

SUPPLEMENTAL DATA

MATERIALS AND METHODS

Image Acquisition

Animal imaging was performed using a Siemens Inveon PET-CT Multimodality System (Siemens Medical Solutions, Inc., Knoxville, TN) with an intrinsic spatial resolution of 1.5 mm. The CT projections (360/rotation) were acquired for 15 minutes with a power of 80 kV, current of 500 μ A, exposure time of 145 ms, and binning size of 4. The CT images were reconstructed using COBRA reconstruction software (Exxim Computing Corporation, Pleasanton, CA). The CT reconstruction protocol, that used a downsampling factor of 2, was set to interpolate bilinearly using a Shepp-Logan filter. PET was performed immediately after CT data acquisition. PET images were acquired for 15 minutes. The acquisition energy window was set to 350-650 keV for ^{124}I . The field of view (FOV) was centered at the shoulder of the mouse. PET images were reconstructed with a 3D Ordered-Subset Expectation Maximization followed by Maximum a Posteriori (OSEM3D/MAP) algorithm using the Siemens Inveon Acquisition Workplace (Siemens Medical Solutions, Inc., Knoxville, TN).

Image Analysis

PET and CT images were co-registered in the AMIDE software package (1). Two ellipsoidal regions of interest (ROIs) were defined manually (Supplemental Figure 1). One ROI encompassed the tumor in all planes. The second ROI was defined in the chest area to measure

the background intensity. The chest ellipsoid axis lengths were fixed to 15 mm, 26 mm and 10 mm in the x, y and z directions, respectively. For each mouse, the mean tumor intensity and the mean background intensity were calculated by averaging the intensity of the voxels within the two ROIs. The dimensionless Contrast Measure (CM) was defined as:

$$CM = \frac{|\bar{I}_T - \bar{I}_b|}{\bar{I}_b},$$

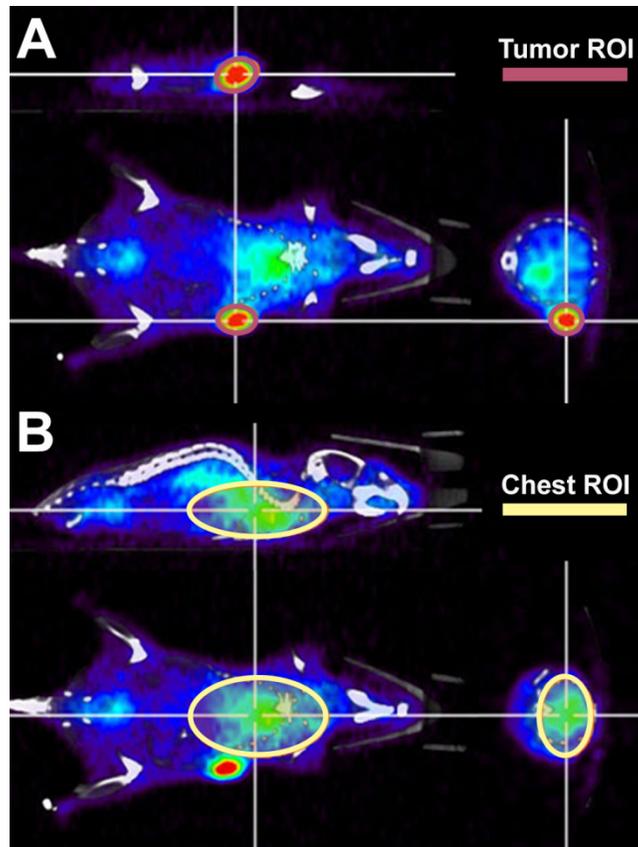
where \bar{I}_T and \bar{I}_b denote the mean tumor intensity and the mean background intensity, respectively. The 3D animal images were created using the 3D volume rendering tool of the AMIDE software. The PET images were displayed using linear intensity adjustments with a zero minimum threshold. For the displays of the images at the same time points, the maximum thresholds were set to show the tumors with equivalent display values.

Statistical Analyses

One-way ANOVA in the statistics toolbox of MATLAB (The Mathworks Inc., Natick, MA) was used to perform statistical analyses. Paired comparisons were carried out using the Tukey-Kramer test (95% or 99% confidence intervals).

REFERENCES

1. Loening AM, Gambhir SS. AMIDE: a free software tool for multimodality medical image analysis. *Mol Imaging*. 2003;2:131-137.



SUPPLEMENTAL FIGURE 1: Different views of the tumor region of interest (ROI) (A) and the chest ROI (B) were overlaid with PET and CT images to determine contrast measures (as an example, the PBS-treated mouse shown in Figure 2 is used).

SUPPLEMENTAL VIDEO LEGENDS

SUPPLEMENTAL VIDEO 1: PET/CT 3D volume rendering of the Abdeg-treated mouse shown in Figure 2 at 24 hours following ^{124}I -pertuzumab injection.

SUPPLEMENTAL VIDEO 2: PET/CT 3D volume rendering of the wild type IgG1-treated mouse shown in Figure 2 at 24 hours following ^{124}I -pertuzumab injection.

SUPPLEMENTAL VIDEO 3: PET/CT 3D volume rendering of the PBS-treated mouse shown in Figure 2 at 24 hours following ^{124}I -pertuzumab injection.