and cell physiological functions ascribed to CCN5-signaling and, which may also counteract the other four-domain CCN proteins (Cyr61 (also known as CCN1), CTGF (also known as CCN2), NOV (also known as CCN3), WISP1 (also known as CCN4) and WISP3 (also known as CCN6)). In other words, proteins are provided, including in the formof fusion proteins, based on domain III, the TSP-1 homology domain, of a CCN protein, which recapitulate, or which have, the biological activity of CCN5, and which are able to antagonize, or inhibit, the effects of the 4-domain CCN proteins, CCN1-4 or CCN6. In particular the proteins herein have anti-fibrotic activity and may also have direct anti-cancer activity.

As noted above, domain III (the TSP-1 homology domain) of the other CCN proteins, namely the 4-domain CCN proteins, when provided as a separate domainin the absence of the other CCN domains, has surprisingly been found to be sufficient to recapitulate the reported activities of CCN5. Accordingly, in other words, domain III of the 4-domain CCN proteins, when provided as a separate domain in the absence of the other CCN domains (i.e. as an isolated domain), has the same activity as CCN5, or, alternatively expressed, as domain III/TSP-1 homology domain of CNN5, (that is an opposite activity to that of full-length 4- domain CCN proteins). Thus from the experiments disclosed in this document it isclear that the isolated TSP-1 homology domain of any CCN protein may exert the same activity as that of the TSP-1 homology domain of CCN5. Other than in the case of CCN5, this may not be the same as the activities exerted by the full-lengthCCN protein.

It has been found that monomeric fusion proteins, wherein the domain III of a CCNprotein is fused to a monomeric fusion partner, are of particular benefit and utility according to the disclosure and disclosure herein.

In one aspect, the present disclosure provides a monomeric fusion protein comprising:
(i) a polypeptide corresponding to at least a portion of the thrombospondin type 1 repeat (TSP-1) homology domain of a CCN family protein;
(ii) a monomeric fusion partner N- or C- terminally fused to the amino acid sequence of (i), wherein said monomeric fusion partner comprises at least 6 amino acids and increases serum half-life of the fusion protein; and
(iii) optionally a peptide linker between the polypeptide of (i) and the monomeric fusion partner of (ii), wherein the polypeptide of (i) is 40 to 60 amino acids in length and comprises an amino acid sequence selected from SEQ ID NOs: 37 or 2 to 6, or a sequence having at least 90% sequence identity to a sequence selected from SEQ ID NOs: 37 or 2 to 6 wherein all of the cysteine residues in said sequence selected from SEQ ID NOs: 37 or 2 to 6 are

conserved, and wherein the monomeric fusion partner of (ii) and the peptide linker of (iii) are not or do not comprise an IGF binding protein homology domain, a von Willebrand factor type C repeat homology domain, or a cysteine knot domain of a CCN family protein.

According to ~~a first aspect~~an example, the present disclosure provides a monomeric fusion protein comprising:

(i) a polypeptide corresponding to at least a portion of the thrombospondin type 1repeat (TSP-1) homology domain of a CCN family protein;

(ii) a monomeric fusion partner N- or C- terminally fused to the amino acid sequence of (i); and

(iii) optionally a peptide linker between the polypeptide of (i) and the monomericfusion partner of (ii),

wherein the polypeptide of (i) is 40 to 60 amino acids in length and comprises an amino acid sequence selected from SEQ ID NOs: 37 or 2 to 6, or a sequence having at least 80% sequence identity to a sequence selected from SEQ ID NOs: 37 or 2 to6 wherein all of the cysteine residues in said sequence selected from

having 80%sequence identity thereto.

In examples of these examples the monomeric fusion partner is selected from serum albumin, transferrin and monomeric Fc fragments, particularly monomeric Fcfragments of IgG, more particularly human IgG.

As noted above, substitution of position 2 of the domain III fragments herein improves the stability of the fragment, in particular resistance to protease degradation. It is believed and proposed herein that such sequence-modified variants of the domain III fragments represent useful proteins in their own right,without being linked to a fusion partner.

Accordingly, another ~~aspect~~example of the disclosure also provides a protein 40 to 60 amino acids in length which comprises, or a protein which consists of, an amino acid sequence as set out in SEQ ID NO: 7, 38, 42 to 46, or 47 to 51 or a sequence with at least 80% sequence identity thereto, wherein the protein comprises an alanine residue at the position corresponding to position 2 of said sequence of SEQ ID NO:7, 38, 42 to 46, 47 to 51, and wherein all of the cysteine residues in said sequence are conserved.

Other proteins and fusion proteins are also provided as further examples of the disclosures, as detailed below.

According to a further example of the disclosure, a recombinant protein is providedcomprising a of formula (I)

Cys - A – Cys – B – Cys – C – Cys – D – Cys – E – Cys – F

(formula (I))wherein

A is a peptide of formula II

A1-A2-A3-A5-A6-A7-A8-A9

wherein A1 is selected from the group consisting of P, A, V, I, and L; A2 is selected from the group consisting of E, D, A, I, L, and V; A3 is selected from the group consisting of G, Q, Y, S, N, W, F; A4 is selected from the group consisting of A, I, L, V, S, T; A5 is an amino acid selected from the group consisting of T, Y, N, G, Q and S; A6 is an amino acid selected from the group consisting of A, V, I, L, P, S, E,D, K, R, and H; A7 is W; A8 is selected from the group consisting of G, T, S, Q, Y, N, P, A, V, I, and L; A9 is an amino acid selected from the group consisting of A, P,L, I, V, Q; and

B is a peptide of formula III

B1-B2-B3

wherein B1 is an amino acid selected from the group consisting of G, Q, N,

108, 109, 112 or 113 and nucleic acid sequences having about 80% sequence identity with SEQ ID NO. 34, SEQ ID No. 35, SEQ ID No. 36 or SEQ ID NOs: 86, 87, 90, 91, 99, 100, 104, 105, 108, 109, 112 or 113.

In another aspect, the present disclosure provides an expression vector comprising a DNA molecule as defined herein. Furthermore, according to another example of the disclosure, an expression vector isprovided comprising a DNA sequence according to the present disclosure. Also a host cell comprising an expression vector according to the disclosure is provided in another aspect.

Finally, a thrombospondin type 1 repeat (TSP-1) homology domain of a CCN family protein, a protein and a fusion protein as defined above is provided for use asa medicament is provided for treatment or prevention of disorders by inhibiting or counteracting the cell signaling, and cell physiological functions ascribed to the four-domain CCN family proteins.

In another aspect, there is provided a fusion protein, or a protein, as defined herein foruse in therapy.

The fusion protein or protein may be for use in the treatment of a condition associated with activity of a 4-domain CCN protein, particularly unwanted or abberant activity of a 4-domain CCN protein. The activity may be associated with a fibrotic effect. The activity may be pro-fibrotic activity.

~~In a further aspect, the present disclosure provides a use of a fusion protein as described herein in the manufacture of a medicament for treating orpreventing a disorder by inhibiting or counteracting the cell signalling and cell physiological functions ascribed to 4-domain CCN family proteins.~~ In a further aspect, the present disclosure provides a use of a fusion protein as described herein in the manufacture of a medicament for the treatment ~~or prevention~~ of fibrosis, or any condition exhibiting fibrosis. In a further aspect, the present disclosure provides a use of a fusion protein as described herein in the manufacture of a medicament for the treatment of cancer.

In another example, there is provided a fusion protein, or a protein, as defined hereinfor use in the treatment or prevention of fibrosis, or any condition exhibiting fibrosis (i.e. a fibrotic condition or disease). In a further example, there is provided a fusion protein, or a protein, as defined herein for use in the treatment of cancer. Also provided is a fusion protein, or a protein, as defined herein for use in the treatment of inflammatory or autoimmune diseases, or metabolic diseases.

Also provided according to such examples of the disclosure is the use of a

CLAIMS

1. A monomeric fusion protein comprising:

(i) a polypeptide corresponding to at least a portion of the thrombospondin type 1 repeat (TSP-1) homology domain of a CCN family protein;

(ii) a monomeric fusion partner N- or C- terminally fused to the amino acid sequence of (i), wherein said monomeric fusion partner comprises at least 6 amino acids and increases serum half-life of the fusion protein; and

(iii) optionally a peptide linker between the polypeptide of (i) and the monomeric fusion partner of (ii),

wherein the polypeptide of (i) is 40 to 60 amino acids in length and comprises an amino acid sequence selected from SEQ ID NOs: 37 or 2 to 6, or a sequence having at least ~~80~~90% sequence identity to a sequence selected from SEQ ID NOs: 37 or 2 to 6 wherein all of the cysteine residues in said sequence selected from SEQ ID NOs: 37 or 2 to 6 are conserved,

and wherein the monomeric fusion partner of (ii) and the peptide linker of (iii) are not or do not comprise an IGF binding protein homology domain, a von Willebrand factor type C repeat homology domain, or a cysteine knot domain of a CCN family protein.

2. The fusion protein of claim 1, wherein the polypeptide of (i) is 44 to 57 amino acids in length.

3. The fusion protein of claim 1 or claim 2, wherein the polypeptide of (i) comprises or consists of:

(a) an amino acid sequence selected from SEQ ID NOs: 1 or 8 to 12; or

(b) an amino acid sequence having at least ~~80~~90% sequence identity to a sequence selected from SEQ ID NOs: 1 or 8 to 12; or

(c) a part of an amino acid sequence of (a) or (b), wherein said part comprises at least the 44 amino acid sequence of SEQ ID NOs: 37, 6, 2, 3, 4 or 5 respectively or a sequence having at least ~~80~~90% sequence identity to a sequence selected from SEQ ID NOs: 37, 6, 2, 3, 4 or 5 respectively.

4. The fusion protein of any one of claims 1 to 3, wherein ~~said~~ the polypeptide of (i) consists of an amino acid sequence selected from SEQ ID NOs: 37 or 2 to 6, or a sequence having at least ~~80~~90% sequence identity to a sequence selected from SEQ ID NOs: 37 or 2 to 6.

5. The fusion protein of any one of claims 1 to 4, wherein the peptide linker of (iii) comprises no more than 50 amino acids.

6. The fusion protein of any one of claims 1 to 5, wherein the polypeptide of (i) comprises an alanine residue at the position corresponding to position 2 of said sequence selected from SEQ ID NOs: 37 or 2 to 6, or SEQ ID NOs: 1 or 8 to 12.

7. The fusion protein of any one of claims 1 to 6, wherein the amino acid sequence of (i) comprises an amino acid sequence selected from SEQ ID NOs: 7, 38, 42 to 46 or 47 to 51, or a sequence with at least ~~80~~90% sequence identity thereto, wherein the protein comprises an alanine residue at the position corresponding to position 2 of said sequence of SEQ ID NO: 7, 38, 42 to 46 or 47 to 51.

8. The fusion protein of any one of claims 1 to 7, wherein said monomeric fusion partner is selected from the group consisting of serum albumin, transferrin, and a monomeric Fc-fragment of human IgG.

9. The fusion protein of claim 8, wherein said monomeric Fc-fragment of human IgG is a monomeric Fc fragment of IgG1, IgG2 or IgG4.

10. The fusion protein of claim 8 or claim 9, wherein the monomeric Fc-fragment is aglycosylated.

11. The fusion protein of any one of claims 8 to 10, wherein the monomeric Fc- fragment comprises a stabilizing disulphide bridge and/or a protease stabilizing mutation.

12. The fusion protein of any one of claims 8 to 11, wherein the monomeric Fc- fragment does not have immune effector function.

13. The fusion protein of any one of claims 1 to 12, wherein the peptide linker between the amino acid sequence of (i) and the monomeric fusion partner has an amino acid sequence selected from the group consisting of SEQ ID NOs: 20 to 25, 39, 57, 63, 65 or 67, or an amino acid sequence having 80% sequence identity thereto.

14. The fusion protein of any one of claims 1 to 8, wherein the fusion protein has an amino acid sequence selected from the group consisting of SEQ ID NOs: 84, 85, 88, 89, 97, 98, 102, 103, 106, 107, 110, and 111, or an amino acid sequence having ~~80~~90% sequence identity thereto.

15. A DNA molecule encoding a monomeric fusion protein as defined in any one of claims 1 to 14.

16. The DNA molecule of claim 15, wherein said molecule further comprises a nucleotide sequence encoding a signal sequence.

17. The DNA molecule of claim 15 or 16, wherein said molecule comprises a nucleotide sequence as set forth in SEQ ID NO: 34, 35, 36, 86, 87, 90, 91, 99, 100, 104, 105, 108, 109, 112 or 113 or a nucleotide sequence having at least 80% sequence identity with any aforesaid sequence.

18. An expression vector comprising a DNA molecule as defined in any one of claims 15 to 17.

19. A host cell comprising a vector as defined in claim 18.

20. A fusion protein according to any one of claims 1 to 14 for use in therapy.

~~21. Use of a fusion protein according to any one of claims 1 to 14 in the manufacture of a medicament for treating or preventing a disorder by inhibiting or counteracting the cell signalling and cell physiological functions ascribed to 4-domain CCN family proteins.~~

~~22~~21. Use of a fusion protein according to any one of claims 1 to 14 in the manufacture of a medicament for the treatment ~~or prevention~~ of fibrosis, or any condition exhibiting fibrosis.

~~23~~22. Use of a fusion protein according to any one of claims 1 to 14 in the manufacture of a medicament for the treatment of cancer.

~~24. A protein 40-60 amino acids in length which comprises or consists of an amino acid sequence as set out in SEQ ID NO: 7, 38, 42 to 46, 47 to 51, or a sequence with at least 80% sequence identity thereto, wherein the protein comprises an alanine residue at the position corresponding to position 2 of said sequence sequence of SEQ ID NO: 7, 38, 42 to 46, 47 to 51, and wherein all of the cysteine residues in said sequence are conserved.~~