**Supplementary Information**

**Material and methods**

**Antibody ELISA**

Maxisorp 96-well microplates (Nalge-Nunc International, Roskilde, Denmark) were coated with 10 μg/mL CH3O-PEG2K-NH2, OH-PEG5K-NH2 or CH3O-PEG20K-NH2 in 50 μL/well 0.1 M NaHCO3/Na2CO3 (adjusted to pH 8.0 with HCl) buffer for 3 h at 37°C and then blocked with 200 μL/well dilution buffer (5% skim milk in PBS) at 4°C overnight. The indicated concentrations of antibodies in 50 μL 2% skim milk in PBS were added to the plates at RT for 1 h. The plates were washed with PBS containing 0.1% CHAPS (Sigma-Aldrich) three times. HRP-conjugated donkey anti-mouse IgG Fc antibody (Jackson ImmunoResearch) in 50 μL dilution buffer were used for detecting bound anti-PEG antibodies on the microplates. After incubating for 1 h at RT, the plates were washed as described above. The bound peroxidase activity was measured by adding 100 μL /well ABTS solution [0.4 mg/mL, 2,2’-azinobis(3-ethylbenzthiazoline-6-sulfonic acid), 0.003% H2O2, and 100 mM phosphate-citrate, pH 4.0) at room temperature. The absorbance (405 nm) of wells was measured in a microplate reader (Molecular Device).

**Flow cytometer analysis**

To compare the surface HER2 expression on different cancer cell lines, 2 μg/mL anti-HER2 antibody was incubated with various live cancer cells including SK-BR-3, BT-474 and MCF-7 breast cancer, HT-29 and SW-480 colon colorectal cancer cells and HCC-36 hepatocarcinoma cells in RPMI medium for 30 min on ice. After washing with cold PBS containing 0.05% BSA, the cells were incubated with 2 μg/mL Alexa Fluor 647 conjugated goat anti-human IgG (H+L) antibody (Invitrogen) for 30 min on ice. After washing twice with cold PBS containing 0.05% BSA, the surface immunofluorescence of 104 viable cells was measured with a FACS LSR II flow cytometer (Becton Dickinson, Mountain View, CA, USA) and fluorescence intensities were analyzed with Flowjo (Tree Star Inc., San Carlos, CA, USA).

**Surface plasmon resonance (SPR)**

HER2 ECD in 10 mM acetate buffer (pH 5.0) was immobilized on a CM5 chip by the standard amine coupling method through the EDC/NHS reaction at immobilization levels of 500 response units. The binding of hybrid antibodies to HER2 ECD was measured by injecting defined concentrations (ranging from 8 nM to 128 nM) of αPEG:αHER2 hybrid antibodies at a constant flow rate of 30 µL/min in HBS-EP+ buffer (GE Healthcare). All the binding assays were carried out at 25°C, and the dissociation steps were monitored for 10 min. The kinetic parameters were calculated by Biacore T200 Evaluation software (GE Healthcare).

## MicroScale thermophoresis (MST) measurements

 CH3O-PEG5K-NH2 was labeled with Alexa Fluor 647-NHS Ester (Invitrogen) according to the manufacturer’s instructions. 0.5 nM CH3O-PEG5K-Alexa Fluor 647 was mixed with different concentrations of hybrid antibodies (ranging from 8 nM to 4 μM) in PBS buffer containing 0.05% BSA. The thermophoresis of the bound and unbound CH3O-PEG5K-Alexa Fluor 647 was measured and the kinetic constant values of interactions between PEG molecules and the hybrid antibodies were calculated on a Monolith NT.115Pico machine **(**NanoTemper Technologies GmbH

Supplementary Table S1. EC50 concentrations of mouse anti-PEG antibodies binding to various PEG molecules*a*

|  |  |  |  |
| --- | --- | --- | --- |
| Antibody | OH-PEG5K-NH2 (nM) | CH3O-PEG2K-NH2 (nM) | CH3O-PEG20K-NH2 (nM) |
| IgG1 control | - | - | - |
| E11 | 6.8±0.5 | 25.3±0.5 | 3.5±0.5 |
| 3.3 | 2.8±1.1 | 2.2±0.1 | 1.2±0.1 |
| 6.3 | 0.2±0.0 | 0.2±0.0 | 0.2±0.0 |

*a* Results represent mean values of duplicate determinations ± SD

Supplementary Table S2. Kinetic constants for binding of humanized hybrid antibodies to HER2 ECD *a* and PEG5K *b*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Hybrid antibody | *k*on (M-1s-1) HER2 ECD | *k*off (s-1) HER2 ECD | K*D* (M) HER2 ECD | K*D* (M) PEG5K |
| αPEG:αHER2 | (1.4±0.3) x 105 | (7.1±0.6) x 10-4 | (5.3±0.8) x 10-9 | (16.8±8.3)x 10-9 |
| αPEG:αCD19 | NA\* | NA\* | NNA\* | (29.5±3.6)x 10-9 |

*a* Results represent mean values of triplicate determinations ± SD by surface plasmon resonance

*b* Results represent mean values of triplicate determinations ± SD by MicroScale thermophoresis (MST) measurements

\* Not applicable

**Supplementary Figures legends**

**Supplementary Figure S1.** **Outline of the hybrid antibody purification process**. Hybrid antibodies from 293 FT cells were affinity purified on protein A to remove mismatched light chain pair and excess light chain, CNBr-PEG to remove αHER2-hole homodimers and anti-HA agarose to remove αPEG-knob homodimers.

**Supplementary Figure S2. Comparison of the binding of mouse anti-PEG antibodies to different PEG molecules.** Graded concentrations of the indicated monoclonal anti-PEG antibodies E11, 3.3, 6.3 and isotype-matched control IgG1 antibody were added to microplates pre-coated with **A**, OH-PEG5K-NH2, **B**, CH3O-PEG2K-NH2 or **C**, CH3O-PEG20K-NH2 and incubated for 1 h at room temperature. After washing with PBS containing 0.1% CHAPS, HRP-conjugated donkey anti-mouse IgG Fc antibody was added to the microplates for 1 h at room temperature. After washing, ABTS substrate was added and the mean absorbance values (405 nm) of duplicate samples were determined. Bars, SD.

**Supplementary Figure S3. Comparison of the surface HER2 expression of different human cancer cell lines.**

 **A**, Surface HER2 expression on SK-BR-3, BT-474, MCF-7, HT-29, SW-480 and HCC-36 cancer cells were compared by incubating cells with anti-HER2 antibody and detected by Alexa Fluor 647 conjugated anti-human IgG Fc antibody. **B**, Mean fluorescence intensities are shown. Mean ± SEM.