Supplemental Materials

Radiolabeling of 5F7 Nanobody with ^{18}F-RL-I, ^{18}F-SFB, and ^{125}I-SGMIB

The prosthetic agent ^{125}I-SGMIB was synthesized by the radioiodination of its tin precursor as reported before (*1*). Briefly, acetic acid in $CHCl_3$ {3% (v/v), 2 µl}, *tert*-butyl hydroperoxide (TBHP) in $CHCl_3$ {30% (w/v), 5 µL}, and 50 µg of tin precursor in $CHCl_3$ (15 µl) were added to a vial containing 37-74 MBq ^{125}I in 2-4 µL of 0.1 N NaOH. The mixture was stirred at 20 °C for 30 min, and ^{125}I-SGMIB was purified by normal phase HPLC. ^{18}F-SFB was synthesized by adapting a reported one pot procedure (*2*) and purified by normal phase HPLC (*3*). The synthesis and purification of ^{18}F-RL-I was performed as described recently by the click reaction of a protected azido precursor with ^{18}F-fluorohexyne (*3*). It was deprotected by treatment with trifluoroacetic acid before reaction with the Nanobody.

The Nanobody 5F7 was radioiodinated using ^{125}I-SGMIB as reported before (*4*) and it was labeled with ^{18}F using ^{18}F-SFB adapting procedures used for labeling other proteins (*5*). Briefly, 50 µl of 5F7 solution in 0.1M borate buffer (2 mg/ml), pH 8.5 was added to the radiolabeled prosthetic agents, and the mixture incubated at 20^{o}C for 20 min. Labeled Nanobody was isolated by gel filtration over a PD10 column (GE Healthcare, Piscataway, NJ) that was eluted with PBS, pH 7.4, and collecting 250 µL fractions; the protein typically eluted in fractions 5-10. For labeling 5F7 with ^{18}F-RL-I, essentially the same procedure was used except 100 µl of the Nanobody solution was used.

Internalization assay

The ability of the prosthetic groups ^{18}F-RL-I and ^{18}F-SFB for trapping ^{18}F activity from radiolabeled 5F7 Nanobody within HER2-expressing cells after internalization was determined by paired label assays using BT474M1 breast carcinoma cells. For this, 5F7 Nanobody labeled with ^{125}I-SGMIB served as common reference. Cells were plated at a density of 8 x 10^5 cells per well in 3 mL medium in 6-well plates. After overnight incubation at 37°C, cells were incubated at 4°C

for 30 min. Medium was replaced with fresh medium containing labeled protein pair (5 nM each of ^{18}F-RL-I-5F7 and ^{125}I-SGMIB-5F7, or ^{18}F-SFB-5F7 and ^{125}I-SGMIB-5F7) and the cells were incubated at 4°C for 1 h. Cell culture supernatants containing unbound radioactivity were removed and cells were supplemented with fresh medium, and brought to 37°C. Cells were incubated at 37°C for 1, 2 and 4 h, and processed as follows. After withdrawing cell culture supernatants, cell membrane-bound radioactivity was removed by incubating with an acidic medium, and cells were solubilized by treatment with 0.1 N NaOH. Cell culture supernatants, acid wash, and cell lysates were counted for ^{18}F and ^{125}I radioactivity using an automated gamma counter (Perkin Elmer Wizard II, Shelton, CT) that corrects for cross-over, decay and dead time. From these data, the percent of initially bound radioactivity that was internalized, membrane-bound and that in supernatants was calculated. Parallel experiments also were performed with a 100-fold molar excess of trastuzumab to determine nonspecific uptake.

BT474M1 xenografts

Sixty-day continuous-release 17-β-estradiol pellets (Innovative Research of America) were implanted in the backs of 10- to 12-wk-old female NOD.CB17-Prkdcscid/J mice (Jackson Laboratories). Two days later, the mice were inoculated in the flank with 5×10^6 BT474M1 cells in 50% Matrigel (BD Biosciences). Biodistribution studies were initiated 5-6 weeks later when tumors reached a volume of 350–500 mm^3.

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