SUPPLEMENTAL MATERIALS AND METHODS

Image J analysis of microSPECT images

The tumor volume on the microSPECT images was first selected by setting the threshold, then measured using "Plugins\3D object counter". The mean intensity of the selected tumor area of 83 slices was determined using "Analyze\Measure", and then summed. The ratio of the summed mean intensity vs. tumor volume was proportional to the relative radioactivity per gram of tumor, since the optical density of the image is proportional to the radioactivity, while the tumor mass is the volume times the density. Then, the relative radioactivity per gram of tumor was calculated by dividing the ratio of the summed mean intensity vs. the tumor volume at 0, 6, 24 and 48 h and plotted vs. the time p.i. of ^{177}Lu-T-AuNP or ^{177}Lu-NT-AuNP.

Radiation dosimetry S-values

The S-value for the whole tumor was calculated using Monte-Carlo N-Particle (MCNP) modeling assuming the tumor as a 6 mm diameter sphere in which ^{177}Lu was distributed homogenously in the sphere. The spectra of negatrons (β^-), Auger and IC electrons, X-rays and γ-rays were taken from MIRD Radionuclide Data (*1*), and were included in the MCNP input file to be directly sampled during radiation transport simulation. The energy deposition per starting particle in the sphere was tallied. The absorbed dose to the tumor per starting particle was calculated by dividing the deposited energy by the mass of the tumor. For each simulation, $1 \times 10^4 - 1 \times 10^6$ particles were launched to reach a standard deviation less than 5% for all energy deposition results. All emitted electrons with energy lower than 1 keV were assumed to deposit all of the energy locally within the tumor sphere.

MCNP was used to simulate the β^- transport and deposition of energy within each voxel of $0.3 \times 0.3 \times 0.3$ mm^3 cube in tumor. Tumor was assumed as a $6.3 \times 6.3 \times 6.3$ mm^3 cube, which was divided into $21 \times 21 \times 21$ voxels of $0.3 \times 0.3 \times 0.3$ mm^3 cube. The center of the central voxel was located at (3.15, 3.15, 3.15 mm). The central voxel was defined as the source in which ^{177}Lu was distributed homogenously, and all the $21 \times 21 \times 21$ voxels as targets. The spectra of negatrons, were taken from MIRD Radionuclide Data (*1*), and were included in the MCNP input file to be directly sampled during radiation transport simulation. The contribution of Auger and IC electrons, X-rays and γ-rays to the absorbed dose to each voxel was negligible compared to negatrons, and were thus neglected. The energy deposition per starting particle in each voxel was tallied. The absorbed dose to each voxel per starting particle was calculated by dividing the deposited energy by the mass of the voxel, assuming the density of tumor as 1.02 g/cm^3. For each simulation, 1×10^6 particles were launched to reach a standard deviation <5%. All emitted electrons with energy lower than 1 keV were assumed to deposit all of this energy locally within the central voxel (source), in which ^{177}Lu was located. This MCNP simulation produces an array of 21 x 21 x 21 S-values with the voxel at the center of the array as the source and the $21 \times 21 \times 21$ voxels as targets (S111111).

The S-values for the sources other than the central voxel, which were 9260 ($=21 \times 21 \times 21$-1), and targets which were the same of $21 \times 21 \times 21$ voxels, were generated using MATLAB R2014b (MathWorks, Natick, MA, USA). First, the $21 \times 21 \times 21$ array (S111111) was set as the center, and then expanded to a 41 x 41 x 41 array by completing the remaining array with zeros. While the position of the source shifted by one voxel along

either x, y or z direction, the corresponding array of S value was obtained by "circshift" of the array one position along the corresponding dimension of the array. At the end, after 9260 steps of circshift along all three dimensions, a total of 9260 arrays of S-values were obtained. The sides of all the 9261 '41 x 41 x 41' arrays were further removed to obtain the central '21 x 21 x 21' arrays.

Calculation of cumulative radioactivity from microSPECT images

MicroSPECT images of tumors treated with ^{177}Lu-T-AuNP and ^{177}Lu-NT-AuNP taken at 48 h p.i. was used to estimate the radioactivity within each voxel of 21 × 21 × 21 array. Each microSPECT image of tumor contained 83 slices (37.20 × 37.20 mm^2) along the Z-axis, parallel to the mouse spine, and with pixel width, height, depth each of 0.3 mm. Raw data of microSPECT images of tumor were saved as a sequence of 83 images in tiff format using ImageJ, then processed using MATLAB. They were first transferred to 83 matrix of 124 × 124 unit 16 using 'imread'. Then 83 matrixes were concatenated along z dimension to form an array of 124 × 124 × 83 unit 16, followed by conversion to double precision. All the elements of this array were summed up as total optical density (440450 for ^{177}Lu-NT-AuNP and 497593 for ^{177}Lu-T-AuNP). Based on the injected dose (4.5 MBq) of ^{177}Lu-NT-AuNP and ^{177}Lu-T-AuNP, as well as their biological elimination (66% and 57 %, respectively) and physical (79%) at 48 h, the total radioactivity retained in the tumor at 48 h p.i. was estimated to be 1.24 and 1.57 MBq, respectively. Thus a conversion factor was further derived as 2.81 and 3.15 Bq/optical density for ^{177}Lu-NT-AuNP and ^{177}Lu-T-AuNP, respectively. Assuming there was only physical decay after 48 h p.i., the accumulated radioactivity from 48 h p.i to infinity was the radioactivity at 48 h p.i. divided

by ^{177}Lu decay constant (1.2 x 10^{-6} s^{-1}). Each element of the array was multiplied by the conversion factor, then divided by the decay constant to give an array of accumulated radioactivity. The 124 × 124 × 83 cumulative radioactivity array was further trimmed down to a 21 × 21 × 21 array with the injection site at the center of the array.

Calculation of dose distribution and potting of tumor dose and radioactivity map

The absorbed dose to 21 × 21 × 21 voxels by ^{177}Lu within each of 21 × 21 × 21 voxel was calculated by multiplying each element of corresponding S-values by the accumulated radioactivity within each voxel, followed by summing the resulting 21 × 21 × 21 arrays. This led to a 21 × 21 × 21 array consisted of absorbed dose values at each voxel. The distribution of radioactivity and the absorbed dose on a planar section of tumor perpendicular to the line of injection was plotted to show the 10 level filled contour of iso-radioactivity and iso-dose using MATLAB.

Dose selection and normal organ toxicity study of ^{177}Lu-T-AuNP

A short-term observation (15 d) study was conducted to select the amount of ^{177}Lu-T-AuNP for a long-term observation (90-120 d) treatment study. Groups of 3-5 CD-1 athymic mice with s.c. MDA-MB-468 tumors were injected i.t. with 0.3-4.5 MBq of ^{177}Lu-T-AuNP (2-6 × 10^{11} AuNP) in 30 μL of normal saline using an Ultra-fine 1cc insulin syringe (U-100 with 29G1/2" needle; Becton Dickinson, Franklin Lakes, NJ, USA). Control mice were injected with unlabeled-T-AuNP or normal saline. Tumor length and width were measured independently twice per week by two observers (S.Y. and Z.C) using

digital calipers (VWR, Mississauga, ON, Canada) and mean values for these dimensions calculated. Tumor volume (mm^3) was calculated from the mean values as volume = (length × $width^2$)/2. The tumor growth index (TGI) for treated and control mice was calculated by dividing the tumor volume at each time point by the initial tumor volume prior to treatment. Similarly, toxicity was assessed by measuring body weight twice per week and calculating a body weight index (BWI) by dividing the body weight at each time point by the initial body weight. The mean TGI and BWI were plotted vs. the time post-treatment. At 15 d, blood samples were collected into EDTA-coated microcapillary tubes (Sarstedt, Nümbrecht Germany) for a complete blood cell (CBC) count and into clot-activating microcapillary tubes (Sarstedt) for measurement of serum alanine aminotransferase (ALT) and creatinine (Cr). CBC was measured on a HEMAVET 950FD analyzer (Drew Scientific, Dallas, TX). Serum Cr and ALT were measured with Infinity Creatinine or ALT clinical chemistry kits (Fisher Diagnostics, Middletown, VA).

SUPPLEMENTAL RESULTS

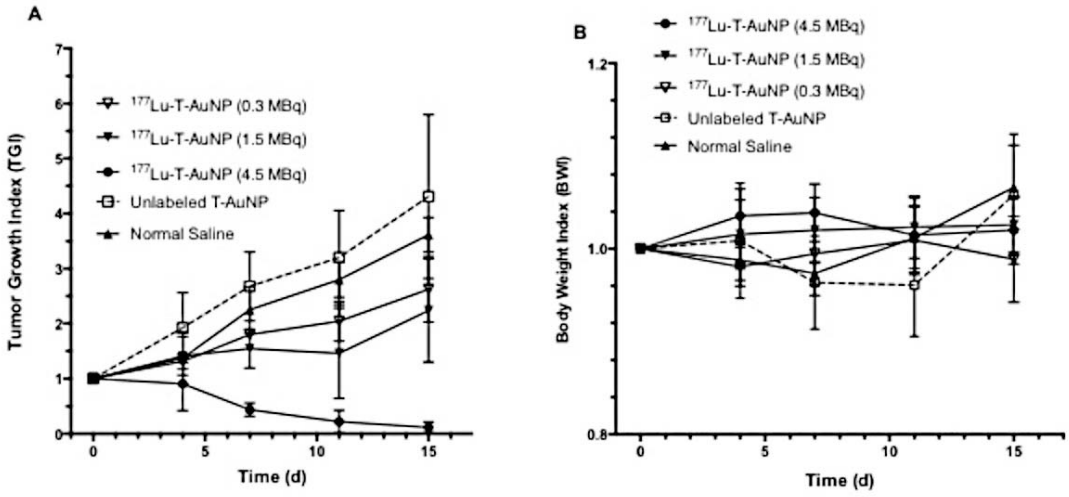
Radiation dosimetry calculations

For a description of the results of radiation dosimetry calculations, see the Results section of the main article.

Dose selection and normal organ toxicity study

The effects of i.t. injection of ^{177}Lu-T-AuNP on the TGI in CD-1 athymic mice bearing s.c. MDA-MB-468 tumor xenografts over a 15 d period is shown in Supplemental

Fig. 1A. The TGI at 15 d in mice injected with 0.3, 1.5 or 4.5 MBq of ^{177}Lu-T-AuNP was 1.4-fold (P=0.02), 1.9-fold (P=0.01) and 32.2-fold (P<0.001) significantly lower than for mice treated with normal saline and 1.6-fold (P=0.1), 1.9-fold (P=0.02) and 38.4-fold (P<0.001) significantly lower than for mice treated with unlabeled T-AuNP (Fig. 5A). There was no significant difference in the BWI at 15 d in mice injected with ^{177}Lu-T-AuNP, unlabeled T-AuNP or normal saline (Supplemental Fig. 1B). All mice maintained or gained weight. There were no significant changes in white blood cell (WBC) or red blood cell (RBC) counts, hemoglobin (Hb) or hematocrit (Hct) or increased serum ALT or Cr in mice injected with any amount of ^{177}Lu-T-AuNP compared to mice receiving unlabeled T-AuNP or normal saline (see Table 2 in the main article).



SUPPLEMENTAL FIGURE 1. (A) Effect of i.t. injection of increasing amounts of ^{177}Lu-T-AuNP, unlabeled AuNP or normal saline on the tumor growth index (TGI) for CD-1 athymic mice with s.c. MDA-MB-468 human breast cancer xenografts. (B) Effect of these treatments on the body weight index (BWI). Values shown represent the mean ± SD (n = 4-6)

SUPPLEMENTAL REFERENCES

1. Weber DA, Eckerman KF, Dillman LT, Ryman JC. *MIRD:Radionuclide data and decay schemes*. New York: The Society of Nuclear Medicine; 1989.