

Supplementary information

Preclinical evaluation of podoplanin-targeted alpha-radioimmunotherapy with the novel antibody NZ-16 for malignant mesothelioma

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Supplementary Methods

Cell binding assay

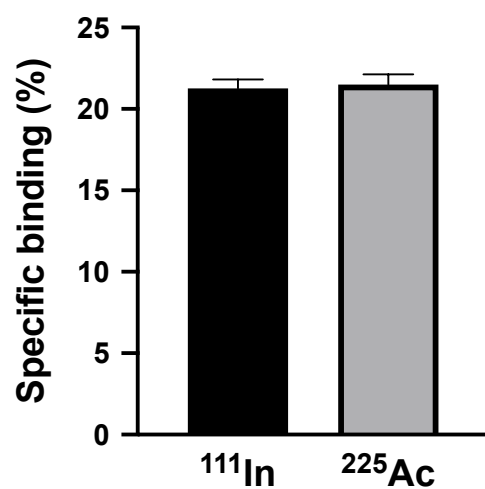
H226 cells (1.3×10^6) in phosphate-buffered saline with 1% BSA were incubated with ²²⁵Ac-labeled NZ-16 antibody on ice for 60 min. After washing, cell-bound radioactivity was measured using a gamma-counter using an energy window of 200–300 keV.

Histologic analysis

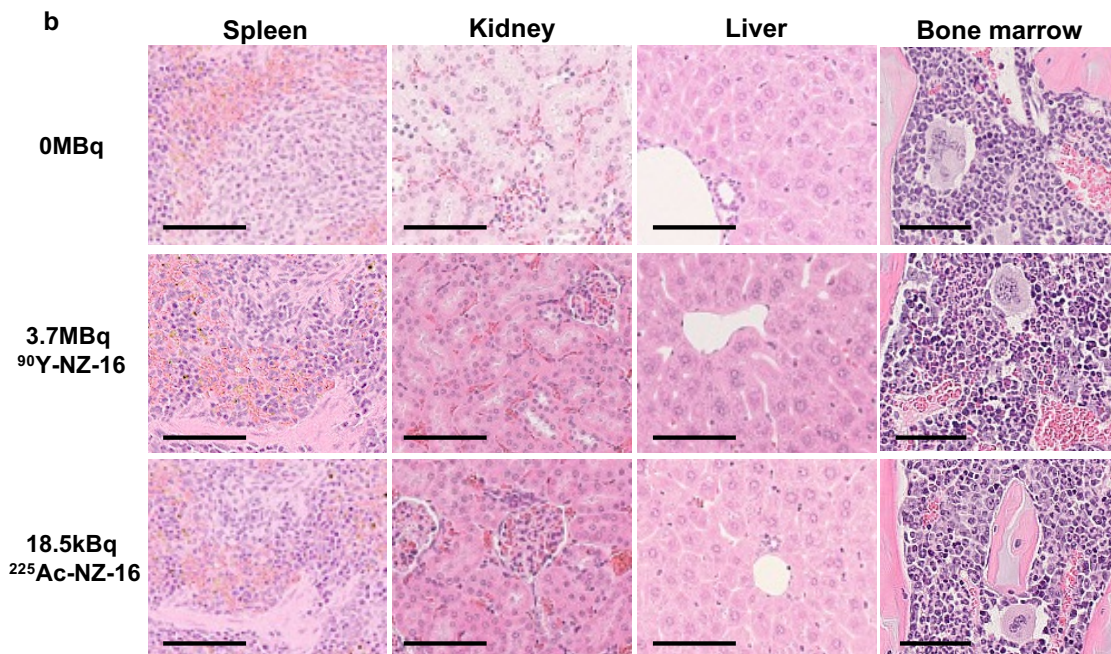
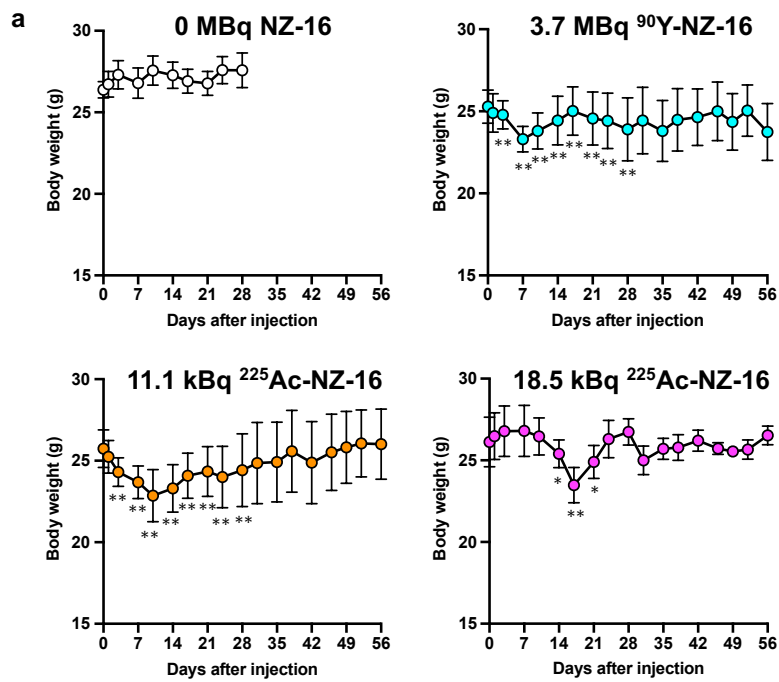
The spleen, kidney, liver and femur (bone marrow) were resected from mice on day 7 after injection with intact NZ-16 (0 MBq, n = 3/time-point), ⁹⁰Y-labeled NZ-16 (3.7 MBq, n = 3/time-point) and ²²⁵Ac-labeled NZ-16 (18.5 kBq, n = 3/time-point). The organs were fixed in 10% neutral-buffered formalin and embedded in paraffin. The organs sections (1-μm thick) were deparaffinized and stained with hematoxylin and eosin (H&E).

TUNEL staining

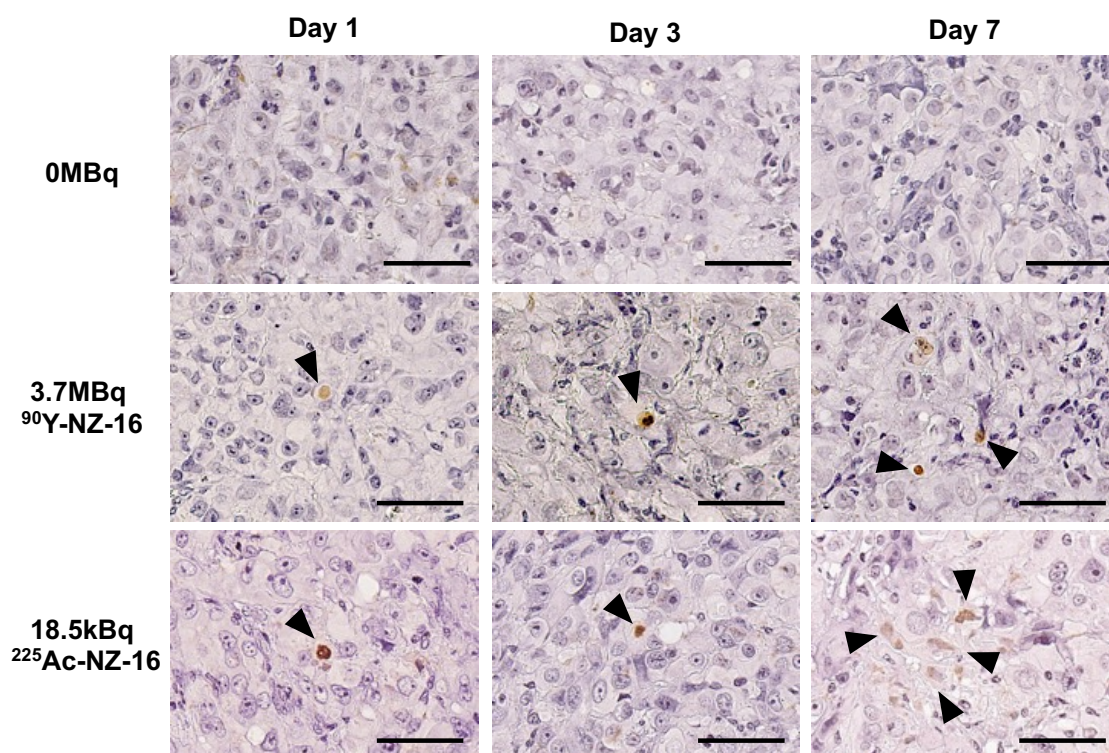
H226 tumors were resected from mice on days 1, 3, and 7 after injection with intact NZ-16 (0 MBq, n = 3/time-point), ⁹⁰Y-labeled NZ-16 (3.7 MBq, n = 3/time-point) and ²²⁵Ac-labeled NZ-16 (18.5 kBq, n = 3/time-point). The tumors were fixed in 10% neutral-buffered formalin and embedded in paraffin. Apoptosis was detected using the DeadEnd Colorimetric TUNEL System (Promega, Madison, WI, USA).



Supplementary Figure 1. Cell binding assay of ^{111}In - and ^{225}Ac - labeled NZ-16 with H226 cells (1.3×10^6 cells). Data indicate the mean and standard deviation. n.s., not significant.



Supplementary Figure 2. Side effects after treatments. (a) Body weight changes after injection with ^{90}Y - and ^{225}Ac -labeled NZ-16. Data indicate mean and standard deviation ($n = 5$). * $P < 0.05$, ** $P < 0.01$ vs. 0 MBq NZ-16. (b) H&E-stained sections of spleen, kidney, liver, and bone marrow. Scale bar, 50 μm .



Supplementary Figure 3. TUNEL-stained H226 tumors at days 1, 3, and 7 after injection with 0 MBq (intact NZ-16 only), 3.7 MBq of ⁹⁰Y-labeled NZ-16, and 18.5 MBq of ²²⁵Ac-labeled NZ-16. Arrowheads indicate TUNEL positive cells. Bar, 50 μm