Supplemental Data

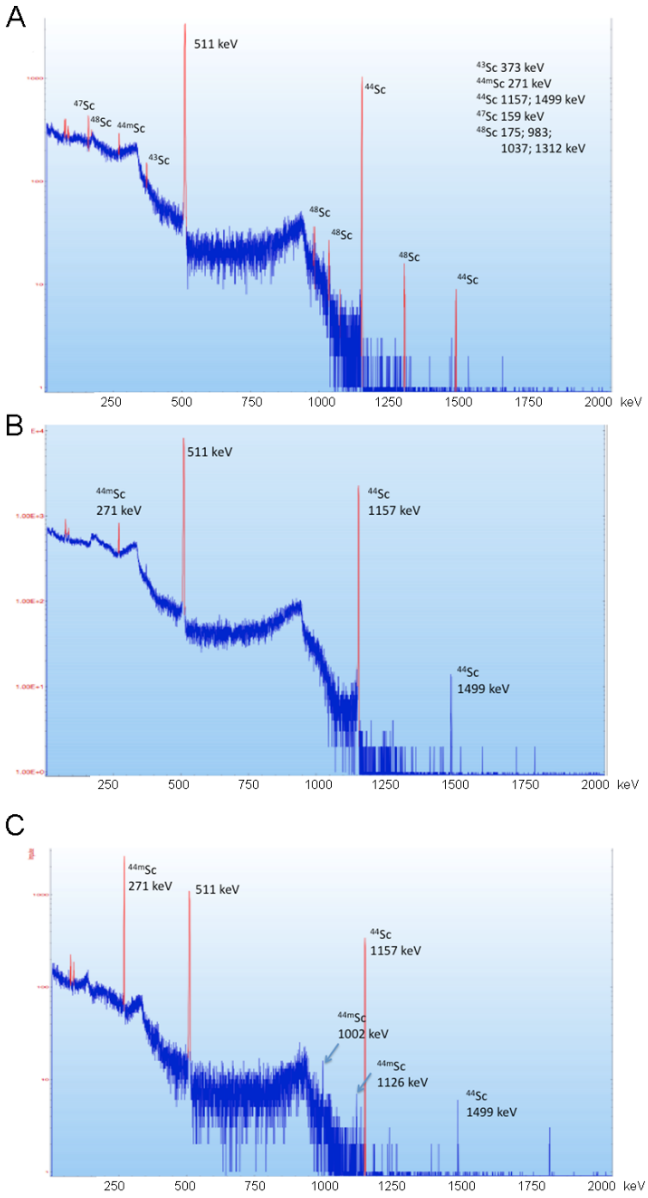
1. γ-Ray Spectrometry of Irradiated ^{44}Ca-Targets

Purpose. To produce ^{44}Sc via the ^{44}Ca(p,n)^{44}Sc-nuclear reaction at a cyclotron, targets that contain either natural calcium (^{nat}Ca, mixture of Ca-isotopes) or enriched calcium (^{44}Ca) can be irradiated (*1-4*). In order to investigate the difference among these possibilities we analyzed the composition of radioactive isotopes upon irradiation of targets composed of either $^{nat}CaCO_3$ or $^{44}CaCO_3$.

Experimental. For preparation of the targets, $^{nat}CaCO_3$ (200 mg, purum p.a., Fluka AG) or $^{44}CaCO_3$ (5-10 mg, 97.0%, Trace Sciences International) combined with graphite powder (150 mg, 99.9999%, Alfa Aesar) was pressed by a target press device and encapsulated in aluminum. Targets were irradiated for 30-40 min at the cyclotron facility at PSI using a proton energy of 17.6 ± 1.8 MeV and a beam current of 50 μA. Next, the irradiated $CaCO_3$-targets were dissolved in 3 M HCl. Five hours (and 48 h) after the end of the irradiation (end of beam) aliquots of 1 mL were taken and analyzed by γ-ray spectrometry using an N-type high-purity germanium (HPGe) coaxial detector (EURISYS MESURES) and the Ortec InterWinner 5.0 software to determine the amount of ^{44}Sc and co-produced radioisotopes.

Results and Conclusion of the γ-Spectrometry. Because of the natural abundances of calcium isotopes in ^{nat}Ca (^{40}Ca: 96.94%; ^{42}Ca: 0.65%; ^{43}Ca: 0.14%, ^{44}Ca: 2.09%; ^{46}Ca: 0.004%; and ^{48}Ca: 0.19%) irradiation of $^{nat}CaCO_3$-targets resulted in the production of ^{44}Sc along with other Sc-radioisotopes (^{43}Sc, 4%, $t_{1/2}$ = 3.89 h; ^{44m}Sc, 0.7%, $t_{1/2}$ = 58.6 h; ^{47}Sc, 0.7%, $t_{1/2}$ = 80.4 h; and ^{48}Sc, 0.7%, $t_{1/2}$ = 43.7 h) as shown on the γ-spectrum obtained from aliquots of dissolved $^{nat}CaCO_3$-target material upon irradiation (Supplemental Fig. 1A). The γ-spectrum of the irradiated enriched $^{44}CaCO_3$-target material showed high radionuclide purity with less than 1% of the co-produced longer-lived ^{44m}Sc (γ-line of 271 keV) as the only by-product (Supplemental Fig. 1B). After 48 h (> 10 half-lives of ^{44}Sc) the γ-spectrum of the same aliquot showed only remaining ^{44m}Sc and its decay product ^{44}Sc which proved the excellent radiochemical purity (Supplemental Fig. 1C).

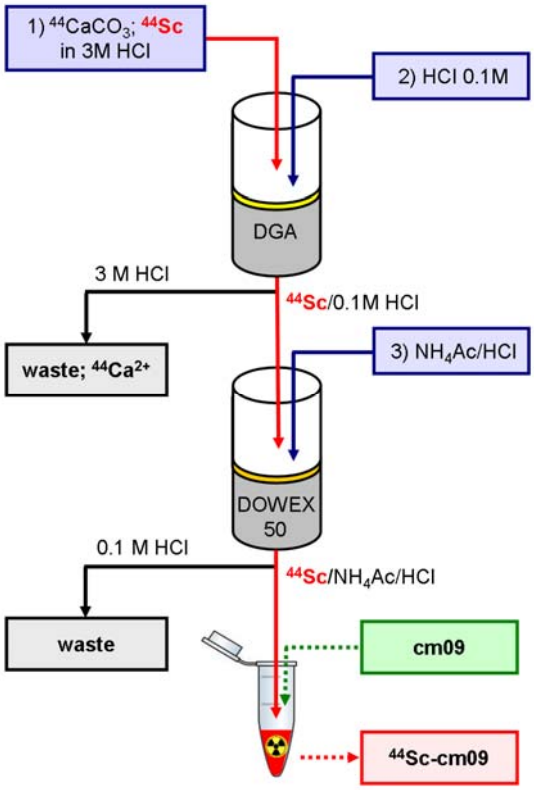
Based on these results it was concluded that the production of ^{44}Sc from enriched ^{44}Ca-targets is the preferred method. As mentioned above, ^{44}Sc which is produced from enriched ^{44}Ca-targets, is obtained in much higher radiochemical purity compared to ^{44}Sc produced from natural Ca-targets. A further advantage of using enriched ^{44}Ca-targets is the much lower amount of $^{44}CaCO_3$ (5-10 mg) which is required to obtain the same amount of ^{44}Sc as would be required if $^{nat}CaCO_3$ (200 mg) was irradiated. A smaller amount of $CaCO_3$ is more favorable with regard to the separation procedure of ^{44}Sc(III) from Ca(II).



SUPPLEMENTAL FIGURE 1. (A) γ-Spectrum of a sample (1 mL) of irradiated $^{nat}CaCO_3$ target material dissolved in HCl (3 M) 5 h after end of beam. (B) γ-Spectrum of a sample (1 mL) of irradiated enriched $^{44}CaCO_3$ target material dissolved in HCl (3 M) 5 h after end of beam and (C) 48 h after end of irradiation.

2. Separation of ^{44}Sc from Irradiated ^{44}Ca-Targets

For separation of ^{44}Sc from irradiated ^{44}Ca-targets we developed a semi-automated separation system which was based on extraction chromatography and cation exchange chromatography. The experimental procedure is described in the main manuscript. A schematic illustration of the separation process is given in Supplemental Figure 2.



SUPPLEMENTAL FIGURE 2. Schematic illustration of the isolation of ^{44}Sc from proton-irradiated ^{44}Ca-targets (DGA = *N,N,N',N'*-tetra-*n*-octyldiglycolamide).

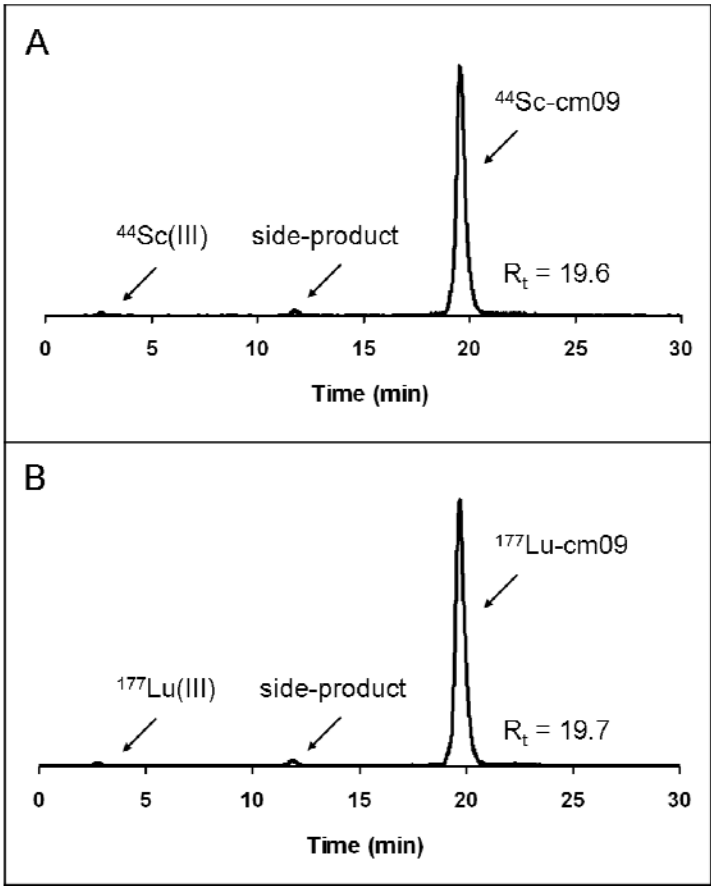
3. In Vitro Comparison of ^{44}Sc-cm09 and ^{177}Lu-cm09

Purpose. Formation of DOTA-complexes with ^{44}Sc(III) and ^{177}Lu(III) are supposed to occur according to the same coordination chemistry (*5*). In contrast to ^{68}Ga-DOTA-complexes, which differ significantly from ^{177}Lu-DOTA complexes, similar chemical characteristics of ^{44}Sc(III) and ^{177}Lu(III) would allow comparison of biomolecules labeled with either of these radioisotopes (*6*). In order to compare the characteristics of ^{44}Sc-cm09 with ^{177}Lu-cm09 we investigated the retention times of the compounds using the same HPLC method and determined the *n*-octanol/PBS distribution coefficients (LogD values). Both parameters are dependent on the hydrophilicity of a specific radioconjugate.

Experimental. High-performance liquid chromatography (HPLC) was performed using a C-18 reversed phase column (Xterra MS C18, 5 μm, 15 cm x 4.6 cm, Waters) with a mobile phase consisting 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 and a flow rate of 1 mL/min. The experimental procedure for the determination of the logD value is reported in the main manuscript and in previous publications (*7, 8*).

Results and Conclusion of the In Vitro Comparison of ^{44}Sc-cm09 and ^{177}Lu-cm09. Representative HPLC chromatograms of ^{44}Sc-cm09 and ^{177}Lu-cm09 are shown in Supplemental Fig. 3. The retention time of ^{44}Sc-cm09 (R_t = 19.6 min, Supplemental Fig. 3A) was about the same as obtained for ^{177}Lu-cm09 (R_t = 19.7 min, Supplemental Fig. 3B). The logD value of ^{44}Sc-cm09 was -4.49 ± 0.12 as reported in the main manuscript. This value was similar to the logD value of ^{177}Lu-cm09 (-4.25 ± 0.41) (*7*).

These results indicate similar characteristics of ^{44}Sc-cm09 and ^{177}Lu-cm09 with regard to their hydrophilicity. These findings are in agreement with the theory of identical coordination chemistry among the two radioisotopes ^{44}Sc and ^{177}Lu.



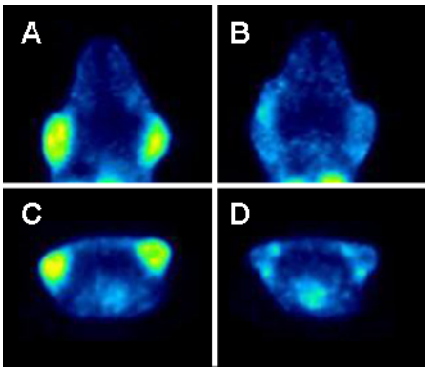
SUPPLEMENTAL FIGURE 3. Representative HPLC chromatograms of ^{44}Sc-cm09 (A) and ^{177}Lu-cm09 (B).

4. Additional PET Imaging Studies with ^{44}Sc-cm09

Purpose. In order to investigate FR specific accumulation of ^{44}Sc-cm09 in KB tumor xenografts, we also performed PET imaging studies with a tumor bearing mouse which was injected with excess folic acid prior to the radiotracer. It was expected that blockade of FRs by folic acid would result in a significant decline of ^{44}Sc-cm09 uptake in the tumor tissue.

Experimental Procedure. Imaging studies were performed with nude mice ~ 14 days after KB tumor cell inoculation. ^{44}Sc-cm09 (~ 25 MBq, ~ 6 nmol per mouse, 100 μL) was injected either as a single agent or immediately after injection of excess folic acid (100 μg in 100 μL PBS). PET scans of 30 min duration were performed with each of the two mice 4 h after injection of the radioconjugate.

Results and Conclusion of Additional PET Imaging Studies with ^{44}Sc-cm09. PET images of a mouse which received folic acid prior to the injection of ^{44}Sc-cm09 showed reduced uptake of radioactivity in the tumor xenografts compared to the tumor uptake of ^{44}Sc-cm09 found in the mouse which received only ^{44}Sc-cm09. These results confirmed FR specific uptake of ^{44}Sc-cm09 since injection of excess folic acid blocked FRs and, hence, prevented uptake of ^{44}Sc-cm09 (Supplemental Fig. 4).



SUPPLEMENTAL FIGURE 4. PET images as coronal (A, B) and transaxial sections (C, D) of KB tumor bearing mice injected with ^{44}Sc-cm09 only (A, C) or with ^{44}Sc-cm09 and preinjected folic acid to block FRs (B, D).

5. In Vivo Biodistribution Data of ^{177}Lu-cm09

The tissue distribution data of ^{177}Lu-cm09 in KB tumor bearing nude mice at different time points after injection are given in Supplemental Table 1.

SUPPLEMENTAL TABLE 1

Biodistribution of ^{177}Lu-**cm09** in KB Tumor Bearing Female Nude Mice (*7*).

	^{177}Lu-cm09			
	% injected dose per gram tissue*			
	1 h p.i.	2 h p.i.	4 h p.i.	24 h p.i.
Blood	8.15 ± 1.21	6.76 ± 0.95	4.38 ± 0.95	1.22 ± 0.19
Lung	4.58 ± 0.83	3.71 ± 0.65	2.70 ± 0.24	1.03 ± 0.20
Spleen	1.59 ± 0.25	1.50 ± 0.42	1.18 ± 0.19	0.63 ± 0.16
Kidneys	15.79 ± 2.13	23.23 ± 1.49	28.05 ± 1.35	30.09 ± 4.04
Stomach	2.04 ± 0.11	1.89 ± 0.59	1.45 ± 0.25	0.70 ± 0.14
Intestines	1.43 ± 0.31	1.07 ± 0.24	0.90 ± 0.20	0.29 ± 0.11
Liver	4.40 ± 0.14	3.98 ± 0.65	3.86 ± 0.65	1.80 ± 1.54
Salivary glands	6.78 ± 1.41	6.35 ± 1.05	6.23 ± 0.69	3.64 ± 0.49
Muscle	1.30 ± 0.06	1.31 ± 0.04	1.26 ± 0.06	0.96 ± 0.22
Bone	1.48 ± 0.08	1.49 ± 0.16	1.23 ± 0.14	0.62 ± 0.13
Tumor	10.84 ± 1.32	14.67 ± 1.65	18.12 ± 1.80	19.46 ± 3.13
Tumor-to-blood	1.36 ± 0.27	2.18 ± 0.18	4.32 ± 1.15	16.02 ± 1.52
Tumor-to-liver	2.47 ± 0.31	3.71 ± 0.30	4.73 ± 0.39	7.77 ± 0.62
Tumor-to-kidney	0.69 ± 0.04	0.63 ± 0.06	0.65 ± 0.07	0.65 ± 0.07

* values shown represent the mean ± S.D. of data from three animals (n=3) per cohort

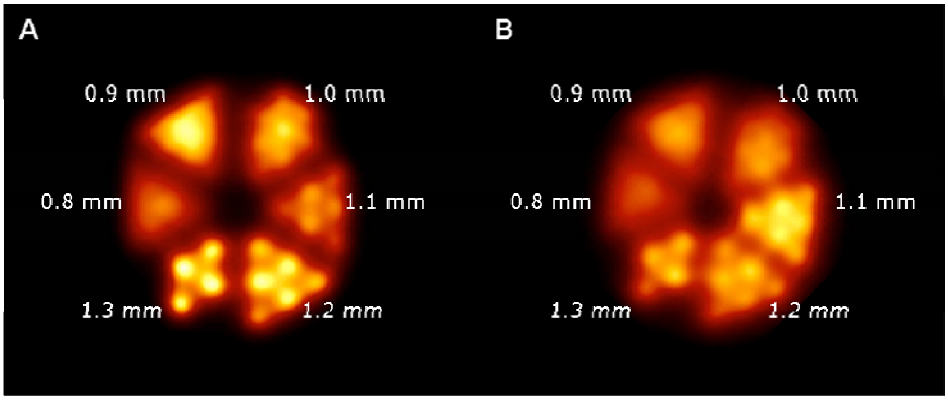
6. Comparison of ^{44}Sc and ^{68}Ga PET Using Derenzo Phantoms

Purpose. In order to investigate the resolution and PET image quality of ^{44}Sc and ^{68}Ga we performed a site-by-site comparison of these two radioisotopes using Derenzo phantoms and a small-animal PET scanner.

Experimental Procedure. PET scans of Derenzo phantoms were performed with a dedicated small-animal PET/CT camera (Vista eXplore, GE Healthcare). Two Derenzo phantoms with hole-diameters ranging from 0.8 to 1.3 mm, in 0.1-mm steps

were filled with ~ 9 MBq of ^{68}Ga(III) and ~ 9 MBq of ^{44}Sc(III), respectively, in a volume of 600 μL. Static PET scans of 30 min duration were acquired of each phantom. Reconstruction of PET data was performed using the instrument's software. For reconstruction, the 2-dimensional ordered-subset expectation maximization (2D-OSEM) algorithm was used.

Results and Conclusion Comparison of ^{44}Sc and ^{68}Ga PET Using Derenzo Phantoms. PET imaging of a Derenzo phantom filled with ^{44}Sc revealed an excellent resolution of ~1.2 mm (Supplemental Fig. 5A). These findings were similar or even superior to those obtained with an equivalent Derenzo phantom filled with about the same amount of ^{68}Ga-radioactivity (Supplemental Fig 5B).



SUPPLEMENTAL FIGURE 5. PET images of Derenzo phantoms. A: ^{44}Sc (~ 9 MBq, scan time: 30 min) and B: ^{68}Ga (~ 9 MBq, scan time: 30 min).

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