MATERIALS AND METHODS

Radiation Dosimetry Estimates

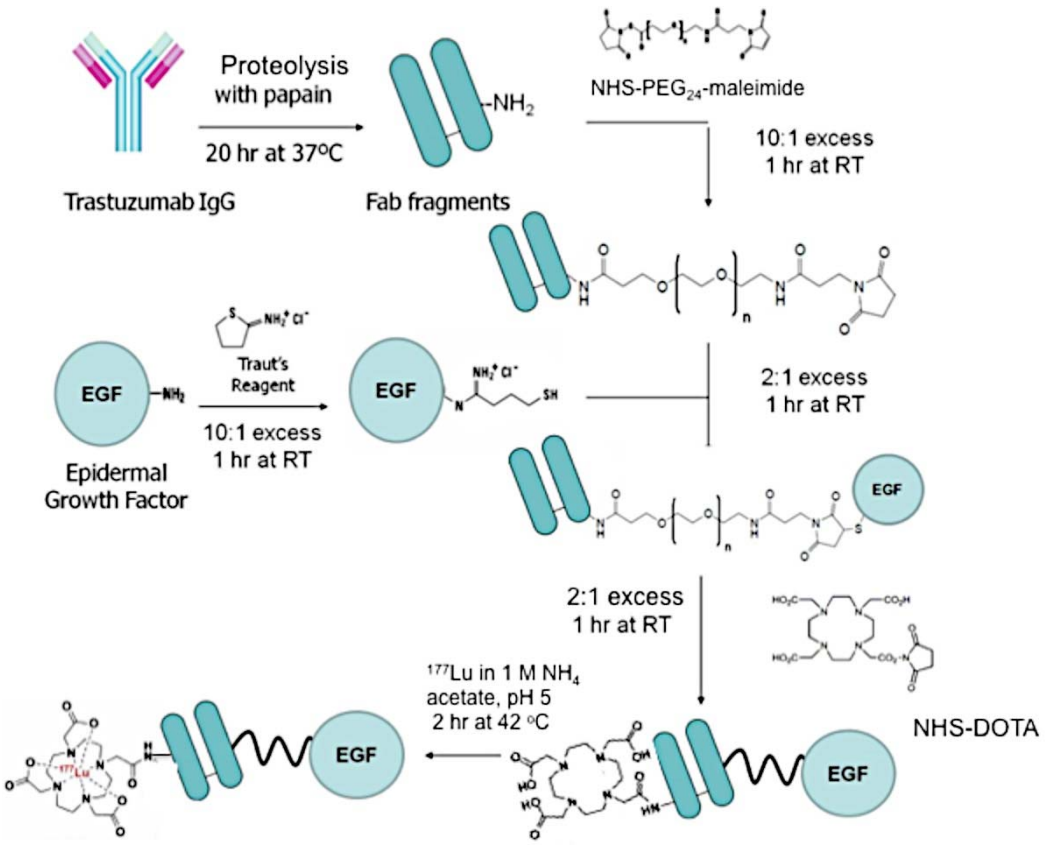
The radiation absorbed doses to the tumor and normal tissues in CD1 athymic mice with MDA-MB-231/H2N xenografts following injection of ^{111}In-DTPA-Fab-PEG$_{24}$-EGF or ^{177}Lu-DOTA-Fab-PEG$_{24}$-EGF were estimated from the previously reported biodistribution of ^{111}In-DTPA-Fab-PEG$_{24}$-EGF (*1*). The radioactivity in source organs at time points from 4 to 72 h p.i. was calculated by multiplying the %ID/g values by standard organ weights in mice (*2*) assuming an injected amount of 11.1 MBq (10 μg). The organ radioactivity was then corrected for radioactive decay by multiplying by $e^{(-\lambda t)}$, where λ is the decay constant (1.03×10^{-2} h^{-1} for ^{111}In and 4.3×10^{-3} h^{-1} for ^{177}Lu) and t is the time p.i. Radioactivity in the tumor was similarly calculated by multiplying the %ID/g values by 0.2 g, assuming a tumour of 200 ± 30 mm^3 measured in a previous study of ^{111}In-DTPA-Fab-PEG$_{24}$-EGF (1) and then correcting for radioactive decay. The cumulative radioactivity for the tumor and normal source organs from 0-72 h ($\tilde{A}_{0\text{-}72h}$) was estimated from the area under the curve (AUC) from 0 h to 72 h [$AUC_{0\text{-}72h}$; Bq × sec] using Prism Ver. 4.0 software (GraphPad Inc., San Diego, CA). The cumulative radioactivity from 72 h to infinity ($\tilde{A}_{72h\text{-}\infty}$) was obtained by dividing the final radioactivity at 72 h by the decay constant, thus assuming elimination after this time point only by radioactive decay. The combined $\tilde{A}_{0\text{-}\infty}$ values for each organ were multiplied by the S-value (Gy/Bq × sec) for ^{111}In and ^{177}Lu (2) to estimate the radiation absorbed doses (Gy). The dose delivered to the tumor was estimated based on the S-

values using the sphere model in OLINDA/EXT radiation dose assessment software, assuming a tumor with diameter of 3.5 mm (*3*).

RESULTS

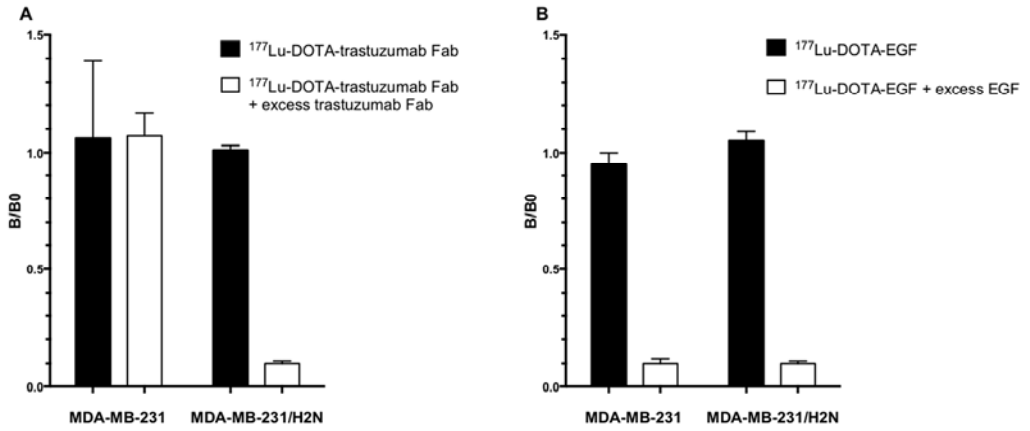
Bispecific Radioimmunoconjugates (bsRICs)

Fig. S1 1 shows the method of synthesis of the trastuzumab Fab-PEG_{24}-EGF bispecific immunoconjugates and their radiolabeling with ^{177}Lu. The binding of ^{177}Lu-DOTA-trastuzumab Fab or ^{177}Lu-DOTA-EGF to MDA-MB-231/H2N cells in the absence or presence of an excess of unlabeled trastuzumab Fab or EGF is shown in Fig. S2.



Supplemental Fig. 1. Synthesis of bispecific ^{177}Lu-DOTA-Fab-PEG$_{24}$-EGF radioimmunoconjugates. Fab fragments of trastuzumab produced by proteolytic digestion of the intact IgG were reacted with a 10-fold mole excess of NHS-PEG$_{24}$-maleimide for 1 hour at room temperature (RT). EGF was thiolated by reaction with a 10-fold mole excess of 2-iminothiolane (Traut's reagent) for 1 hour at RT. Then a 2-fold mole excess of thiolated EGF was reacted with maleimide functionalized PEG$_{24}$-Fab for 1 hour at RT. Fab-PEG$_{24}$-EGF immunoconjugates were purified and reacted with a 2-fold mole excess of NHS-DOTA for 1 hour at RT. Following re-purification, DOTA-

Fab-PEG_{24}-EGF was labeled with ^{177}Lu by incubation with $^{177}LuCl_3$ in 1 M ammonium acetate buffer, pH 5.0 for 2 hours at 42 °C. The synthesis of the bispecific radioimmunoconjugates was adapted from Razumienko et al. (*1*).



Supplemental Fig. 2. Binding of (A) ^{177}Lu-DOTA-trastuzumab Fab (1 nmol/L) or (B) ^{177}Lu-DOTA-EGF (6.4 nmols/L) to MDA-MB-231 cells ($HER2^{low}$/$EGFR^{mod}$) and MDA-MB-231/H2N cells ($HER2^{mod}$/$EGFR^{mod}$) in the absence or presence of an excess of unlabeled trastuzumab Fab (69 nmols/L) or EGF (1,659 nmols/L). B/B_0: Binding in the presence of competitor divided by the binding in the absence of competitor. Values shown represent the mean ± SD (n=3).

REFERENCES

1. Razumienko E DL, Scollard D, Reilly RM. MicroSPECT/CT imaging of co-expressed HER2 and EGFR on subcutaneous human tumor xenografts in athymic mice using ^{111}In-labeled bispecific radioimmunoconjugates. *Breast Cancer Res Treat*. 2013;138:709-718.
2. Bitar A, Lisbona A, Thedrez P, et al. A voxel-based mouse for internal dose calculations using Monte Carlo simulations (MCNP). *Phys Med Biol*. 2007;52:1013-1025.
3. 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.