Supplemental Materials and Methods

HER2 expression of cell lines using flow cytometry

All incubation steps were performed on ice and in 100 µl final volume. 1-2x10^5 cells were incubated for 20 min with 10 ng of PE mouse anti-HER-2/neu (clone Neu 24.7, BD Biosciences) or PE mouse IgG1, κ isotype control (clone MOPC-31C, BD Biosciences). Tubes were washed twice with PBS-BSA (0.5%). Samples were measured on a FACS Canto II analyzer (BD Biosciences) and analyzed with FlowJo 10 (TreeStar). Cells were gated for singlets before binding analysis.

Crystallization and structure determination of the HER2-2Rs15d complex

The top candidate for large-scale protein production identified by PROTEROS encodes HER2 residues 24 to 646 and equips HER2 with a C-terminal His-tag. HER2(24-646)His was purified from the supernatant of transfected HEK293F cells using a protocol comprising affinity and SEC steps and subsequently deglycosylated. This procedure yielded homogeneous protein with a purity greater than 95% as judged by SDS-PAGE analysis. The HER2-2Rs15d complex was formed by mixing equimolar amounts of HER2(24-646)His with 2Rs15d. Crystallization conditions were screened using a standard screen with approximately 1200 different conditions. Hits were further optimized by systematically varying the incubation temperature, protein concentration, drop ratio, pH, and precipitant concentrations. The His tag of HER2(24-646)His was retained for crystallization.

Diffraction-quality crystals of the complex were obtained in optimized crystallization conditions (100 mM HEPES pH 7.25, 10% 2-propanol, 22% PEG 4000). The HER2-2Rs15d crystals were cryocooled in liquid nitrogen with the addition of 25%(v/v) glycerol to the mother liquor as a cryoprotectant. Data were collected at the Swiss Light Source (SLS, Villigen, Switzerland) and were processed with XDS (1). The quality of the collected data sets was verified by close inspection of the XDS output files and through phenix.xtriage in the PHENIX package (2). Analysis of the unit-cell contents was performed with the program MATTHEWS_COEF, which is part of the CCP4 package (3). The structure of the HER2-2Rs15d complex was determined by molecular replacement with PHASER-MR (4) using the structure of HER2 as a search model. From here, refinement cycles using the maximum likelihood target function cycles of phenix.refine (5) were alternated with manual building using Coot (6). The final refinement cycle included TLS (Translation/Libration/Screw) refinement, for which the optimal TLS groups were determined using the TLSMD Web server (7). Details on data collection and structure determination are given in Supplementary Table S1.

Preparation of [^{131}I]-labeled sdAbs

[^{131}I]SGMIB was synthesized and purified as reported previously (8,9). Sodium [^{131}I] iodide was reacted with its trimethylstannyl precursor in chloroform for 30 min at RT, after which Boc-protected [^{131}I]SGMIB was purified on normal phase HPLC, using an ethyl acetate/hexane gradient. Deprotection was acchieved with a 10 min incubation at RT with trifluoroacetic acid. Finally, [^{131}I]SGMIB was incubated with 100 µg sdAb in 0.1 M borate buffer pH 8.5 for 20 min at RT, and the labeled conjugate was purified using a PD-10 column. Quality control (QC) was performed by instant thin layer chromatography (iTLC), using glass microfiber sheet impregnated silica gel strips (Agilent, Lake Forest, CA, USA), run with PBS, pH 7.4. In parallel, radio-size exclusion chromatography (SEC, 0.5mL/min, 0.02 M phosphate buffer and 0.28 M NaCl, pH 7.4, Superdex 75 5/150 GL, 5 bar) was performed.

In vitro targeting of sAbs using Flow cytometry

All incubation steps were performed on ice and in 100 µl final volume. 2x10^5 cells were incubated for 1 h with either 1 µM sdAb or Trastuzumab, or buffer (stain control). sdAb binding was detected by sequential incubation with 1 µg mouse anti-Histidine tag (clone AD1.1.10, Bio-Rad) for 1 h and 0.2 µg PE rat anti-mouse IgG1 (clone A85-1, BD Biosciences) for 30 min. Trastuzumab binding was detected by incubation with 1 µg PE mouse anti-human IgG1 (clone IS11-12E4.23.20, Miltenyi Biotec). Tubes were washed once (after sdAb binding) or twice with PBS-BSA (0.5%) in between steps. Samples were measured on a FACS Canto II analyzer (BD Biosciences) and analyzed with FlowJo 10 (TreeStar). Cells were gated for singlets before binding analysis.

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