

CLAIMS

1. Immunoglobulin single variable domain (ISVD), having:

- a CDR1 (according to Abm) that is the amino acid sequence GFTFSTADMG (SEQ ID NO: 5); and
- a CDR2 (according to Abm) that is the amino acid sequence RISGIDGTTY (SEQ ID NO: 6); and
- a CDR3 (according to Abm) that is the amino acid sequence PRYADQWSAYDY (SEQ ID NO: 4);

and having:

- a degree of sequence identity with the amino acid sequence of SEQ ID NO: 1 (in which any C-terminal extension that may be present as well as the CDRs are not taken into account for determining the degree of sequence identity) of at least 85%, preferably at least 90%, more preferably at least 95%;

and/or

- no more than 7, such as no more than 5, preferably no more than 3, such as only 3, 2 or 1 “amino acid differences” (as defined herein, and not taking into account any of the above-listed mutations at position(s) 11, 89, 110 or 112 that may be present and not taking into account any C-terminal extension that may be present) with the amino acid sequence of SEQ ID NO: 1 (in which said amino acid differences, if present, may be present in the frameworks and/or the CDRs but are preferably present only in the frameworks and not in the CDRs);

and optionally having:

- a C-terminal extension (X)_n, in which n is 1 to 10, preferably 1 to 5, such as 1, 2, 3, 4 or 5 (and preferably 1 or 2, such as 1); and each X is an (preferably naturally occurring) amino acid residue that is independently chosen, and preferably independently chosen from the group consisting of alanine (A), glycine (G), valine (V), leucine (L) or isoleucine (I);

in which:

- the amino acid residue at position 11 is preferably chosen from L or V; and
- the amino acid residue at position 89 is preferably suitably chosen from T, V or L; and
- the amino acid residue at position 110 is preferably suitably chosen from T, K or Q; and
- the amino acid residue at position 112 is preferably suitably chosen from S, K or Q;

such that (i) position 89 is T; or (ii) position 89 is L and position 11 is V; or (iii) position 89 is L and position 110 is K or Q; or (iv) position 89 is L and position 112 is K or Q; or (v) position 89 is L and position 11 is V and position 110 is K or Q; or (vi) position 89 is L and position 11 is V and position 112 is K or Q; or (vii) position 11 is V and position 110 is K or Q; or (viii) position 11 is V and position 112 is K or Q.

2. The ISVD according to claim 1, wherein the amino acid residue at position 49 is an alanine; and/or wherein the amino acid residue at position 74 is serine; and/or wherein the amino acid residue at position 73 is N and the amino acid residue at position 75 is K.
3. The ISVD according to any one of claims 1 to 2, wherein said ISVD is chosen from the group consisting of SEQ ID NO:s 40, 39, 36, 37, 38, 41 and 61-68, in particular SEQ ID NO:s 40, 39 and 36, most particularly SEQ ID NO: 40.
4. Immunoglobulin single variable domain according to claim 3, having a D at position 1 and a C-terminal extension X(n), which is preferably a C-terminal alanine residue.
5. Compound comprising at least one ISVD according to any one of claims 1 to 4.
6. The compound according to claim 5 that comprises at least two ISVDs according to any one of claims 1 to 4, wherein said at least two ISVDs can be the same or different.
7. The compound according to claim 6, wherein said at least two ISVDs are independently chosen from the group consisting of SEQ ID NO:s 8-41, 61-66 and 69.
8. The compound according to any one of claims 5-7, wherein said compound further comprises a serum protein binding moiety, wherein said serum protein binding moiety binds serum albumin.
9. The compound according to claim 8, wherein said serum protein binding moiety is a serum albumin binding ISVD, and wherein said serum albumin binding ISVD essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3 respectively) , in which CDR1 is SFGMS, CDR2 is SISGSGSDTLYADSVKG and CDR3 is GGSLSR.
10. The compound according to claim 9, wherein said serum albumin binding ISVD comprises Alb8, Alb23, Alb129, Alb132, Alb11, Alb11 (S112K)-A, Alb82, Alb82-A, Alb82-AA, Alb82-AAA, Alb82-G, Alb82-GG, Alb82-GGG, Alb92 and Alb223.
11. A construct that comprises or essentially consists of an ISVD according to any one of claims 1 to 4 or a compound according to any one of claims 5-10, and which further comprises one or more other groups, residues, moieties or binding units, optionally linked via one or more peptidic linkers, in which said one or more other groups, residues, moieties or binding units is chosen from the group

consisting of a polyethylene glycol molecule, serum proteins or fragments thereof, binding units that can bind to serum proteins, an Fc portion, and small proteins or peptides that can bind to serum proteins.

12. A composition comprising an ISVD according to any one of claims 1-4, a compound according to any one of claims 5-10 and/or the construct according to claim 11.

13. The composition according to claim 12, which is a pharmaceutical composition, which further comprises at least one pharmaceutically acceptable carrier, diluent or excipient and/or adjuvant, and optionally comprises one or more further pharmaceutically active polypeptides and/or compounds.

14. The composition according to any one of claims 12-13, the construct according to claim 11, the ISVD according to any one of claims 1 to 4, or the compound according to any one of claims 5-10 for use as a medicament.

15. The composition according to any one of claims 12-14, the construct according to claim 11, the ISVD according to any one of claims 1 to 4, or the compound according to any one of claims 5-10 for use in the treatment of a disease and/or disorder of the digestive tract.

16. The composition according to any one of claims 12-15, the construct according to claim 11, the ISVD according to any one of claims 1 to 4, or the compound according to any one of claims 5-10 for use in the treatment of inflammatory bowel disease (IBD), irritable bowel syndrome, Crohn's disease, ulcerative colitis, mucositis, aphthous stomatitis, celiac disease, trauma to the digestive tract and/or cancers to the digestive tract.

17. A method of identifying an amino acid residue that imparts stability of an ISVD to gastric degradation comprising the steps of:

- a) degrading an ISVD by one or more proteases into fragments; and
- b) analysing the fragments of step a) by a suitable means such as e.g. LC-MS;

thereby identifying the amino acid residue(s) imparting stability of an ISVD to gastric degradation.

18. A method of enhancing the stability of an ISVD to gastric degradation comprising the steps a) and b) of claim 17, followed by:

- c) mutating the amino acid residue(s) imparting stability of an ISVD to gastric degradation; and
- d) repeating steps a) and b) of claim 17;

whereby the absence of one or more fragments indicates an enhanced stability of the ISVD to gastric degradation.