

^{68}Ga -Labeled Peptides in Tumor Imaging

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Radiolabeled peptides are of increasing interest in nuclear oncology. Special emphasis has been given to the development of peptides labeled with positron emitters. Among these, ^{68}Ga deserves special attention, because it is available from an in-house generator rendering ^{68}Ga radiopharmacy independent of an onsite cyclotron. ^{68}Ga has a half-life of 68 min and decays by 89% through positron emission. The parent, ^{68}Ge , is accelerator produced and decays with a half-life of 270.8 d by electron capture. Currently, at least 1 commercial and several in-house generators are available. ^{68}Ge is strongly absorbed on metal oxides or organic material, making a ^{68}Ge -breakthrough highly unlikely. Several groups continue to further develop generators to remove cationic impurities from the eluate. Several bifunctional chelators based on 1,4,7-triazacyclononane-*N,N',N''*-tri-acetic acid and 1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid (DOTA) macrocycles are available for coupling to peptides and other biomolecules. In addition to these hydrophilic chelators, a lipophilic tetradentate S_3N ligand was developed. Radiopeptides for ^{68}Ga labeling have been developed and tested preclinically for the targeting of somatostatin receptors, the melanocortin 1 receptor, and the bombesin receptor. Clinical studies were performed with ^{68}Ga -DOTA, Tyr³-octreotide, localizing neuroendocrine tumors with higher sensitivity than ^{111}In -diethylenetriaminepentaacetic acid-octreotide. In addition, ^{68}Ga -DOTA-based bombesin derivatives are being investigated with some success in patients with prostate cancer. **Conclusion:** Generator-produced ^{68}Ga and the development of small chelator-coupled peptides (and other small biomolecules) may open a new generation of freeze-dried, good manufacturing practice-produced, kit-formulated PET radiopharmaceuticals similar to $^{99\text{m}}\text{Mo}/^{99\text{m}}\text{Tc}$ -generator-based, $^{99\text{m}}\text{Tc}$ -labeled radiopharmaceuticals.

Key Words: oncology; ^{68}Ga ; peptides; tumor imaging

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Radiopeptides are of increasing interest in imaging and targeted radiotherapy of tumors (1–5). The major goal in the past was the development of radiopeptides labeled with $^{99\text{m}}\text{Tc}$ and ^{111}In for SPECT and with ^{90}Y and ^{177}Lu for targeted radiotherapy (6–13).

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Recently, peptides labeled with positron emitters based on ^{68}Ga , ^{66}Ga , ^{18}F , ^{86}Y , and ^{64}Cu have been investigated (12–19). Interest in using nonphysiologic metallic positron emitters for clinical PET comes mainly from the availability and additional advantages of ^{68}Ga and from the use of ^{86}Y to quantitate the biodistribution of ^{90}Y -labeled vector-targeted radiotherapy. ^{68}Ga is available from an inhouse generator, rendering it independent of an onsite cyclotron. With a half-life of 68 min, it decays by 89% through positron emission of 1.92 MeV (max. energy) and 11% orbital electron capture. In addition, ^{68}Ga can be labeled to a chelator-conjugated biomolecule, allowing kit production and enabling wide availability. Deutsch (20) proposed in a 1993 editorial in *The Journal of Nuclear Medicine* that the time had come to use the $^{68}\text{Ge}/^{68}\text{Ga}$ generator and cold kit formulations for labeling with ^{68}Ga , similar to the successfully used $^{99\text{m}}\text{Mo}/^{99\text{m}}\text{Tc}$ generator system in $^{99\text{m}}\text{Tc}$ radiopharmacy. This, he suggested, would allow PET centers to benefit from the generator for important clinical studies. Deutsch's editorial was included as a commentary on an article by Tsang et al. (21) on a cationic $^{68}\text{Ga}(\text{III})$ complex with a $\text{N}_4\text{O}_2^{2-}$ Schiff-base ligand that showed promising properties as a radiopharmaceutical for cardiac PET. Unfortunately, little activity was focused on ^{68}Ga -based PET radiopharmaceuticals over the last 10 y. With the appearance of small radiolabeled peptides as a new class of radiopharmaceuticals, however, a renaissance in ^{68}Ga -based PET radiopharmaceuticals is underway. These peptides show very fast blood clearance and fast target localization, making the short half-life ideal for clinical studies.

THE $^{68}\text{Ge}/^{68}\text{Ga}$ GENERATOR

Data on the $^{68}\text{Ge}/^{68}\text{Ga}$ generator system have recently been summarized by Mirzadeh and Lambrecht (22). The parent ^{68}Ge is accelerator produced on Ga_2O_3 targets by a (p,2n) reaction. It decays with a half-life of 270.8 d by electron capture. ^{68}Ge is strongly absorbed to different solid supports, such as metal oxides and organic pyrogallol-formaldehyde resins.

An important aspect for wide use of ^{68}Ga in clinical PET is its chemical form and concentration after elution from the generator. In addition, there is concern about ^{68}Ge -breakthrough and contamination of the generator column material. As indicated, different ^{68}Ge -carrier column materials were proposed and used in the past, among them inorganic

oxides such as Al_2O_3 , TiO_2 , or SnO_2 . Recently, a TiO_2 -based generator (Cyclotron Co.) has become commercially available and is being eluted with 0.1 mol/L HCl. Several groups are currently using this generator and modifying it to allow safe handling and remove potential cationic impurities.

Meyer et al. (23) used a microchromatography column filled with a strong basic anion-exchange resin to purify and concentrate the generator eluate. They designed a very sophisticated system to monitor the accumulation of radioactivity in the microcolumn and to survey ^{68}Ge breakthrough. Their final labeling solution contained 10–20 nmol of 1,4,7,10-tetraazacyclododecane- N,N',N'',N''' -tetraacetic acid (DOTA)-peptides in 400 μL N -(2-hydroxyethyl)piperazine- N' -(2-ethanesulfonic acid) buffer.

Velikyan et al. (24) used microwave heating and, like Meyer et al. (23), purified and concentrated the eluate from potential cationic impurities, such as a breakthrough of ^{68}Ge , using $^{68}\text{GaCl}_4^-$ to be adsorbed on anion exchange resins from HCl solutions. The adsorption was found to be 100% in 3.8 mol/L HCl. Deionized water was used to elute. The parent $^{68}\text{Ge}^{4+}$ was not retained on the anion exchange column. The concentration step was also seen as a purification of $^{68}\text{Ga}^{3+}$ from $^{68}\text{Ge}^{4+}$ breakthrough.

Under microwave heating, labeling yields of >99% were obtained at 1 min with as low as 0.5 nmol DOTA-Tyr³-octreotide (DOTATOC).

In addition, Breeman et al. (25) reported using the same TiO_2 generator to label specific activities of 1 GBq/nmol DOTATOC within 5 min at 80°C without the need for purification of the eluate.

Schuhmacher et al. (26) used a pyrogallol-formaldehyde copolymer, an organic ion-exchange resin, as a matrix for their homemade generator. They eluted the generator with 5.5 mol/L HCl and purified and concentrated the ^{68}Ga by adsorbing it on a small Dowex anion-exchange column as $^{68}\text{GaCl}_4^-$, which they eluted with 0.5 mol/L HCl.

AQUEOUS COORDINATION CHEMISTRY OF GA(III) AND BIFUNCTIONAL CHELATORS

Ga(III) chemistry in radiopharmaceutical applications has been reviewed elsewhere in the literature (27).

In aqueous solution, gallium occurs solely in the oxidation state +3. Ga^{3+} is classified as a hard acid metal, bonding to highly ionic hard base ligand donors, such as carboxylic acids, amino nitrogens, hydroxamates, and phenolates. Thiols also have been shown to be good coordinating groups. In addition, the aqueous solution chemistry is determined by the easy hydrolysis of the aquo ion when the pH is raised above about 3 (depending on concentration). Ultimately, hydrolysis leads to the precipitation of $\text{Ga}(\text{OH})_3$. The other important competitor in physiologic fluid for Ga^{3+} radiometals is transferrin. This Fe^{3+} -carrying protein binds Ga^{3+} with high affinity ($\log K = 23.7$). Any application of a ^{68}Ga radiopharmaceutical, then, needs to

resist the exchange of Ga^{3+} from its chelate with transferrin and OH^- ions.

Several suitable bifunctional chelators were proposed, developed, and coupled to biomolecules for gallium labeling. For radiopharmaceutical studies, ^{67}Ga is often used as a surrogate for ^{68}Ga .

One suitable chelator is desferal, which has high affinity for Fe^{3+} and also for Ga^{3+} . It has 3 hydroxamate groups as metal binding sites. Desferal was coupled with ^{67}Ga to octreotide via a succinyl spacer and studied in tumor-bearing rats and in vitro (28–30). Promising preclinical data prompted investigators to perform clinical studies. In humans, Bihl et al. (H. Bihl, unpublished data, 1994) studied the radiopeptide along with ^{111}In -diethylenetriaminepentaacetic acid (DTPA)-octreotide (Octreoscan; Mallinckrodt, Inc.) in 8 patients with somewhat disappointing results because of slow blood clearance (31).

DOTA was also used as a monofunctional version for linking to peptides. The synthesis of a DOTA-tris(tBu) ester allowed convenient linking to the N -terminus of peptides assembled on a solid support (8). If coupled to Tyr³-octreotide to yield DOTA⁰-Tyr³-octreotide (Fig. 1) and labeled with ^{67}Ga , the resulting radiopeptide showed not only 5 times higher binding affinity to the somatostatin-receptor subtype 2 (SSTR2) but also about 2.5 times higher tumor uptake in a mouse model and lower kidney uptake than the $^{111}\text{In}^{90}\text{Y}$ -DOTATOC. Clinical data with this PET radiopharmaceutical are very promising.

Other small molecules coupled to DOTA have also been developed for ^{68}Ga labeling. Roivainen et al. (32) synthesized DOTA-coupled 17-mer-oligonucleotides as imaging agents for tumors containing K-ras point mutations in codon 12. In addition, hybridization properties were not altered by the ^{68}Ga -DOTA coupling, and labeling was possible with high specific activity. Griffiths et al. (33) synthesized and studied a bivalent peptidic hapten, again coupled to DOTA, and labeled it with ^{68}Ga . They used it along with a bispecific antibody against carcinoembryonic antigen and against the hapten in a 2-step pretargeting approach. The preclinical study was summarized by noting that the outstanding target-to-tissue ratios obtained with this approach encourage its development for improved cancer imaging in the future.

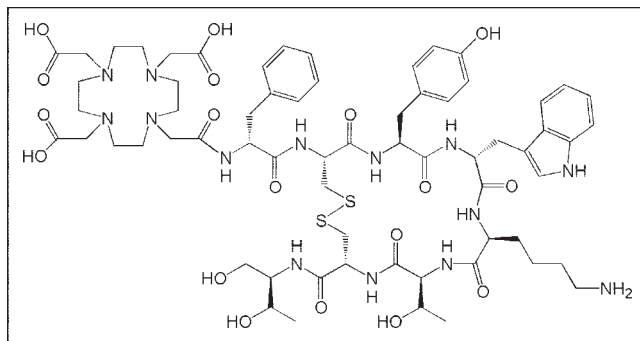


FIGURE 1. Structural formula of DOTATOC.

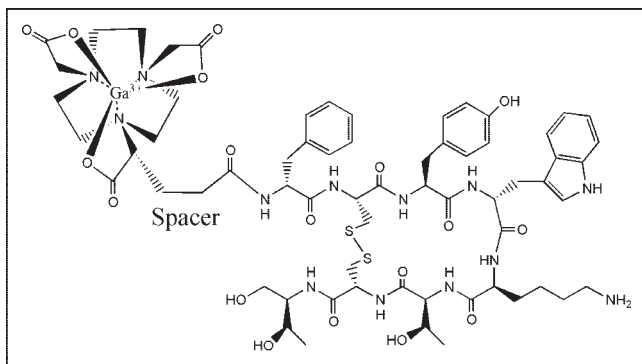


FIGURE 2. Structural formula of NODAGATOC.

In addition to DOTA as a chelator for ^{68}Ga , 1,4,7-tricarboxymethyl-1,4,7-triazacyclononane (NOTA) was coupled to Tyr³-octreotide as the bifunctional version, (1-(1-carboxy-3-carboxy-propyl)-4,7-(carboxy-methyl)-1,4,7-triazacyclononane (NODAGA); Fig. 2). NODAGATOC has not been tested clinically yet but is at least equivalent to ^{68}Ga -DOTATOC in preclinical studies (34).

A tetradentate tripodal S_3N ligand was described forming lipophilic tetrahedral Ga(III) complexes (35). The bifunctional version bis(2-(benzylthio)benzyl)(2-(benzylthio)-4-aminobenzyl)amine was coupled to model peptides to show the usefulness of the approach. The bioconjugate complex was found to be stable to ligand exchange, indicating its suitability for in vivo use.

PRECLINICAL DEVELOPMENT OF DOTA-COUPLED PEPTIDES FOR ^{68}Ga -LABELING AND DIAGNOSTIC PET IMAGING

Targeting of the Somatostatin Receptors

As indicated previously, ^{68}Ga -DOTATOC is the gold standard for ^{68}Ga -based PET peptide radiopharmaceuticals, and other chelator-based somatostatin analogs are being developed for clinical studies.

Targeting of Melanoma Using the Melanocortin System

The melanocortin system consists of the melanocortin peptides α -, β -, and γ -melanocyte-stimulating hormone (α -, β -, and γ -MSH) and adrenocorticotrophic hormone. The melanocortins are involved in diverse physiologic functions, including pigmentation, steroidogenesis, energy homeostasis, exocrine secretion, sexual function, analgesia, inflammation, immunomodulation, temperature control, cardiovascular regulation, and neuromuscular regeneration. Their action is mediated by a family of 5 7-transmembrane G-protein-coupled melanocortin receptors and the endogenous melanocortin antagonists agouti and agouti-related protein.

MC1R is expressed by cutaneous melanocytes, where it has a key role in determining skin and hair pigmentation, in keratinocytes, fibroblasts, endothelial cells, and antigen-presenting cells. The receptor is also expressed by leukocytes, where it mediates the antiinflammatory and immunomodulatory properties of melanocortins.

Because both melanotic and amelanotic melanomas overexpress MC1R, radiolabeled α -MSH analogs were developed for tumor imaging and staging but also with the intention of using them for peptide-based radionuclide therapy (36).

Studies using α -MSH analogs labeled with ^{111}In after conjugation to the metal chelator DTPA were performed preclinically and in patients (37,38). They revealed targeting of melanomas, however, with high nonspecific accumulation of these compounds in several tissues including the liver.

Another α -MSH analog, [Nle⁴, Asp⁵, D-Phe⁷]- α -MSH (4–11) (NAPamide), was conjugated to DOTA. After labeling with ^{67}Ga and ^{68}Ga , DOTA-NAPamide was characterized in vitro and in vivo in the mouse B16F1 melanoma model. Both the ^{111}In -DOTA-NAPamide and ^{67}Ga -DOTA-NAPamide showed high tumor and relatively low kidney uptake. PET studies using ^{68}Ga -DOTA-NAPamide revealed high contrast images even at 1 h after tracer administration (39) (Fig. 3). However, receptor density in human melanomas is expected to be much lower than in the murine tumor model. Consequently, the first clinical scans in 5 patients with melanoma were negative.

Bombesin Derivatives and Bombesin Receptors

Bombesin receptors belong to the group of G-protein-coupled receptors and are overexpressed on major human tumors such as prostate and breast cancer (40,41). There-

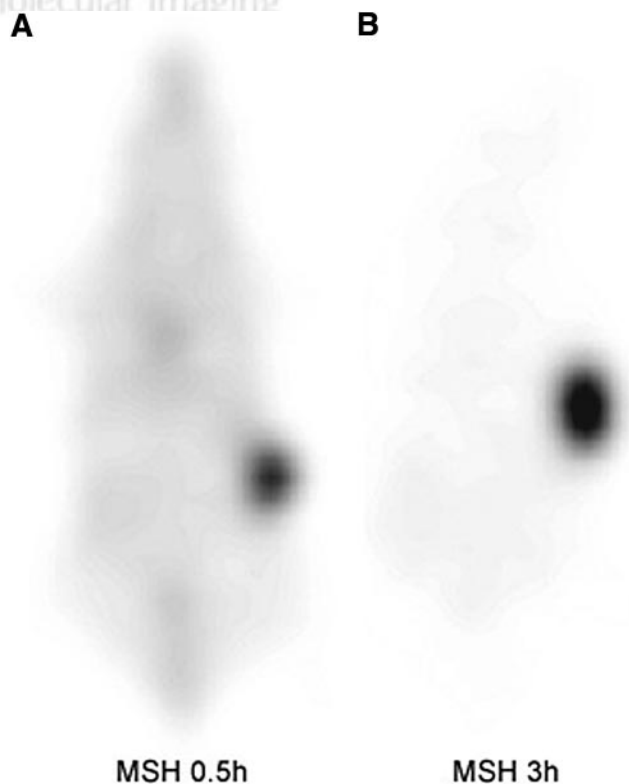


FIGURE 3. Coronal images at 0.5 h (A) and 3 h (B) after administration of ^{68}Ga -DOTA-NAPamide in a melanoma-bearing mouse. Tracer uptake is seen predominantly in tumor and bladder.



FIGURE 4. Coronal PET images of AR42J-tumor bearing mice at 90 min after administration of 0.5 MBq ^{68}Ga -BZH3. Tracer uptake in tumors, pancreas, and duodenum.

fore, radioligands based on bombesin are currently being developed and studied preclinically and in patients (42–45). Several of these new bombesin-based radiopeptides are conjugated to DOTA and can be labeled with ^{68}Ga . As an example, in a pancreatic carcinoma model (AR42J), DOTA-PEG₂-[D-Tyr⁶,βAla¹¹,Thi¹³,Nle¹⁴]bombesin(6–14) was studied after radiolabeling with ^{67}Ga or ^{68}Ga . The compound showed high affinity and rapid internalization in vitro with >85% endocytosed radioactivity after a 1-h incubation period. Tumor uptake in vivo ranged between 5.5 and 11 %ID/g (depending on the injected peptide mass) at 1 h after tracer administration, with a biologic half-life of 15 h.

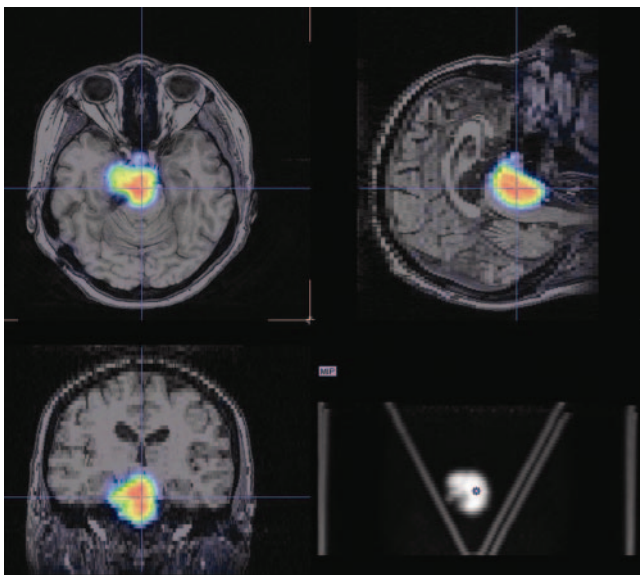


FIGURE 5. Fusion image of MR scan and ^{68}Ga -DOTATOC PET in patient with meningioma. PET scan was acquired 1 h after tracer administration.

Scintigraphic images at 1 h after injection showed specific accumulation in the tumor, kidneys, bowel, and pancreas (Fig. 4), which was confirmed by biodistribution data including blocking studies (46).

FUTURE DIRECTIONS: PHAGE DISPLAY FOR THE IDENTIFICATION OF NEW TUMOR TARGETING PEPTIDES

Bioengineering will lead to the design of new biomolecules by methods such as phage display, which may be used for new approaches in isotope-based diagnosis and treatment of disease. The principle behind phage-displayed peptide libraries is the display of these libraries fused with

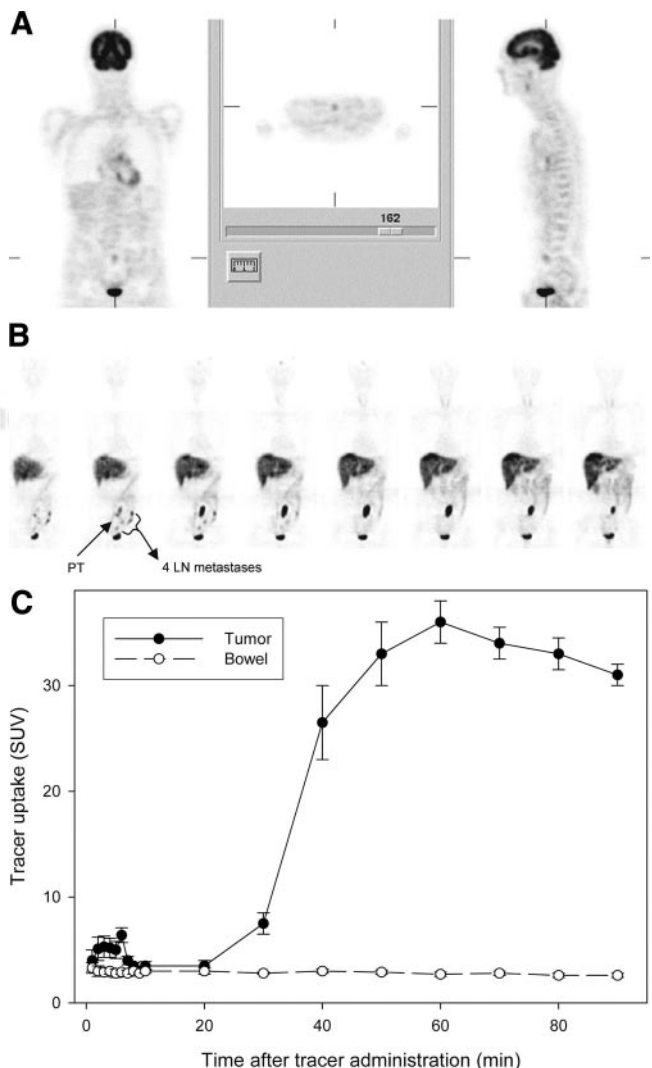


FIGURE 6. ^{18}F -FDG PET (320 MBq injected dose) (A) and ^{68}Ga -DOTATOC PET (169 MBq) (B) images obtained at 60 min after tracer administration in patient with abdominal carcinoid. ^{68}Ga -DOTATOC scan showed higher accumulation of tracer in tumor (SUV in primary tumor [PT] = 38) than did ^{18}F -FDG (SUV = 1.6). Four lymph node (LN) metastases are shown with ^{68}Ga that were missed with ^{18}F -FDG. PT and LN metastases were proven histologically. (C) Tracer kinetics for ^{68}Ga -DOTA-TOC in primary tumor and bowel.

the carboxy terminal domain of the minor coat protein (gene III protein fragment) on the surface of a filamentous phage. The relevant molecule is then directly detected and screened using the target molecules and amplified after infection with *E. coli*. This allows a rapid selection (within weeks) of specific clones from large pools ($>10^{10}$ clones) and determination of the amino acid sequence of a peptide displayed on a phage by sequencing the relevant section of the phage genome. This technique has been used for searching antibodies, receptors for new drug discovery and cancer therapy (either as an antagonist or an agonist of a natural ligand-receptor interaction), and custom-made enzymes for gene therapy (47).

The breakthrough with regard to clinical PET studies with ^{68}Ga was the development of ^{68}Ga -DOTATOC (48). Three communications and an "image of the month" using ^{68}Ga -DOTATOC have been published recently (14–16,49). In 3 patients with meningiomas, Henze et al. (15) showed very fast uptake of the tracer with standardized uptake values (SUVs) reaching a plateau at 60–120 min after injection (mean SUV = 10.6). No tracer was found in the healthy surrounding brain tissue. All meningiomas, including the smallest (<7 mm diameter), showed high tracer uptake and were clearly visualized. These studies provided useful information about the extent of meningiomas located beneath osseous structures, especially at the base of the skull. An example of a PET image fused with a CT image is shown in Figure 5.

Hofmann et al. (14) studied 8 patients with histologically proven carcinoid tumors. They found a biexponential blood clearance with half-lives of 2 ± 0.3 min and 48 ± 7 min. Tumor accumulation reached a maximum after 70 ± 20

min. In all, 40 lesions were predefined by CT or MR imaging, and all lesions were found on ^{68}Ga -DOTATOC PET images. In addition, $>30\%$ more lesions were detected with this tracer. ^{111}In -DTPA-octreotide showed a lower detection rate. The authors concluded that ^{68}Ga -DOTATOC PET showed a high tumor-to-nontumor contrast at early times and a detection rate superior to ^{111}In -DTPA-octreotide. Kowalski et al. (16) also studied 4 patients with metastasizing neuroendocrine tumors. The pharmacokinetic data were found to be very similar to the studies mentioned here and the diagnostic sensitivity higher than that with ^{111}In -DTPA-octreotide.

Figures 6A–6C compare ^{18}F -FDG PET and ^{68}Ga -DOTATOC in a patient with abdominal carcinoid. Unlike ^{18}F -FDG, ^{68}Ga -DOTATOC PET shows high uptake in the primary tumor and in several lymph nodes. Figure 6C shows the tracer uptake in the primary tumor and the bowel with high SUVs at early time points.

Because small cell lung cancer also shows expression of SSTR, therapeutic uses for ^{90}Y -DOTATOC may be promising. However, for the identification of patients who are likely to receive therapeutically sufficient doses, pretherapeutic dosimetry with ^{68}Ga -DOTATOC is useful. Figures 7A–7B show an example of a patient with metastasizing small cell lung cancer imaged with ^{18}F -FDG and ^{68}Ga -DOTATOC.

PET studies with a ^{68}Ga -labeled bombesin analog were performed in 11 patients with prostate cancer (50). In 4 of these patients, mild reversible systolic blood pressure reduction was observed in the first 2 min after a slow intravenous administration. Renal secretion was rapid, with $>75\%$ of injected dose recovered in the urine at 60 min

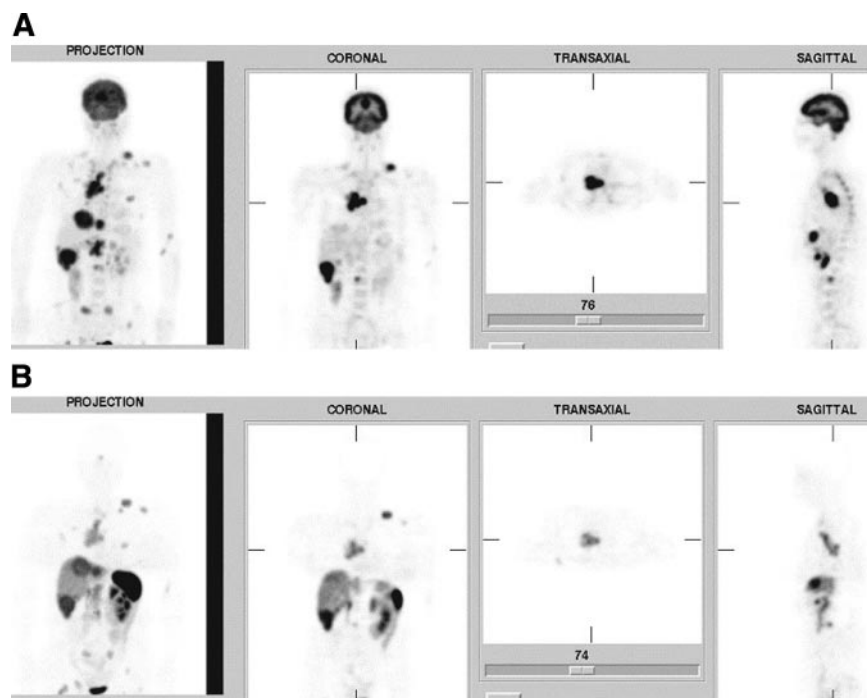


FIGURE 7. ^{18}F -FDG PET (350 MBq injected dose) (A) and ^{68}Ga -DOTATOC PET (250 MBq injected dose) (B) images obtained at 60 min after tracer administration in a patient with a small cell lung carcinoma. ^{18}F -FDG shows higher accumulation (SUV = 10.6) than ^{68}Ga -DOTATOC (SUV = 6.0).

after injection. In all patients, primary tumors were visible, with the smallest tumor size being 5 mm and a plateau of tumor uptake at 15–25 min after injection. In 3 of these patients lymph node metastases also were found. However, in 4 patients a significant nonspecific enrichment was observed in the upper abdomen, which was interpreted as the pancreas. A typical example of primary tumor and lymph node metastases is shown in Figure 8.

CONCLUSION

Generator-produced ^{68}Ga and the development of small chelator-coupled peptides with affinity to receptors overexpressed on a variety of human tumors may open a new generation of kit-formulated PET radiopharmaceuticals. This would allow good manufacturing practice–produced kits and an onsite generator to produce radiopharmaceuticals similar to routinely used $^{99\text{m}}\text{Tc}$ -based radiopharmaceuticals. Along with the long half-life of the generator, which can be used for more than a year, ^{68}Ga -based radiopharmaceuticals may also become a very cost-effective alternative to cyclotron-based tracers.

The short half-life of ^{68}Ga and the fast localization of small peptides make this an ideal combination to study receptor regulation in patients. The 68-min half-life allows generator elution every 2–3 h and several applications in patients per day. This allows, for example, determination of the ideal time point for a therapeutic application of ^{90}Y -DOTATOC or ^{177}Lu -DOTA-Tyr³-Thr⁸-octreotide after discontinuation of cold octreotide therapy in patients with neuroendocrine tumors.

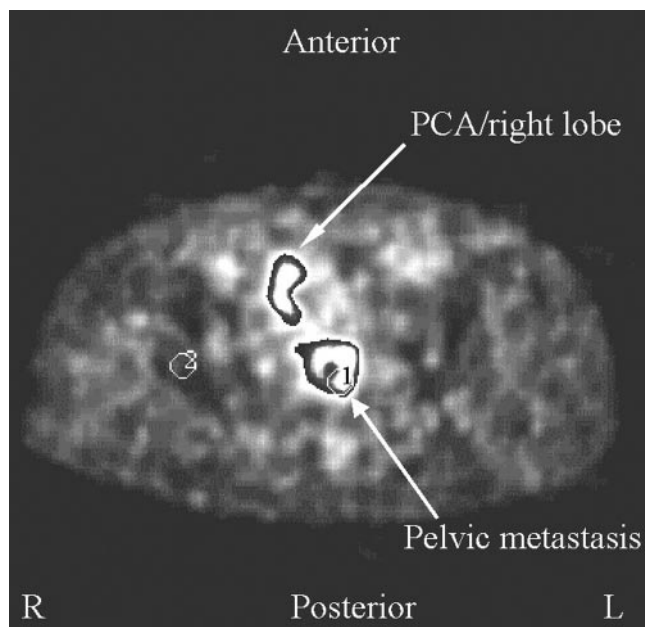


FIGURE 8. ^{68}Ga -labeled bombesin analog image showing primary prostate carcinoma (PCA) and a pelvic metastasis (region of interest [ROI] 2:ROI 1 = 1:202).

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REFERENCES

1. Heppeler A, Froidevaux S, Eberle AN, Maecke HR. Receptor targeting for tumor localisation and therapy with radiopeptides. *Curr Med Chem.* 2000;7:971–994.
2. Behr TM, Gotthardt M, Barth A, Behe M. Imaging tumors with peptide-based radioligands. *Q J Nucl Med.* 2001;45:189–200.
3. Breeman WA, de Jong M, Kwekkeboom DJ, et al. Somatostatin receptor-mediated imaging and therapy: basic science, current knowledge, limitations and future perspectives. *Eur J Nucl Med.* 2001;28:1421–1429.
4. Okarvi SM. Recent developments in $^{99\text{m}}\text{Tc}$ -labelled peptide-based radiopharmaceuticals: an overview. *Nucl Med Commun.* 1999;20:1093–1112.
5. Weiner RE, Thakur ML. Radiolabeled peptides in the diagnosis and therapy of oncological diseases. *Appl Radiat Isot.* 2002;57:749–763.
6. Stolz B, Weckbecker G, Smith-Jones PM, Albert R, Raulf F, Bruns C. The somatostatin receptor-targeted radiotherapeutic [^{90}Y -DTPA-D-Phe¹, Tyr³]octreotide (^{90}Y -SMT 487) eradicates experimental rat pancreatic CA 20948 tumours. *Eur J Nucl Med.* 1998;25:668–674.
7. de Jong M, Bakker WH, Krenning EP, et al. Yttrium-90 and indium-111 labeling, receptor binding and biodistribution of [DOTA⁰, D-Phe¹, Tyr³]octreotide, a promising somatostatin analogue for radionuclide therapy. *Eur J Nucl Med.* 1997;24:368–371.
8. Heppeler A, Froidevaux S, Mäcke HR, et al. Radiometal-labelled macrocyclic chelator-derivatised somatostatin analogue with superb tumour-targeting properties and potential for receptor-mediated internal radiotherapy. *Chem A Eur J.* 1999;5:1016–1023.
9. Kwekkeboom DJ, Bakker WH, Kooij PP, et al. [^{177}Lu -DOTA⁰Tyr³]octreotate: comparison with [^{111}In -DTPA⁰]octreotide in patients. *Eur J Nucl Med.* 2001;28:1319–1325.
10. Waldherr C, Pless M, Maecke H, et al. Tumor response and clinical benefit in neuroendocrine tumors after 7.4 GBq ^{90}Y -DOTATOC. *J Nucl Med.* 2002;43:610–616.
11. Bushnell D, O’Dorisio T, Menda Y, et al. Evaluating the clinical effectiveness of ^{90}Y -SMT 487 in patients with neuroendocrine tumors. *J Nucl Med.* 2003;44:1556–1560.
12. Förster GJ, Engelbach M, Brockmann J, et al. Preliminary data on biodistribution and dosimetry for therapy planning of somatostatin receptor positive tumours: comparison of ^{86}Y -DOTATOC and ^{111}In -DTPA-octreotide. *Eur J Nucl Med.* 2001;28:1743–1750.
13. Jamar F, Barone R, Mathieu I, et al. ^{86}Y -DOTA⁰-D-Phe¹-Tyr³-octreotide (SMT487)—a phase I clinical study: pharmacokinetics, biodistribution and renal protective effect of different regimens of amino acid co-infusion. *Eur J Nucl Med Mol Imaging.* 2003;30:510–518.
14. Hofmann M, Maecke H, Börner A, et al. Biokinetics and imaging with the somatostatin receptor PET radioligand ^{68}Ga -DOTATOC: preliminary data. *Eur J Nucl Med.* 2001;28:1751–1757.
15. Henze M, Schuhmacher T, Hipp P, et al. PET imaging of somatostatin receptors using [^{68}Ga]DOTA-D-Phe¹-Tyr³-octreotide: first results in patients with meningiomas. *J Nucl Med.* 2001;42:1053–1056.
16. Kowalski J, Henze M, Schuhmacher J, Macke HR, Hofmann M, Haberkorn U. Evaluation of positron emission tomography imaging using [^{68}Ga]DOTA-D-Phe¹-Tyr³-Octreotide in comparison to [^{111}In]-DTPAOC SPECT. First results in patients with neuroendocrine tumors. *Mol Imaging Biol.* 2003;5:42–48.
17. Ugur O, Kothari PJ, Finn RD, et al. Ga-66 labeled somatostatin analogue DOTA-D-Phe¹-Tyr³-octreotide as a potential agent for positron emission tomography imaging and receptor mediated internal radiotherapy of somatostatin receptor positive tumors. *Nucl Med Biol.* 2002;29:147–157.
18. Wester H-J, Schottelius M, Scheidhauer K, et al. PET imaging of somatostatin receptors: design, synthesis and preclinical evaluation of a novel ^{18}F -labelled, carbohydrate analogue of octreotide. *Eur J Nucl Med Mol Imaging.* 2002;30:117–122.
19. Anderson CJ, Pajean TS, Edwards WB, Sherman EL, Rogers BE, Welch MJ. In vitro and in vivo evaluation of copper-64-octreotide conjugates. *J Nucl Med.* 1995;36:2315–2325.

20. Deutsch E. Clinical PET: its time has come? *J Nucl Med.* 1993;34:1132–1133.
21. Tsang BW, Mathias CJ, Green MA. A gallium-68 radiopharmaceutical that is retained in myocardium: $^{68}\text{Ga}[(4,6\text{-MeO}_2\text{sal})_2\text{BAPEN}]^+$. *J Nucl Med.* 1993;34:1127–1131.
22. Mirzadeh S, Lambrecht R. Radiochemistry of germanium. *J Radioanal Nucl Chem.* 1996;202:7–102.
23. Meyer GJ, Maecke H, Schuhmacher J, Knapp WH, Hofmann M. ^{68}Ga -labelled DOTA-derivatised peptide ligands. *Eur J Nucl Med Mol Imaging.* 2004;31:1097–1104.
24. Velikyan I, Beyer GJ, Langstrom B. Microwave-supported preparation of ^{68}Ga bioconjugates with high specific radioactivity. *Bioconjug Chem.* 2004;15:554–560.
25. Breeman WA, de Jong M, Krenning E. Preclinical aspects of Lu-177 labelled DOTA-peptides. Paper presented at: COST D18 Working Group Meeting on Lanthanides in Therapy. May 20, 2004; Athens, Greece.
26. Schuhmacher J, Maier-Borst W. A new $^{68}\text{Ge}/^{68}\text{Ga}$ radioisotope generator system for production of ^{68}Ga in dilute HCl. *Int J Appl Radiat Isot.* 1981;32:31–36.
27. Green MA, Welch MJ. Gallium radiopharmaceutical chemistry. *Int J Rad Appl Instrum B.* 1989;16:435–448.
28. Smith-Jones PM, Stolz B, Bruns C, et al. Gallium-67/gallium-68-[DFO]-octreotide—a potential radiopharmaceutical for PET imaging of somatostatin receptor-positive tumors: synthesis and radiolabeling in vitro and preliminary in vivo studies. *J Nucl Med.* 1994;35:317–325.
29. Stolz B, Smith-Jones PM, Albert R, Reist H, Macke H, Bruns C. Biological characterisation of [^{67}Ga] or [^{68}Ga] labelled DFO-octreotide (SDZ 216–927) for PET studies of somatostatin receptor positive tumors. *Horm Metab Res.* 1994;26:453–459.
30. Maecke HR, Smith-Jones P, Maina T, et al. New octreotide derivatives for in vivo targeting of somatostatin receptor-positive tumors for single photon emission computed tomography (SPECT) and positron emission tomography (PET). *Horm Metab Res Suppl.* 1993;27:12–17.
31. Maecke H, Heppeler A, Nock B. Somatostatin analogues labeled with different radionuclides. In: Nicolini, U Mazzi eds. *Technetium, Rhenium and Other Metals in Chemistry and Nuclear Medicine.* Padua, Italy: SGE Ditoriali; 1999:77–91.
32. Roivainen A, Tolvanen T, Salomaki S, et al. ^{68}Ga -labeled oligonucleotides for in vivo imaging with PET. *J Nucl Med.* 2004;45:347–355.
33. Griffiths GL, Chang CH, McBride WJ, et al. Reagents and methods for PET using bispecific antibody pretargeting and ^{68}Ga -radiolabeled bivalent hapten-peptide-chelate conjugates. *J Nucl Med.* 2004;45:30–39.
34. Eisenwiener KP, Prata MI, Buschmann I, et al. NODAGATOC, a new chelator-coupled somatostatin analogue labeled with [^{67}Ga] and [^{111}In] for SPECT, PET, and targeted therapeutic applications of somatostatin receptor (hsst2) expressing tumors. *Bioconjug Chem.* 2002;13:530–541.
35. Luyt LG, Katzenellenbogen JA. A trithiolate tripodal bifunctional ligand for the radiolabeling of peptides with gallium(III). *Bioconjug Chem.* 2002;13:1140–1145.
36. Eberle AN. Proopiomelanocortin and the melanocortin peptides. In: RD Cone, ed. *The Melanocortin Receptors.* Totowa, NJ: Humana Press Inc.; 2000:3–67.
37. Bagutti C, Stolz B, Albert R, Bruns C, Pless J, Eberle AN. [^{111}In]-DTPA-labeled analogues of alpha-melanocyte-stimulating hormone for melanoma targeting: receptor binding in vitro and in vivo. *Int J Cancer.* 1994;58:749–755.
38. Wraight EP, Bard DR, Maughan TS, Knight CG, Page-Thomas DP. The use of a chelating derivative of alpha melanocyte stimulating hormone for the clinical imaging of malignant melanoma. *Br J Radiol.* 1992;65:112–118.
39. Froidevaux S, Calame-Christe M, Schuhmacher J, et al. A gallium-labeled DOTA-alpha-melanocyte-stimulating hormone analog for PET imaging of melanoma metastases. *J Nucl Med.* 2004;45:116–123.
40. Gugger M, Reubi JC. Gastrin-releasing peptide receptors in non-neoplastic and neoplastic human breast. *Am J Pathol.* 1999;155:2067–2076.
41. Markwalder R, Reubi JC. Gastrin-releasing peptide receptors in the human prostate: relation to neoplastic transformation. *Cancer Res.* 1999;59:1152–1159.
42. Van de Wiele C, Dumont F, Vanden Broecke R, et al. Technetium-99m RP527, a GRP analogue for visualisation of GRP receptor-expressing malignancies: a feasibility study. *Eur J Nucl Med.* 2000;27:1694–1699.
43. Breeman WA, de Jong M, Erion JL, et al. Preclinical comparison of ^{111}In -labeled DTPA- or DOTA-bombesin analogs for receptor-targeted scintigraphy and radionuclide therapy. *J Nucl Med.* 2002;43:1650–1656.
44. Smith CJ, Sieckman GL, Owen NK, et al. Radiochemical investigations of gastrin-releasing peptide receptor-specific [$^{99\text{m}}\text{Tc}(\text{X})(\text{CO})_3\text{-Dpr-Ser-Ser-Ser-Gln-Trp-Ala-Val-Gly-His-Leu-Met-(NH}_2\text{)]$ in PC-3, tumor-bearing, rodent models: syntheses, radiolabeling, and in vitro/in vivo studies where Dpr = 2,3-diaminopropionic acid and X = H_2O or $\text{P}(\text{CH}_2\text{OH})_3$. *Cancer Res.* 2003;63:4082–4088.
45. Nock B, Nikolopoulou A, Chiotellis E, et al. [$^{99\text{m}}\text{Tc}$]demobesin 1, a novel potent bombesin analogue for GRP receptor-targeted tumour imaging. *Eur J Nucl Med Mol Imaging.* 2003;30:247–258.
46. Schuhmacher J, Maecke H, Hauser H, et al. In vivo and in vitro characterization of a $^{67,68}\text{Ga}$ -labeled bombesin(6–14) analog for receptor scintigraphy with PET [in German][abstract]. *Nuklearmedizin.* 2004;43:A145.
47. Zwick M, Shen J, Scott J. Phage-displayed peptide libraries. *Curr Opin Biotech.* 1998;9:427–436.
48. Béhé M, Maecke H. New somatostatin analogs labelled with technetium-99m [abstract]. *Eur J Nucl Med.* 1995;22:791.
49. Henze M, Schuhmacher J, Dimitrakopoulou-Strauss A, et al. Exceptional increase in somatostatin receptor expression in pancreatic neuroendocrine tumour, visualised with ^{68}Ga -DOTATOC PET [image of the month]. *Eur J Nucl Med Mol Imaging.* 2004;31:466.
50. Hofmann M, Machtens S, Stief C, Maecke H, Boerner AR, Knapp WH. Feasibility of Ga-68-DOTABOM PET in prostate carcinoma patients [abstract]. *J Nucl Med.* 2004;45:449P.