Supplementary materials

Production and selection of αHGF-Nanobodies

Anti-HGF Nanobodies were generated at Ablynx NV (Ghent, Belgium). Two *Llama glama* were immunized with 100 and 50 µg human HGF (Peprotech, cat# 100-39) in Stimmune adjuvant (Cedi Diagnostics) and blood was collected. These animal experiments were conducted with the approval of the Ethical Committee of the Faculty of Veterinary Medicine, University of Ghent, Belgium.

Anti-HGF Nanobodies were isolated using phage display. To this end, 2 libraries were constructed and panned on human HGF. Principles in short; peripheral blood mononuclear cells were prepared from blood samples using Ficoll-Hypaque according to the manufacturer's instructions. Next, total RNA was extracted from these cells and used as starting material for RT-PCR to amplify Nanobody encoding gene fragments. These fragments were cloned into phagemid vector pAX50. Phage was prepared according to standard methods and stored after filter sterilization at 4^{o}C for further use. Phage library size from both animals was 0.7 x 10^{8} and 2.1 x 10^{8}, and percentages of insert 100 and 91.3%, respectively. Phage libraries were used for selection on immobilized human HGF, and the best selections, with the highest enrichment factor, were chosen for further analysis. Bound phage was eluted by addition of trypsin and rescued via infection of *E. coli*. Individual colonies were picked and grown in 96 deep well plates (1 mL).

Nanobody expression was induced by addition of IPTG and periplasmic extracts (80 µL) were prepared according to standard methods. Alternatively, selected Nanobodies were expressed in the periplasmic space of E. coli as c-myc and His6 tagged proteins in a culture volume of 50 mL and purified via immobilized metal affinity chromatography (IMAC). Nanobodies were eluted from the column with 250 mM imidazole followed by gel filtration and buffer exchange to PBS.

Selected human HGF–specific Nanobodies were converted into a bispecific format by genetic fusion to the albumin-binding Nanobody Alb8 using a flexible Gly-Ser linker (Gly4-Ser-Gly3-Ser).