Methods and Material

Production and preparation of ^{89}Zr-panitumumab

Production of ^{89}Zr

Pressed pellets of yttrium metal (200 mg, 99.99 % purity; American Elements, USA) were irradiated with a proton beam of 15 MeV and a current of 20 μA for 2–4 h on a GE PETtrace cyclotron located at the Clinical Center, National Institutes of Health, Bethesda, MD, USA. ^{89}Zr was separated from the yttrium target material by the use of hydroxamate resin. The target material was dissolved in 4 x 0.5 mL fraction of 6M HCl. After 1 h, the undissolved solid residue from the target material was separated by filtration, the resulting solution diluted to 5 mL with de-ionized water and loaded onto the hydroxamate resin column. The column was then washed with 4 x 2.5 mL of 2 M HCl and 4 x 2.5 mL de-ionized water. After the solution was removed from the column, the ^{89}Zr was eluted with successive portions of 1 M oxalic acid. The first 0.4 mL fraction was discarded and the next 0.7 mL fraction collected for further use.

Preparation of ^{89}Zr labeled panitumumab

The bifunctional chelator, *p*-isothiocyanatobenzyl-desferrioxamine B (Df-Bz-NCS) (Macrocylics, Dallas, TX, USA) was conjugated to

panitumumab for radiolabeling with ^{89}Zr. Panitumumab (Vectibix®, Amgen) was dialyzed against 0.1 M sodium acetate, pH 4, using a 10 kD molecular-weight cut-off (MWCO) dialysis cassette (Pierce, Rockford, IL, USA). The solution was changed three times a day over the course of 4 days. The total quantity of recovered protein was determined by absorbance at 280 nm. Before conjugation, panitumumab solution (typically 10 mg, 66.7 nmol) was buffered to pH 8.5 with 0.1 M $NaHCO_3$. A 5-fold molar excess of Df-Bz-NCS, dissolved in DMSO solution was added to panitumumab drop by drop with gentle shaking. The reaction mixture was then incubated for 1 h at 37 °C with stirring at 750 rpm. After incubation, the reaction was transferred to a 3 mL Slide-A-Lyzer Dialysis Cassette G2 (Thermo Fisher Scientific, Rockford, IL) and dialyzed against PBS buffer at 4 °C for three days with nine buffer changes (9 L total). Protein recovery during conjugation was determined by measuring the UV absorption at 280 nm. The chelate to protein product ratio was determined by the isotope dilution method as previously described using high purity non-radioactive $ZrCl_4$ solution. For radiolabeling, 37 -370 MBq of the ^{89}Zr oxalate solution (pH ≤1) was neutralized to pH 7-7.5 by slow addition of 2 M Na_2CO_3 followed by 0.5 M HEPES buffer. Alternatively 5 M ammonium acetate was used instead of 0.5 M HEPES buffer to neutralize the solution after slow addition of 2 M Na_2CO_3. When precipitation of the oxalate salt was observed, de-ionized water was added to the solution. A freshly prepared solution of gentisic acid

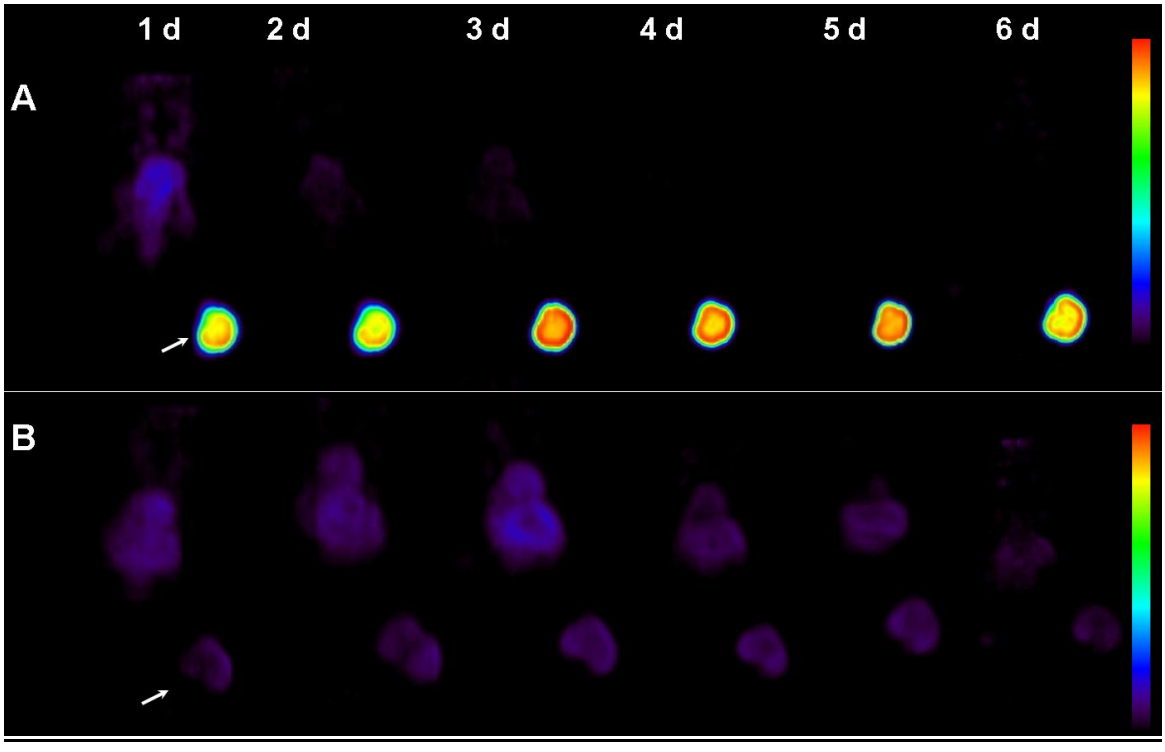
(50 µL, 220 µg/µL) was then added to the solution to prevent radiolysis of the mAb followed by 0.1 mg of panitumumab in 0.15 M ammonium acetate buffer. The reaction mixture was gently stirred and incubated at room temperature for 1 h. The reaction was quenched by the addition of ethylenediaminetetraacetic acid solution (4 µL, 0.1 M). The radiolabeled product was purified using a PD-10 desalting column (GE Healthcare, Piscataway, NJ). Size-exclusion high-performance liquid chromatography (SE-HPLC) and cell based immunoreactivity assays were performed to ascertain the purity and biological integrity of the radioimmunoconjugate using previously described methods.

PET imaging

Small animal PET studies were performed using the Siemens Focus 120 scanner at the National Institutes of Health, Bethesda, MD, USA. Whole body imaging studies (singe bed positions, total acquisition time of 1 h per mouse) were carried out on mice anesthetized with 1.5-1.7% isoflurane on a temperature-controlled bed. Subcutaneous and pulmonary tumors bearing female athymic mice were injected i.v. with 1.7-1.9 MBq (< 5 µg) of ^{89}Zr labeled panitumumab. While for mice bearing intraperitoneal tumors, 1.7-1.9 MBq (< 5 µg) of ^{89}Zr labeled panitumumab was injected (i.p.). The RIC was injected in two set of mice, one set comprised mice bearing intraperitoneal tumors with 3 d tumor burden and another set comprised of 7 d tumor burden, therefore representing relatively early stage and late stage

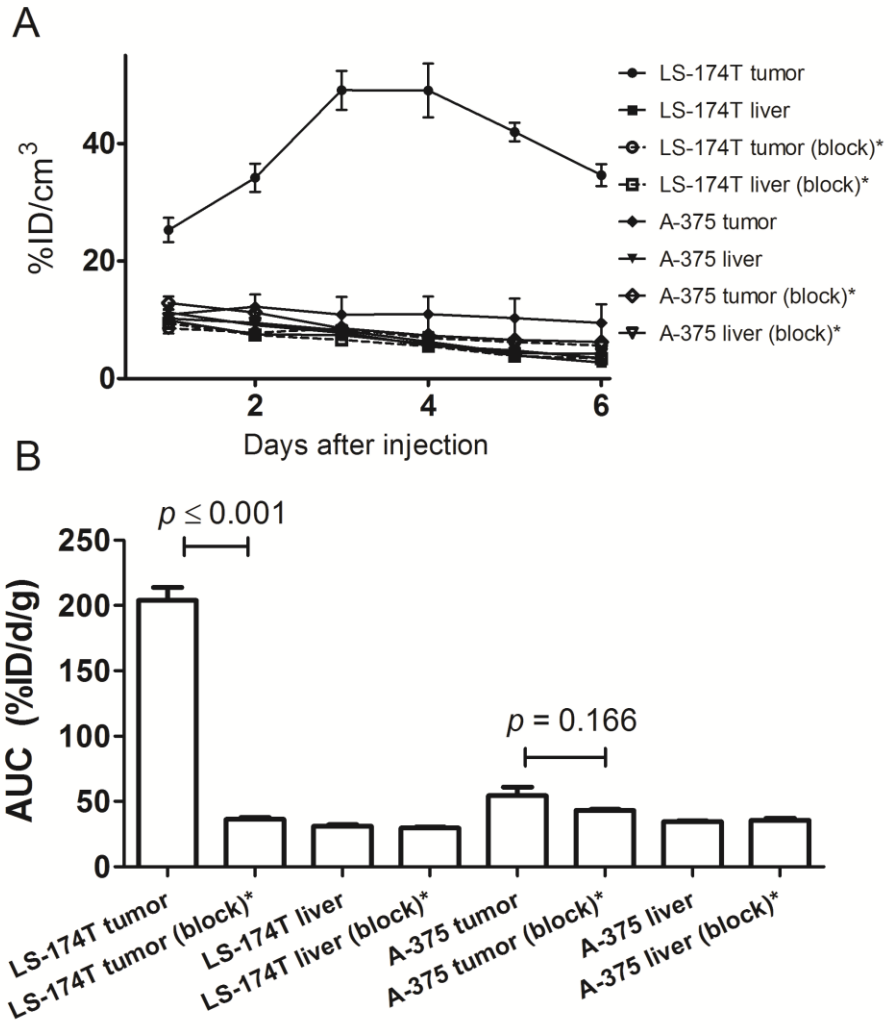
disease. Additionally, non-tumor bearing mice were injected (i.v. or i.p.) with the equivalent RIC as controls. To determine HER1-specificity in mice bearing subcutaneous tumor xenografts, excess unmodified mAb (0.1 mg) was co-injected with the ^{89}Zr labeled panitumumab. A 10 min transmission scan was performed with precalibrated ^{57}Co sealed source for attenuation correction for mice bearing pulmonary or intraperitoneal tumors. ^{89}Zr-filled cylinder phantoms were imaged during each imaging session for normalization and quantitative analysis. The energy window for PET acquisition of ^{89}Zr was set between 350 and 650 keV. The imaging data were reconstructed using a 2-dimensional Fourier rebinned ordered-subsets expectation maximization method with scatter correction (linear background subtraction). Additional dead time, decay, attenuation and background corrections were applied for quantitative analysis. The reconstructed images were processed and analyzed using the AMIDE: A Medical Image Data Examiner software program. To minimize spillover effects, regions of interest were drawn to enclose approximately 80%–90% of the organ of interest to avoid the edges. To minimize partial-volume effects caused by nonuniform distribution of the radioactivity in the containing volume, smaller regions of interest were consistently drawn to enclose the organ. After imaging, select mice were euthanized and biodistribution studies were performed to determine the correlation between PET-assessed *in vivo* % ID/cm^3 and biodistribution determined *ex vivo* % ID/g.

Supplemental Figure 1



(A) Representative reconstructed and processed maximum-intensity projections of female athymic (NCr) *nu/nu* mouse bearing s.c LS-174T tumor xenograft intravenously injected via tail vein with 1.7–1.9 MBq of ^{89}Zr labeled panitumumab or (B) 1.7–1.9 MBq of ^{89}Zr labeled panitumumab co-injected with 0.1 mg panitumumab for receptor blocking. Scale represents percentage of maximum and minimum threshold intensity. Tumors are indicated with white arrows.

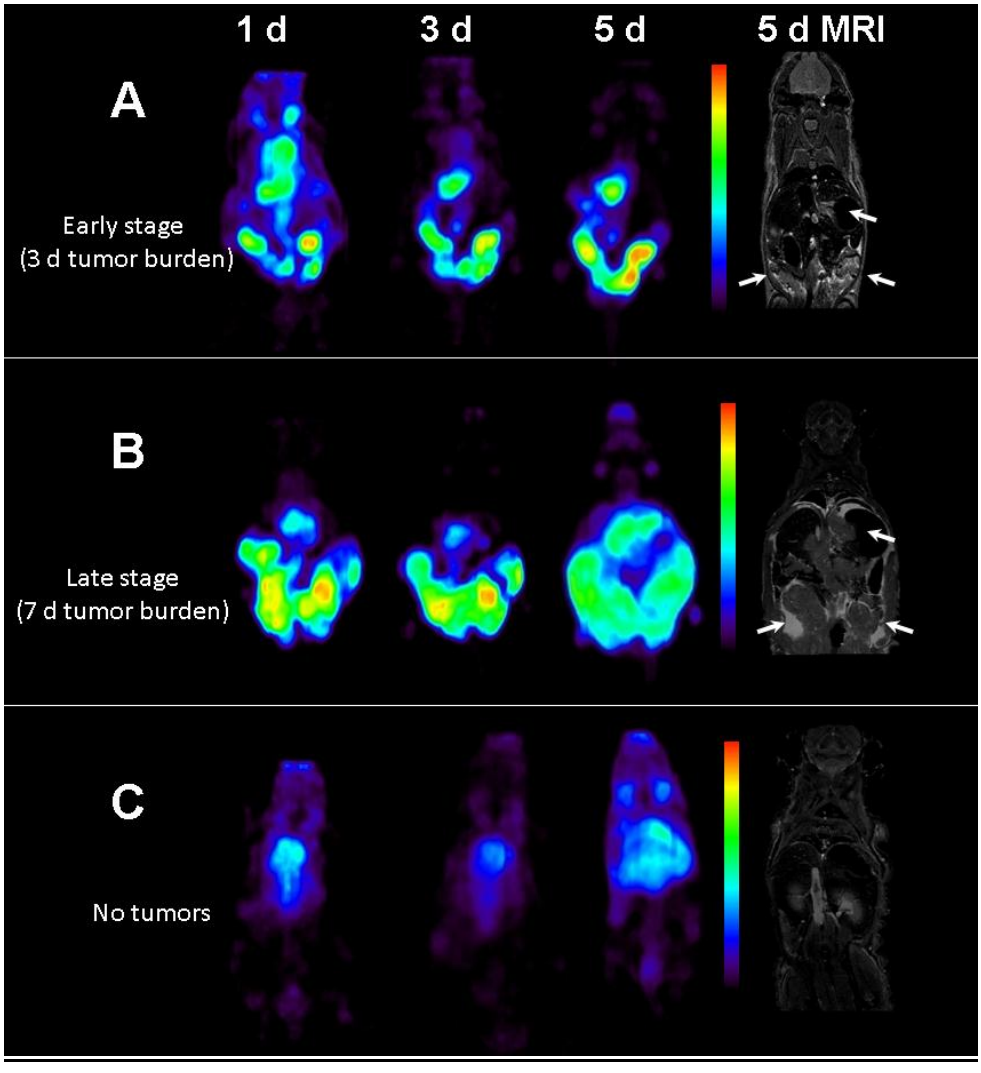
Supplemental Figure 2



(A) Time–activity curve and uptake values of ^{89}Zr labeled panitumumab in selected organs of female athymic (NCr) *nu/nu* mice bearing HER1-positive LS-174T and HER1-negative A375 tumor xenografts assessed through quantitative small-animal PET. (B) Comparative AUC values derived from quantitative small-animal PET.

* Receptor blocking studies were performed by co-injecting 0.1 mg of panitumumab with radiolabeled antibody.

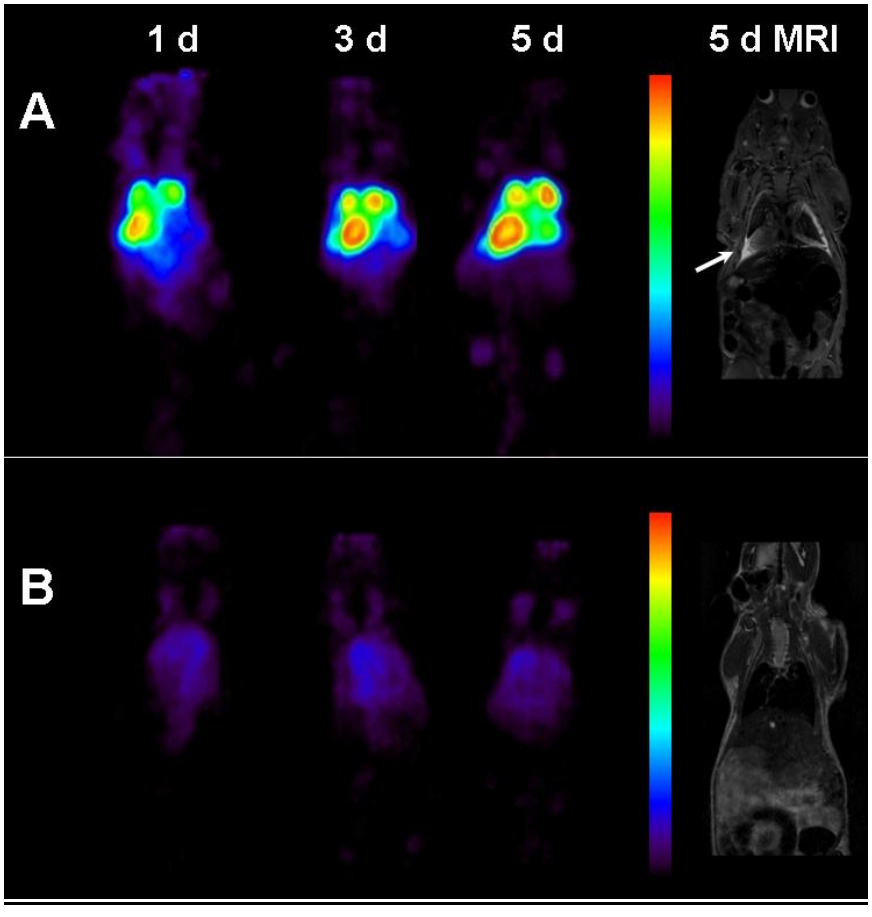
Supplemental Figure 3



(A) Representative reconstructed and processed maximum-intensity PET projections and corresponding T2-weighed coronal MRI slice of female athymic

(NCr) *nu/nu* mouse bearing intraperitoneal LS-174T tumors (3 d burden), (B) mouse bearing intraperitoneal LS-174T tumors (7 d burden) and (C) non-tumor bearing mouse. Tumors are indicated with white arrows. All mice were imaged after i.p. injection of 1.7–1.9 MBq ^{89}Zr labeled panitumumab.

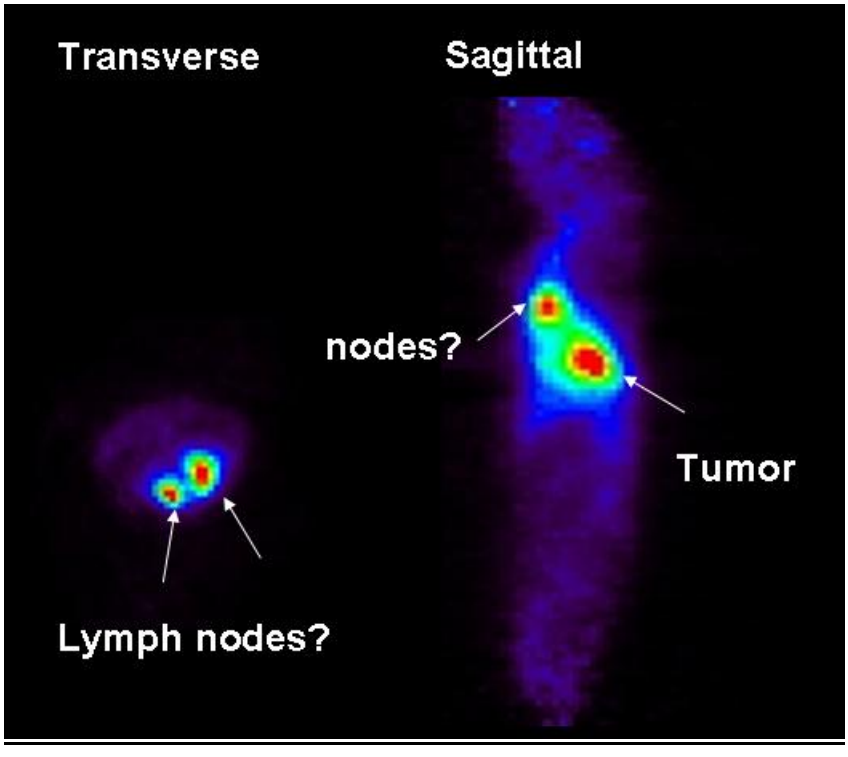
Supplemental Figure 4



(A) Representative reconstructed and processed maximum-intensity PET projections and corresponding T2-weighed coronal MRI slice of female athymic (NCr) *nu/nu* mouse bearing pulmonary metastatic LS-174T tumors and (B) non-

tumor bearing mouse. Scale represents percentage of maximum and minimum threshold intensity. Tumors are indicated with white arrows. All mice were imaged after i.v. injection of 1.7–1.9 MBq ^{89}Zr labeled panitumumab.

Supplemental Figure 5



PET imaging of suspected lymph node metastases in mice bearing metatstatic pulmonary LS-174T tumors injected with ^{89}Zr labeled panitumumab.