Supplemental Data

αGPC3 IgG1 Generation

RBF/DnJ mice were immunized with recombinant carrier-free human GPC3 protein in Freund's adjuvant solution. Following several boost injections, antiserum ELISAs confirmed presence of the αGPC3 IgG. Additional boost injections were delivered to ensure IgM/IgG switch, which was verified on ELISA with IgG titrated to 1:10,000. Following final pre-fusion boost injections, mice were euthanized, their spleens were harvested, and 10^8 splenocytes were fused 1:1 with FOX-NY myeloma cells, and resultant hybridomas were resuspended in adenine/aminopterin/thymidine FBS solution. Clones producing high titers of GPC3 IgG1 were selected using capture ELISA with goat anti-mouse IgG1 for isotyping.

Production of ^{89}Zr-labeled αGPC3

All reagents were purchased from Sigma-Aldrich unless otherwise indicated. PD-10 and Superdex-200 (10/300 GL) chromatography columns were purchased from GE-Healthcare. In process radioactivity (>0.2 MBq) was measured using a Capintec ion-chamber (CRC-25R, setting 465) and radio-HPLC was performed with a Carroll Ramsey and Associates unit (105-S) equipped with Si-PIN diode radiation detector. ITLC was performed with paper strips from Biodex (cat. no. 150-001) eluted with buffered 0.02 M sodium citrate.

A polypropylene conical Falcon tube (50 mL, Blue-Max, Becton-Dickinson) was successively charged with: Zr-89 in 1 M oxalate (400 μL, 270 MBq (7.28 mCi)); 2M sodium carbonate (180 μL, slowly); 1M HEPES buffer (300 μL); water (210 μL); Df-(NCS)-antibody (P5F8) conjugate (200 μL, 1 mg protein in 0.25 M sodium acetate trihydrate buffer with 5 mg/ml gentisic acid); 1M HEPES buffer (355 μL) and water (355 μL). The final mixture (2.0 mL) was incubated at room temperature for 1 h, with intermittent swirling, then loaded onto a pre-washed (0.25 M sodium acetate trihydrate with 5 mg/mL gentisic acid, pH 5.4) PD-10 column, in addition to 2 × 0.5 mL reaction tube rinses (0.25 M NaOAc-gentisic acid buffer). The column was next eluted with the acetate-gentisic buffer and fractions (0.5 mL) were serially collected.

Micro-PET

A transmission scan was collected for each mouse as part of each imaging session. The vendor's three-dimensional ordered subset, maximum a posteriori (3D OSEM-MAP) image reconstruction with attenuation and scatter correction was used to create the mouse images. The image resolution was specified at 1.5 mm full width at half maximum (FWHM) for the 3D OSEM-MAP reconstruction. Data were exported via DICOM and region of interest (ROI) analysis was conducted off-line using Osirix (open source software, http://www.osirix-viewer.com/).