MATERIALS AND METHODS

Image Analysis

Lesions visualized relative to adjacent tissue on PET were considered PET-positive. The radiologist (Dr. Conti) visually aligned the image sets from the FDG, trastuzumab Day 1 and trastuzumab Day 2 scans in the axial direction. He then simultaneously reviewed them for potential true-positive findings on PET (non-physiologic focal or extended regions of elevated intensity relative to immediate surroundings; cold spots within the livers of patients not pre-infused with cold trastuzumab). When a potential true-positive PET lesion was identified on any of the 3 scans, Dr. Conti examined the coregistered CT images and fused PET-CT images for a corresponding anatomic feature consistent with tumor (non-physiologic mass, lymph node, sclerotic or lytic bone lesion, shadow in liver, lung nodule, pleural effusion). Because of the high blood activity, potential true-positive ^{64}Cu-DOTA-trastuzumab lesions were disregarded if they were judged likely due to blood pool or large blood vessels. If a correlated CT feature consistent with tumor was identified, the potential PET lesion was scored as a true positive, and the other 2 PET scans were scored as true positive or false negative, depending on whether the lesion was visualized in those scans. If no correlated CT lesion was found, the potential PET lesion was disregarded unless it was seen only on 1 of or both the ^{64}Cu scans, in which case it was recorded as a false positive for ^{64}Cu-DOTA-trastuzumab. (The possibility of ^{18}F-FDG false-positive lesions was not considered.) However, potential PET lesions were always disregarded if CT was judged inconclusive (as for the small hot spots seen in the ribs of the patient depicted in Fig. 1A of the main text). Examples of a

^{64}Cu-DOTA-trastuzumab false-negative lesion and a ^{64}Cu-DOTA-trastuzumab true-positive intrahepatic cold-spot lesion are illustrated in Figs. 2B, and 3A of the main text, respectively.

Radiation Dose Estimates

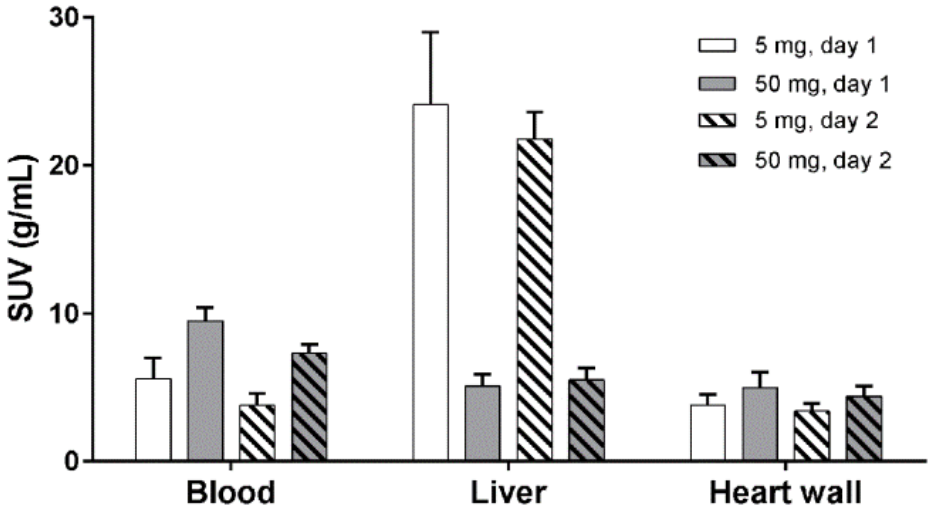
We previously calculated organ and tissue doses for 8 HER2-positive metastatic breast cancer patients imaged with ^{111}In-MxDTPA-trastuzumab (*1*). Data were derived from serial blood samples, urine collection, and conjugate-view, whole-body gamma camera images obtained 0-168 h post-injection. Details of the dosimetry calculations are given in the cited reference.

To adapt the ^{111}In-MxDTPA-trastuzumab calculations to the current study, we first repeated the analysis using the ^{111}In-derived data corrected for the difference in physical decay rate between ^{111}In and ^{64}Cu to obtain fraction-of-injected activity vs. time [FIA(t)] curves and residence times (integral FIAs) for ^{64}Cu-MxDTPA-trastuzumab in whole body, blood, liver, heart + blood content, kidneys and spleen. Next, we converted blood and PET-derived SUV data for liver, heart wall, kidney and spleen from the current ^{64}Cu-DOTA-trastuzumab study (50 mg trastuzumab dose) to FIAs for blood, liver, heart + blood content, kidneys and spleen using a published algorithm to estimate total blood volume from patient height and weight (*2*) and an adult female reference model to estimate organ volumes (*3*). We found the slopes of the patient-averaged, ^{111}In-derived ^{64}Cu FIA(t) curves in the Day 1 - Day 2 time range of the ^{64}Cu-DOTA-trastuzumab study to be similar to those of the patient-averaged, ^{64}Cu-derived FIA(t)s. Given the much shorter half life of ^{64}Cu, any differences in radiolabel pharmacokinetics

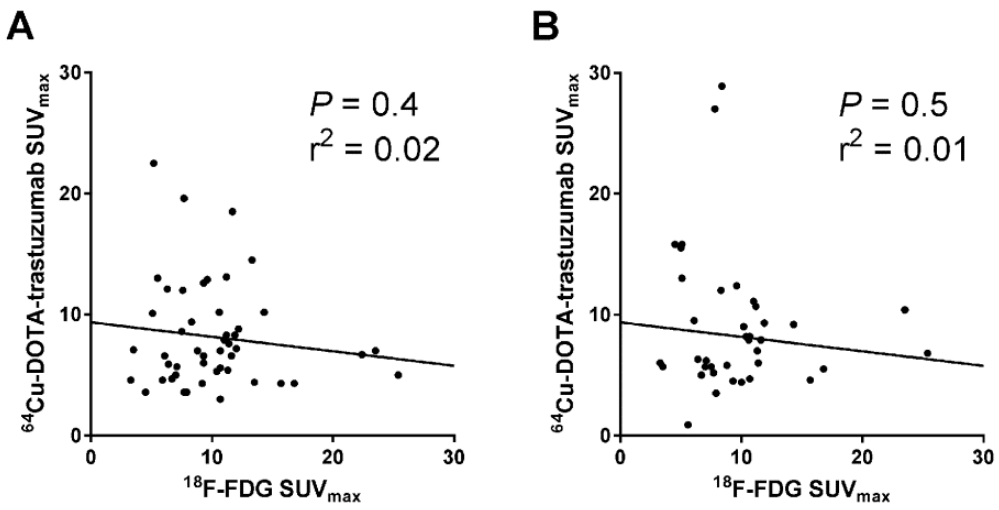
between ^{64}Cu-DOTA-trastuzumab and ^{111}In-MxDTPA-trastuzumab beyond 48 h would have little effect on dosimetry. Thus, we assumed the shapes of the decay-corrected FIA curves to be same for ^{111}In-MxDTPA-trastuzumab and ^{64}Cu-DOTA-trastuzumab. To obtain residence times for ^{64}Cu-DOTA-trastuzumab, we used linear interpolation to determine the ratios of the patient-averaged, ^{111}In-derived ^{64}Cu FIA(t) curves to the patient-averaged, ^{64}Cu-derived FIA(t)s at the mid-time of the PET study (36 h), and multiplied those ratios by the corresponding ^{111}In-derived ^{64}Cu residence times. Residence time for red marrow was calculated from the estimated ^{64}Cu-DOTA-trastuzumab blood residence time assuming a red marrow-to-blood activity concentration ratio of 0.31 (*4*) and a red marrow total volume-to-blood total volume ratio of 0.21 (*3*). Residence time for the body "remainder" (i. e., whole body minus the sum of red marrow, heart + contents, liver, kidney and spleen) was assumed to equal that for ^{111}In-MxDTPA-trastuzumab, adjusted to ^{64}Cu decay. Residence times for ^{64}Cu-DOTA-trastuzumab were input to OLINDA/EXM v.1.0 (*5*) to obtain tissue and organ equivalent dose and effective dose estimates.

REFERENCES

1. Wong JYC, Raubitschek A, Yamauchi D, et al. A pretherapy biodistribution and dosimetry study of indium-111-radiolabeled trastuzumab in patients with human epidermal growth factor receptor 2-overexpressing breast cancer. *Cancer Biother Radiopharm.* 2010;25:387-394.
2. Nadler SB, Hidalgo JH, Bloch T: Prediction of blood volume in normal human adults. *Surgery.* 1962;51:224-232.
3. International Commission on Radiological Protection. *Basic anatomical and physiological data for use in radiological protection: reference values*. ICRP Publication 89. New York, NY: Pergammon Press; 2003.
4. Sgouros, G. Bone marrow dosimetry for radioimmunotherapy: theoretical considerations. *J Nucl Med.* 1993;34:689-694.
5. Stabin MG, Sparks RB, Crowe E. OLINDA/EXM: the second-generation personal computer software for internal dose assessment in nuclear medicine. *J Nucl Med.* 2005;46:1023-1027.



SUPPLEMENTAL FIGURE 1. Effect of trastuzumab protein dose on the pharmacokinetics and biodistribution of ^{64}Cu-DOTA–trastuzumab. Error bars indicate standard deviations. 5 mg trastuzumab dose: n = 2. 50 mg trastuzumab dose: Day 1, n = 5; Day 2, n= 6.



SUPPLEMENTAL FIGURE 2. Tumor uptakes of ^{64}Cu-DOTA-trastuzumab and ^{18}F-FDG are uncorrelated. The plots compare maximum single-voxel standardized uptake values (SUV_{max}) within the same tumor between ^{18}F-FDG and ^{64}Cu-DOTA-trastuzumab Day 1 (A) and Day 2 (B). Slopes of the indicated linear regression lines are not significantly different from zero (P = 0.4, correlation coefficient = -0.1 for Day 1; P = 0.5, correlation coefficient = -0.1 for Day 2).