Supplementary Methods

Construction of expression plasmids. The plasmids for the periplasmic expression of scFv-barnase and barstar fusion proteins are based on the vector pIG6-4D5 (refs 1-3). The barstar and barnase genes were amplified out of the plasmid pMT413 (ref. 4) using the primers *Asc*I_bs-fwd 5'-TGGCGCGCCGAAAAAAGCAGTCATTAACGGG, bs_*Asc*I-rev 5'-CGGCGCGCCAGAAAGTATGATGGTGATGTC, *Asc*I_bn-fwd 5'-GTGGCGCGCCTGCACAGGTTATCAACACGTTTG and bn_*Asc*I-rev 5'-GTGCGGCGCGCCTCTGATTTTTGTAAAGGTCTG. The *Asc*I fragments were ligated into the vector pIG6-4D5. The gene for the plasmid-encoded fusion protein V_L-linker-V_H-hinge-barstar-His_5-tag was easily obtained. In contrast, no clones with the correct barnase gene were found initially. To obtain 4D5 scFv-barstar derivative without a His-tag, the *Bsp*EI/*Hin*dIII fragment was cut out from the 4D5 scFv-barstar-His_5 encoding plasmid and replaced by the *Bsp*EI/*Hin*dIII fragment from the plasmid pMT413. To introduce the barstar gene for bicistronic transcription downstream of the scFv-barnase encoding gene, the barstar gene was amplified from the plasmid pMT413 using primers *Hin*dIII_bs-fwd 5'-CGTCTAAGCTTGATGAAAAAAGCAGTCATTAACG and bs_*Hin*dIII-rev 5'-AACAGCTATGACCATGATTACG and ligated into the *Hin*dIII-digested plasmid pIG6-4D5. The resulting plasmid was used for cloning the barnase gene flanked by *Asc*I sites. To introduce in the vector pIG6-4D5 the barstar gene under the control of its own promotor the fragment was amplified from the plasmid pMT413 using primers *Hin*dIII_pbs-fwd 5'-ATCAGACCTTTACAAAAAGCTTATAAC and bs_*Hin*dIII-rev and cloned in the *Hin*dIII site. The vector so obtained was used to fuse the barnase gene to the scFv fragment 4D5 by using the *Asc*I site. The resulting plasmid had no mutations and encoded the fusion protein V_L-linker-V_H-hinge-barnase-His_5-tag. The plasmid carrying the dimeric barnase fragment instead of barnase was derived analogously by using overlap extension PCR and primers dibn-fwd 5'-CAGACCTTTACAAAAATCAGAGACACGTTTGACGGGGTTGC, dibn-rev 5'-GCAACCCCGTCAAACGTGTCTCTGATTTTTGTAAAGGTCTG, *Asc*I_bn-fwd and bn_*Asc*I-rev.

Antigen-binding ELISA. MaxiSorp Nunc-Immuno Plates (Life Technologies) were coated with 75 μl of 15 μg/ml $p185^{HER2-ECD}$ antigen (kindly provided by Genetech Inc.) in PBS buffer, pH 7.4, for 16 h at 4° C. The respective scFv constructs at different dilutions (see Supplementary Material Fig.1) were applied in a volume of 100 μl in PBS buffer to each well for 1 h at room temperature and detected with an anti-His-tag antibody[5] as described.

References

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