

CLAIMS

1. An immunoglobulin single variable domain (ISV) that specifically binds to Aggrecan, preferably human aggrecan [SEQ ID NO: 125], and optionally dog Aggrecan (SEQ ID NO: 126), bovine Aggrecan (SEQ ID NO: 127), rat Aggrecan (SEQ ID NO: 128); pig Aggrecan (SEQ ID NO: 129); mouse Aggrecan (SEQ ID NO: 130), rabbit Aggrecan (SEQ ID NO: 131); cynomolgus Aggrecan (SEQ ID NO: 132) and/or rhesus Aggrecan (SEQ ID NO: 133).
2. The ISV according to claim 1, that essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:
 - CDR1 is chosen from the group consisting of SEQ ID NOs: 24, 20, 21, 22, 23, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37 and 109; preferably chosen from the group consisting of SEQ ID NOs: 24, 20, 21, 25, 27, 29, 31, 34, 35, 36, 37 and 109;
 - CDR2 is chosen from the group consisting of SEQ ID NOs: 42, 38, 39, 40, 41, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55 and 110; preferably chosen from the group consisting of SEQ ID NOs: 42, 38, 39, 43, 45, 47, 49, 50, 53, 54, 55, and 110; and
 - CDR3 is chosen from the group consisting of SEQ ID NOs: 60, 56, 57, 58, 59, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74 and 111; preferably chosen from the group consisting of SEQ ID NOs: 60, 56, 57, 61, 63, 65, 67, 71, 72, 73, 74, and 111.
3. The ISV according to claim 1 or 2, that essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:
 - i) CDR1 is chosen from the group consisting of:
 - a) SEQ ID NOs: 24, 20, 21, 109; and
 - b) amino acid sequences that have 5, 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 24, wherein
 - at position 2 the S has been changed into R, F, I, or T;
 - at position 3 the T has been changed into I;
 - at position 5 the I has been changed into S;
 - at position 6 the I has been changed into S, T, or M;
 - at position 7 the N has been changed into Y, or R;
 - at position 8 the V has been changed into A, Y, T, or G;
 - at position 9 the V has been changed into M; and/or

- at position 10 the R has been changed into G, K, or A;

and/or

ii) CDR2 is chosen from the group consisting of:

c) SEQ ID NOs: 42, 38, 39 and 110; and

d) amino acid sequences that have 5, 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 42, wherein

- at position 1 the T has been changed into A, or G;
- an S or N is inserted between position 3 and position 4;
- at position 3 the S has been changed into R, W, N, or T;
- at position 4 the S has been changed into T or G;
- at position 5 the G has been changed into S;
- at position 6 the G has been changed into S, or R;
- at position 7 the N has been changed into S, T, or R;
- at position 8 the A has been changed into T; and/or
- at position 9 the N has been changed into D or Y;

and/or

iii) CDR3 is chosen from the group consisting of:

e) SEQ ID NO: 60, 56, 57 and 111; and

f) amino acid sequences that have 5, 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 60, wherein

- at position 1 the P has been changed into G, R, D, or E, or is absent;
- at position 2 the T has been changed into R, L, P, or V, or is absent;
- at position 3 the T has been changed into M, S, or R, or is absent;
- at position 4 the H has been changed into D, Y, G, or T;
- at position 5 the Y has been changed into F, V, T or G;
- at position 6 the G has been changed into L, D, S, Y, or W;
- an R, T, Y or V is inserted between position 6 and position 7;
- at position 7 the G has been changed into P, or S;
- at position 8 the V has been changed into G, T, H, R, L, or Y;
- at position 9 the Y has been changed into R, A, S, D or G;
- at position 10 the Y has been changed into N, E, G, W, or S;
- a W is inserted between position 10 and position 11;
- at position 11 the G has been changed into S, K, or Y;
- at position 12 the P has been changed into E, or D, or is absent; and/or
- at position 13 the Y has been changed into L, or is absent.

4. The ISV according to claim 1 or 2, that essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

5 i) CDR1 is chosen from the group consisting of:

a) SEQ ID NO: 32, 30 and 23; and

b) amino acid sequences that have 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 32, wherein

- at position 2 the R has been changed into L;
- 10 - at position 6 the S has been changed into T; and/or
- at position 8 the T has been changed into A;

and/or

ii) CDR2 is chosen from the group consisting of:

c) SEQ ID NO: 50, 41, 48 and 51; and

15 d) amino acid sequences that have 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 50, wherein

- at position 7 the G has been changed into S or R; and/or
- at position 8 the R has been changed into T;

and/or

20 iii) CDR3 is chosen from the group consisting of:

e) SEQ ID NO: 68, 59, 66 and 69; and

f) amino acid sequences that have 5, 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 68, wherein

- at position 4 the R has been changed into V, or P;
- 25 - at position 6 the A has been changed into Y;
- at position 7 the S has been changed into T;
- at position 8 the S is absent;
- at position 9 the N has been changed into P;
- at position 10 the R has been changed into T or L;
- 30 - at position 11 the G has been changed into E; and/or
- at position 12 the L has been changed into T or V.

5. The ISV according to claim 1 or 2, that essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

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i) CDR1 is chosen from the group consisting of:

a) SEQ ID NO: 28; and

b) amino acid sequences that have 5, 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 28, wherein

- at position 1 the G has been changed into R;
- at position 2 the P has been changed into S or R;
- at position 3 the T has been changed into I;
- at position 5 the S has been changed into N;
- at position 6 the R has been changed into N, M, or S;
- at position 7 the Y has been changed into R or is absent;
- at position 8 the A has been changed into F or is absent; and/or
- at position 10 the G has been changed into Y;

and/or

ii) CDR2 is chosen from the group consisting of:

c) SEQ ID NO: 46; and

d) amino acid sequences that have 5, 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 46, wherein

- at position 1 the A has been changed into S, or Y;
- at position 4 the W has been changed into L;
- at position 5 the S has been changed into N;
- at position 6 the S is absent;
- at position 7 the G is absent;
- at position 8 the G has been changed into A;
- at position 9 the R has been changed into S, D, or T; and/or
- at position 11 the Y has been changed into N or R;

and/or

iii) CDR3 is chosen from the group consisting of:

e) SEQ ID NO: 64; and

f) amino acid sequences that have 5, 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 64, wherein

- at position 1 the A has been changed into R, or F;
- at position 2 the R has been changed into I, or L;
- at position 3 the I has been changed into H, or Q;
- at position 4 the P has been changed into G, or N;
- at position 5 the V has been changed into S;

- at position 6 the R has been changed into G, N, or F;
- at position 7 the T has been changed into R, W, or Y;
- at position 8 the Y has been changed into R, or S, or is absent;
- at position 9 the T has been changed into S, or is absent;
- 5 - at position 10 the S has been changed into E, K or is absent;
- at position 11 the E has been changed into N, A, or is absent;
- at position 12 the W has been changed into D, or is absent;
- at position 13 the N has been changed into D, or is absent;
- at position 14 the Y is absent; and/or
- 10 - D and N are added after position 14 of SEQ ID NO: 64.

6. The ISV according to any one of claims 2 to 5, wherein said ISV is chosen from the group of ISVs, wherein:

- CDR1 is SEQ ID NO: 24, CDR2 is SEQ ID NO: 42, and CDR3 is SEQ ID NO: 60;
- 15 - CDR1 is SEQ ID NO: 20, CDR2 is SEQ ID NO: 38, and CDR3 is SEQ ID NO: 56;
- CDR1 is SEQ ID NO: 21, CDR2 is SEQ ID NO: 39, and CDR3 is SEQ ID NO: 57;
- CDR1 is SEQ ID NO: 25, CDR2 is SEQ ID NO: 43, and CDR3 is SEQ ID NO: 61;
- CDR1 is SEQ ID NO: 27, CDR2 is SEQ ID NO: 45, and CDR3 is SEQ ID NO: 63;
- CDR1 is SEQ ID NO: 29, CDR2 is SEQ ID NO: 47, and CDR3 is SEQ ID NO: 65;
- 20 - CDR1 is SEQ ID NO: 31, CDR2 is SEQ ID NO: 49, and CDR3 is SEQ ID NO: 67;
- CDR1 is SEQ ID NO: 34, CDR2 is SEQ ID NO: 50, and CDR3 is SEQ ID NO: 71;
- CDR1 is SEQ ID NO: 35, CDR2 is SEQ ID NO: 53, and CDR3 is SEQ ID NO: 72;
- CDR1 is SEQ ID NO: 36, CDR2 is SEQ ID NO: 54, and CDR3 is SEQ ID NO: 73;
- CDR1 is SEQ ID NO: 37, CDR2 is SEQ ID NO: 55, and CDR3 is SEQ ID NO: 74;
- 25 - CDR1 is SEQ ID NO: 32, CDR2 is SEQ ID NO: 50, and CDR3 is SEQ ID NO: 68;
- CDR1 is SEQ ID NO: 32, CDR2 is SEQ ID NO: 51, and CDR3 is SEQ ID NO: 69;
- CDR1 is SEQ ID NO: 30, CDR2 is SEQ ID NO: 48, and CDR3 is SEQ ID NO: 66;
- CDR1 is SEQ ID NO: 23, CDR2 is SEQ ID NO: 41, and CDR3 is SEQ ID NO: 59;
- CDR1 is SEQ ID NO: 28, CDR2 is SEQ ID NO: 46, and CDR3 is SEQ ID NO: 64;
- 30 - CDR1 is SEQ ID NO: 22, CDR2 is SEQ ID NO: 40, and CDR3 is SEQ ID NO: 58;
- CDR1 is SEQ ID NO: 26, CDR2 is SEQ ID NO: 44, and CDR3 is SEQ ID NO: 62; and
- CDR1 is SEQ ID NO: 33, CDR2 is SEQ ID NO: 52, and CDR3 is SEQ ID NO: 70.

7. The ISV according to any one of claims 1 to 6, wherein said ISV is chosen from the group consisting of ISVs with consisting of SEQ ID NO:s 1-19 and 114-118, preferably SEQ ID NOs: 5,

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1, 2, 6, 8, 10, 12, 16, 17, 18, and 19, and ISVs which have more than 80%, such as 90% or 95% sequence identity with any one of SEQ ID NO:s 1-19 and 114-118, preferably SEQ ID NOs: 5, 1, 2, 6, 8, 10, 12, 16, 17, 18, and 19.

- 5 8. A domain antibody, an immunoglobulin that is suitable for use as a domain antibody, a single domain antibody, an immunoglobulin that is suitable for use as a single domain antibody, a dAb, an immunoglobulin that is suitable for use as a dAb, a Nanobody, a VHH sequence, a humanized VHH sequence, a camelized VH sequence, a VHH sequence that has been obtained by affinity maturation that binds to epitope bin 1 of the G1-domain of Aggrecan, a VHH
10 sequence that has been obtained by affinity maturation that binds to the G1-IGD-G2 domain of Aggrecan, or a VHH sequence that has been obtained by affinity maturation that binds to the G2-domain of Aggrecan, and which competes for binding to Aggrecan with the ISV according to any one of claims 1-7.
- 15 9. A polypeptide comprising at least one ISV according to any one of claims 1-8, and possibly a second ISV, possibly a third ISV, and possibly a fourth ISV.
- 20 10. The polypeptide that comprises at least two ISVs according to any one of claims 1-8, and possibly a third ISV, and possibly a fourth ISV.
- 25 11. The polypeptide according to claim 10, wherein said at least two ISVs can be the same or different, preferably said at least two ISVs are independently chosen from the group consisting of SEQ ID NOs: 1-19 and 114-118.
- 30 12. The polypeptide according to any one of claims 9-11, wherein said at least one ISV according to any one of claims 1-8 or said at least two ISVs according to any one of claims 1-8, bind to a member of the serine protease family, cathepsins, matrix metalloproteinases (MMPs)/Matrixins or A Disintegrin and Metalloproteinase with Thrombospondin motifs (ADAMTS), preferably MMP8, MMP13, MMP19, MMP20, ADAMTS5 (Aggrecanase-2),
ADAMTS4 (Aggrecanase-1) and/or ADAMTS11.
13. The polypeptide according to any one of claims 9-12, wherein said polypeptide further comprises a serum protein binding moiety or a serum protein.

14. A construct that comprises or essentially consists of an ISV according to any one of claims 1-8 or a polypeptide according to any one of claims 9-13, and which further comprises one or more other groups, residues, moieties or binding units, optionally linked via one or more peptidic linkers.

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15. A composition comprising at least one ISV according to any one of claims 1-8, a polypeptide according to any one of claims 9-13, or a construct according to claim 14, said composition is preferably pharmaceutical composition, optionally further comprising 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.

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16. The composition according to claim 15, the ISV according to any one of claims 1-8, the polypeptide according to any one of claims 9-13, or the construct according to claim 14 for use as a medicament.

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17. The composition according to claim 15, the ISV according to any one of claims 1-8, the polypeptide according to any one of claims 9-13, or the construct according to claim 14 for use in preventing or treating arthropathies and chondrodystrophies, arthritic disease, such as osteoarthritis, rheumatoid arthritis, gouty arthritis, psoriatic arthritis, traumatic rupture or detachment, achondroplasia, costo-chondritis, Spondyloepimetaphyseal dysplasia, spinal disc herniation, lumbar disk degeneration disease, degenerative joint disease, and relapsing polychondritis.

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