SUPPLEMENTAL DATA

SUPPLEMENTAL METHODS

Conjugation Of Monoclonal Antibody With Chelator

The antibody in phosphate buffered saline (PBS) was first adjusted to pH 9.2 using 0.07 M sodium borate buffer (Sigma Aldrich). The sample was concentrated using an Amicon Ultra-2 centrifugal filter (2 mL, 100 K, Millipore). The chelator CHX-A″-DTPA, dissolved in borate buffer, was added to the protein solution in a molar ratio of 3:1 chelator to antibody, and the mixture was incubated with gentle shaking at 40°C *(1)*. The reaction was terminated after 4 h, and the conjugates, from now on called DTPA-m11B6 or DTPA-h11B6, were separated from free chelator by size-exclusion chromatography on a NAP-5 column (GE Healthcare) equilibrated with 20 mL 0.2 M ammonium acetate buffer, pH 5.5 (Sigma Aldrich). Conjugated 11B6 was eluted with 1 mL ammonium acetate buffer (Sigma Aldrich) and was stored at -20°C.

Radiolabeling

Radiolabeling was acomplished by mixing, typically 150 μL of conjugated 11B6 (~1 μg/μL in 0.2 M in ammonium acetate buffer, pH 5.5) with a predetermined amount (~150–200 MBq) of $^{177}LuCl_3$ (IDB Petten BV), and adjusted to a total volume of 500 μL. After incubation at room temperature for 2 h, the labeling was terminated, and the mixture was purified on a NAP-5 column equilibrated with PBS (Thermo Scientific). Labeling efficiency and labeling kinetics were monitored with instant thin-layer chromatography strips, eluted with 0.2 M citric acid (Sigma Aldrich). The radioactivity distribution was determined with a PhosphorImager system using Optiquant as the quantification software (Perkin Elmer).

Cell Line

LNCaP (hK2+) cells were purchased from American Type Culture Collection. Cells (ATCC) were cultured in RPMI 1640 medium (Thermo Scientific) supplemented with 10% fetal bovine serum (Thermo Scientific) with 100 IU/mL penicillin and 100 µg/mL streptomycin (Thermo Scientific). The cells were maintained at 37°C in a humidified incubator at 5% CO_2 and were detached with trypsin-EDTA solution (Thermo Scientific).

Intensity calibration of optical imaging system

The imaging system was intensity-calibrated using a Tungsten light source (OceanOptics LS-1, Dunedin, FL, USA) connected to a fiber patch cord. A fiber tip with a known diameter and numerical aperture was imaged using the camera setup. The power emitted from the fiber tip within a known spectral interval was measured using a power meter (Ophir Nova II, Ophir, Israel). Using these measurements, the radiance emitted from the fiber tip could be calculated. A calibration coefficient that relates the camera counts (ADU) to radiance was then retrieved from the image of the fiber tip.

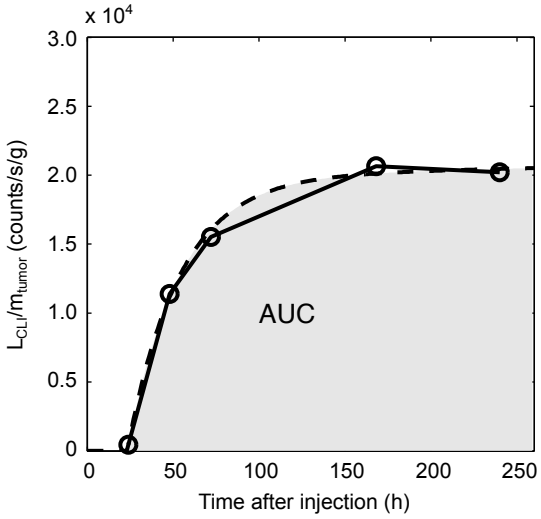
Supplemental Table 1. Experimental details for the different animal groups

Group	Strain	n	Radioimmuno-conjugate	Administered Activity (MBq)	CLI time points (h p.i.)	SPECT time points (h p.i.)
1	Balb/c-nu	2	^{177}Lu-m11B6	20	24,48,72,168,264,336	24,48,72,168,264,336
1	NMRI-nu	3	^{177}Lu-m11B6	20	24,48,72	24,48,72
2	Balb/c-nu	4	^{177}Lu-h11B6	20	24,48,72,168,ςφτ	NA

SUPPLEMENTAL RESULTS

Tumor Response Monitoring Using CLI

A representative plot of the CLI radiance, normalized with the tumor weight, is plotted against time p.i. in Supplemental Figure 1. The shaded area in Supplemental Figure 1 represents the AUC.



Supplemenal Figure 1: A representative plot of the CLI radiance normalized with the tumor weight. The shaded area represents the area-under-curve (AUC).

SUPPLEMENTAL REFERENCES

1. Tolmachev V, Wallberg H, Andersson K, Wennborg A, Lundqvist H, Orlova A. The influence of Bz-DOTA and CHX-A''-DTPA on the biodistribution of ABD-fused anti-HER2 Affibody molecules: implications for (114m)In-mediated targeting therapy. *Eur J Nucl Med Mol Imaging.* 2009;36:1460-1468.