

Promises of Cyclotron-Produced ^{44}Sc as a Diagnostic Match for Trivalent β^- -Emitters: In Vitro and In Vivo Study of a ^{44}Sc -DOTA-Folate Conjugate

Cristina Müller^{*1}, Maruta Bunka^{*2,3}, Josefine Reber¹, Cindy Fischer⁴, Konstantin Zhernosekov², Andreas Türler^{2,3}, and Roger Schibli^{1,4}

¹Center for Radiopharmaceutical Sciences ETH-PSI-USZ, Paul Scherrer Institute, Villigen-PSI, Switzerland; ²Laboratory of Radiochemistry and Environmental Chemistry, Paul Scherrer Institute, Villigen-PSI, Switzerland; ³Laboratory of Radiochemistry and Environmental Chemistry, Department of Chemistry and Biochemistry, University of Bern, Bern, Switzerland; and ⁴Department of Chemistry and Applied Biosciences, ETH Zurich, Zurich, Switzerland

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In recent years, implementation of ^{68}Ga -radiometalated peptides for PET imaging of cancer has attracted the attention of clinicians. Herein, we propose the use of ^{44}Sc (half-life = 3.97 h, average β^+ energy [$E\beta^+_{\text{av}}$] = 632 keV) as a valuable alternative to ^{68}Ga (half-life = 68 min, $E\beta^+_{\text{av}}$ = 830 keV) for imaging and dosimetry before ^{177}Lu -based radionuclide therapy. The aim of the study was the preclinical evaluation of a folate conjugate labeled with cyclotron-produced ^{44}Sc and its in vitro and in vivo comparison with the ^{177}Lu -labeled pendant. **Methods:** ^{44}Sc was produced via the $^{44}\text{Ca}(p,n)^{44}\text{Sc}$ nuclear reaction at a cyclotron (17.6 ± 1.8 MeV, 50 μA , 30 min) using an enriched ^{44}Ca target (10 mg $^{44}\text{CaCO}_3$, 97.00%). Separation from the target material was performed by a semiautomated process using extraction chromatography and cation exchange chromatography. Radiolabeling of a DOTA-folate conjugate (cm09) was performed at 95°C within 10 min. The stability of ^{44}Sc -cm09 was tested in human plasma. ^{44}Sc -cm09 was investigated in vitro using folate receptor–positive KB tumor cells and in vivo by PET/CT imaging of tumor-bearing mice. **Results:** Under the given irradiation conditions, ^{44}Sc was obtained in a maximum yield of 350 MBq at high radionuclide purity (>99%). Semiautomated isolation of ^{44}Sc from ^{44}Ca targets allowed formulation of up to 300 MBq of ^{44}Sc in a volume of 200–400 μL of ammonium acetate/HCl solution (1 M, pH 3.5–4.0) within 10 min. Radiolabeling of cm09 was achieved with a radiochemical yield of greater than 96% at a specific activity of 5.2 MBq/nmol. In vitro, ^{44}Sc -cm09 was stable in human plasma over the whole time of investigation and showed folate receptor–specific binding to KB tumor cells. PET/CT images of mice injected with ^{44}Sc -cm09 allowed excellent visualization of tumor xenografts. Comparison of cm09 labeled with ^{44}Sc and ^{177}Lu revealed almost identical pharmacokinetics. **Conclusion:** This study presents a high-yield production and efficient separation method of ^{44}Sc at a quality suitable for radiolabeling of DOTA-functionalized biomolecules. An in vivo proof-of-concept study using a DOTA-folate conjugate demonstrated the excellent features of ^{44}Sc for PET imaging. Thus, ^{44}Sc is a valid alternative to ^{68}Ga for imaging and dosimetry before ^{177}Lu -radionuclide tumor therapy.

In the past decade, applications of radiometal-based PET have rapidly increased, particularly for oncologic imaging purposes (1). Clinical implementation of ^{68}Ga -labeled somatostatin analogs (e.g., ^{68}Ga -DOTATATE, ^{68}Ga -DOTATOC) for imaging of neuroendocrine tumors has raised attention because of the excellent imaging quality that can be achieved and the on-site availability of ^{68}Ga (half-life [$T_{1/2}$] = 68 min, average β^+ energy [$E\beta^+_{\text{av}}$] = 830 keV, intensity = 89%) by the $^{68}\text{Ge}/^{68}\text{Ga}$ generator (2–6). Somatostatin receptor–targeted PET is currently used for dosimetry before application of ^{177}Lu -based radionuclide therapy (7). These facts have established the basis of a new era of PET applications using radiometal-based radiopharmaceuticals. An advantage of ^{68}Ga is its easy availability by the $^{68}\text{Ge}/^{68}\text{Ga}$ generator system. However, because of their short half-lives, ^{68}Ga -based radiopharmaceuticals are predominantly used in-house. Also, in most countries radiochemical laboratories in compliance with good-manufacturing practice and qualified personnel are required (8). These circumstances lead to high overall cost and render ^{68}Ga radiopharmaceuticals of limited interest for centralized production and commercial distribution. An alternative is the cyclotron-produced ^{64}Cu ($T_{1/2}$ = 12.7 h, $E\beta^+_{\text{av}}$ = 278 keV, intensity = 17.6%), which has been used in a large number of preclinical and clinical PET studies (9–13). The half-life of 12.7 h provides the flexibility to use it in combination with small molecules and with slow-clearing targeting agents and for transportation of ^{64}Cu radiopharmaceuticals to hospitals without cyclotrons and radiopharmacies (14). However, ^{64}Cu possesses a complex redox chemistry, which has to be taken into consideration when choosing the chelating agents. In addition, coemission of β^- -particles (~39% branch ratio) contribute to additional dose burden to the patient without diagnostic benefit. Recently, ^{44}Sc was proposed as an alternative radionuclide for PET imaging using radiometalated peptides and other small-molecular-weight biomolecules (15). ^{44}Sc is a rare earth metal, which decays by emission of positrons ($E\beta^+_{\text{av}}$ = 632 keV, intensity = 94.3%) with a half-life of 3.97 h. This almost 4-fold-longer half-life of ^{44}Sc , compared with ^{68}Ga , potentially allows centralized production and cost-efficient

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For correspondence or reprints contact: Prof. Dr. Roger Schibli, Center for Radiopharmaceutical Sciences ETH/PSI-USZ, Paul Scherrer Institute, 5232 Villigen-PSI, Switzerland.

E-mail: roger.schibli@psi.ch

*Contributed equally to this work.

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distribution of ^{44}Sc radiopharmaceuticals to even remote PET centers. Moreover, the application of ^{44}Sc may be appealing if applied with biomolecules of longer biologic half-lives and for applications, which should cover several hours after administration of the radiopharmaceutical. These advantages have to be carefully traded off against the coemission of high-energy γ -rays by ^{44}Sc ($E_\gamma = 1,157$ keV, intensity = 99.9% and 1,499 keV, intensity = 0.9%).

^{44}Sc can be produced via a $^{44}\text{Ti}/^{44}\text{Sc}$ generator systems (15–17). However, ^{44}Ti ($T_{1/2} = 60$ y) can be produced only at a small number of facilities in the world, with limited yields and at high costs (18). Therefore, the accessibility of cyclotron-produced ^{44}Sc by proton irradiation of natural calcium targets has recently been proposed as an inexpensive alternative by Severin et al. (19). Also, the idea of using enriched calcium targets to optimize radionuclide purity of the produced ^{44}Sc was reported in the literature (20–22).

The present study is the first report, to our knowledge, on the cyclotron production of ^{44}Sc at a quality and quantity sufficient for radiolabeling of a biomolecule, including preclinical evaluation. We demonstrate the feasibility of the production of ^{44}Sc via the $^{44}\text{Ca}(p,n)^{44}\text{Sc}$ nuclear reaction. Moreover, we present a semiautomated process, which allowed isolation of ^{44}Sc in a short time and at high radionuclide purity for direct radiolabeling of biomolecules. For the in vivo proof-of-concept study, we chose a recently published folic acid conjugate with a DOTA chelator (cm09) (23). The ^{177}Lu -labeled version (^{177}Lu -cm09) proved to have excellent characteristics with regard to the uptake in folate receptor (FR)-positive tumors in a well-established tumor mouse model (23). In vivo imaging of tumor-bearing mice after the administration of ^{44}Sc -cm09 demonstrated the promising features of ^{44}Sc for PET imaging purposes.

MATERIALS AND METHODS

Production of ^{44}Sc

^{44}Sc was produced via the $^{44}\text{Ca}(p,n)^{44}\text{Sc}$ nuclear reaction at the PSI Injector 2 cyclotron facility. Targets for irradiation were prepared with enriched $^{44}\text{CaCO}_3$ (5–10 mg; 97.00% ^{44}Ca ; 2.89% ^{40}Ca ; 0.06% ^{42}Ca ; 0.03% ^{43}Ca ; 0.02% ^{48}Ca ; and <0.002% ^{46}Ca [Trace Sciences International]). Graphite powder (150 mg, 99.9999%; Alfa Aesar) was used as matrix material. The enriched $^{44}\text{CaCO}_3$ powder was pressed on the surface of the prepressed graphite disk, followed by encapsulation of the target in aluminum. The targets were irradiated with protons of 17.6 ± 1.8 MeV (degraded from an initial beam of 72 MeV) at a beam current of 50 μA for 30–40 min. For the chemical separation of $^{44}\text{Sc(III)}$ from Ca(II) , a semiautomated separation system was developed. The irradiated target (with typical activities of ~ 350 MBq) was transferred into a conical glass vial and dissolved in HCl (3 M, prepared from 30% HCl and Suprapur and MilliQ water; Merck KGaA). A small aliquot of dissolved $^{44}\text{CaCO}_3$ was analyzed by γ -ray spectrometry. The unsolved graphite remained in the conical glass vial, and the radioactive solution mixture was pumped onto the first column (1-mL column of 55 mm \times 5.65 mm, fitted with a 20- μm frit; ISOLUTE SPE Accessories) filled with 50–70 mg of N,N,N',N' -tetra-*n*-octyldiglycolamide (DGA) (50–100 μm ; Triskem International) resin. The adsorbed $^{44}\text{Sc(III)}$ was eluted from the DGA resin with HCl (0.1 M, 2–3 mL). Afterward, the acidic $^{44}\text{Sc(III)}$ solution was loaded on a second column (1-mL column of 55 mm \times 5.65 mm, fitted with 20- μm frit; ISOLUTE SPE Accessories) filled with 100–140 μL of a cation exchange resin in water (DOWEX 50, hydrogen form, 200–400 mesh; Fluka Analytic). $^{44}\text{Sc(III)}$ was eluted in ammonium acetate ($\text{CH}_3\text{COONH}_4/\text{HCl}$ 1 M, 200–400 μL , pH 3.5–4.0; $\text{CH}_3\text{COONH}_4$; Trace SELECT, $\geq 99.9999\%$ [Fluka Analytic]) at a radioactivity concentration of up to 980 MBq/mL. For the adjustment

of the pH to 3.5–4.0, HCl (0.1 M, 10–30 μL) was added before the use of the $^{44}\text{Sc(III)}$ solution for the radiolabeling procedure.

Radiosynthesis

A stock solution of cm09 (25–35 μL , 10^{-3} M) was added to 200–400 μL of the ^{44}Sc solution (130–180 MBq, pH 3.5–4.0) and incubated at 95°C for 10 min. Then, Na-diethylenetriaminepentaacetic acid (Na-DTPA, 10 μL , 5 mM, pH 5) was added to the reaction mixture for complexation of traces of free $^{44}\text{Sc(III)}$. Quality control was performed by high-performance liquid chromatography (HPLC) using a C-18 reversed-phase column (Xterra MS C18, 5 μm , 15 \times 4.6 cm; Waters). The mobile phase consisted of MilliQ water with 0.1% trifluoroacetic acid (A) and methanol (B) with a linear gradient from 95% A and 5% B to 20% A and 80% B over 25 min with a flow rate of 1 mL/min. For in vitro and in vivo application, the labeling mixture containing ^{44}Sc -cm09 was diluted with water to reduce the osmolality.

^{177}Lu -cm09 was prepared for in vitro cell experiments, which were performed in parallel with ^{44}Sc -cm09. The radiosynthesis and quality control of ^{177}Lu -cm09 was performed as previously reported (23). The specific activity of ^{177}Lu -cm09 was adapted to allow its application at the same molar amount, which was used for ^{44}Sc -cm09.

In Vitro Stability

For the determination of the stability of ^{44}Sc -cm09 in human plasma, a solution of the radiotracer (50 μL , ~ 1.0 MBq) was mixed with a sample of human plasma (250 μL) and incubated at 37°C. After 1, 2, and 4 h, aliquots (40 μL) were taken, and the plasma proteins were precipitated by the addition of methanol (200 μL). The suspension was centrifuged (8,000 rcf, 3 min) twice and the supernatants analyzed by HPLC. To investigate potential radiolysis, ^{44}Sc -cm09 was incubated at a high-radioactivity concentration (150–160 MBq in a volume of 500 μL) over several hours at room temperature. After 1, 2, 3, and 4 h, aliquots (5 μL) were taken and analyzed by HPLC.

Determination of Octanol/Phosphate-Buffered Saline (PBS) Distribution Coefficient (LogD)

For determination of the distribution coefficient (logD), ^{44}Sc -cm09 was purified via HPLC using the same system as described above for analytic purposes. The logD value of ^{44}Sc -cm09 was determined by the shake-flask method as previously published in the literature (23, 24). In brief, a sample of ^{44}Sc -cm09 (25 μL , ~ 0.25 MBq) was added to a mixture of *n*-octanol (1,500 μL) and PBS (pH 7.4, 1,475 μL). The resulting biphasic system was mixed again by vortex for 1 min at room temperature, followed by centrifugation (2,500 rpm, 6 min). Aliquots of each layer were measured for radioactivity in a γ -counter. The logD value was expressed as the ratio of counts per minute measured in a defined volume of the *n*-octanol phase to the counts per minute measured in an equal volume of the PBS phase. The results represent the average \pm SD of quintuplets of 2 independent experiments.

Cell Culture

KB cells (human cervical carcinoma cell line, HeLa subclone; ACC-136) were purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ). The cells were cultured as monolayers at 37°C in a humidified atmosphere containing 5% CO_2 . Importantly, KB cells were cultured in a folate-free cell culture medium, FFRPMI (modified RPMI, without folic acid, vitamin B_{12} , and phenol red; Cell Culture Technologies GmbH). The FFRPMI medium was supplemented with 10% heat-inactivated fetal calf serum, L-glutamine, and antibiotics (penicillin/streptomycin/fungizone). Routine culture treatment was performed twice a week using 0.25% trypsin-ethylenediamine tetraacetic acid (1 \times) (Gibco by Life Technologies) to release cells from cell culture flasks.

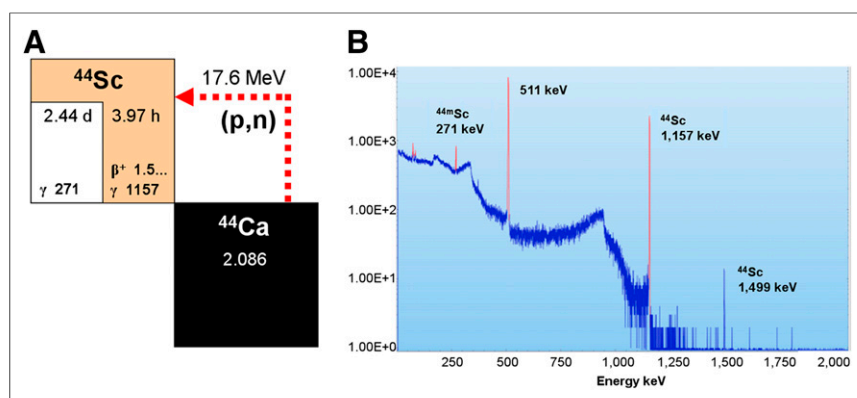


FIGURE 1. (A) Nuclear reaction for production of ^{44}Sc from enriched ^{44}Ca targets. (B) γ -ray spectrum of ^{44}Sc obtained from irradiated ^{44}Ca targets 5 h after end of beam.

Cell Experiments

KB cells were seeded in 12-well plates ($\sim 700,000$ cells in 2 mL of FFRPMI medium per well), allowing cell adhesion and growth overnight at 37°C . After removal of the supernatant, cells were washed once with PBS before the addition of FFRPMI medium (975 μL /well) without supplements. ^{44}Sc -cm09 (25 μL , ~ 38 kBq, ~ 8 pmol) or ^{177}Lu -cm09 (25 μL , ~ 38 kBq, ~ 8 pmol) was added to each well. In some cases, cells were incubated with an excess of folic acid (100 μM) to block FRs on the surface of KB cells. After incubation of the well plates for different time periods (0, 5, 15, 30, 60, 120, and 240 min) at 37°C , the cells were washed twice with ice-cold PBS to determine total uptake of ^{44}Sc -cm09. To assess the fraction of internalized ^{44}Sc -cm09, KB cells were additionally washed with a stripping buffer (aqueous solution of 0.1 M acetic acid and 0.15 M NaCl, pH 3 (25)) to release FR-bound radiofolates from the cell surface (26). Cell samples were lysed by the addition of NaOH (1 M, 1 mL) to each well. The cell suspensions were then transferred to 4-mL tubes, and each sample was counted for radioactivity in a γ -counter. After homogenization by vortex, the concentration of proteins was determined for each sample by a Micro BCA Protein Assay kit (Pierce, Thermo Scientific) to standardize measured radioactivity to the average content of 0.2 mg of protein in a single well.

Biodistribution Studies

In vivo experiments were approved by the local veterinarian department and conducted in accordance with the Swiss law of animal protection. Six- to 8-wk-old female, athymic nude mice (CD-1 Foxn-1/nu) were purchased from Charles River Laboratories. The animals were fed with a folate-deficient rodent diet (ssniff Spezialdiäten GmbH) starting 5 d before tumor cell inoculation (27). Mice were inoculated with KB cells (5×10^6 cells in 100 μL of PBS) into the subcutis of each shoulder. Biodistribution studies were performed in triplicate approximately 14 d after cell inoculation. ^{44}Sc -cm09 (2 MBq/mouse in a volume of 100 μL) at a specific activity of approximately 4 MBq/nmol was injected into a lateral tail vein. Importantly, the same molar amount of cm09 (0.5 nmol/mouse) was used as previously used for the tissue distribution of ^{177}Lu -cm09 (23) because alteration of the molar amount of the biomolecule may critically affect its tissue distribution. Blocking studies were performed by the injection of excess folic acid (100 μg in a volume of 100 μL of PBS, pH 7.4) immediately before administration of ^{44}Sc -cm09. The animals were sacrificed at predetermined time points after administration of ^{44}Sc -cm09. Selected tissues and organs were collected, weighed, and counted for radioactivity in a γ -counter. The results were listed as a percentage of the injected dose per gram of tissue weight (%ID/g), using counts of defined volume of the original injection solution counted at the same time.

PET/CT Imaging

PET/CT scans were obtained with a dedicated small-animal PET/CT camera (Vista eXplore, Sedecal Spain; GE Healthcare). Reconstruction of PET and CT data was performed using the instrument's software. For PET reconstruction, the 2-dimensional ordered-subset expectation maximization algorithm was used. PET and CT data were fused using PMOD software (version 3.3; PMOD Technologies Ltd.).

Imaging studies were performed with nude mice approximately 14 d after KB tumor cell inoculation. ^{44}Sc -cm09 (25 MBq, 6 nmol per mouse) was intravenously injected. During the PET scans, mice were anesthetized by inhalation of isoflurane in an air-oxygen mixture approximately

5 min before PET data acquisition. Static PET scans were obtained with each of the mice from 240 to 270 min after injection of ^{44}Sc -cm09. All PET scans were followed by a CT scan.

RESULTS

Production of ^{44}Sc

^{44}Sc was produced via the $^{44}\text{Ca}(p,n)^{44}\text{Sc}$ nuclear reaction (Fig. 1A) in typical yields of approximately 350 MBq under the given irradiation conditions (10 mg of $^{44}\text{CaCO}_3$ on the surface of a graphite disk). This resulted in an apparent production cross section of about 300 millibarn at an initial proton beam energy of 17.6 ± 1.8 MeV. Significantly higher quantities of ^{44}Sc can readily be obtained by optimization of the beam energy, by increasing the amount of ^{44}Ca , and by extending the time of irradiation. This procedure requires a fully automated separation process in shielded hot cells to avoid high radiation doses to the technical personnel. (The high-energy γ -radiation [$E_\gamma = 1,157$ keV, 99.9% and 1,499 keV, 0.9%] coemitted with the β^+ -particles have to be taken into consideration for radiation protection precautions.) These optimizations are the topic of ongoing investigations in our laboratories.

The γ -ray spectrum of the dissolved target material showed almost exclusively the γ -lines of ^{44}Sc (511, 1,157, and 1,499 keV), proving the high radionuclide purity of greater than 99%, which is achievable by this method (Fig. 1B). As the only coproduced scandium isotope of enriched ^{44}Ca targets, the longer-lived $^{44\text{m}}\text{Sc}$ ($T_{1/2} = 2.4$ d) was identified by its 271-keV γ -line as a radioisotopic impurity ($<1\%$) (supplemental data, Supplemental Fig. 1 [supplemental materials are available at <http://jnm>).

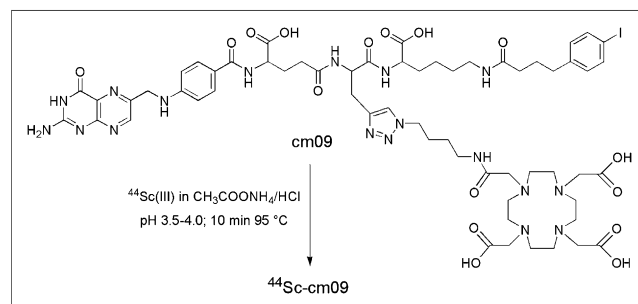


FIGURE 2. Chemical structure of DOTA-folate conjugate (cm09) and preparation of ^{44}Sc -cm09.

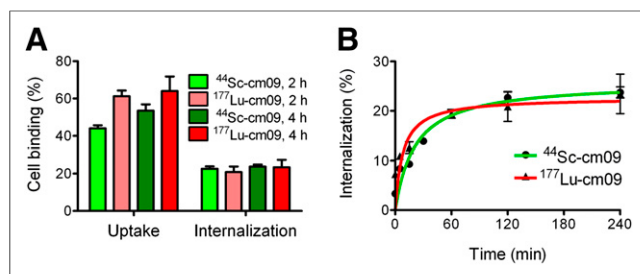


FIGURE 3. (A) Cell uptake and internalization of ^{44}Sc -cm09 (green) and ^{177}Lu -cm09 (red) after incubation time of 2 or 4 h at 37°C , respectively. (B) Time-dependent internalization of ^{44}Sc -cm09 (green) and ^{177}Lu -cm09 (red) over 4 h.

snmjournals.org]). A schematic illustration of the separation process of ^{44}Sc from the ^{44}Ca /graphite target is given in the supplemental data (Supplemental Fig. 2). DGA-based extraction chromatography resulted in adsorption of $^{44}\text{Sc}(\text{III})$ on the column and hence separation from $\text{Ca}(\text{II})$, which was not retained on the column. ^{44}Sc was almost quantitatively eluted from the DGA resin by 0.1 M HCl. Cation-based exchange chromatography was used to concentrate the ^{44}Sc solution to 200–400 μL (1 M ammonium acetate). The purification process from target dissolution to the elution of ^{44}Sc was accomplished within 10 min. Before use, the pH value of the ^{44}Sc solution was adjusted to 3.5–4.0.

Radiolabeling and In Vitro Characterization

^{44}Sc -cm09 was prepared by radiolabeling of cm09 (25 nmol) with $^{44}\text{Sc}(\text{III})$ (130 MBq, 200 μL) in an ammonium acetate/HCl solution at a pH of 3.5–4.0 (Fig. 2). Quality control performed by HPLC showed the product peak of ^{44}Sc -cm09 with a retention time of 19.6 min, which was largely identical to ^{177}Lu -cm09 (retention time = 19.7 min) (supplemental data, Supplemental Fig. 3). The radiochemical yield was greater than 96% at a specific activity of up to 5.2 MBq/nmol, representing a ^{44}Sc -to-ligand molar ratio of 1:5,600. If the molar amount of cm09 was reduced, the labeling yield dropped rapidly. Neither varying the pH value of the labeling solution nor increasing the incubation time improved

these results. For comparison, we prepared ^{177}Lu -cm09 at the same specific activity (~ 5.2 MBq/nmol) and calculated a metal-to-ligand ratio of about 1:138.

Investigation of the stability of ^{44}Sc -cm09 in blood plasma showed no decomposition product or free $^{44}\text{Sc}(\text{III})$ over the time of investigation. Also, radiolysis of ^{44}Sc -cm09 was not observed over at least 4 h, even at a radioactivity concentration of up to 400 MBq/mL. The distribution coefficient (logD) of ^{44}Sc -cm09 between *n*-octanol and PBS pH 7.4 revealed a value of -4.49 ± 0.12 . This value was comparable to the logD value obtained for ^{177}Lu -cm09 (-4.25 ± 0.41) (23).

Cell Experiments

Uptake and internalization of ^{44}Sc -cm09 into FR-positive KB tumor cells was determined in vitro over a period of 4 h. The internalized fraction of ^{44}Sc -cm09 accounted for 30%–50% of the total FR-bound fraction of ^{44}Sc -cm09. Analysis of cell samples, which were coincubated with excess folic acid to block FRs, showed a decline of ^{44}Sc -cm09 uptake to less than 1%. These results were comparable to those of other folate radioconjugates previously developed in our group (28). Direct comparison of ^{44}Sc -cm09 and ^{177}Lu -cm09 revealed slight variations of the values for the total cell uptake (Fig. 3A) but almost identical results for the internalized fraction over 4 h (Fig. 3B).

Biodistribution Studies

The tissue distribution of ^{44}Sc -cm09 in athymic nude mice showed an excellent accumulation of radioactivity in KB tumor xenografts (Table 1). Already 2 h after injection of ^{44}Sc -cm09, the tumor uptake was high (8.37 ± 0.41 %ID/g) and reached a value that was almost doubled at 20 h after injection (14.05 ± 2.29 %ID/g). The enhanced blood circulation time of ^{44}Sc -cm09 as a consequence of the albumin-binding entity integrated in the structure of cm09 was reflected by a relatively high amount of radioactivity in the blood pool (3.74 ± 0.88 %ID/g) at 2 h after injection. In nontargeted tissues and organs such as liver, stomach, lung, muscle, and bones, accumulation of ^{44}Sc -cm09 was low and no higher than background levels 20 h after injection. Specific uptake of ^{44}Sc -cm09 was found in the salivary glands and in the

TABLE 1
Biodistribution of ^{44}Sc -cm09 in KB Tumor-Bearing Female Nude Mice

Organ	^{44}Sc -cm09 (% injected dose per gram tissue*)			
	2 h after injection	4 h after injection	8 h after injection	20 h after injection
Blood	3.74 ± 0.88	2.67 ± 0.35	1.77 ± 0.10	0.87 ± 0.08
Lung	2.29 ± 0.42	1.84 ± 0.21	1.40 ± 0.10	0.90 ± 0.12
Spleen	1.05 ± 0.19	0.90 ± 0.12	0.77 ± 0.01	0.70 ± 0.14
Kidneys	19.21 ± 1.70	22.61 ± 4.52	21.30 ± 4.62	22.22 ± 1.94
Stomach	1.19 ± 0.17	1.01 ± 0.06	0.81 ± 0.03	0.58 ± 0.02
Intestines	0.59 ± 0.16	0.53 ± 0.09	0.38 ± 0.05	0.36 ± 0.12
Liver	3.63 ± 0.19	3.12 ± 0.38	3.56 ± 0.55	2.31 ± 0.15
Salivary glands	6.49 ± 0.52	6.48 ± 0.29	4.51 ± 0.53	3.48 ± 0.57
Muscle	1.07 ± 0.03	1.30 ± 0.61	1.12 ± 0.37	0.76 ± 0.28
Bone	0.98 ± 0.04	1.04 ± 0.16	0.92 ± 0.09	0.68 ± 0.05
Tumor	8.37 ± 0.41	12.51 ± 1.74	12.43 ± 1.04	14.05 ± 2.29
Tumor to blood	2.50 ± 0.59	3.79 ± 1.88	7.03 ± 0.64	16.07 ± 1.71
Tumor to liver	2.31 ± 0.11	3.25 ± 1.62	3.34 ± 0.43	6.07 ± 0.72
Tumor to kidney	0.44 ± 0.03	0.45 ± 0.23	0.59 ± 0.06	0.63 ± 0.07

*Values shown represent mean \pm SD of data from 3 animals ($n = 3$) per cohort.

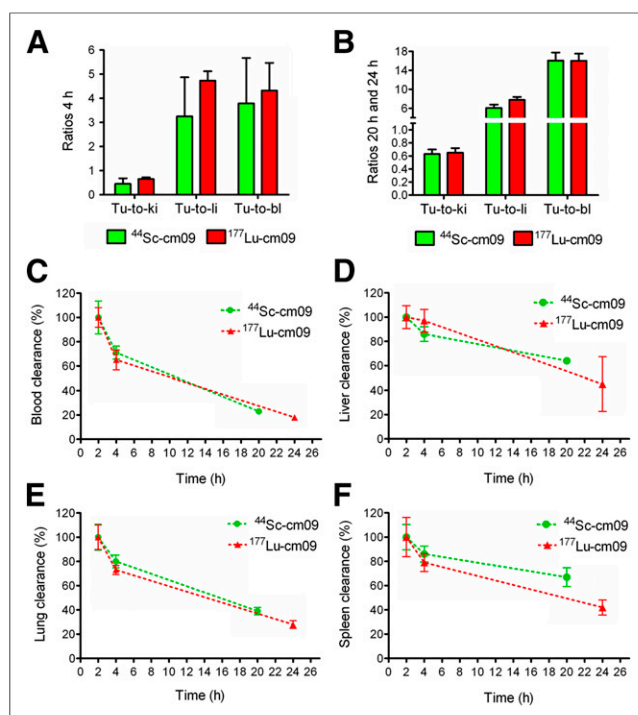


FIGURE 4. (A) Tumor-to-background ratios 4 h after injection of ^{44}Sc -cm09 (green) and ^{177}Lu -cm09 (red). (B) Tumor-to-background ratios 20 h after injection of ^{44}Sc -cm09 (green) and 24 h ^{177}Lu -cm09 (red). Blood clearance (C), liver clearance (D), lung clearance (E), and spleen clearance (F) of ^{44}Sc -cm09, compared with ^{177}Lu -cm09 (the %ID/g values at 2 h after injection were set to 100%).

kidneys, which are known to express the FR. Renal retention of ^{44}Sc -cm09 was in the range of 19–23 %ID/g at all investigated time points. The excellent accumulation of ^{44}Sc -cm09 in the tumor tissue resulted in high tumor-to-kidney ratios, which reached values between 0.45 and 0.65.

The tissue distribution data of ^{44}Sc -cm09 were also compared with our previous data obtained with ^{177}Lu -cm09 (23). Retention of ^{44}Sc -cm09 in the blood circulation was somewhat lower than found for the ^{177}Lu -labeled radioconjugate (supplemental data, Supplemental Table 1) (23). However, the tumor-to-background ratios were comparable for both ^{44}Sc -cm09 and ^{177}Lu -cm09 at early time points after injection and almost identical at 1 d after injection (Figs. 4A and 4B). Also, the relative clearances from blood and nontargeted organs such as liver, lung, and spleen (with the 2-h-after-injection time point set to 100%) revealed equivalent values for ^{44}Sc -cm09 and ^{177}Lu -cm09 (Figs. 4C–4F).

In Vivo PET Imaging Studies Using ^{44}Sc -cm09

PET/CT imaging studies were performed 4 h after injection of approximately 25 MBq of ^{44}Sc -cm09. The enhanced blood circulation time of ^{44}Sc -cm09 led to a high tumor uptake and a reduced renal retention, resulting in an excellent tumor-to-kidney ratio of about 1. On the other hand, the tumor-to-nontarget ratio was somewhat lower than what is usually observed for fast clearing small-molecular-weight molecules. However, the PET image allowed excellent visualization of tumor xenografts located on each shoulder of the mouse and in the kidneys, which were the 2 major sites of accumulated radioactivity (Fig. 5A). Besides the uptake of ^{44}Sc -cm09 in these FR-positive organs and tissue, radioactivity

was found only in the urinary bladder. Studies with a KB tumor-bearing mouse injected with excess folic acid immediately before injection of ^{44}Sc -cm09 resulted in a significant reduction of accumulated radioactivity in tumor xenografts and in the kidneys (supplemental data, Supplemental Fig. 4). The PET scans obtained 4 h after injection of ^{44}Sc -cm09 showed the same distribution profile of radioactivity, which was previously observed on SPECT/CT images of a mouse 4 h after injection of ^{177}Lu -cm09 (Fig. 5B).

DISCUSSION

Proton irradiation of enriched ^{44}Ca targets resulted in the production of ^{44}Sc in radiochemical purity higher than 99%. As the only radioactive side product, traces of the longer-lived isotope $^{44\text{m}}\text{Sc}$ were determined by γ -ray spectrometry. In contrast, the quality of ^{44}Sc obtained from irradiation of natural calcium targets was much lower because of several coproduced scandium radioisotopes as reported by Severin et al. (supplemental data, Supplemental Fig. 1) (19). The semiautomated separation system allowed isolation of more than 85% of the initial activity of ^{44}Sc (III) at high radioactivity concentrations (>900 MBq/mL) within only 10 min. For the in vivo assessment of ^{44}Sc , we have chosen a folate derivative (cm09) comprising an albumin-binding entity. This novel folate conjugate reveals an enhanced blood circulation time and, hence, slower pharmacokinetic properties, making it suitable for combining with a longer-lived PET and therapeutic radiometal. ^{44}Sc radiolabeling of cm09 was achieved at a specific activity of up to 5.2 MBq/nmol, which is an unprecedentedly high value, compared with other ^{44}Sc -labeling results reported in the literature (29–31).

In vitro ^{44}Sc -cm09 was tested in parallel to ^{177}Lu -cm09 on FR-positive KB tumor cells. The results were largely in agreement with the results previously obtained with ^{177}Lu -cm09 (23). In vivo, we found an excellent tissue distribution of ^{44}Sc -cm09, with increasing accumulation of radioactivity in FR-positive tumor xenografts over time (8.37 ± 0.41 %ID/g at 1 h after injection to 14.05 ± 2.29 %ID/g at 20 h after injection). Accumulation in nontargeted tissue and organs such as liver, lung, intestinal tract, muscle, and bone was largely absent.

Although the radius of Sc(III) is similar to that of Ga(III), its chemical properties resemble more closely those of Y(III) (pseudolanthanide) and lanthanides such as ^{177}Lu (III) with common coordination numbers of 8 and 9 (32). This allows the formation of stable ^{44}Sc complexes with DOTA chelators. Recently, it was shown that DOTA was the most preferred chelator for ^{44}Sc (III) from a series of macrocyclic ligands (33). All 4 amine and carboxyl groups of a DOTA chelator contribute to the complexation of ^{44}Sc (III), resulting in a stability constant comparable to those of Y(III) and lanthanides (33). In theory, comparable results for ^{44}Sc -labeled and ^{177}Lu -labeled biomolecules would be expected.

The reason why the values for uptake in the tumor tissue and in the kidneys were somewhat lower for ^{44}Sc -cm09 than previously published values for ^{177}Lu -cm09 can presumably be attributed to interexperimental variability (Table 1; supplemental data, Supplemental Table 1) (23). However, the tumor-to-background ratios of ^{44}Sc -cm09 and ^{177}Lu -cm09 were almost the same, particularly at late time points (20 and 24 h, respectively) after application.

Similar to what has been published for ^{44}Sc - and ^{177}Lu -DOTA-TATE complexes (33), we determined largely the same hydro-

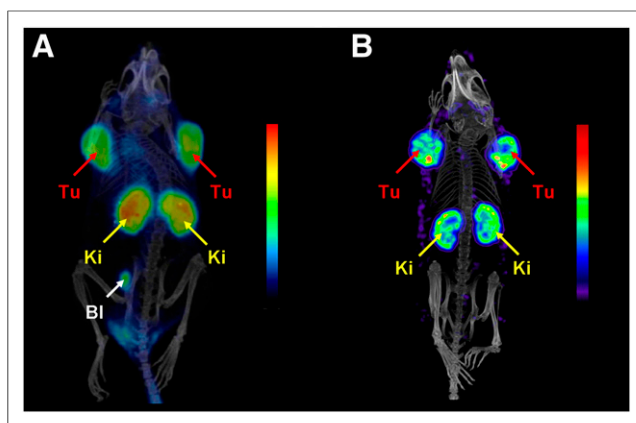


FIGURE 5. (A) PET/CT image of KB tumor-bearing mouse 4 h after injection of approximately 25 MBq of ^{44}Sc -cm09. (B) SPECT/CT image of KB tumor-bearing mouse 4 h after injection of approximately 35 MBq of ^{177}Lu -cm09 (23). Tumors (Tu) are indicated with red arrows, kidneys (Ki) are indicated with yellow arrows, and urinary bladder (Bl) is indicated with white arrow.

philicity for ^{44}Sc -cm09 ($\log D$, -4.49 ± 0.12) and ^{177}Lu -cm09 ($\log D$, -4.25 ± 0.41) (23). Our findings and those of recent literature reports (30,33) corroborated the hypothesis of a similar in vivo behavior for ^{44}Sc - and ^{177}Lu -labeled biomolecules rather than for a ^{68}Ga -labeled pendant. Moreover, because of the reduced β^+ -energy of ^{44}Sc ($E\beta^+_{\text{av}} = 632$ keV), compared with ^{68}Ga ($E\beta^+_{\text{av}} = 830$ keV), the resolution of PET images obtained with ^{44}Sc is expected to be equal or even superior to that of ^{68}Ga . This has been demonstrated by PET imaging studies using Derenzo phantoms (supplemental data, Supplemental Fig. 5). Moreover, the longer half-life makes ^{44}Sc an attractive alternative to ^{68}Ga , allowing distribution of ^{44}Sc -labeled biomolecules over long distances.

It should also be recognized that ^{47}Sc exists as a chemically absolute identical therapeutic match to ^{44}Sc (34). ^{47}Sc decays by the emission of low-energy β^- -particles (average β^- energy [$E\beta^-_{\text{av}}$] = 162 keV) similar to ^{177}Lu ($E\beta^-_{\text{av}} = 134$ keV). The much shorter half-life of ^{47}Sc ($T_{1/2} = 3.35$ d), compared with ^{177}Lu ($T_{1/2} = 6.65$ d), may result in a lower dose burden to the patient. Moreover, it is important to recognize that ^{47}Sc also emits γ -rays of an ideal energy ($E\gamma = 159$ keV) for SPECT imaging. Direct monitoring of a therapeutic application of ^{47}Sc radiopharmaceuticals would be accessible as it is currently performed with ^{177}Lu -labeled compounds.

CONCLUSION

In this study, we demonstrated the feasibility to use cyclotron-produced ^{44}Sc of a high quality for the preparation of a ^{44}Sc -labeled folate conjugate, which was evaluated in a proof-of-concept in vivo study. Optimizations of the production process of ^{44}Sc to obtain higher quantities of activity is the topic of ongoing investigations. On the basis of the excellent features of ^{44}Sc for PET, it is likely that this radioisotope will find application in clinical nuclear imaging and pretherapeutic dosimetry of cancer patients, who would subsequently be treated with the ^{177}Lu -labeled pendant. Moreover, clinical implementation of ^{47}Sc as a therapeutic match would allow application of identical radiopharmaceuticals with equal pharmacokinetics. Future prospects with the matched pair $^{44}\text{Sc}/^{47}\text{Sc}$ for PET imaging and radionuclide therapy are de-

pendent on the realization of the theranostic principle and routine availability of these novel radioisotopes.

DISCLOSURE

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REFERENCES

- Kramer-Marek G, Capala J. Can PET imaging facilitate optimization of cancer therapies? *Curr Pharm Des*. 2012;18:2657–2669.
- Gabriel M, Decristoforo C, Kendler D, et al. ^{68}Ga -DOTA-Tyr³-octreotide PET in neuroendocrine tumors: comparison with somatostatin receptor scintigraphy and CT. *J Nucl Med*. 2007;48:508–518.
- Haug AR, Cindea-Drimus R, Auernhammer CJ, et al. The role of ^{68}Ga -DOTA-TATE PET/CT in suspected neuroendocrine tumors. *J Nucl Med*. 2012;53:1686–1692.
- Putzer D, Kroiss A, Waitz D, et al. Somatostatin receptor PET in neuroendocrine tumours: ^{68}Ga -DOTA⁰,Tyr³-octreotide versus ^{68}Ga -DOTA⁰-lanreotide. *Eur J Nucl Med Mol Imaging*. 2013;40:364–372.
- Kroiss A, Putzer D, Decristoforo C, et al. ^{68}Ga -DOTA-TOC uptake in neuroendocrine tumour and healthy tissue: differentiation of physiological uptake and pathological processes in PET/CT. *Eur J Nucl Med Mol Imaging*. 2013;40:514–523.
- Wild D, Bomanji JB, Benkert P, et al. Comparison of ^{68}Ga -DOTANOC and ^{68}Ga -DOTATATE PET/CT within patients with gastroenteropancreatic neuroendocrine tumors. *J Nucl Med*. 2013;54:364–372.
- Kwekkeboom DJ, de Herder WW, Kam BL, et al. Treatment with the radio-labeled somatostatin analog [^{177}Lu -DOTA⁰,Tyr³]octreotate: toxicity, efficacy, and survival. *J Clin Oncol*. 2008;26:2124–2130.
- Breeman WA, de Blois E, Sze Chan H, Konijnenberg M, Kwekkeboom DJ, Krenning EP. ^{68}Ga -labeled DOTA-peptides and ^{68}Ga -labeled radiopharmaceuticals for positron emission tomography: current status of research, clinical applications, and future perspectives. *Semin Nucl Med*. 2011;41:314–321.
- McCarthy DW, Shefer RE, Klinkowstein RE, et al. Efficient production of high specific activity ^{64}Cu using a biomedical cyclotron. *Nucl Med Biol*. 1997;24:35–43.
- Lewis JS, Lewis MR, Srinivasan A, Schmidt MA, Wang J, Anderson CJ. Comparison of four ^{64}Cu -labeled somatostatin analogues in vitro and in a tumor-bearing rat model: evaluation of new derivatives for positron emission tomography imaging and targeted radiotherapy. *J Med Chem*. 1999;42:1341–1347.
- Lewis JS, Lewis MR, Cutler PD, et al. Radiotherapy and dosimetry of ^{64}Cu -TETA-Tyr³-octreotate in a somatostatin receptor-positive, tumor-bearing rat model. *Clin Cancer Res*. 1999;5:3608–3616.
- Anderson CJ, Dehdashti F, Cutler PD, et al. ^{64}Cu -TETA-octreotide as a PET imaging agent for patients with neuroendocrine tumors. *J Nucl Med*. 2001;42:213–221.
- Wadas TJ, Eiblmaier M, Zheleznyak A, et al. Preparation and biological evaluation of ^{64}Cu -CB-TE2A-sst₂-ANT, a somatostatin antagonist for PET imaging of somatostatin receptor-positive tumors. *J Nucl Med*. 2008;49:1819–1827.
- Anderson CJ, Ferdani R. Copper-64 radiopharmaceuticals for PET imaging of cancer: advances in preclinical and clinical research. *Cancer Biother Radiopharm*. 2009;24:379–393.
- Roesch F. Scandium-44: benefits of a long-lived PET radionuclide available from the $^{44}\text{Ti}/^{44}\text{Sc}$ generator system. *Curr Radiopharm*. 2012;5:187–201.
- Filosofov DV, Loktionova NS, Rösch F. A $^{44}\text{Ti}/^{44}\text{Sc}$ radionuclide generator for potential application of ^{44}Sc -based PET-radiopharmaceuticals. *Radiochim Acta*. 2010;98:149–156.

17. Pruszyński M, Loktionova NS, Filosofov DV, Rösch F. Post-elution processing of $^{44}\text{Ti}/^{44}\text{Sc}$ generator-derived ^{44}Sc for clinical application. *Appl Radiat Isot.* 2010;68:1636–1641.
18. Zhernosekov K, Bunka M, Hohn A, Schibli R, Türlér A. Development and evaluation of ^{44}Ti production on high energy protons. *J Labelled Compd Rad.* 2011;54(suppl):S239.
19. Severin GW, Engle JW, Valdovinos HF, Barnhart TE, Nickles RJ. Cyclotron produced ^{44}Sc from natural calcium. *Appl Radiat Isot.* 2012;70:1526–1530.
20. Krajewski S, Cydzik I, Abbas K, et al. Simple procedure of DOTATATE labelling with cyclotron produced ^{44}Sc and ^{43}Sc . *Nucl Med Rev.* 2012;15: A22–A46.
21. Kamel A, Izabela C, Federica S, Seweryn K, Agata K, Aleksander B. Cyclotron production of ^{44}Sc : new radionuclide for PET technique. *J Labelled Compd Rad.* 2011;2011(suppl):S53.
22. Zhernosekov K, Bunka M, Schibli R, Türlér A. Development of ^{44}Sc production for radiopharmaceutical applications. *Radiother Oncol.* 2012;102(suppl 1): S141.
23. Müller C, Struthers H, Winiger C, Zhernosekov K, Schibli R. DOTA conjugate with an albumin-binding entity enables the first folic acid-targeted ^{177}Lu -radionuclide tumor therapy in mice. *J Nucl Med.* 2013;54:124–131.
24. Müller C, Mindt TL, de Jong M, Schibli R. Evaluation of a novel radiofolate in tumour-bearing mice: promising prospects for folate-based radionuclide therapy. *Eur J Nucl Med Mol Imaging.* 2009;36:938–946.
25. Ladino CA, Chari RVJ, Bourret LA, Kedersha NL, Goldmacher VS. Folate-maytansinoids: target-selective drugs of low molecular weight. *Int J Cancer.* 1997;73:859–864.
26. Parker N, Turk MJ, Westrick E, Lewis JD, Low PS, Leamon CP. Folate receptor expression in carcinomas and normal tissues determined by a quantitative radioligand binding assay. *Anal Biochem.* 2005;338:284–293.
27. Mathias CJ, Wang S, Lee RJ, Waters DJ, Low PS, Green MA. Tumor-selective radiopharmaceutical targeting via receptor-mediated endocytosis of gallium-67-deferoxamine-folate. *J Nucl Med.* 1996;37:1003–1008.
28. Müller C, Vlahov IR, Santhapuram HK, Leamon CP, Schibli R. Tumor targeting using ^{67}Ga -DOTA-Bz-folate: investigations of methods to improve the tissue distribution of radiofolates. *Nucl Med Biol.* 2011;38:715–723.
29. Pruszyński M, Majkowska-Pilip A, Loktionova NS, Eppard E, Roesch F. Radio-labeling of DOTATOC with the long-lived positron emitter ^{44}Sc . *Appl Radiat Isot.* 2012;70:974–979.
30. Koumariou E, Loktionova NS, Fellner M, et al. ^{44}Sc -DOTA-BN[2-14] NH_2 in comparison to ^{68}Ga -DOTA-BN[2-14] NH_2 in pre-clinical investigation: is ^{44}Sc a potential radionuclide for PET? *Appl Radiat Isot.* 2012;70:2669–2676.
31. Eigner S, Vera DR, Fellner M, et al. Imaging of protein synthesis: in vitro and in vivo evaluation of ^{44}Sc -DOTA-puromycin. *Mol Imaging Biol.* 2013;15: 79–86.
32. Liu S, Edwards DS. Bifunctional chelators for therapeutic lanthanide radiopharmaceuticals. *Bioconjug Chem.* 2001;12:7–34.
33. Majkowska-Pilip A, Bilewicz A. Macrocyclic complexes of scandium radionuclides as precursors for diagnostic and therapeutic radiopharmaceuticals. *J Inorg Biochem.* 2011;105:313–320.
34. Srivastava SC. Paving the way to personalized medicine: production of some promising theragnostic radionuclides at Brookhaven National Laboratory. *Semin Nucl Med.* 2012;42:151–163.



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Promises of Cyclotron-Produced ^{44}Sc as a Diagnostic Match for Trivalent β^- -Emitters: In Vitro and In Vivo Study of a ^{44}Sc -DOTA-Folate Conjugate

Cristina Müller, Maruta Bunka, Josefine Reber, Cindy Fischer, Konstantin Zhernosekov, Andreas Türlér and Roger Schibli

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