Materials

Lipids 1,2-diarachidoyl-sn-glycero-3-phosphocholine (20PC), 1,2-dipalmitoyl-sn-glycero-3-phospho-L-serine (sodium salt) (DPPS), 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-(Lissamine Rhodamine B Sulfonyl) (Ammonium Salt) (DPPE-Rhodamine), and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000](ammonium salt) (DSPE-PEG) were purchased from Avanti Polar lipids (Alabaster, AL). Cholesterol, Sepharose 4B, Sephadex G50, sodium bicarbonate, diethylenetriaminepentaacetic acid (DTPA), calcium ionophore A23187, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), poly(2-hydroxyethyl methacrylate) (PolyHEMA), ascorbic acid, sodium carbonate, Trizma base, crystal violet, sucrose, Matrigel™ were purchased from Sigma-Aldrich Chemicals (Atlanta, GA). Phosphate buffered saline (PBS) was purchased from Quality Biological (Gaithersburg, MD). Dimethylformamide (DMF) was purchased from Avantor Performance Materials (Center valley, PA). Ethylenediaminetetraacetic acid, disodium salt dihydrate (EDTA), BCA protein assay, trypsin, penicillin-streptomycin, and 2-propanol (IPA) were purchased from Fisher Scientific (Pittsburgh, PA). Hybricare medium and the BT474 cell line were both obtained from ATCC. Fetal Bovine Serum (FBS) was obtained from Omega Scientific (Tarzana, CA).

Methods

Liposome formation and radiolabeling

Liposomes composed of 20PC:DPPS:cholesterol:DSPE-PEG-DAP:DPPE-Rhodamine at 0.61:0.26:0.04:0.09:0.001 mole ratio and encapsulating 12.5mM DOTA in Citric acid buffer (150mM, pH 5.0) were formed using the thin film hydration method (6). Briefly, lipids (5 μmoles) were dried in round bottom flasks, and the film was hydrated with 375 μL Citric acid buffer (150 mM, pH 5.0), DOTA (125 μL, 20 mg/mL) and ascorbic acid (15 μL, 0.8M) and annealed for two hours at a temperature above 75°C. For loading of ^{111}In, liposomes encapsulated 2mM DTPA and

0.12mM Ascorbic acid in PBS (300mOsm pH 7.6). Lipid suspensions were then extruded 21 times through two stacked polycarbonate membranes with pore sizes of 100 nm and were then passed through a Sepharose 4B column equilibrated with HEPES buffer (20 mM, pH 7.4, 300 mOsm) to remove unencapsulated DOTA (or DTPA).

Then, the sample was incubated with a mixture of ^{225}Ac (dissolved in 0.2 N HCl) or ^{111}In, 25 µL of calcium ionophore A23187 in ethanol at 15.6 mg/mL, and 25 µL of water. The liposomes were incubated at 75°C for 1 hour, after which, the sample was allowed to cool. Upon reaching room temperature, the liposomes were passed through a Sephadex G-50 column to remove unentrapped ^{225}Ac or ^{111}In. The loading yield was calculated from a ratio of the activities before and after the Sephadex G-50 column, after reaching secular equilibrium (for ^{225}Ac), using an automated γ-counter (Cobra II Auto-Gamma, Packard Instrument Co., Inc).

Antibody radiolabeling

Trastuzumab was labeled with ^{225}Ac (or ^{111}In) using the “one step” method, and all buffers were prepared using metal-free water (6). First DOTA-SCN was conjugated to the antibody; in particular, DOTA-SCN was dissolved in DMF at 10 mg/mL and added drop wise to 0.5 mg/mL ice cold antibody solution in 0.1 M sodium carbonate buffer at pH 9.0 to achieve a 40:1 chelator/antibody ratio (DTPA-SCN at 20 mg/mL in DMSO at a 15:1 chelator:antibody ratio for ^{111}In labeling). This reaction was left to continue overnight at room temperature on a plate shaker. Upon completion of the reaction, non-conjugated DOTA-SCN was separated from DOTA-SCN-Trastuzumab conjugates using a 10-DG size exclusion column equilibrated with 0.1M Tris buffer at pH 9.0. Similarly, non-conjugated DTPA-SCN was separated from DTPA-SCN-Trastuzumab using a 10-DG column equilibrated with 1M acetate buffer, pH 4.5. Final antibody concentration was confirmed using BCA Assay.

Actinium-225 dissolved in 0.2M HCl was added to at least 100 µg antibody in 500 µL of 0.1M Tris buffer at pH 9.0 (or 1M acetate buffer at pH 4.5 for ^{111}In) and was allowed to incubate for 1 hour at 37°C. Free/non-chelated ^{225}Ac was removed by the addition of 50 µL of 10 mM DTPA. After 5 minutes, the sample was purified by a 10-DG column equilibrated with PBS and 1mM EDTA at pH 7.4 (6).

Radiochemical purity of ^{225}Ac-DOTA-SCN-labeled antibody was assessed using iTLC. Briefly, a spot of ^{225}Ac-DOTA-SCN-antibody was added towards the bottom of a strip of filter paper and allowed to dry completely. The iTLC strip was then vertically placed in a beaker with 10 mM EDTA in water (mobile phase), ensuring that the spot was not below the fluid level (6). The immunoreactivity was evaluated by incubation for 1 hour, on ice, with BT474 cells at 100x excess of HER2 receptors in the presence and absence of blocking by the cold antibody. Stability of radiolabeling was assessed by measuring the retained activity of the antibody after incubation in cell culture media at 37°C for 24 hours. Unbound activity was separated using a 10-DG column equilibrated with PBS at pH 7.4.

Antibody labeling with fluorophores

Trastuzumab was labeled with Alexa Fluor 647 NHS Ester (ex 651 nm; em 672 nm) in a 5:1 dye to antibody molar ratio in accordance with Thermo Fisher. Briefly, 2 mg/ml of Trastuzumab in 0.1M sodium bicarbonate at pH 8.3 was incubated with Alexa 647 for 2 hours. Unlabeled Alexa Fluor 647 was separated from labeled antibody using a 10DG column equilibrated with PBS at pH 7.4.

Clonogenic survival

Cell monolayers of 500,000 cells/well were plated in 6-well plates and were allowed to adhere overnight. After incubation for 6 hours with varying concentrations of radioactivity, the

cells were washed, scraped and resuspended in media in 3 different densities, and were plated in dishes to grow until the untreated cells formed colonies of > 50 cells per colony. Colonies were stained and counted (6), and the survival fraction was determined by normalizing the counted number of treated colonies to the number of untreated colonies, accounting for the plating efficiency (6).

Spatiotemporal Profiles in Spheroids

Liposomes labeled with 1 mole% DPPE-Rhodamine (ex/em: 550/590 nm) were loaded with CFDA-SE (ex/em: 497/517nm; used as the hydrophilic drug surrogate) and incubated with spheroids at 5mM total lipid. Trastuzumab was labeled with AlexaFluor647 NHS Ester (ex/em: 651/672nm) as described in the Supporting Information, and was incubated with spheroids at 20μg/ml.

Spheroids were incubated with fluorescently-labeled liposomes and/or the antibody for 6 and/or 24 hours, respectively. At different time points during incubation and after being moved to wells with fresh media, spheroids were sampled, immediately frozen in cryochrome gel, cryosectioned in 20 μm-thickness slices, and the equatorial slices were imaged and analyzed using an eroding code. The spatial distributions at each time point were integrated (using the trapezoid rule) to evaluate the are-under-the-curve (AUC) for each position within the spheroids. Spheroids without treatment were frozen similarly and used as background. Calibration curves for the concentration of liposomes, CFDA-SE, and/or the fluorescently-labeled antibody were performed with known concentrations in a quartz cuvette of 20 μm optical pathlength using the same microscope.

An inhouse eroding code in Matlab was used to determine the average intensity of each 5 μm concentric ring of the spheroid, moving from the edge to the core of the spheroid.

MRI-based measurement of tumor pH_e (verbatim from SI of (13))

In vivo MRI images were acquired on a Bruker BioSpec 9.4 T horizontal MR scanner using a home-built solenoid coil placed around the subcutaneous tumors. Animals were anaesthetized by inhalation of 2% isoflurane in oxygen and maintained with 1.0 to 1.5% isoflurane during the experiment. Anesthetized animals were fixed with tape to the plexiglass animal-holder to minimize breathing-related motion. The physiological state of the animal was monitored throughout the MRI experiment using a pneumatic pillow sensor recording the respiratory rate of the animal, that was kept around 30-50 bpm. This setup was then positioned in the isocenter of the magnet.

Briefly, anatomical T2 weighted spin-echo images were acquired in the three orthogonal directions using Rapid Acquisition Relaxation Enhancement (RARE) sequence. The following parameters were used: repetition time (TR) = 2000 ms, echo time (TE) = 48 ms, RARE factor = 8, 1 averages, field of view (FOV) = 40 x 40 mm, acquisition matrix (Mtx) = 128 × 128; 156 × 156 μm^2 in plane resolution, slice thickness = 1 mm and 12 slices. These slices were used to localize the 4 mm thick ^1H magnetic resonance spectroscopic imaging (MRSI) coronal slice. MRSI was acquired using a chemical shift imaging (CSI) sequence following administration of 200 μl i.p. of the pHe probe [(±)2-(imidazol-1-yl)succinic acid] (ISUCA) at a dose of 3 mg/g. Acquisition parameters were: FOV = 16 × 16 mm, CSI matrix = 8 × 8 zero-filled to 16 × 16, (final in-plane resolution 1000 x 1000 μm^2), slice thickness = 4 mm, TR = 1000 ms, TE =48 ms and 16 repetitions acquired during 17.2 min. Water suppression was performed with a Variable Pulse Power and Optimized Relaxation Delays (VAPOR) sequence. An anatomical T2 weighted image was acquired with the same geometry than the MRSI to be used as a reference. The following parameter were used: RARE sequence, TR = 2000 ms, TE = 32 ms, RARE factor = 8, 1 averages, FOV = 16 x 16 mm, acquisition matrix (Mtx) = 128 × 128; 125 × 125 μm^2 in plane resolution, slice thickness = 4 mm.

pHe map analysis

MRSI images were reconstructed using 3DiCSI 1.9.9 software (Hatch Center for MR Research, Columbia University, New York, NY). pHe maps were obtained as previously described (11). In the presence of ISUCA, Lorentzian line shapes were fitted to the H2 peaks to obtain the H2 chemical shifts using the endogenous choline resonance as reference (3.21 ppm). Only those voxels with signal-to-noise ratio (SNR) higher than two were considered. Extracellular pH was then determined in each voxel from the chemical shift of the H2 peak using the Henderson-Hasselbalch equation:

$$pH = pk_a - log\frac{\delta_1 - \delta}{\delta - \delta_2}$$

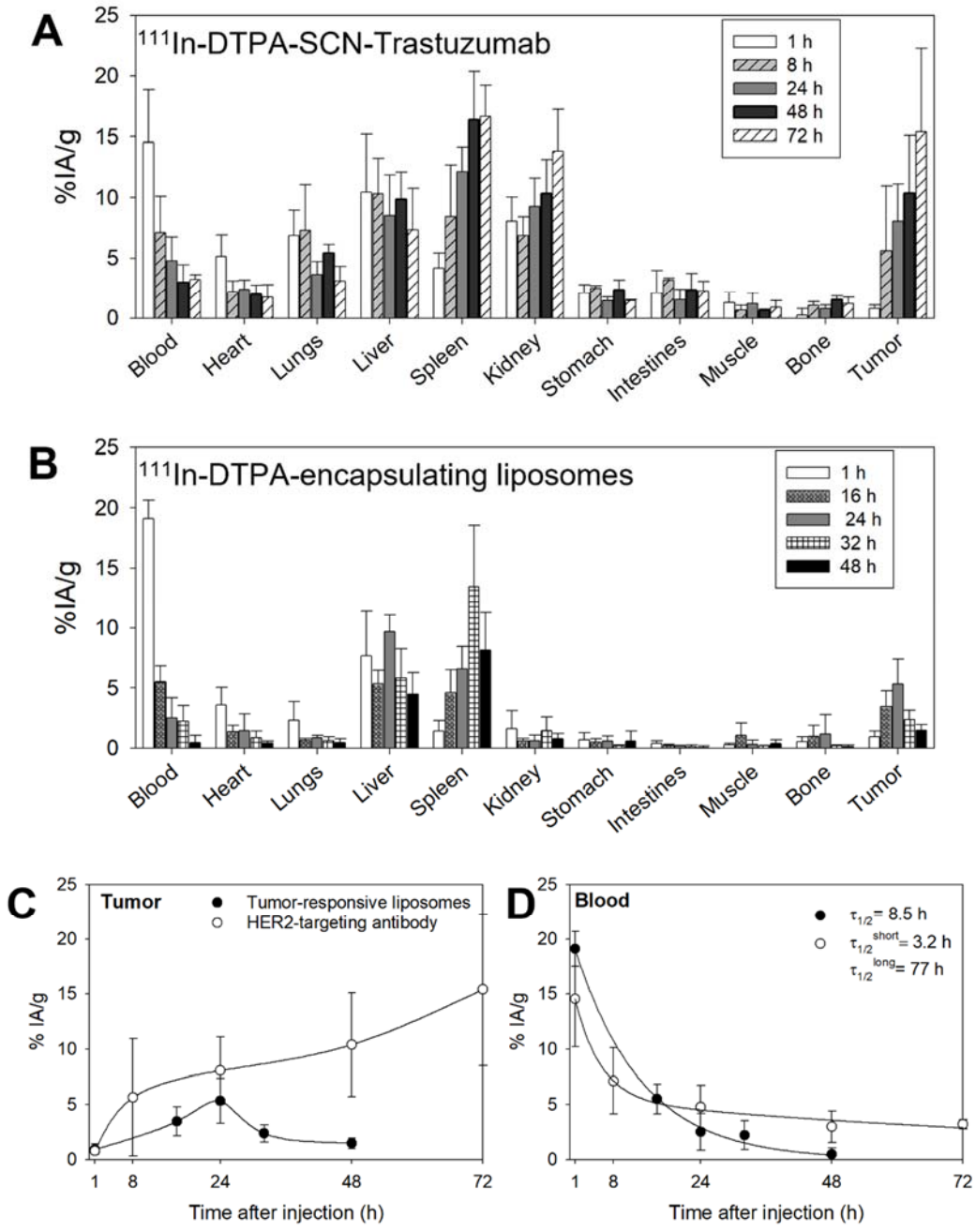
where, pk_a = 7.07, δ is the measured ISUCA H2 chemical shift and δ1 (8.75) and δ2 (7.68) are the alkaline and acidic asymptotic values of the ISUCA titration curve.

	^{111}In-DTPA-SCN-Trastuzumab				
	% IA/g				
	Time points (hours)				
	1	**8**	**24**	**48**	**72**
Heart	5.15 ± 1.75	2.19 ± 0.90	2.39 ± 0.78	1.95 ± 0.80	1.72 ± 1.06
Lung	6.87 ± 2.08	7.30 ± 3.79	3.64 ± 1.05	5.42 ± 0.73	3.11 ± 1.20
Liver	10.47 ± 4.73	10.34 ± 2.89	8.52 ± 3.36	9.87 ± 2.22	7.33 ± 3.42
Spleen	4.15 ± 1.29	8.44 ± 4.25	12.16 ± 2.00	16.40 ± 3.95	16.67 ± 2.54
Kidney	8.07 ± 1.99	6.87 ± 1.53	9.26 ± 2.35	10.32 ± 2.81	13.83 ± 3.43
Stomach	2.10 ± 0.68	2.52 ± 0.18	1.47 ± 0.29	2.35 ± 0.82	1.47 ± 0.08
Intestine	2.08 ± 1.90	3.17 ± 0.15	1.52 ± 0.88	2.35 ± 1.38	2.26 ± 0.79
Bone	0.22 ± 0.56	1.07 ± 0.29	0.77 ± 0.31	1.52 ± 0.31	1.22 ± 0.51
Muscle	1.31 ± 0.83	0.69 ± 0.35	1.21 ± 0.89	0.61 ± 0.14	0.91 ± 0.53
Blood	14.52 ± 4.34	7.12 ± 2.99	4.77 ± 1.98	2.99 ± 1.43	3.22 ± 0.41
Tumor	0.78 ± 0.32	5.64 ± 5.31	8.07 ± 3.07	10.39 ± 4.69	15.40 ± 6.89

SUPPLEMENTAL TABLE 1. **Biodistributions in mice** bearing orthotopic BT474 xenografts of I.V. administered ^{111}In-DTPA-SCN-trastuzumab used as surrogate of ^{225}Ac-DOTA-SCN-trastuzumab. Error bars correspond to standard deviations of measurements averaged over n=3 mice per time point.

	^{111}In-DTPA-encapsulating liposomes				
	% IA/g				
	Time points (hours)				
	1	**16**	**24**	**32**	**48**
Heart	3.61 ± 1.43	1.37 ± 0.55	1.45 ± 1.40	0.88 ± 0.53	0.46 ± 0.15
Lung	2.32 ± 1.56	0.66 ± 0.17	0.86 ± 0.21	0.63 ± 0.32	0.48 ± 0.32
Liver	7.67 ± 3.71	5.36 ± 1.13	9.66 ± 1.39	5.85 ± 2.42	4.52 ± 1.78
Spleen	1.41 ± 0.88	4.60 ± 1.93	6.62 ± 1.82	13.44 ± 5.12	8.14 ± 3.11
Kidney	1.63 ± 1.49	0.63 ± 0.20	0.65 ± 0.47	1.46 ± 1.13	0.78 ± 0.43
Stomach	0.71 ± 0.61	0.50 ± 0.28	0.59 ± 0.46	0.21 ± 0.08	0.61 ±0.83
Intestine	0.40 ± 0.25	0.25 ± 0.09	0.18 ± 0.07	0.13 ± .016	0.08 ± 0.12
Bone	0.54 ± 0.43	1.00 ± 0.91	1.17 ± 1.63	0.20 ± 0.06	0.17 ± 0.12
Muscle	0.29 ± 0.13	1.07 ± 1.01	0.31 ± 0.35	0.19 ± 0.04	0.38 ± 0.32
Blood	19.10 ± 1.57	5.50 ± 1.33	2.52 ± 1.68	2.24 ± 1.32	0.48 ± 0.59
Tumor	0.94 ± 0.48	3.48 ± 1.30	5.34 ± 2.03	2.38 ± 0.78	1.48 ± 0.48

SUPPLEMENTAL TABLE 2. **Biodistributions in mice** bearing orthotopic BT474 xenografts of I.V. administered tumor-responsive liposomes encapsulating ^{111}In-DTPA, used as surrogate of ^{225}Ac-DOTA. Error bars correspond to standard deviations of measurements averaged over n=3 mice per time point.

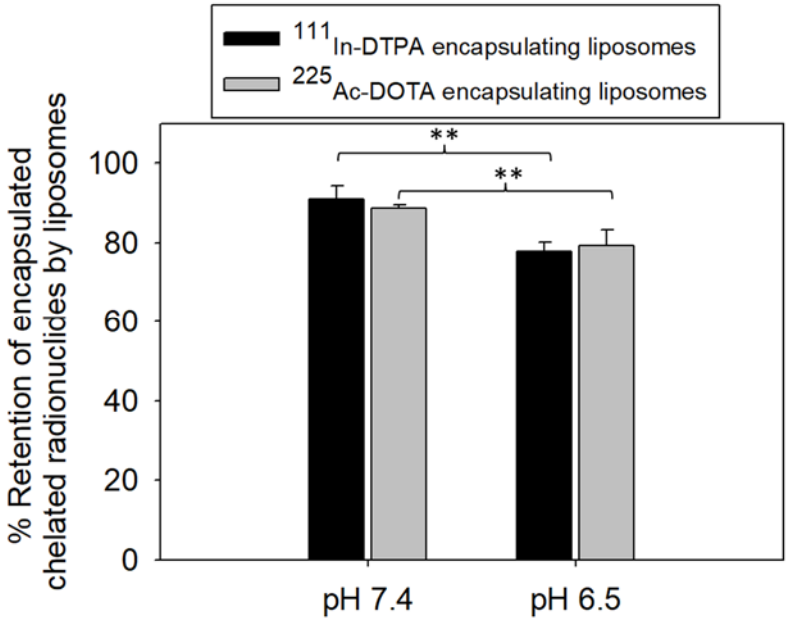


SUPPLEMENTAL FIGURE 1. Biodistributions in mice bearing orthotopic BT474 xenografts of I.V. administered (A) ^{111}In-DTPA-SCN-Trastuzumab and (B) tumor-responsive liposomes encapsulating ^{111}In-DTPA. (C) Comparison of blood clearance and tumor uptake/clearance of

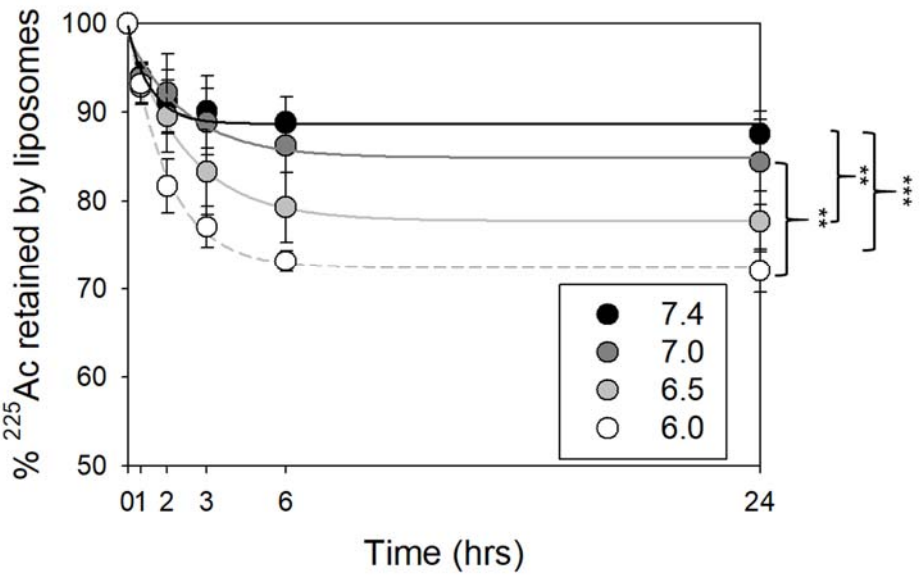
each carrier. Error bars correspond to standard deviations of measurements averaged over n=3 mice per time point.

Radiolabeling stability of *t*rastuzumab with ^{111}In (89.3±4.4%, 24-hour radioactivity retention) and retention of ^{111}In-DTPA by liposomes was similar to trastuzumab radiolabeling with ^{225}Ac and to retention of ^{225}Ac-DOTA by liposomes, respectively (Tables 1 and 2 in main text, Supplemental Figure 2 *(13)*).

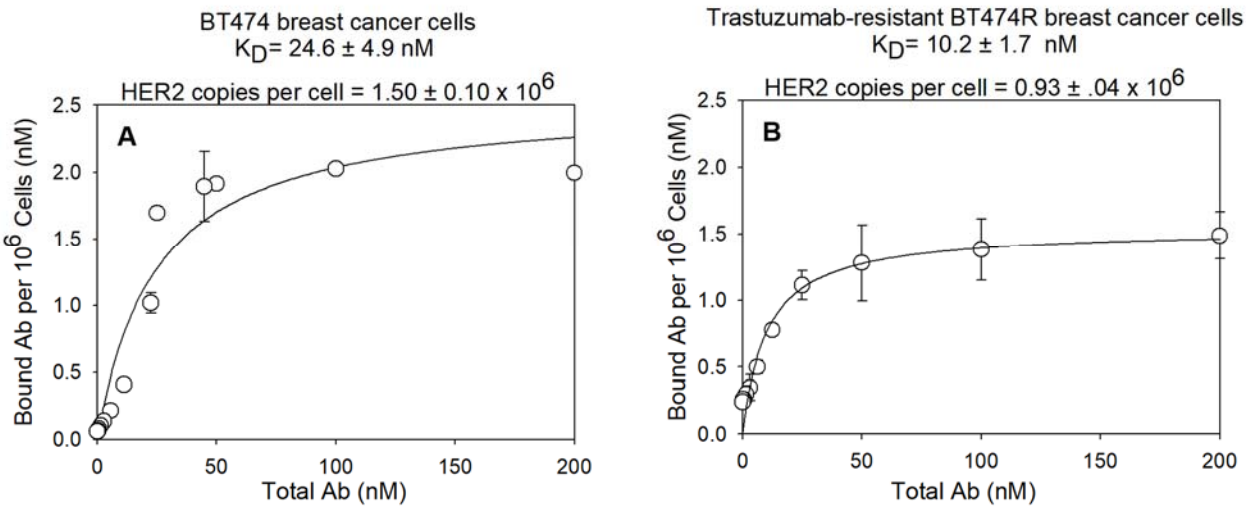
The biodistributions demonstrated the significantly greater mean tumor uptake of injected radioactivity when delivered by trastuzumab compared to the tumor uptake when radioactivity was delivered by liposomes (Supplemental Fig. 1C). ^{111}In has been confirmed as a surrogate of ^{225}Ac biodistribution (14). In liposomes, the same retention of ^{111}In and ^{225}Ac by tumor-responsive liposomes (Supplemental Fig. 2), justified the use of ^{111}In as surrogate of ^{225}Ac (13).



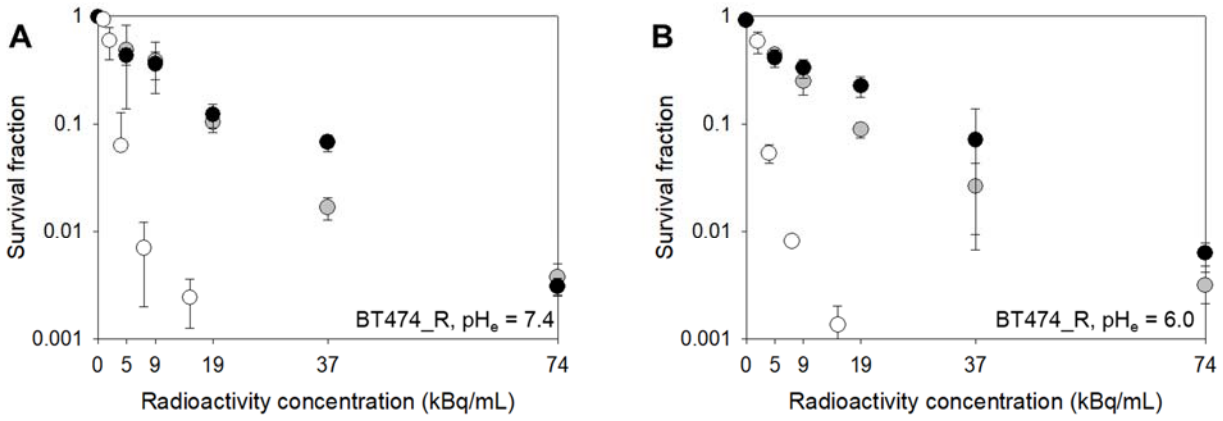
SUPPLEMENTAL FIGURE 2. Comparison of the retention of encapsulated ^{111}In-DTPA (black bars) and ^{225}Ac-DOTA (gray bars) by liposomes at neutral pH (left), and at acidic pH values (right) six hours post incubation at the corresponding pH conditions, at 37^{o}C. Errors correspond to standard deviations of n=3 independent liposome preparations. ** *p*-value < 0.05.



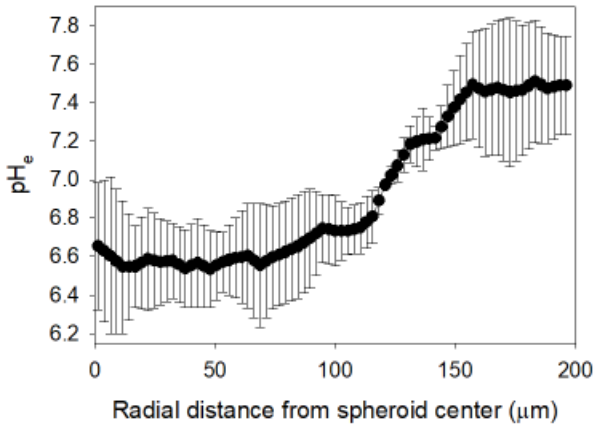
SUPPLEMENTAL FIGURE 3. **Kinetics of increasing release of ^{225}Ac from liposomes with lowering pH**. Liposomes were incubated in serum supplemented media (in the presence of cells) at 37^{o}C. At different time points, samples from the parent suspension were run through a size exclusion chromatography column to separate liposome-encapsulated radioactivity from released radioactivity, and the γ-emissions of Bismuth-213, the last radioactive daughter of ^{225}Ac, were counted after separated fractions reached secular equilibrium. The fitted kinetic constants are listed on Table 1 (main text). Errors correspond to standard deviations of n=4 independent liposome preparations. ** indicates 0.01< *p*-values < 0.05; *** indicates *p*-values ≤0.001.



SUPPLEMENTAL FIGURE 4. **Binding curves of ^{111}In-DTPA-SCN-trastuzumab** with **(A)** the BT474 breast cancer cells and **(B)** the Trastuzumab-resistant BT474-R breast cancer cells. Immunoreactivity of the radiolabeled antibody was: 90.4 ± 5.3 %. All measurements were collected from suspended cells at a fixed concentration and maintained, constantly shaking, at ice-cold temperatures. Ab stands for antibody in above plots. Error bars correspond to standard deviations of repeated measurements (2 samples per preparation, 2 separate preparations).



SUPPLEMENTAL FIGURE 5. Colony survival of trastuzumab-resistant BT474-R (0.93±0.04 x 10^6 HER2 copies/cell) breast cancer cells following a 6 hour incubation at 37°C with free ^{225}Ac-DOTA (gray symbols), tumor-responsive liposomes loaded with ^{225}Ac-DOTA (black symbols) and radiolabeled trastuzumab (^{225}Ac-DOTA-SCN-Ab) (white symbols) at extracellular pH values of 7.4 (A) or 6.0 (B), as the lowest expected acidic value of the tumor interstitial pH_e. Radiolabeled trastuzumab's specific activity was 2.9MBq/mg at the highest radioactivity concentration. Cold conditions of liposomes and the antibody are indicated at zero radioactivity concentration. Error bars correspond to standard deviations of repeated measurements (4-6 samples per radioactivity concentration).

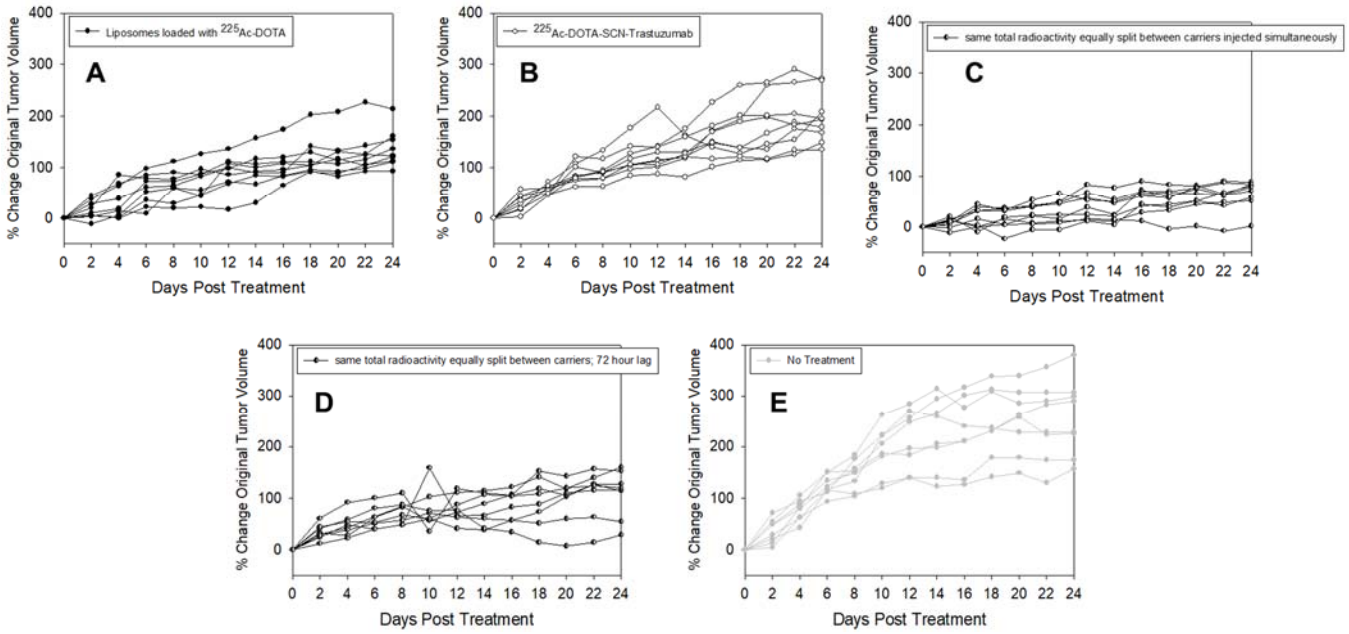


SUPPLEMENTAL FIGURE 6. Radial profile of the interstitial pH (pH_e) in BT474 spheroids of 400μm in diameter. The mean pH_e at the spheroid edge (r=200μm) ranged from 7.42 to 7.46, and the pH close to the spheroid center (r=0) was approximately 6.5.

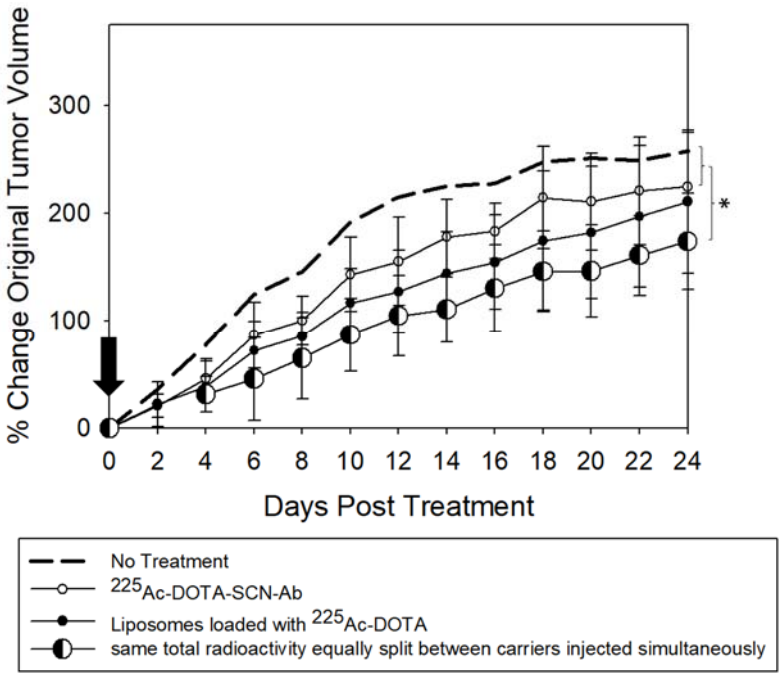
Method for pH_e profile measurement

The pH_e of the interstitium of multicellular 400 μm-in-diameter spheroids as a function of spheroid radius was measured by incubating spheroids with 350μM solution of the membrane impermeant pH-indicator SNARF-4F in media overnight at 37°C. The ratio of SNARF-4F's emission intensities at 640 nm and 580 nm (ex: 514 nm) varies linearly with pH between the values 6 to 8 independent of the fluorophore's concentration. Right before using a Zeiss LSM 780 confocal microscope under a 10x dry objective to image spheroids, the latter were transferred into fresh media and optical slices (z=10μm) were acquired immediately (to prevent significant diffusion of the fluorophore out of the spheroid). For simplicity, only radial dependence was assumed. An in-house developed erosion algorithm (with ring width equal to 4μm) was applied on the images to evaluate the mean radial intensities and to generate the ratios of the intensities as a function of position. Using the calibration curve of SNARF-4F in media at known pH values at identical imaging conditions, the ratios were then converted to pH_e values in spheroids. Errors correspond

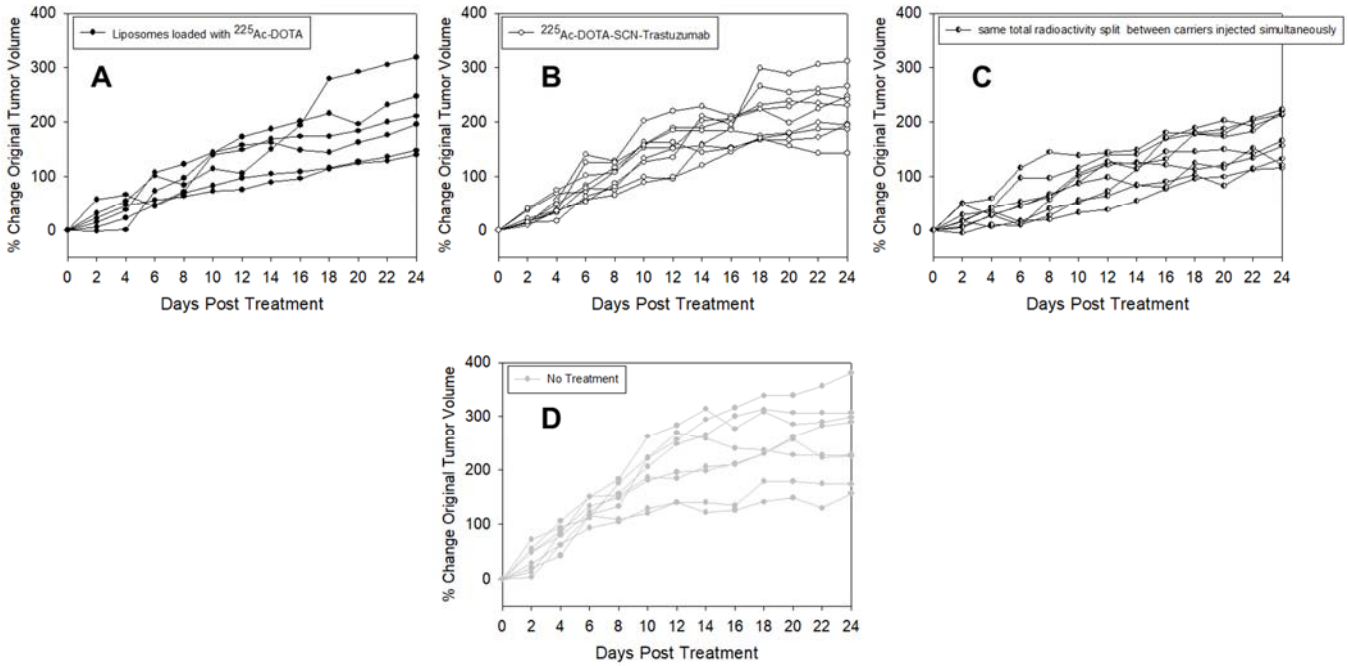
to standard deviations of n=3 independent liposome preparations. Errors correspond to standard deviations of n=3 independent spheroid runs of n=3 spheroids per run.



SUPPLEMENTAL FIGURE 7. Volume progression of HER2-positive BT474 orthotopic xenografts on individual NCR nu/nu female mice following a single I.V. administration (at t = 0) of 9.25 kBq per 20g mouse of ^{225}Ac delivered **(A)** by the tumor-responsive liposomes loaded with ^{225}Ac-DOTA alone (black circles), **(B)** by the radiolabeled Trastuzumab alone (^{225}Ac-DOTA-SCN-antibody, 2.96 MBq/mg specific radioactivity in injectate) (white circles), **(C)** by both carriers at equally split (same total) radioactivity injected simultaneously (half-black-half-white circles), and **(D)** by both carriers at equally split (same total) radioactivity with the radiolabeled antibody being administered 72 hours *after* the liposomes (to largely allow for the clearance of the latter from the liver and spleen) (half-black-half-gray circles). Tumors of animals in the non-treated group are indicated by gray circles in plot **(E)**.



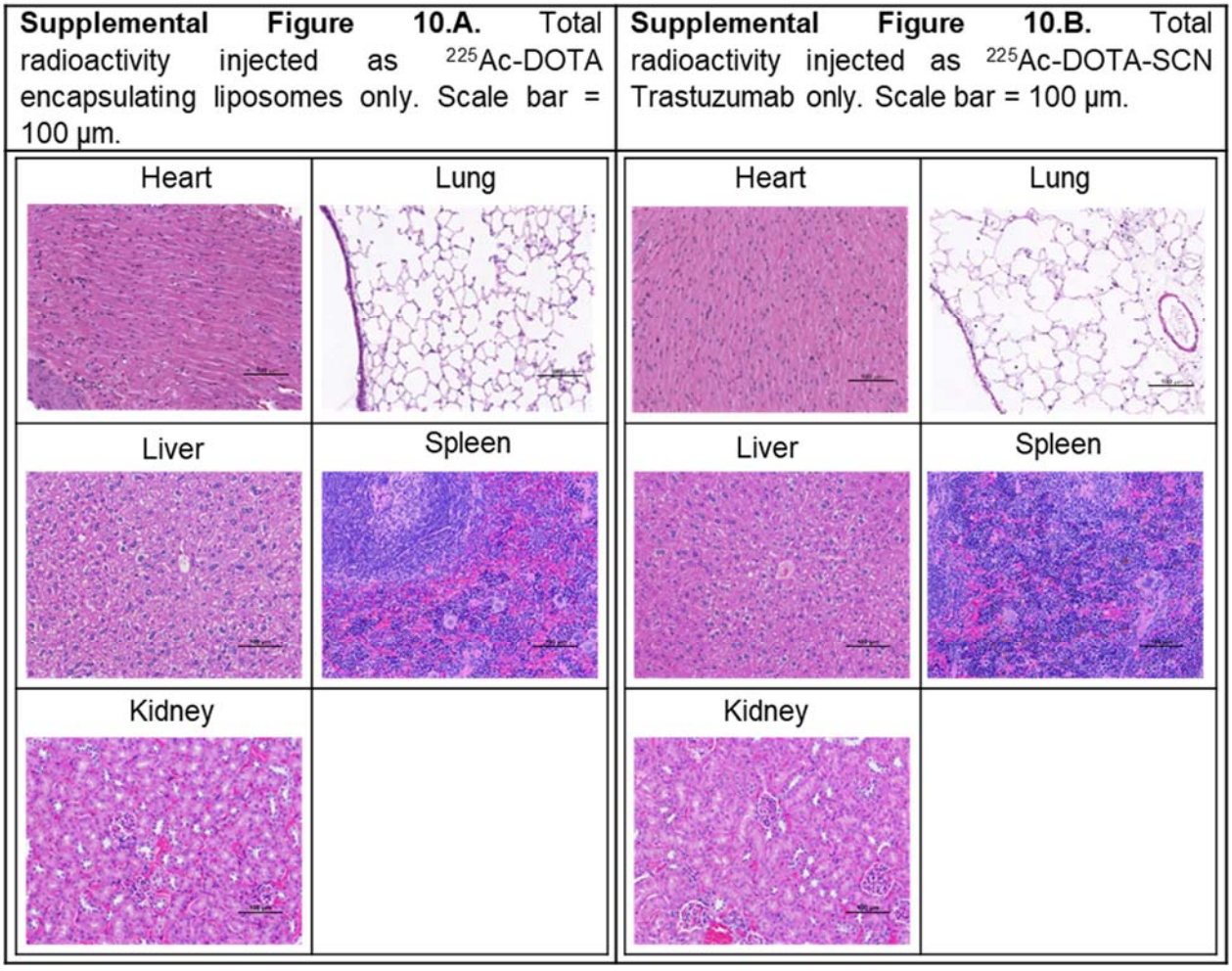
SUPPLEMENTAL FIGURE 8. Volume progression of HER2-positive BT474 orthotopic xenografts on NCR nu/nu female mice following a single I.V. administration (at t=0 indicated by the black arrow) of 4.63 kBq per 20g mouse of ^{225}Ac delivered by the radiolabeled trastuzumab alone (^{225}Ac-DOTA-SCN-antibody, 2.96 MBq/mg specific radioactivity in injectate) (white circles), the tumor-responsive liposomes loaded with ^{225}Ac-DOTA alone (black circles), and by both carriers at equally split (same total) radioactivity injected simultaneously (half-black-half-white circles). Non-treated group is indicated by the dashed line. Data points are the mean values and error bars the standard deviations of n=8–9 animals per group. Significance was calculated with one-way ANOVA (p-value<0.05). * indicates 0.01< p-values <0.05.



SUPPLEMENTAL FIGURE 9. Volume progression of HER2-positive BT474 orthotopic xenografts on individual NCR nu/nu female mice following a single I.V. administration (at t = 0) of 4.63 kBq per 20g mouse of ^{225}Ac delivered **(A)** by the tumor-responsive liposomes loaded with ^{225}Ac-DOTA alone (black circles), **(B)** by the radiolabeled Trastuzumab alone (^{225}Ac-DOTA-SCN-antibody, 2.96 MBq/mg specific radioactivity in injectate) (white circles), and **(C)** by both carriers at equally split (same total) radioactivity injected simultaneously (half-black-half-white circles). Tumors of animals in the non-treated group are indicated by gray circles in plot **(D)**.

SUPPLEMENTAL FIGURE 10. Treatment study: Normal tissue H&E images from BT474 tumor-bearing NCR nu/nu female mice treated with tumor-responsive liposomes loaded with ^{225}Ac-DOTA and/or ^{225}Ac-DOTA-SCN HER2-targeting Trastuzumab at a total (single I.V.) dose equal to 9.25 kBq per 20g animal.

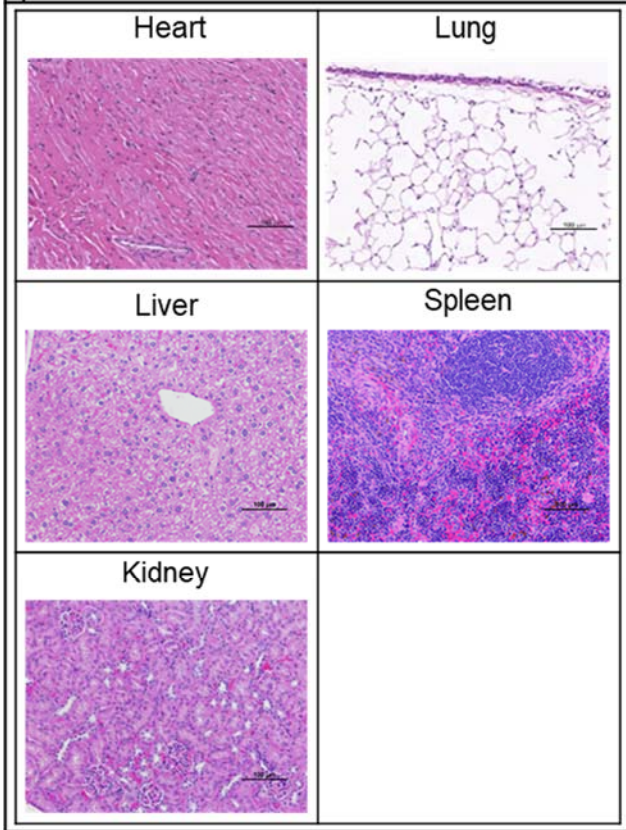
Histopathology analysis of H&E stained sections of the organs of tumor-bearing mice showed no noteworthy hepatic, cardiac, or renal toxicities across all constructs at the time of sacrifice. Slight inflammation in the diaphragm of the liposome-only treatment group was observed, but otherwise there was no visible lung inflammation. Additionally, increased cell death in and reduced size of the spleen was observed in the liposome-only condition.



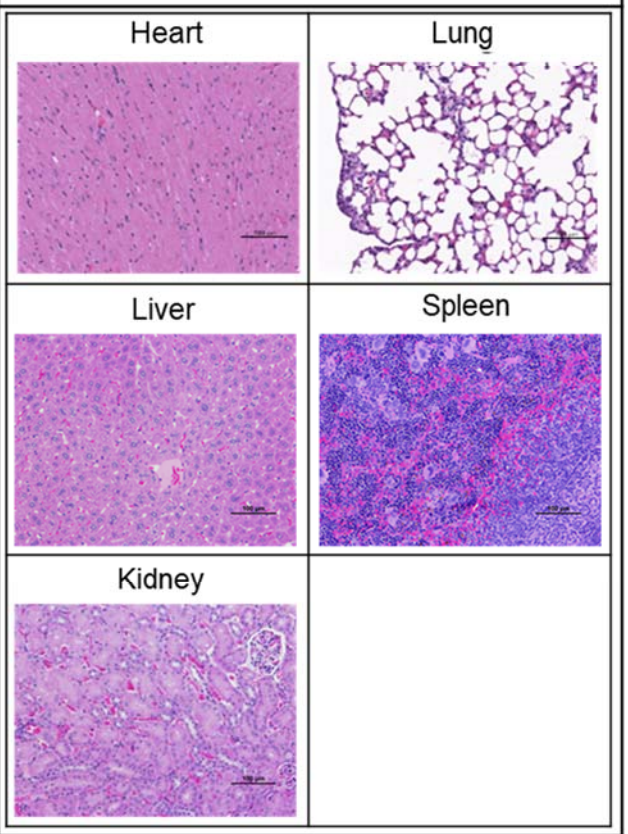
Supplemental Figure 10.A. Total radioactivity injected as ^{225}Ac-DOTA encapsulating liposomes only. Scale bar = 100 µm.

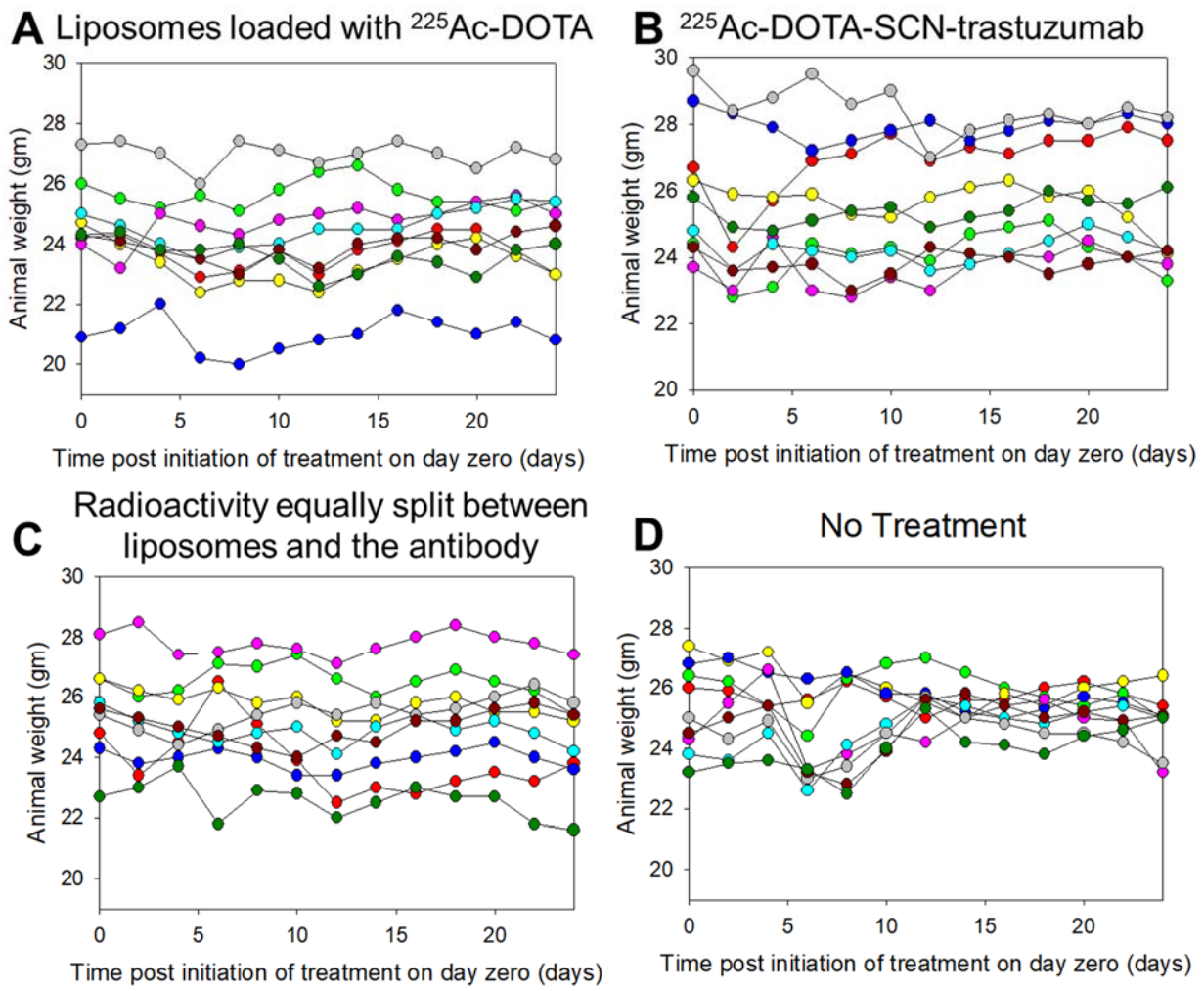
Supplemental Figure 10.B. Total radioactivity injected as ^{225}Ac-DOTA-SCN Trastuzumab only. Scale bar = 100 µm.

Supplemental Figure 10.C. Total radioactivity injected 50% as ^{225}Ac-DOTA encapsulating liposomes and 50% as ^{225}Ac-DOTA-SCN-Trastuzumab. Scale bar = 100 µm.

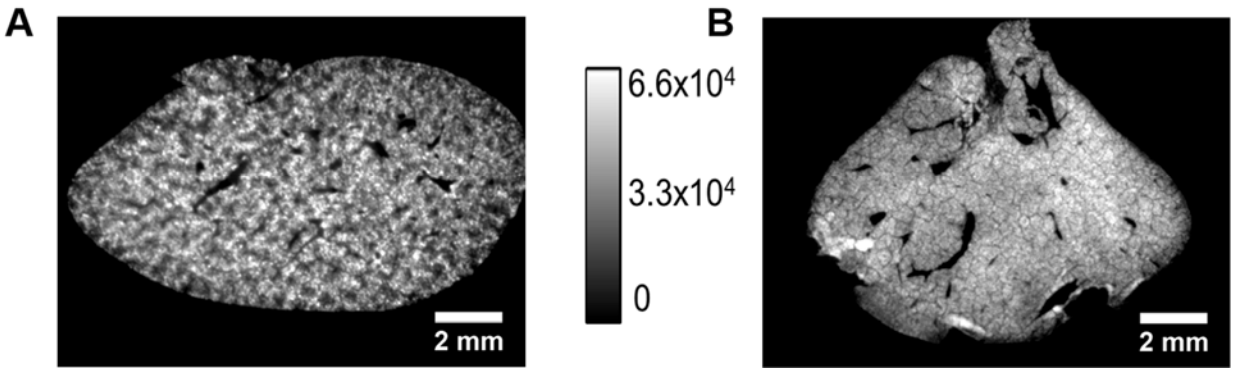


Supplemental Figure 10.D. No treatment. Scale bar = 100 µm.

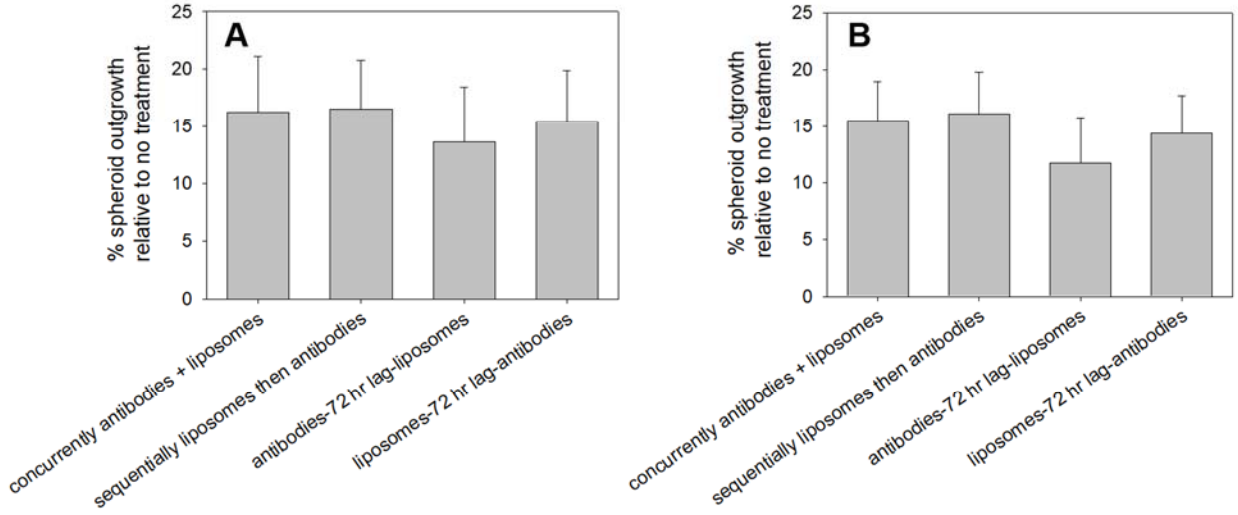




SUPPLEMENTAL FIGURE 11. Treatment study: weight of individual animals during the tumor growth control study. BT474 tumor-bearing NCR nu/nu female mice were injected I.V. with a single total administration equal to 9.25kBq per 20g animal on day 0 in the following forms: **(A)** ^{225}Ac-DOTA encapsulating tumor-responsive liposomes, **(B)** ^{225}Ac-DOTA-SCN Trastuzumab, and **(C)** no treatment, and **(D)** same total radioactivity equally split between ^{225}Ac-DOTA encapsulating liposomes and ^{225}Ac-DOTA-SCN Trastuzumab. Animal weight was monitored every other day, and did not decrease beyond 10% of original weight during the treatment study.



SUPPLEMENTAL FIGURE 12. Decay-corrected α-Camera images of liver sections from NCR nu/nu female mice collected 24 hours upon I.V. administration showing the spatial distributions of ^{225}Ac delivered by **(A)** ^{225}Ac-DOTA encapsulating tumor-responsive liposomes, and **(B)** ^{225}Ac-DOTA-SCN-trastuzumab. Scale bar = 2mm. The spatial micro-distributions of ^{225}Ac were less uniform (and more grainy) when delivered by liposomes, compared to when delivered by the antibody.



SUPPLEMENTAL FIGURE 13. Variation in the delivery schedule of same total radioactivity and same radioactivity split ratio between the ^{225}Ac-DOTA encapsulating tumor-responsive liposomes and ^{225}Ac-DOTA-SCN-Trastuzumab did not result in statistically different extents of spheroid outgrowth control in 400 μm-in diameter spheroids following treatment with either **(A)** 13.75kBq/mL or **(B)** 18.5kBq/mL. (n = 4-5 spheroids per condition, 2 independent preparations.)