groups involved in the metal coordination. Moreover, the control of the labeling site and the in vivo stability of the resulting complexes are often poor. Nevertheless, it has been used for some peptide radiopharmaceuticals.[9]

The preformed chelate approach involves the synthesis of ^{99m}Tc/$^{186/188}$Re complexes with a so called bifunctional chelating agent (BFCA). Purification of this complex is followed by activation of the functional group, used for coupling and conjugation to a peptide. This approach results in conjugates of high specific activity. The drawback is that it is time consuming, involves several synthetic steps, and does not allow the development of an instant kit formulation.[1, 10] Furthermore, the presence of over one reactive group in the biomolecule can result in different species of similar chromatographic behavior (complicating their separation and/or purification), but with variable biological and pharmacological characteristics.

The post conjugation labeling approach is the most desirable approach because it allows kit formulation. The difficulty is to develop bifunctional chelators, which complex Tc/Re efficiently and selectively to achieve uniform products of high specific activities. In addition the Tc/Re-labeled radioconjugate must be thermodynamically stable and kinetically inert to survive under physiological conditions.

Several metal cores of technetium and rhenium are nowadays available for the radiolabeling of peptides (Fig.1). The most common core is the metal-oxo core ($[M^{+V}=O]^{3+}$; M = ^{99m}Tc, $^{186/188}$Re), where the metal center is in the oxidation state +V. Alternatives to the M=O core are the metal-nitrido core ($[M^{+V}\equiv N]^{2+}$), the HYNIC core (M=N=NR) and the organometallic tricarbonyl core ($[M^{+I}(CO)_3]^+$) (Fig.1). All these systems require the introduction of BFCA to firmly attach the radiometal to a peptide, for example. The in vivo stability of the rhenium conjugates or the rhenium complexes respectively is crucial because of the particle emitting radiation. Loss of the particle emitting radionuclide in vivo could lead to uncontrollable biodistribution and potentially to damage of healthy tissue. Therefore, much attention has been dedicated to develop chelating systems, which stabilize the rhenium-cores in vivo and at the same time give rise to good pharmacokinetic properties of the radioconjugates and potential metabolites thereof.

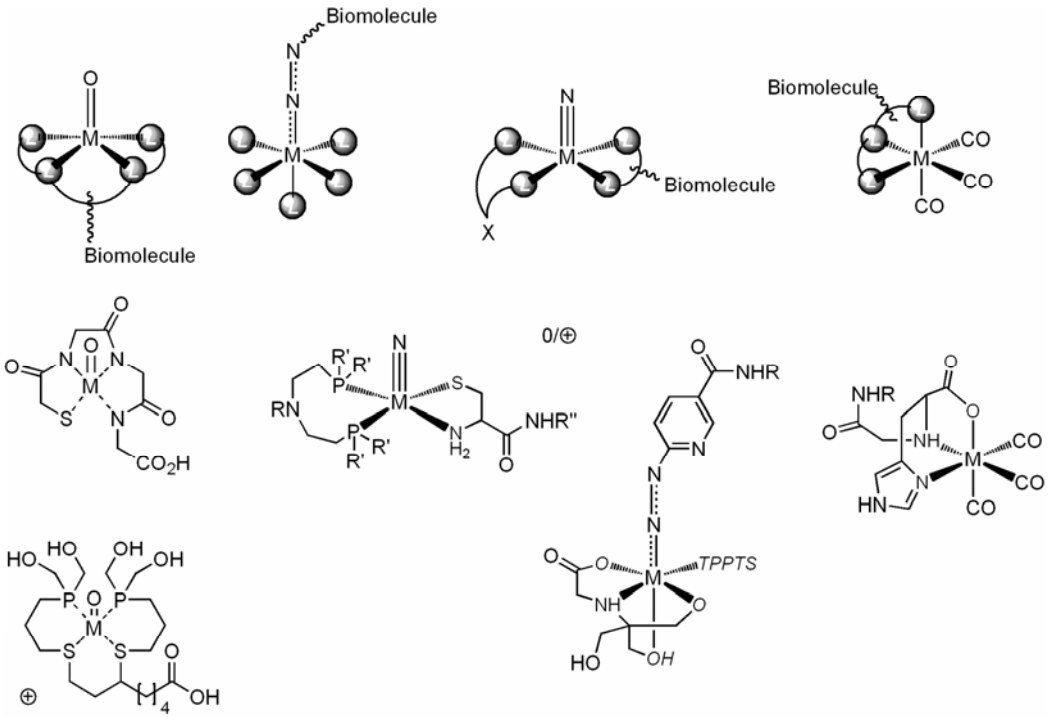


Fig.1 (Top) General structure of Tc/Re-cores and fragments; (from left to right) Tc/Re(V)-oxo complexes with tetradentate N/N-, N/S- and S/P-chelators, HYNIC-system (Hydrazino nicotinitc acid) with coligands, Tc/Re(V)-nitrido complexes and organometallic Tc/Re(I)-tricarbonyl complexes with tridentate chelator. (Bottom) Selected examples of Tc/Re BFCAs conjugates used for labeling of biomolecules.

A variety of BFCAs have been evaluated upon conjugation to peptides, to achieve this goal. They are of the type N_3S triamidethiols,[11] N_2S_2 diamidedithiols, N_4 tetraamines,[12] PnAO propylenediamine dioxime[13], and hydrazine nicotinic acid (HYNIC).[14, 15] Relevant chelator structures are shown in Fig.1. The development of hydrophilic chelating moieties to facilitate efficient clearance of the ^{99m}Tc/^{188}Re activity from the blood pool through the kidneys and into the urine, with low retention of radioactivity in the liver and kidneys is an important endeavor in the development of effective radiopharmaceuticals particularly for therapy. With this aim, Katti and coworkers developed BFCAs, based on the dithia-bis (hydroxymethyl) phosphine (Fig.1) or a diamino-bis (hydroxymethyl) phosphine framework that could be linked to peptides or other biomolecules to produce ^{99m}Tc-labeled conjugates.[16 - 18] For the same reason different hydrophilic coligands have been combined with the HYNIC-core and tested in vitro and in vivo.[15]

A slightly different approach was more recently introduced by the Tisato/Duatti group and the Schubiger/Alberto/Schibli group. Both groups proposed the use of stable, water-soluble and water stable Tc/Re-precursors with substitution labile coordination sites for the effective coupling of the metal center under mild reaction conditions. Tisato and Duatti used a tetra/penta-coordinated nitrido core with two coordination sites, which could readily be exchanged by bidentate chelating systems attached to the peptide. The group of Schubiger developed an organometallic Tc(+I)/Re(+1)-tricarbonyl precursor with three tightly coordinated, facially arranged CO ligands and three substitution labeling water molecules in transposition. The low-spin d^6 configuration renders the metal center (and thus the complexes) very inert. $[M(H_2O)_3(CO)_3]^+$ is not prone to hydrolysis and is shelf-stable on the n.c.a. level for several hours. Kit formulations for the preparation of the precursor, $[*M(H_2O)_3(CO)_3]^+$ for both Tc-99m and Re-188 have been developed (Fig.2).[19 - 22] The kit-preparation of the rhenium homolog $[^{188}Re(OH_2)_3(CO)_3]^+$ needs a prereduction step or addition of a stronger or more stable reducing agent, such as, $H_3B{\cdot}NH_3$ (eventually in combination with another polymer-bound reducing agent), whereas the Tc-99m analog can be synthesized in one step directly from $[^{99m}TcO_4]^-$.[23,24]

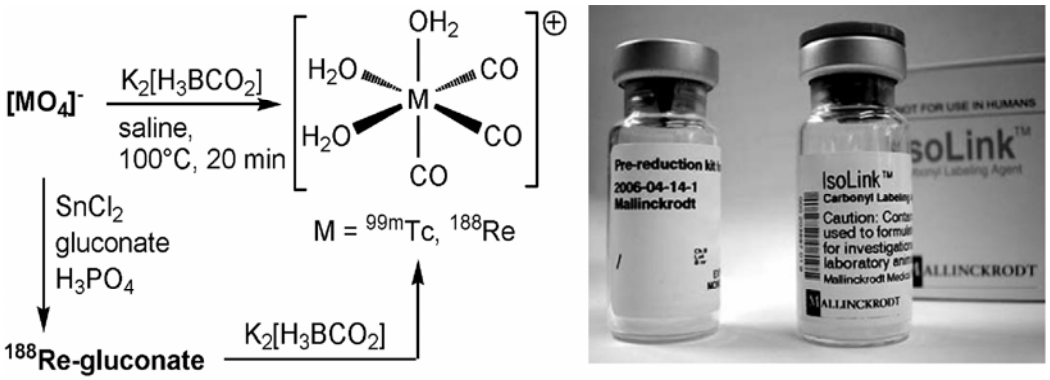


Fig.2 Kit-formulation (one-step for ^{99m}Tc and two-step for ^{188}Re) for the synthesis of the organometallic precursors $[M(OH_2)_3(CO)_3]^+$ (M=^{99m}Tc, ^{188}Re).

Alternative to the above mentioned "pendent-approach", where the artificial Tc/Re chelating moiety is attached to the C- or N-terminus of the peptides, is the "integrated approach". Here the metal coordinates directly via donor atoms provided by the amino acid side chains of the peptide. Thereby the metal center forms the template (e.g., a square planar template), which significantly modulates the ternary structure of the peptide. An excellent example is the ^{188}Re-labeled α-melanocyte stimulation hormone derivative (Fig.3). Here the rhenium-(V)-oxo core is coordinated via three sulfur atoms of the three Cys, as well as an amid nitrogen of the amino acid back bone[25] (see chapter 4.3).

3 Strategies for effective tumor targeting with peptides

By definition peptides are small proteins, whose size varies from ~2-50 amino acids. Peptides offer several advantages over bigger proteins and antibodies (Table 1). They are readily synthesized, chemically modified, and radiolabeled. They lack immunogenicity and provide low toxicity. Natural peptides regulate a large variety of physiological functions in the human body, exerting their action through membrane-bound receptors which mostly belong to the superfamily of the G-protein coupled receptors. They show high affinity for their receptors, usually to more than one subtype. Naturally occurring peptides are necessary molecules in many fundamental biological processes. They can act as hormones, neurotransmitters, neuromodulators, and growth factors.

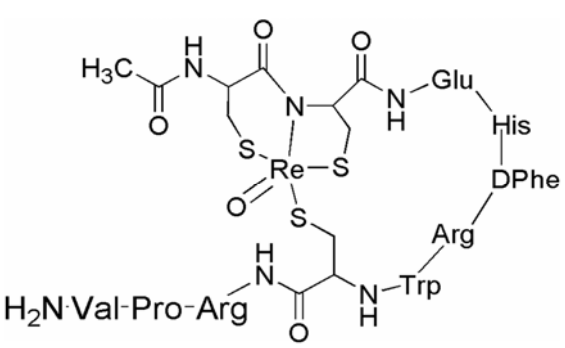


Fig.3 Structure of 188Re(V)-(Arg11)CCMSH: Ac-Cys-Cys-Glu-His-DPhe-Arg-Trp-Cys-Arg-Pro-Val-NH2.

Table 1 General characteristics of regulatory peptides as radiotracers

Advantages	Disadvantages
+ Small molecules	– Rapid degradation
+ Easy to synthesize, modify, and label	– Small changes may alter binding properties
+ Low immunogenicity and low toxicity	– High kidney uptake
+ Favorable pharmacokinetics permeability, rapid excretion	
+ High affinity for the receptors	
+ Modifiable rate and route of excretion	

Their molecular characteristics (small size and usually hydrophilic) give rise to their good permeability, rapid degradation, fast clearance from the blood pool and nontarget tissues, and efficient excretion (renal and/or hepatobiliar). Moreover, it is also well documented that peptides play a role in pathological conditions. They may be involved in tumor growth and tumor progression, inflammation, platelet aggregation or apoptosis. Peptide receptors are overexpressed in a variety of human cancers (Table 2). Therefore, both peptide and peptide receptors represent important tools for in vivo tumor imaging and therapy with radiolabeled peptides and their truncated analogs. Such peptide-based radioconjugates should fulfill the following conditions to become good candidates for tumor targeting: (a) rapid and efficient labeling, (b) high specific activity, (c) sufficient in vivo stability, (d) unaltered binding properties after labeling, and (e) favorable pharmacokinetics (high tumor uptake, rapid clearance from nontarget organs, and retention in the tumor tissue). The favorable pharmacokinetics of peptides, together with their usually high affinity and specificity for their corresponding receptors, contribute to the accumulation of the radiolabeled peptide in receptor-positive tissues, allowing early imaging.[26] Peptides can be synthesized by solution-phase synthesis and solid-phase peptide synthesis.[27, 28] Both methods allow the insertion of metal chelators and spacer groups for radiolabeling with radiometals for diagnosis and therapy.[1, 28] Site-specificity of modification and radiolabeling is crucial for peptides, to prevent loss of activity and binding affinity.

Table 2 Peptide receptors overexpressed in tumors

Receptor	Tumor type
Somatostatin	Neuroendocrine, non-Hodgkin's lymphoma, melanoma, breast, pancreatic, gastric, colon, prostate, lung, SCLC, MTC
VIP/PACAP	SCLC, colon, gastric, breast, pancreatic, prostate, urinary bladder, lymphoma, meningioma
Bombesin/GRP	SCLC, glioblastoma, colorectal, gastric, prostate, breast, ovarian
CCK-B/gastrin	MTC, SCLC, pancreatic, astrocytoma, ovarian
Neurotensin	SCLC, colon, exocrine pancreatic, prostate
α-MSH	Melanoma
Neuropeptide Y	Neuroblastoma, glioblastoma, breast
GnRH/LHRH	Prostate, breast, endometrial, ovarian, melanoma
Substance P	SCLC, MTC, glioblastoma, astrocytoma, breast
Opioid	SCLC, neuroblastoma, breast

Small peptides generally reveal a short biological half-life because of a rapid degradation by endogenous peptidases and proteases. Different strategies are commonly used to increase the stability of peptides, such as, end-capping, substitution of peptide bonds by other chemical bonds, use of D- or β-amino acids, or cyclization (Table 3). The structural modifications have to be carefully designed to avoid a loss of binding affinity. Another drawback of radiolabeled peptides is the high uptake in the kidneys. This would be especially important in peptide receptor mediated therapy as potential radiotoxicity to renal tissue could compromise the therapeutic efficacy.[29] The introduction of hydrophilic and/or lipophilic amino acids can modify the rate and the route of excretion without altering binding properties.

Table 3 Frequent methods of stabilizing peptides

Method	Description
End-capping	N-methylation, amide at C-terminal position
Group reduction	Peptidomimetics
Substitution of peptide bonds	
Replacement of amino acids	Use of D-, β- or unnatural amino acids
Cyclization	
Replacement of groups	Amino moieties with imino groups

In this short review, the focus will be on the representative peptides, which have been successfully radiolabeled with both Tc-99m and Re-186/188 till today.

4 Tumor imaging and therapy with ^{99m}Tc/$^{186/188}$Re-radiolabeled peptides

4.1 Somatostatin (SST) analogs

Somatostatin and Vasoactive Intestinal Peptide (VIP) analogs have been successfully applied for scintigraphic imaging of receptor overexpressing tumors and this has boosted the interest in the development of other peptide radiopharmaceuticals for potential peptide receptor targeting in vivo.[30, 31] ^{111}In-DTPA- Octreotide (OctreoScan) was first introduced in the year 1994. SST comprises of a family of two peptides, one cyclic of 14 and one of 28 amino acids. SST exerts many physiological functions both at the central nervous system and the gastrointestinal tract. Moreover, it also plays a role in cancer inhibiting tumor growth. The actions of SST are mediated by specific SST receptors. To date, five different subtypes have been identified (SST_1-SST_5). It is well known that many tumors, mainly of the neuroendocrine origin, overexpress SST receptors and are therefore potential targets for radiolabeled SST analogs.[32] Both SST-14 and SST-28 show high affinity for their receptors. The rapid degradation in vivo leads to the development of a cyclic analog (Octreotide), which shows increased plasma stability, preserving the biological activity of the original peptide. However, the use of Octreoscan has some limitations, primarily because ^{111}In is expensive (cyclotron-produced radionuclide) and its physical characteristics are not optimal. To overcome these limitations, several SST analogs have been developed for labeling with ^{99m}Tc. Some examples are depreotide, HYNIC-octreotide, demotate or vapreotide. Depreotide (P829) is a 10-amino acid peptide with affinity for SST_2, SST_3, and SST_5.

High in vivo uptake and retention in tumors, as well as, good imaging in mice bearing tumors was observed for ^{99m}Tc-Demotate.[12] The peptide P829 functionalized with N_3S and ^{99m}Tc-labeled allowed imaging of nonsmall cell lung cancer.[33] ^{99m}Tc-P829 has been approved by the FDA and is commercially available as a kit (NeoTect) for the assessment of indeterminate pulmonary nodules.[33,34] ^{99m}Tc-EDDA/HYNIC-TOC was found to be similar to ^{111}In-Octreoscan with comparable pharmacokinetics and biodistribution in man. Tumor uptake was rapid and specific, and allowed good imaging in patients.[35] Moreover, kidney retention and gastrointestinal activity were low. ^{99m}Tc-tricine-HYNIC-TOC also showed a comparable tumor targeting pattern to ^{111}In-DTPA octreotide with a high and rapid uptake in the tumor.[36] Excretion was mainly via the kidneys and it also showed favorable pharmacokinetics in humans. Vapreotide (^{99m}Tc-MAG_3-RC-160), with high affinity for SST_2 and SST_5 and moderate affinity for SST_3 and SST_4 has been tested in mice, but the pharmacokinetics did not seem to be so favorable.[9] ^{0}Tyr^{3}octreotate analogs were functionalized with various BFCA and radiolabeled with the organometallic precursor $[M(H_2O)_3(CO)_3]^+$. The BFCA gave rise to metal conjugate complexes of different overall charge (+ 1 to -3).[37, 38] Wester et al. have coupled picoline-amino acetic acid to a carbohydrated octreotide. The carbohydrate rendered the conjugate to be much more hydrophilic, and an excellent biodistribution in humans was observed.[39, 40] Fewer studies, however, have been performed with ^{188}Re-radiolabeled SST analogs. The labeling of P829 with Re-188 is shown in Fig.4.[41, 42] A kit-formulation has been presented containing 50 μg of this peptide, sodium ethylene-diaminetetraacetic acid dihydrate, sodium gluco-heptonate, and $SnCl_2$. Reconstitution with about 1 mL $^{188}ReO_4$ eluant followed by boiling for 15 min provides a labeling efficiency of greater than 95%. Gentistic acid was introduced as an antioxidant.

Two other interesting ^{188}Re-analogs, ^{188}Re-RC-160 and ^{188}Re-P2045 have been reported. ^{188}Re-RC-160 reduced tumor burden after intratumoral or intrathoracic administration in three different animal models.[43] The analog ^{188}Re-P2045 significantly suppressed tumor growth, in a mouse model, of small cell lung cancer after i.v. injection.[44]

4.2 Bombesin (BBS)/Gastrin-releasing peptide (GRP) analogs

BBS is a 14-amino acid neuropeptide, first isolated from frog skin. GRP is its mammalian counter-

part, a peptide of 27 amino acids, with which it shares the C-terminal sequence. BBS and GRP play an important role in tumor cell proliferation. Four different BBS/GRP receptor subtypes have been identified (GRP, NMB, BBS3, and bb4). BBS receptors are also overexpressed in a variety of human tumors and are then potential targets for imaging receptor-positive tumors. Several analogs radiolabeled with ^{99m}Tc have been developed. ^{99m}Tc-RP-527 shows specific binding and internalization in PC-3 cells.[45] In mice with tumor xenografts the high accumulation in tumor has resulted in high tumor-to-muscle ratios and good imaging properties allowing tumor localization in patients. Nock et al., have reported preclinical results of several analogs (Demobesins), with high affinity to PC-3 cells in vitro as well as high and specific uptake in PC-3 tumor xenografts and pancreas in mice.[46, 47]

The analogs based on the parent tetradecapeptide were excreted through the kidneys, whereas, those based on the truncated 7-14 sequence were mainly excreted through the hepatobiliar system. Smith et al. tested the influence of different spacers.[48] The best in vitro properties corresponded to the analogs with spacers of 3- to 8-carbon atoms. In mice, the analog with the 5-carbons spacer showed a high uptake and retention in pancreas, and excretion was both renal and hepatobiliar.

Moustapha et al. presented an N_3S-5-Ava-BBS(7-14)NH_2 conjugate radiolabeled with $^{186/188}$Re, which retained high in vitro and in vivo specificity for GRP receptor-expressing cells (Fig.5).[49] This study showed that N_3S-5-Ava-BBS(7-14)NH_2 could be labeled with $^{186/188}$Re following the post-labeling approach. The stable $^{186/188}Re^{V}O$-N_3S-5-Ava-BBS(7–14)NH_2 was formed following the reduction of perrhenate with stannous chloride at room temperature, as verified by HPLC and stability studies. The radiolabeling yield was found to be > 90%. The biodistribution studies demonstrated that the carrier-added and no-carrier added $^{186/188}$Re conjugates behaved similarly, raising the question of whether n.c.a. $^{186/188}$Re is necessary for specific tumor receptor targeting.

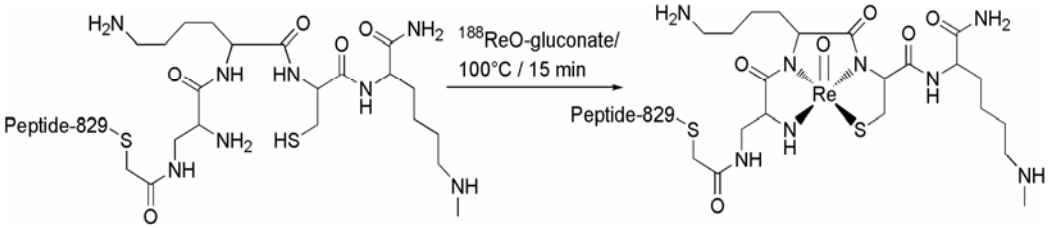


Fig.4 Radiolabeling strategy used for peptide P829 with ^{188}Re via Re(V)-gluconate.

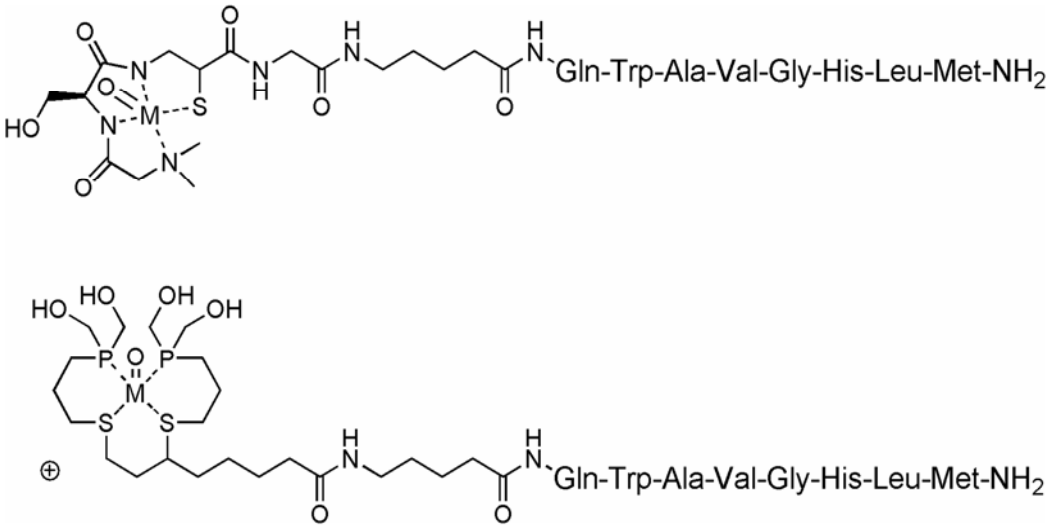


Fig.5 (Top) Structure of $^{186/188}Re^{V}O$-N_3S-5-Ava-BBS(7–14)NH_2; (Bottom) Structure of ^{188}Re-P_2S_2-5-Ava-BBS(7-14).

The same group also developed the bombesin analog ^{188}Re-P_2S_2-5-Ava-BBS(7-14) with chelating systems comprising of highly hydrophilic hydroxyl methyl phosphine groups.[17] The analog was found to be stable both in vitro an in vivo. Preliminary in vivo tests in mice bearing PC-3 tumors demonstrated that

the conjugate retained high in vivo targeting specificity for GRP receptor-positive tissues with minimal decomposition to $^{188}ReO_4$. Another analog which includes a spacer in the molecule, ^{188}Re-Dpr-SSS-BBS(7-14), showed high uptake and retention in mice with PC-3 tumor xenografts for time-point less or equal to 24 h.[48] However, no preclinical therapeutic studies have been reported yet.

Bombesin was also derivatized at the C-terminus with bidentate chelators and reacted with $[M(H_2O)_3(CO)_3]^+$ (Fig.6).[50, 51] The labeled peptide fully retained the biological activity and was stable in vitro and in vivo. As the bidentate coordination was not optimal with respect to pharmacokinetics, the coordination sphere of the metal tricarbonyl core had been saturated with a highly hydrophilic phosphine. This resulted in Tc-99m/Re-188 radioconjugates with significantly higher hydrophilicity and an improved biodistribution profile.[50]

For $[M(H_2O)_3(CO)_3]^+$ labeled BBS analogs, this group found that there was an increase in tumor-to-blood ratios after stabilization of the peptide sequence, although tumor uptake was not improved.[52] The additional insertion of a spacer, however, increased both tumor and pancreatic uptake and tumor to nontumor ratios (Fig.7).

At the moment BBS-analogs with both stabilization and a hydrophilic spacer group seem to be very promising for tumor targeting of BBS/GRP receptor-positive cancers.

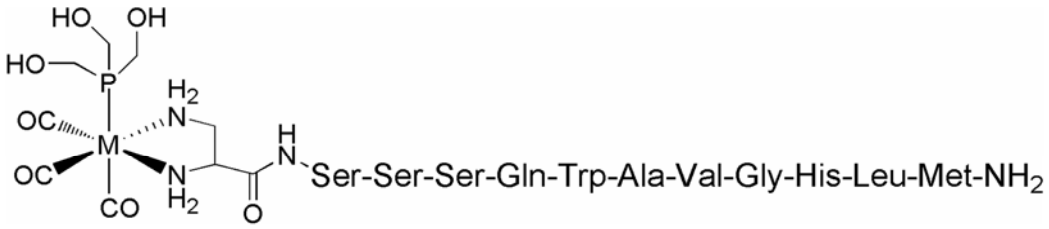


Fig.6 Structure of organometallic $^{188}Re(CO)_3$-labeled BBS(7-14) with a $(Ser)_3$-spacer, a bidentate chelate and a monodentate hydroxymethyl phosphine coligand.

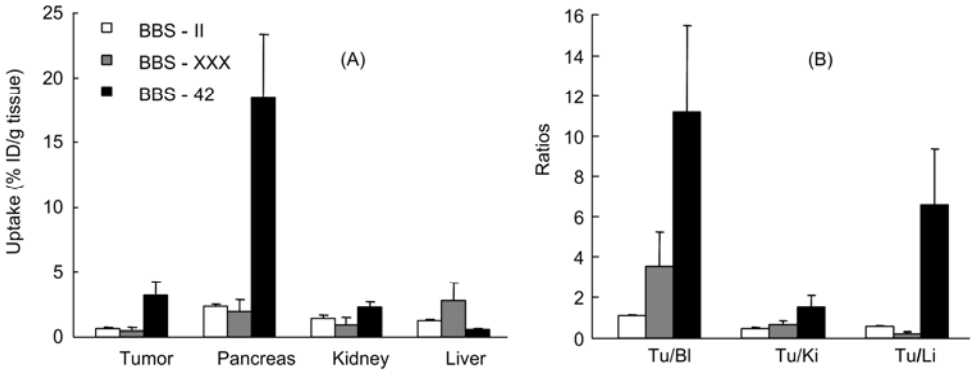


Fig.7 Biodistribution of BBS analogs in mice with PC-3 xenografts. (A) Tissue accumulation; (B) Tumor to nontumor ratios. BBS-II (unmodified sequence with: (N -His)Ac-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2); BBS-XXX (stabilized: (N -His)Ac-Gln-Trp-Ala-Val-Gly-His-CyHAla-Nle-NH_2); BBS-42 (stabilized and with hydrophilic spacer: (N -His)Ac-Lys (Shikimic-acid)-βAla-βAla-Gln-Trp-Ala-Val-Gly-His-CyHAla-Nle-NH_2). (N -His)Ac is a tridentate chelating system suitable for $[M(H_2O)_3(CO)_3]^+$.

4.3 Alpha-melanocyte stimulating hormone (α-MSH) analogs

α-MSH is a 13-amino acid peptide mainly responsible for the regulation of skin pigmentation. Receptors for α-MSH have been found in murine and human melanoma cells, as well as in most human melanoma samples from patients with metastatic lesions. The rapid in vivo degradation of α-MSH has led to the development of synthetic peptides with increased stability, such as, the potent analog (Nle^4-D-Phe^7)α- MSH(NDP). For ^{99m}Tc-labeling, different chelating systems have been used, such as Ac-Cys-Gly-Cys-Gly (CGCG) or MAG_2. In in vivo studies with mice, bearing melanoma xenografts,

^{99m}Tc-CGCG-α-MSH showed a higher tumor uptake than ^{99m}Tc-MAG_2- α-MSH, but the retention in tumor cells was poor for both analogs.[53] Another approach has been the use of a cyclic analog (^{99m}Tc-CCMSH). A rapid uptake in tumors was found for this analog, in melanoma-bearing mice. Moreover, compared to linear peptides, both tumor uptake and tumor activity retention were higher.[54] α-MSH analogs have also been radiolabeled with ^{188}Re and tested for their potential therapeutic use. A high accumulation in the tumor was found with analog ^{188}Re-CCMSH, but kidney uptake was higher than that of the ^{99m}Tc-labeled analog. As nephrotoxicity may be a serious problem after peptide receptor radiotherapy, Lys^{11} was replaced by an Arg to reduce kidney uptake. Interestingly, this substitution contributed to not only a significant decrease in kidney uptake, but also improved tumor uptake, leading to much higher tumor to nontumor ratios in mice with murine melanoma tumors.[25] More recently a study demonstrated the therapeutic efficacy of ^{188}Re-(Arg^{11}) CCMSH both in murine and human melanoma-bearing mice.[55]

Dadachova et al. reported experimental melanoma therapy with ^{188}Re-labeled melanin-binding decapeptide (^{188}Re-HYNIC-4B4) and a comprehensive safety evaluation of this treatment.[56] Radiolabeling of the peptides with ^{188}Re resulted in 55% - 65% yields. Administration of 3.7×10^7Bq of ^{188}Re-HYNIC-4B4 (once 3.7×10^7Bq or twice 3.7×10^7Bq, 20 days apart) to MNT1 tumor-bearing mice significantly slowed tumor growth, with the therapeutic effect being a result of specific binding to tumor melanin, as irrelevant ^{188}Re-labeled decapeptide did not produce therapeutic gain. Repeated administration of ^{188}Re-HYNIC-4B4 had a more profound effect on tumor growth rather than a single dose. Treatment of tumors with 0.3-0.4 cm diameter was more effective than that of larger ones (0.5-0.7 cm). The dose delivered to the MNT1 tumor of 3.7×10^7Bq ^{188}Re-HYNIC-4B4 was estimated to be 300 cGy. These results indicated that radiolabeled melanin-binding peptides were efficient and safe in treatment of melanoma and could be potentially useful against this tumor type.

4.4 Neurotensin (NT) analogs

NT is a 13-amino acid peptide localized in the central nervous system and in the peripheral tissues (gastrointestinal tract) (Table 4). To date, three NT receptor subtypes have been characterized: NT1, NT2, and NT3. Overexpression of NT receptors has been described in different human tumors.[57] As NT is also rapidly degraded in vivo, this group has synthesized different stabilized analogs and tested them in vitro and in vivo after radiolabeling with ^{99m}Tc. Modifications at positions 8-9 and 11-12 have led to stable analogs with preserved binding affinity (Table 4).

In a clinical study with the analog ^{99m}Tc-NT-XI the tumor of a patient with ductal pancreatic adenocarcinoma could be detected. The main drawback of this analog was the high kidney uptake.[58] More recently, the analog ^{99m}Tc-/^{188}Re-NT-XII showed better biodistribution with lower kidney uptake and higher tumor uptake. Tumor activity retention was also longer.[59] Modifications at the three cleavage bonds conferred on the new analog (NT-XIX) an even higher stability. Although affinity to NT receptors was reduced, both in vivo tumor uptake and tumor activity retention remained similar to that of NT-XII. In addition, both kidney and liver uptakes were much reduced resulting in better tumor-to-background ratios. SPECT/CT experiments have been performed with ^{99m}Tc$(CO)_3$-labeled NT-XII and NT-XIX (Fig.8).

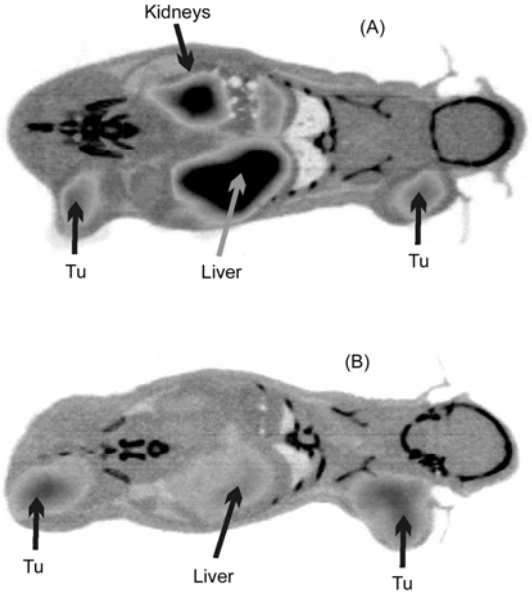


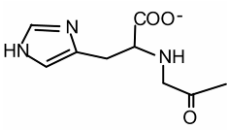
Fig. 8 Coronal SPECT/CT pictures of ^{99m}Tc$(CO)_3$-labeled NT-XII (A) and NT-XIX (B).

Stabilized NT analogs (NT-XI, NT- XII, and NT-XIX) were radiolabeled with ^{188}Re$(CO)_3$. The in vitro binding properties were similar to those of their

corresponding ^{99m}Tc compounds. Moreover, the in vivo biodistributions in mice with HT-29 tumor xenografts were also comparable.[60] Similar to ^{99m}Tc-NT-XI, ^{188}Re-NT-XI showed high kidney uptake, which would limit its potential utility as a radiotherapeutic agent. On the other hand, both ^{188}Re-NT-XII and ^{188}Re-NT-XIX revealed more favorable biodistribution patterns. They inhibited the growth of HT-29 tumor xenografts in mice without significant side effects in the studies (Fig.9). Although the therapeutic effect of ^{188}Re-NT-XII was similar to that of ^{188}Re-NT-XIX, the latter was the most promising as it showed the lowest uptake in kidney and liver.

Table 4 Amino acid sequence of NT(8-13) and NT-analogs

Analog	Amino acid sequence	Plasma half-life	Affinity /nmol•L^{-1}
NT(8-13)	pGlu^1-Leu^2-Tyr^3-Gly^4-Asn^5-Lys^6-Pro^7-Arg^8-Arg^9-Pro^{10}-Tyr^{11}-Ile^{12}-Leu^{13}	—	1.6
NT-II	(N_αHis)Ac-Arg-Arg-Pro-Tyr-Ile-Leu	5 min	0.3
NT-X	(N_αHis)Ac-Arg-Arg-Pro-Tyr-Tle-Leu	4 h	0.2
NT-XI	(N_α-His)Ac-Lys-(ψCH_2NH)-Arg-Pro-Tyr-Tle-Leu	21 d	2.0
NT-XII	(N_αHis)Ac-Arg-(N-CH_3)-Arg-Pro-Tyr-Tle-Leu	20 d	2.0
NT-XIX	(N_αHis)Ac-Arg-(N-CH_3)-Arg-Pro-Dmt-Tle-Leu(Dmt=dimethyl tyrosine)	28 d	15.0



tridentate (N_αHis)Ac= chelator suitable for the [M(CO)$_3$]-core

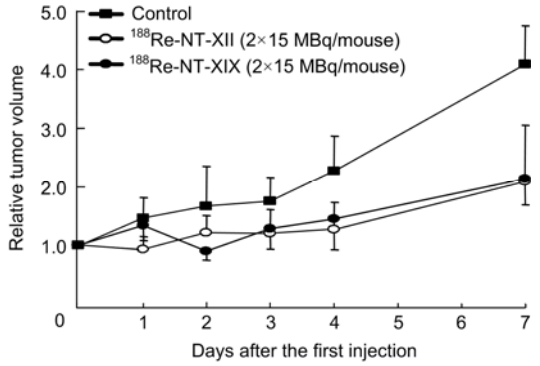


Fig.9 Therapeutic effect of ^{188}Re-NT-XII/NT-XIX analogs injected i.v. at day 0 and 2 in mice with HT-29 xenografts.

4.5 Miscellaneous peptides

The amino acid sequence Arg-Gly-Asp (RGD) is considered to be the common epitope that many members of the integrin family of cell surface receptors recognize on their ligands.[61] Integrins are the main receptors by which cells attach to extracellular matrices, and they also mediate important cell–cell adhesion processes including platelet aggregation and thrombus formation.[62] In addition, they appear to play a key role in tumor invasion, dissemination, and cell proliferation of various neoplasias including malignant melanoma, osteosarcoma, and glioblastoma. A number of technetium-99m-labeled RGD-containing molecules have been designed, synthesized, and evaluated for the detection and imaging of thrombi. Within this framework, the synthetic linear decapeptide Arg-Gly-Asp-Ser-Cys-Arg-Gly-Asp-Ser-Tyr (RGD-SCRGDSY), which contains two RGD moieties in its sequence, was complexed with the ^{99m}Tc=O^{3+}-core and applied successfully for the detection of experimentally induced thrombi in rabbits. Furthermore, it was administered to patients with malignant melanoma, a disease of high metastatic potential with cells expressing RGD-binding integrins, and was found to bind specifically to adhesion molecules on tumors, thus permitting the in vivo detection of neoplastic metastases.[63] The ^{188}Re-complex of the decapeptide was also prepared with the ultimate goal of preparing a radiotherapeutic agent. The decapeptide Arg-Gly-Asp-Ser-Cys-Arg-Gly-Asp-Ser-Tyr, which contains two Arg-Gly-Asp (RGD) moieties in its se-

quence, has been successfully labeled with Re-188 yielding a single, stable oxorhenium(V) complex (Fig.10).[64] This complex is being evaluated for possible application in oncology as a target-specific radiotherapeutic agent, because its radioactive technetium-99m analog has already been applied to the scintigraphic detection of malignant melanoma in humans.

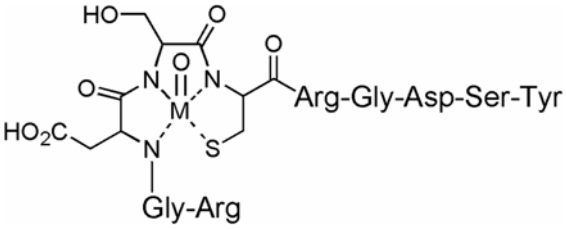


Fig.10 Structure of the Tc/Re-labeled RGD peptide Arg-Gly-Asp-Ser-Cys-Arg-Gly-Asp-Ser-Tyr.

The peptide AG 8.0 used in tumor pretargeting is an example of a peptide with a N_3S chelator coupled with the N terminus.[65] MAG_3 was conjugated to the peptide as shown in Fig.11. The peptide was reacted with ^{188}Re-gluheptonate at 100℃. Purification was necessary because the labeling efficiency was ≤ 70%. After purification, the immunoreactive fraction was found to be 91% at 5 min, but decreased to 18% 5 h later for unknown reasons.

The $^{186/188}$Re-labeled peptide IMP-192 was also used for tumor pretargeting (Fig.12).[66] A labeling efficiency of > 95% with a specific activity greater than 18.5 TBq/mmol was reported using a lyophilized kit.

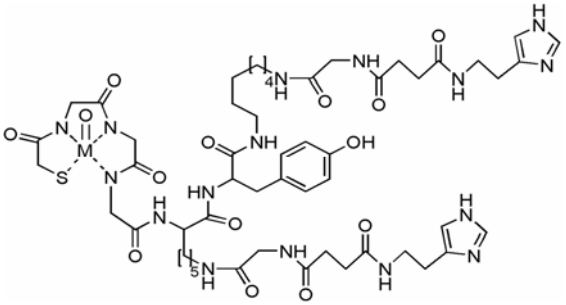


Fig.11 Structure of the Tc/Re-labeled peptide AG 8.0 with N_3S (MAG_3) chelator.

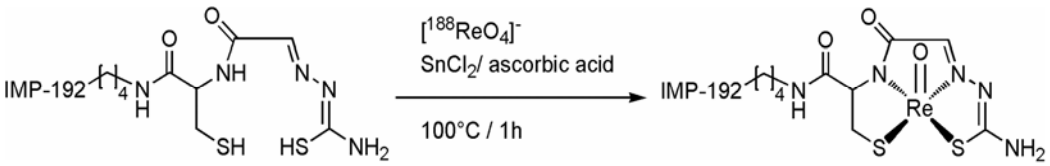


Fig.12 Strategy for the labeling of peptide IMP-192 with Re-188 via an S_2N_2-chelator.

5 Conclusions

^{99m}Tc-radiolabelled peptides are an important class of tumor targeting agents with potential application in nuclear medicine. Clinical studies have already shown the potential utility of some of them as diagnostic agents. In the future they may be suitable not only for localization of the lesions, but also for assessment of prognosis. Furthermore, recent in vitro studies have demonstrated that many human cancers can overexpress several peptide receptors concomitantly.[67, 68] This coexpression of different peptide receptors in tumor cells can be useful for multi-receptor tumor targeting. The combination of several peptide analogs would improve the clinical therapeutic efficacy of radiolabeled peptides, especially in tumors with a nonhomogenous expression of receptors or in tumors which have lost some of the peptide receptors after dedifferentiation.[68] The possibility of labeling these peptides with the β-emitting radionuclides $^{186/188}$Re, for use as potential therapeutic agents may increase their significance for patient treatment in the future. In addition, the treatment of tumors with cocktails of peptides radiolabeled with different β-emitters (e.g., ^{186}Re/^{188}Re and others) would lead to an optimal radiotherapy for tumors of various sizes.

Acknowledgments

We thank Christian Schweinsberg for helping to prepare this review article.

References

1 Liu S, Edwards D S. Chem Revs, 1999, **99**: 2235-2268.

2 Shigeta N, Matsuoka H, Osa A, *et al*. Journal of Radioanalytical and Nuclear Chemistry-Articles, 1996, **205**: 85-92.

3 Ehrhardt G J, Blumer M E, Su F M, *et al*. Applied Radiation and Isotopes, 1997, **48**: 1-4.

4 Lapi S, Ressler J J, Cox M E, *et al*. Technetium and rhe-

nium and other metals in chemistry and nuclear medicine 7. Padua: SGE Editoriali, 2006: 593-596.

5 Hohn A, Hirzel W, Schibli R. Technetium and rhenium and other metals in chemistry and nuclear medicine 7. Padua, 2006: 609-610.

6 Knapp F F, Mirzadeh S, Beets A L, *et al*. Technetium and rhenium in chemistry and nuclear medicine 4. Padua: SGE Editoriali, 1995: 319-324.

7 Liu G Z, Zhang S R, He J, *et al*. Bioconjugate Chem, 2002, **13**: 893-897.

8 Liu G Z, Dou S P, He J, *et al*. Applied Radiation and Isotopes, 2006, **64**: 971-978.

9 Thakur M L, Kolan H, Li J, *et al*. Nucl Med Biol, 1997, **24**: 105-113.

10 Okarvi S M. Medicinal Research Reviews, 2004, **24**: 357-397.

11 Liu S, Edwards D S, Looby R J, *et al*. Bioconjugate Chem, 1996, **7**: 63-71.

12 Maina T, Nock B, Nikolopoulou A, *et al*. Eur J Nucl Med Mol Imaging, 2002, **29**: 742-753.

13 Maina T, Stolz B, Albert R, *et al*. Eur J Nucl Med, 1994, **21**: 437-444.

14 Harris T D, Sworin M, Williams N, *et al*. Bioconjugate Chem, 1999, **10**: 808-814.

15 Edwards D S, Liu S, Barrett J A, *et al*. Bioconjugate Chem, 1997, **8**: 146-154.

16 Kothari K K, Gali H, Prabhu K R, *et al*. Nucl Med Biol, 2002, **29**: 83-89.

17 Gali H, Hoffman T J, Sieckman G L, *et al*. Bioconjugate Chem, 2001, **12**: 354-363.

18 Karra S R, Schibli R, Gali H, *et al*. Bioconjugate Chem, 1999, **10**: 254-260.

19 Alberto R, Schibli R, Egli A, *et al*. J Organomet. Chem, 1995, **493**: 119-127.

20 Alberto R, Schibli R, Egli A, *et al*. J Am Chem Soc, 1998, **120**: 7987-7988.

21 Alberto R, Schibli R, Schubiger A P, *et al*. J Am Chem Soc, 1999, **121**: 6076-6077.

22 Egli A, Hegetschweiler K, Alberto R, *et al*. Organometallics, 1997, **16**: 1833-1840.

23 Schibli R, Schwarzbach R, Alberto R, *et al*. Bioconjugate Chem, 2002, **13**: 750-756.

24 Park S H, Seifert S, Pietzsch H J. Bioconjugate Chem, 2006, **17**: 223-225.

25 Miao Y B, Owen N K, Whitener D, *et al*. Int J Cancer, 2002, **101**: 480-487.

26 Boerman O C, Oyen W J G, Corstens F H M. Semin Nucl Med, 2000, **30**: 195-208.

27 Merrifield B. Solid-Phase Peptide Synthesis. 1997: 3-13.

28 Wentworth Jr P. Trends Biotechnol, 1999, **17**: 448-452.

29 Lambert B, Cybulla M, Weiner S M, *et al*. Radiation Research, 2004, **161**: 607-611.

30 Krenning E P, Kwekkeboom D J, Bakker W H, *et al*. Eur J Nucl Med, 1993, **20**: 716-731.

31 Virgolini I, Raderer M, Kurtaran A, *et al*. New England Journal of Medicine, 1994, **331**: 1116-1121.

32 Kwekkeboom D J, Krenning E P. Semin Nucl Med, 2002, **32**: 84-91.

33 Menda Y, Kahn D. Semin Nucl Med, 2002, **32**: 92-96.

34 Blum J, Handmaker H, Lister-James J, *et al*. Chest, 2000, **117**: 1232-1238.

35 Decristoforo C, Mather S J, Cholewinski W, *et al*. Eur J Nucl Med, 2000, **27**: 1318-1325.

36 Bangard M, Behe M, Guhlke S, *et al*. Eur J Nucl Med, 2000, **27**: 628-637.

37 Marmion M E, Alberto R, Bugaj J, *et al*. J Labelled Compd Radiopharm, 1999, **42**: S231-S233.

38 Schibli R, Schubiger A P. Eur J Nucl Med, 2002, **29**: 1529-1541.

39 Wester H J. Cancer Biotherapy and Radiopharmaceuticals, 2003, **18**: 277-277.

40 Wester H J, Schottelius M, Schwaiger M. J Nucl Med, 2001, **42**: 115P-115P.

41 Pearson D A, Lister-James J, McBride W J, *et al*. J Med Chem, 1996, **39**: 1372-1382.

42 Zinn K R, Buchsbaum D J, Chaudhuri T R, *et al*. J Nucl Med, 2000, **41**: 887-895.

43 Zamora P O, Bender H, Gulhke S, *et al*. Anticancer Res, 1997, **17**: 1803-1808.

44 Bugaj J E, Bickel E M, Azure M T, *et al*. J Nucl Med, 2002, **43**: 123P-123P.

45 Van de Wiele C, Dumont F, Van den Broecke R, *et al*. Eur J Nucl Med, 2000, **27**: 1694-1699.

46 Nock B, Nikolopoulou A, Chiotellis E, *et al*. Eur J Nucl Med Mol Imaging, 2003, **30**: 247-258.

47 Nock B A, Maina T, Behe M, *et al*. J Nucl Med, 2005, **46**: 1727-1736.

48 Smith C J, Gali H, Sieckman G L, *et al*. Bioconjugate Chem, 2003, **14**: 93-102.

49 Moustapha M E, Ehrhardt G J, Smith C J, *et al*. Nucl Med Biol, 2006, **33**: 81-89.

50 Smith C J, Sieckman G L, Owen N K, *et al*. Anticancer

Res, 2003, **23**: 63-70.

51 Smith C J, Sieckman G L, Owen N K, *et al*. Cancer Res, 2003, **63**: 4082-4088.

52 Garcia-Garayoa E, Ruegg D, Bläuenstein P, *et al*. Nucl Med Biol, 2006, in press.

53 Chen J Q, Giblin M F, Wang N N, *et al*. Nucl Med Biol, 1999, **26**: 687-693.

54 Chen J Q, Cheng Z, Hoffman T J, *et al*. Cancer Res, 2000, **60**: 5649-5658.

55 Miao Y B, Owen N K, Fisher D R, *et al*. J Nucl Med, 2005, **46**: 121-129.

56 Dadachova E, Moadel T, Schweitzer A D, *et al*. Cancer Biotherapy and Radiopharmaceuticals, 2006, **21**: 117-129.

57 Reubi J C. Endocr Rev, 2003, **24**: 389-427.

58 Buchegger F, Bonvin F, Kosinski M, *et al*. J Nucl Med, 2003, **44**: 1649-1654.

59 Garcia-Garayoa E, Maes V, Blauenstein P, *et al*. Nucl Med Biol, 2006, **33**: 495-503.

60 Blauenstein P, Garayoa E G, Ruegg D, *et al*. Cancer Biotherapy and Radiopharmaceuticals, 2004, **19**: 181-188.

61 Ruoslahti E, Pierschbacher M D. Science, 1987, **238:** 491-497.

62 Hynes R O. Cell, 1992, **69**: 11-25.

63 Sivolapenko G B, Skarlos D, Pectasides D, *et al*. Eur J Nucl Med Mol Imaging, 1998, **10**: 1383-1389.

64 Costopoulos B, Benaki D, Pelecanou M, *et al*. Inorg Chem, 2004, **43**: 5598-5602.

65 Gestin J F, Loussouarn A, Bardies M, *et al*. J Nucl Med, 2001, **42**: 146-153.

66 Karacay H, McBride W J, Griffiths G L, *et al*. Bioconjugate Chem, 2000, **11**: 842-854.

67 Reubi J C, Gugger M, Waser B. Eur J Nucl Med Mol Imaging, 2002, **29**: 855-862.

68 Reubi J C, Waser B. Eur J Nucl Med Mol Imaging, 2003, **30**: 781-793