

Use of CCN domain-containing proteins for the treatment of disease

This application claims priority from GB application number 2400055.6 filed on 3 January 2024, the contents and elements of which are herein incorporated by reference for all purposes.

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Field of the Invention

The present invention relates to the treatment of disease, and particularly to the use of active agents containing CCN dIII domain sequences for the treatment of obesity, a condition associated with insulin resistance or glucose intolerance, and polycystic kidney disease.

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Background

CCN proteins are a family of extracellular proteins that are associated with the extracellular matrix (ECM). The CCN denomination is both an abbreviation for Cellular Communication Network factors, and acronym for the original names of the first three members: Cyr61 (Cysteine Rich Angiogenic Inducer 61); CTGF (Connective Tissue Growth Factor) and NOV (nephroblastoma overexpressed) (Perbal B., J Cell Commun Signal. 2018 Dec;12(4):625-629). The CCN family comprises CCN1 (previously known as Cyr61), CCN2 (previously known as CTGF), CCN3 (previously known as NOV), CCN4 (previously known as WNT1 inducible signalling pathway protein 1/WISP1), CCN5 (previously known as WNT1 inducible signalling pathway protein 2/WISP2) and CCN6 (previously known as WNT1 inducible signalling pathway protein 3/WISP3).

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The expression of both CCN2 and the other CCN-family proteins are largely confined to the embryonic state in healthy organisms. However, the CCN proteins are often reactivated during disease states, in particular wound healing processes, fibrosis, and carcinomas (Bradham, DM et al., J Cell Biol 114, 1285-1294 (1991)). In being extracellular proteins mechanistically involved in the development of fibrosis and of limited expression in healthy organisms, CCN proteins appear as attractive therapeutic targets. CCN1-4 and CCN6 are composed of four structural homology domains, while CCN5 is atypical in lacking the fourth domain. CCN5 has previously been reported to oppose the actions of CCN2 and display anti-fibrotic activities (Jeong D et al., JACC VOL. 67, NO. 13, 2016; Xu H. et al., Clin Exp Pharmacol Physiol. 2015 Nov;42(11):1207-19; Zhang L et al., Int J Mol Med. 2014 Feb;33(2):478-86; Yoon PO, et al. J Mol Cell Cardiol 2010).

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CCN proteins are reported to be involved in the pathogenesis of multiple different diseases, although the underlying mechanism is generally not well characterised.

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Grünberg et al. (Sci Rep. 2017 Feb 27;7:43515.) reported that overexpression of CCN5 led to a reduced body fat percentage in mice stressed with a high-fat diet while preserving lean body mass. Grünberg et al. (*supra*) also found that CCN5 overexpression increased insulin sensitivity and improved glucose tolerance. In corroboration, Kim et al., (PLoS One. 2018 Nov 28;13(11):e0207228) reported that CCN5

knock-out in mice results in increased body weight, impaired glucose tolerance, increased insulin resistance and increased subcutaneous white adipose tissue. However neither of these reports investigates whether these activities can be ascribed to a specific portion of CCN5, and if so, which.

- 5 A paper by Dwivedi et al. (JASN 31: 1697–1710, 2020) reported that conditional knockout of CCN2 attenuated kidney growth, cystic index and α -SMA and Collagen-1a mRNA expression in a model of polycystic kidney disease, thus showing that CCN2 contributes to the pathogenesis of polycystic kidney disease. US 2008/193443 proposes that connective tissue growth factor (CTGF, i.e. CCN2) is a driver of cyst growth.

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Zolfaghari et al. (J. Biol. Chem. (2023) 299(1) 102803) recently reported that fusion proteins comprising domain III of CCN5 are bioactive and inhibit migration of CCN2-stimulated fibroblast cells. A fusion protein containing domain III of CCN3 also displayed a similar bioactivity as the fusion proteins comprising domain III of CCN5, thus showing that CCN domain III in CCN5 and CCN3 share at least some properties. However, the extent to which domain III of CCN5 shares the activities of CCN5 remains unknown.

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Summary of the Invention

It has surprisingly been found that an active agent containing a CCN3 domain III ("dIII"), separate from other CCN protein domains (domains I, II and IV; referred to as "dI", "dII" and "dIV" respectively), has beneficial therapeutic effects in murine models of obesity, insulin resistance and/or glucose intolerance and polycystic kidney disease. The similarity between dIII domains across the CCN protein family, combined with previous findings that CCN3 dIII is also to mimic certain physiological activities of CCN5, suggests that similar therapeutic activity may be shared by dIII domains from other CCN proteins.

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Thus, in a first aspect, the invention provides an active agent for use in inhibiting weight gain or promoting weight loss, e.g. for use in the treatment of obesity, wherein the active agent comprises a CCN dIII domain sequence or a functionally active fragment thereof, and lacks a CCN dI domain sequence, a CCN dII domain sequence and a CCN dIV domain sequence.

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The invention further provides a method of inhibiting weight gain or promoting weight loss in an individual, comprising administering to the individual an active agent which comprises a CCN dIII domain sequence or a functionally active fragment thereof, and lacks a CCN dI domain sequence, a CCN dII domain sequence and a CCN dIV domain sequence. The effect on body weight may be cosmetic or therapeutic.

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The method may constitute a method of treating obesity in the individual.

The invention further provides the use of an active agent in the preparation of a medicament for inhibiting weight gain or promoting weight loss in an individual, e.g. for the treatment of obesity, wherein the active

agent comprises a CCN dIII domain sequence or a functionally active fragment thereof, and lacks a CCN dI domain sequence, a CCN dII domain sequence and a CCN dIV domain sequence.

Also provided is the use of an active agent in inhibiting weight gain or promoting weight loss in an individual, wherein the active agent comprises a CCN dIII domain sequence or a functionally active fragment thereof, and lacks a CCN dI domain sequence, a CCN dII domain sequence and a CCN dIV domain sequence. The use may be therapeutic or non-therapeutic.

Further provided is an agent for use in the prophylaxis or treatment of a condition associated with insulin resistance or glucose intolerance in a subject, wherein the subject is obese, and wherein the active agent comprises a CCN dIII domain sequence or a functionally active fragment thereof, and lacks a CCN dI domain sequence, a CCN dII domain sequence and a CCN dIV domain sequence.

The invention further provides a method of treatment or prophylaxis of a condition associated with insulin resistance or glucose intolerance in a subject, comprising administering to the subject an active agent which comprises a CCN dIII domain sequence or a functionally active fragment thereof, and lacks a CCN dI domain sequence, a CCN dII domain sequence and a CCN dIV domain sequence, and wherein the subject is obese.

The invention further provides the use of an active agent in the preparation of a medicament for treatment or prophylaxis of a condition associated with insulin resistance or glucose intolerance in a subject, wherein the active agent comprises a CCN dIII domain sequence or a functionally active fragment thereof, and lacks a CCN dI domain sequence, a CCN dII domain sequence and a CCN dIV domain sequence, and wherein the subject is obese.

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In some embodiments, the condition is selected from diabetes, pre-diabetes, insulin resistance syndrome and impaired glucose tolerance (IGT).

The invention further provides an active agent for use in prophylaxis or treatment of a condition associated with obesity or excess body weight, wherein the active agent comprises a CCN dIII domain sequence or a functionally active fragment thereof, and lacks a CCN dI domain sequence, a CCN dII domain sequence and a CCN dIV domain sequence.

The invention further provides a method of prophylaxis or treatment of a condition associated with obesity or excess body weight in an individual, comprising administering to the individual an active agent which

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comprises a CCN dIII domain sequence or a functionally active fragment thereof, and lacks a CCN dI domain sequence, a CCN dII domain sequence and a CCN dIV domain sequence.

5 The invention further provides the use of an active agent in the preparation of a medicament for prophylaxis or treatment of a condition associated with, or caused by, obesity or excess body weight in an individual, wherein the active agent comprises a CCN dIII domain sequence or a functionally active fragment thereof, and lacks a CCN dI domain sequence, a CCN dII domain sequence and a CCN dIV domain sequence.

10 The condition may be selected from obesity-linked inflammation, obesity-linked gallbladder disease, diabetes (particularly type 2 diabetes), pre-diabetes, insulin resistance syndrome, impaired glucose tolerance (IGT), metabolic syndrome, hyperglycemia, diabetic heart disease, hypertension, cardiovascular disease (including ischemic heart disease and arrhythmia), heart failure, myocardial infarction, coronary heart disease, stroke, atherogenic dyslipidemia, hepatic steatosis ("fatty liver",
15 including non-alcoholic fatty liver disease (NAFLD, also known as metabolic dysfunction associated steatotic liver disease (MASLD)), non-alcoholic steatohepatitis (NASH, also known as metabolic dysfunction associated steatohepatitis (MASH)), and metabolic dysfunction and alcohol associated steatotic liver disease (MetALD)), kidney disease, kidney failure, arteriosclerosis (e.g. atherosclerosis), osteoarthritis, gout, reproductive disorders (e.g. polycystic ovary syndrome (PCOS)) and respiratory
20 disorders (including obstructive sleep apnea (or obesity-induced sleep apnea) and asthma).

In a second aspect, the invention provides an active agent for use in the treatment of polycystic kidney disease (PKD), wherein the active agent comprises a CCN dIII domain sequence or a functionally active fragment thereof, and lacks a CCN dI domain sequence, a CCN dII domain sequence and a CCN dIV
25 domain sequence.

The invention further provides a method of treating polycystic kidney disease in an individual, comprising administering to the individual an active agent which comprises a CCN dIII domain sequence or a functionally active fragment thereof, and lacks a CCN dI domain sequence, a CCN dII domain sequence
30 and a CCN dIV domain sequence.

The invention further provides the use of an active agent in the preparation of a medicament for the treatment of polycystic kidney disease, wherein the active agent comprises a CCN dIII domain sequence or a functionally active fragment thereof, and lacks a CCN dI domain sequence, a CCN dII domain
35 sequence and a CCN dIV domain sequence.

The polycystic kidney disease may be autosomal dominant polycystic kidney disease (ADPKD) or autosomal recessive polycystic kidney disease (ARPKD). The treatment may involve the inhibition of cyst growth or a reduction in the volume of existing cysts.

- 5 The CCN dIII domain may comprise or consist of the sequence:

CIVQTTWSQCSKTCGTGISTRVTNDNPECRLVKETRICEVRPC (SEQ ID NO: 1, from CCN1);
 CLVQTTEWSACSKTCGMGISTRVTNDNASCRLKQSRCLCMVRPC (SEQ ID NO: 2, from CCN2);
 CIEQTTEWTACSKSCGMGFSTRVTNRNRQCEMLKQTRLCMVRPC (SEQ ID NO: 3, from CCN3);
 10 CIAYTSPWSPCSTSCGLGVSTRISNVNAQCWPEQESRLCNLRPC (SEQ ID NO: 4, from CCN4);
 CPEWSTAWGPCSTTCGLGMATRVSNQNRFCRLETQRRCLCLSRPC (SEQ ID NO: 5, from CCN5); or
 CLVQATKWTPCSRTCGMGISNRVTNENSNCCEMRKEKRLCYIQPC (SEQ ID NO: 6 from CCN6);

or may have at least 70%, 75%, 80%, 85%, 90%, or 95% identity to one of those sequences.

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In preferred embodiments, the 6 cysteine residues are conserved. Preferably, the spacing between them is also conserved.

The CCN dIII domain may comprise or consist of the sequence:

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CIVQTTWSQCSKTCGTGISTRVTNDNPECRLVKETRICEVRPCGQPVYSSLKKGKK (SEQ ID NO: 7, from CCN1);
 CLVQTTEWSACSKTCGMGISTRVTNDNASCRLKQSRCLCMVRPCEADLEENIKKGKK (SEQ ID NO: 8, from CCN2);
 25 CIEQTTEWTACSKSCGMGFSTRVTNRNRQCEMLKQTRLCMVRPCEQEPEQPTDKKGKK (SEQ ID NO: 9, from CCN3);
 CIAYTSPWSPCSTSCGLGVSTRISNVNAQCWPEQESRLCNLRPCDVIHTLIKAGKK (SEQ ID NO: 10, from CCN4);
 CPEWSTAWGPCSTTCGLGMATRVSNQNRFCRLETQRRCLCLSRPCPPSRGRSPQNSAF (SEQ ID NO: 11, from CCN5); or
 30 CLVQATKWTPCSRTCGMGISNRVTNENSNCCEMRKEKRLCYIQPCDSNILKTIKIPKGKT (SEQ ID NO: 12, from CCN6);

or may have at least 70%, 75%, 80%, 85%, 90%, or 95% identity to one of those sequences.

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In preferred embodiments, the 6 cysteine residues are conserved. Preferably, the spacing between them is also conserved.

In some embodiments, the residue immediately following the first cysteine residue (residue 2 of SEQ ID NOs: 1 to 12) is not I (e.g. for CCN1, CCN3 or CCN4), L (e.g. for CCN2 or CCN6) or P (e.g. for CCN5). For example, it may be alanine or Aib.

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Thus, for example, the CCN dIII domain may comprise or consist of the sequence:

CAEQTTEWTACSKSCGMGFSTRVTNRNRQCEMLKQTRLCMVRPC (SEQ ID NO: 13) or

10 CAEQTTEWTACSKSCGMGFSTRVTNRNRQCEMLKQTRLCMVRPCEQEPEQPTDKKGKK (SEQ ID NO: 14).

The active agent may comprise a heterologous moiety. In some embodiments, the heterologous moiety increases the stability of the active agent. In some embodiments, the heterologous moiety increases the serum half-life of the active agent as compared to a similar agent in the absence of the heterologous moiety.

For example, the heterologous moiety may be selected from an Fc fragment, serum albumin (e.g., human serum albumin (HSA)), fibrinogen, glutathione S-transferase, transferrin, or streptavidin. In some embodiments, the heterologous moiety is a monomeric Fc. In some embodiments, the heterologous moiety is dimeric Fc. The Fc fragment may be an Fc fragment of any subclass or chimera of any subclasses. In some embodiments, the Fc fragment is a IgG1, IgG2, or IgG4 Fc fragment.

The heterologous moiety may comprise or consist of amino acids 25-609 of human serum albumin (HSA) having the sequence:

DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVADESAE
NCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPRLPRLVRPEV
DVMCTAFHDNEETFLKKYLYEIAARRHPYFYAPELLFFAKRYKAAFTECCQAADKAACLLP
30 KLDELRLDEGKASSAKQRLKCASLQKFGERAFAKAWAVARLSQRFPAEFAEVSKLVTDLTK
VHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPA
DLPSLAADFVESKDVCKNYAEAKDVFLGMFLYFYARRHPDYSVVLRLAKTYETTLEKC
CAAADPHECYAKVFDEFKPLVEEPQNLIKQNCLEFQLGLEYKFQNALLVRYTKKVPQVST
PTLVEVSRNLGKVGSKCKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTES
35 LVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKAT
KEQLKAVMDDFAAFVEKCKADDKETCFAEEGKKLVAASQAALGL (SEQ ID NO: 15)

or may have at least 70% identity thereto, e.g. at least 75% identity, at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to that sequence.

The active agent may further comprise a linker between the CCN dIII domain sequence (or fragment thereof) and any heterologous moiety. The linker may be a peptide linker. The linker may comprise no more than 50 amino acids, e.g. no more than 25 amino acids, e.g. no more than 10 amino acids. The linker may be 1-50, 1-45, 1-40, 1-30, 1-25, 1-20, 1-15, 1-12, 1-10, e.g. 1-8, 1-6, 1-5, or 1-4, amino acids long. The linker may comprise one or more iterations of the amino acid sequence EAAAK, e.g. it may comprise or consist of the sequence EAAAK.

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In certain embodiments, the active agent may have the mature sequence:

CAEQTTTEWTACSKSCGMGFSTRVTNRNQCEMLKQTRLQCMVRPCEAAAKDAHKSEVAHRFKDLGEENFKA
LVLIIFAQYLYQQCPFEDHVKLNVNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCC
AKQEPERNECFLOHKDDNPPLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAKRY
15 KAAFTCECCQAADKAACLLPKLDELRLDEGKASSAKQRLKQSLQKFGERAFAKAWAVARLSQRFPAEFAEV
SKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLEKSHCIAEVENDEMPAD
LPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLRLAKTYETTLEKCCAAADPHECYA
KVFDEFKPLVEEPQNLKQNCLEFQLGLEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCKHP
EAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHA
20 DICTLSEKERQIKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCKKADDKETCFAEEGKKLVAASQA
ALGL (SEQ ID NO: 16);

being composed of (i) the human CCN3 dIII domain (underlined) with an I2A substitution ; (ii) a short linker sequence (italics); and (iii) amino acids 25-609 of human serum albumin (HSA) (double underlined).

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Where the heterologous moiety and any linker are peptidic, it (or they) may be provided as part of a fusion protein with the CCN dIII domain; i.e. the CCN dIII domain, the heterologous moiety, and any linker, are all part of the same peptide chain. Thus the active agent may be a fusion protein.

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Whether the active agent consists only of the CCN dIII domain, or also comprises other components (such as a heterologous moiety and optionally a linker), a peptidic active agent which comprises only naturally occurring (proteinogenic) amino acids encoded by the standard genetic code may be capable of being expressed recombinantly, i.e. from a nucleic acid encoding the active agent and capable of driving
35 expression of the active agent in a suitable target cell. Such a nucleic acid may be described as an expression vector.

The invention further extends to the therapeutic use of such nucleic acids or expression vectors. Therapeutic methods which employ delivery of nucleic acid or an expression vector to achieve therapeutic expression of an active agent within a target cell may be described as "gene therapy".

5 Thus the invention further provides a nucleic acid encoding active agent for use in inhibiting weight gain or promoting weight loss, wherein the active agent comprises a CCN dIII domain sequence or a functionally active fragment thereof, and lacks a CCN dI domain sequence, a CCN dII domain sequence and a CCN dIV domain sequence

10 The invention further provides a method of inhibiting weight gain or promoting weight loss in an individual, comprising administering to the individual a nucleic acid encoding an active agent which comprises a CCN dIII domain sequence or a functionally active fragment thereof, and lacks a CCN dI domain sequence, a CCN dII domain sequence and a CCN dIV domain sequence.

15 The invention further provides the use of a nucleic acid encoding an active agent in the preparation of a medicament for inhibiting weight gain or promoting weight loss in an individual, e.g. for the treatment of obesity, wherein the active agent comprises a CCN dIII domain sequence or a functionally active fragment thereof, and lacks a CCN dI domain sequence, a CCN dII domain sequence and a CCN dIV domain sequence.

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The invention further provides a nucleic acid encoding an active agent for use prophylaxis or treatment of a condition associated with insulin resistance or glucose intolerance in a subject, wherein subject is obese, wherein the active agent comprises a CCN dIII domain sequence or a functionally active fragment thereof, and lacks a CCN dI domain sequence, a CCN dII domain sequence and a CCN dIV domain

25 sequence.

The invention further provides a method of prophylaxis or treatment of a condition associated with insulin resistance or glucose intolerance in a subject, comprising administering to the individual a nucleic acid encoding an active agent which comprises a CCN dIII domain sequence or a functionally active fragment thereof, and lacks a CCN dI domain sequence, a CCN dII domain sequence and a CCN dIV domain

30 sequence, wherein the subject is obese.

The invention further provides the use of a nucleic acid encoding an active agent in the preparation of a medicament for prophylaxis or treatment of a condition associated with insulin resistance or glucose

35 intolerance in a subject, wherein the active agent comprises a CCN dIII domain sequence or a

functionally active fragment thereof, and lacks a CCN dI domain sequence, a CCN dII domain sequence and a CCN dIV domain sequence, wherein the subject is obese.

5 The invention further provides a nucleic acid encoding an active agent for use in prophylaxis or treatment of a condition associated with obesity or excess body weight, wherein the active agent comprises a CCN dIII domain sequence or a functionally active fragment thereof, and lacks a CCN dI domain sequence, a CCN dII domain sequence and a CCN dIV domain sequence.

10 The invention further provides a method of prophylaxis or treatment of a condition associated with obesity or excess body weight in an individual, comprising administering to the individual a nucleic acid encoding an active agent which comprises a CCN dIII domain sequence or a functionally active fragment thereof, and lacks a CCN dI domain sequence, a CCN dII domain sequence and a CCN dIV domain sequence.

15 The invention further provides the use of a nucleic acid encoding an active agent in the preparation of a medicament for prophylaxis or treatment of a condition associated with obesity or excess body weight in an individual, wherein the active agent comprises a CCN dIII domain sequence or a functionally active fragment thereof, and lacks a CCN dI domain sequence, a CCN dII domain sequence and a CCN dIV domain sequence.

20 The invention further provides a nucleic acid encoding an active agent for use in treatment of polycystic kidney disease, wherein the active agent comprises a CCN dIII domain sequence or a functionally active fragment thereof, and lacks a CCN dI domain sequence, a CCN dII domain sequence and a CCN dIV domain sequence.

25 The invention further provides a method of treatment of polycystic kidney disease in an individual, comprising administering to the individual a nucleic acid encoding an active agent which comprises a CCN dIII domain sequence or a functionally active fragment thereof, and lacks a CCN dI domain sequence, a CCN dII domain sequence and a CCN dIV domain sequence.

30 The invention further provides the use of a nucleic acid encoding an active agent in the preparation of a medicament for treatment of polycystic kidney disease, wherein the active agent comprises a CCN dIII domain sequence or a functionally active fragment thereof, and lacks a CCN dI domain sequence, a CCN dII domain sequence and a CCN dIV domain sequence.

It will be apparent that other features of the active agent and the therapeutic methods as described elsewhere in this specification may be employed mutatis mutandis in the context of these gene therapy approaches where the context permits.

- 5 The invention includes the combination of the aspects and preferred features described except where such a combination is clearly impermissible or expressly avoided.

Summary of the Figures

- Embodiments and experiments illustrating the principles of the invention will now be discussed with
10 reference to the accompanying figures in which:

Figure 1. Effect of TRX-45 on body weight in obesity. Figure 1 show measurements of absolute body weight (panel (A)) and relative body weight (panel (B)) over a period of 12 weeks during which TRX-45 or vehicle was administered to obese mice showing that TRX-45 dose-dependently attenuated body weight
15 gain (* $p < 0.05$ vs. Vehicle of endpoint values: t-test not corrected for multiple testing). Curves represent means, $n = 23-27$ animals per group.

Figure 2. Effect of TRX-45 on adipose tissue mass in obesity. Figure 2 shows measurements of inguinal white adipose tissue (WAT) fat pad weights from obese mice after 12 weeks of administration of TRX-45
20 or vehicle showing that TRX-45 dose-dependently reduces the weight of inguinal WAT in obese mice (* $p < 0.05$ Vs. Vehicle: t-test not corrected for multiple testing). Bars represent mean + SEM, $n = 23-27$ animals per group.

Figure 3. Effect of TRX-45 on attenuating cyst growth in ADPKD. Figure 3 shows in vivo magnetic
25 resonance imaging (MRI) measurements of total kidney volume growth corrected for body weight growth from baseline until after 11 weeks of drug treatment. The data demonstrate TRX-45 mediated reductions in kidney growth in male mice with polycystic kidney disease (* $p < 0.05$, ** $p < 0.01$ vs. Vehicle: t-test not corrected for multiple testing). The bars represent mean + SEM, $n = 9$ animals per group.

Figure 4. Effect of TRX-45 on glycated hemoglobin in insulin-resistance ("diabetes"). Figure 4 shows
30 HbA1c levels after 12 weeks of drug treatment with TRX-45 in insulin resistant ("diabetic") mice, demonstrating a significant reduction in HbA1c with TRX-45 administration. (*TRX-45 (20 mg/kg) vs. Vehicle: $p = 0.0427$). Bars represent mean + SEM, $n = 28-29$ animals per group.

Detailed Description of the Invention

Aspects and embodiments of the present invention will now be discussed with reference to the accompanying figures. Further aspects and embodiments will be apparent to those skilled in the art. All documents mentioned in this text are incorporated herein by reference.

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Sequence identity

The identity between a candidate sequence and a reference sequence may be measured in terms of percentage identity.

- 10 Percent (%) amino acid sequence identity with respect to a reference sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. % identity values may be determined by WU-BLAST-2 (Altschul et al.,
15 Methods in Enzymology, 266:460-480 (1996)). WU-BLAST-2 uses several search parameters, most of which are set to the default values. The adjustable parameters are set with the following values: overlap span = 1, overlap fraction = 0.125, word threshold (T) = 11. A % amino acid sequence identity value is determined by the number of matching identical residues as determined by WU-BLAST-2, divided by the total number of residues of the reference sequence (gaps introduced by WU-BLAST-2 into the reference
20 sequence to maximize the alignment score being ignored), multiplied by 100.

- Reference to "corresponding" reference sequence should be taken to mean the portion of reference sequence which aligns with a candidate sequence when that candidate sequence is optimally aligned with the full length reference sequence. Thus, for example, a 10 amino acid sequence of a targeting
25 peptide which is identical to a contiguous 10 amino acid stretch of N18 would be considered to have 100% identity to that stretch of corresponding N18 sequence.

Amino acid classification

The normal 20 proteinogenic amino acids may be grouped according to the following classification:

- 30 I. Asp and Glu (acidic amino acids);
II. Arg, Lys and His (basic amino acids);
III. Asn, Gln, Ser, Thr and Tyr (uncharged polar amino acids);
IV. Ala, Gly, Val, Leu, Ile, Pro, Phe, Met, Trp and Cys (non-polar amino acids).
- 35 For the avoidance of doubt, the term "uncharged amino acid" is used herein to encompass both uncharged polar and non-polar amino acids.

A “conservative substitution” may be defined as a substitution within an amino acid class, or a substitution that scores positive in the BLOSUM62 matrix.

Substitutions which score positive in the BLOSUM62 matrix are as follows:

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Original residue	C	S	T	P	A	G	N	D	E	Q	H	R	K	M	I	L	V	F	Y	W
Substitution	-	T A N	S	-	S	-	S D H	N E	D Q K	E R K	N Y	Q K	E Q R	I L V	M L V	M I V	M I L	Y W	H F W	F Y

Non-proteinogenic amino acids (i.e. those not encoded by the standard genetic code) may also find use in the context of the present invention. These include 2-aminoisobutyric acid (Aib), sarcosine (Sar), norleucine (Nle) and ornithine (Orn).

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The terminal functional groups (amino and carboxyl) of the peptide chain of the CCN dIII domain, or of the active agent, may remain as free amino and carboxyl groups. Alternatively, either or both may be linked to a heterologous moiety, such as a half-life extending moiety, especially where the active agent does not contain such a moiety as part of the peptide chain. Alternatively, either or both may be derivatised to increase stability. Thus the N-terminal amino group may be represented by -NH-Y1, where Y1 may be hydrogen (H), acetyl (Ac) or a heterologous moiety such as a half-life extending moiety. The C-terminal carboxyl group may be represented by -C(O)-Y2, where Y2 may be OH (forming a terminal carboxylic acid group), NH₂ (forming a terminal amide), or a heterologous moiety such as a half-life extending moiety.

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CCN dIII domain sequences

Members of the CCN proteins share a relatively conserved domain structure, having (from N- to C-terminus an insulin-like growth factor binding domain (IGFBP; dI), a Von Willebrand factor type C domain (VWC; dII), a thrombospondin type 1 repeat domain (TSP type-1; dIII), and a C-terminal domain (CT; dIV, except in CCN5) containing a cysteine knot motif.

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The active agents of the present invention comprise a CCN dIII domain or an active fragment thereof, particularly a human CCN dIII domain or an active fragment thereof. The term “domain III” or “dIII” is used to refer to the TSP type-1 domain, regardless of its position in the corresponding wild type molecule.

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The sequence corresponding to dII in CCN6 lacks four cysteine residues which are conserved in other members of the family so may not strictly be a “VWC” domain. However it can still be considered as a

"dII" domain and so the TSP type-1 domain of CCN6 is still designated "dIII" in that protein. However, the term "TSP type-1 domain" or equivalents may be used if preferred.)

5 The dIII domains of the human CCN proteins (CCN1 to CCN6) are from 52 to 55 amino acids in length and have 6 conserved cysteine residues with identical spacing, at residues 1, 11, 15, 30, 39 and 44. These cysteine residues are believed to form 3 intramolecular disulfide bonds which are important for folding and activity of the domain. Thus, it may be desirable that these 6 cysteine residues, and optionally also the spacing between them, are conserved.

10 Thus the active fragment may comprise or consist of the 44 amino sequence defined by the first and sixth cysteine residues, inclusive.

It may have a sequence according to Formula (I):

Cys - A - Cys - B - Cys - C - Cys - D - Cys - E - Cys - F (Formula (I))

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wherein

A is a peptide of 9 amino acids;

B is a peptide of 3 amino acids;

C is a peptide of 14 amino acids;

20 D is a peptide of 8 amino acids;

E is a peptide of 4 amino acids;

F is absent or is a peptide of any desired length, e.g. of up to 20 amino acids, e.g. of up to 15 amino acids, e.g. of up to 11 amino acids, e.g. of 8 to 11 amino acids.

25 In some embodiments:

A is a peptide of Formula II:

A1-A2-A3-A5-A6-A7-A8-A9 (Formula II)

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wherein A1 is an amino acid selected from P, A, V, I, and L;

- A2 is an amino acid selected from E, D, A, I, L, and V;
A3 is an amino acid selected from G, Q, Y, S, N, W and F;
A4 is an amino acid selected from A, I, L, V, S and T;
A5 is an amino acid selected from T, Y, N, G, Q and S;
5 A6 is an amino acid selected from A, V, I, L, P, S, E, D, K, R, and H;
A7 is W;
A8 is selected from G, T, S, Q, Y, N, P, A, V, I, and L; and
A9 is an amino acid selected from A, P, L, I, V and Q.

- 10 In some embodiments, B is a peptide of Formula III:

B1-B2-B3 (Formula III)

wherein B1 is an amino acid selected from G, Q, N, S, Y, and T;

- 15 B2 is an amino acid selected from T, S, N, F, Q, H, R and K; and
B3 is an amino acid selected from G, Q, N, S, Y and T;

In some embodiments, C is a peptide of Formula IV:

- 20 C1-C2-C3-C4-C5-C6-C7-C8-C9-C10-C11-C12-C13-C14 (Formula IV)

wherein C1 is an amino acid selected from G, Q, N, S, Y, and T;

C2 is an amino acid selected from K, R, H, M, T, S, A, L, I, and V;

C3 is an amino acid selected from G, Q, N, S, Y and T;

- 25 C4 is an amino acid selected from M, F, A, I, L, V and W;
C5 is an amino acid selected from G, Q, N, S, T, Y, A, I, L and V;
C6 is an amino acid selected from G, Q, N, S, and T;
C7 is an amino acid selected H, R and L;
C8 is an amino acid selected from A, L, I, and V;
30 C9 is an amino acid selected from G, Q, N, S, T and Y;

- C10 is an amino acid selected from G, Q, N, S, T, Y (preferably N);
- C11 is an amino acid selected from V, P, A, I, L, G, Q, N, S, T, Y, R, K, D and E;
- C12 is an amino acid selected from G, Q, N, S, Y and T;
- C13 is an amino acid selected from H, K, R, A, L, I, V, P, G, Q, N, S, Y and T; and
- 5 C14 is an amino acid selected from F, P, W, G, Q, N, S, Y, T, E and D;

In some embodiments, D is a peptide of Formula V:

- 10 D1-D2-D3-D4-D5-D6-D7-D8 (Formula V)

wherein D1 is an amino acid selected from R, K, H, D, E, W and P;

D2 is an amino acid selected from P, A, L, I, V, M, W, D and E;

D3 is an amino acid selected from D, E, A, L, I, V, R, K and H;

- 15 D4 is an amino acid selected from G, Q, S, Y, T, R, L, K and H;

D5 is an amino acid selected from G, Q, N, S, Y, T, D and E;

D6 is an amino acid selected from H, R, K, G, Q, N, S, Y and T;

D7 is an amino acid selected from L, H and R; and

D8 is an amino acid selected A, L, I and V.

20

In some embodiments, E is a peptide of Formula VI

E1-E2-E3-E4 (Formula VI)

- 25 wherein E1 is an amino acid selected from P, A, L, I, V, M, W, G, Q, N, S, T, Y, D and E;

E2 is an amino acid selected from P, A, L, I, V, M, W, G, Q, N, S, T and Y;

E3 is an amino acid selected from R, K, H, G, Q, N, S, T and Y; and

E4 is an amino acid selected from P, A, L, I and V.

- 30 In some embodiments, the active fragment satisfies all of Formulae I – VI set out above.

In further embodiments:

A1 is selected from the group consisting of P, A, I and L;

5 A2 is selected from E, V and A;

A3 is selected from W, Q, and Y;

A4 is selected from S, T and A;

A5 is selected from T and S;

A6 is selected from A, E, P, S and K;

10 A7 is W;

A8 is selected from G, S and T; and

A9 is selected from P, Q and A;

B1 is S (serine);

15 B2 is selected from T, K and R; and

B3 is selected from T and S;

C1 is G (glycine);

C2 is selected from T, L and M;

20 C3 is G (glycine);

C4 is selected from M, F, I and V;

C5 is selected from S and A;

C6 is selected from T and N;

C7 is R (arginine);

25 C8 is selected from V and I;

C9 is selected from S and T;

C10 is N (asparagine);

C11 is selected from Q, R, D, V, and E;

C12 is N (asparagine);

30 C13 is selected from R, A, P, and S; and

C14 is selected from F, Q, S, E, and N;

D1 is selected from R, E and W;

D2 is selected from L, M, and P;

5 D3 is selected from E, L, V and R;

D4 is selected from T, K, and Q;

D5 is selected from Q and E;

D6 is selected from R, T, S, and K;

D7 is R (arginine); and

10 D8 is selected from L and I;

E1 is selected from L, M, E, N and Y;

E2 is selected from S, V, L and I;

E3 is selected from Q and R; and

15 E4 is P;

F is absent or a peptide of up to 20 amino acids, e.g. up to 15 amino acids, e.g. up to 13 amino acids, and may comprise an amino acid sequence selected from

20 GQPVYSSLKKGKK, EADLEENIKKGKK, EQEPEQPTDKKGKK, DVDIHTLIKAGKK, PPSRGRSPQNSAF and DSNILKTIKIPKGKT (SEQ ID NOs: 17 to 22 respectively) or a fragment thereof, e.g. an N-terminal fragment thereof lacking one or more consecutive residues from the C-terminal end of the relevant sequence.

25 When present, F may comprise or consist of the sequence EQEPEQPTDKKGKK (SEQ ID NO: 19), or a fragment thereof.

In preferred embodiments, F may be absent.

30 The active agent may comprise or consist of one of the following 44 amino acid sequences from the human CCN dIII domains:

- CIVQTTWSQCSKTCGTGISTRVTNDNPECRLVKETRICEVRPC (SEQ ID NO: 1, from CCN1);
 CLVQTTEWSACSKTCGMGISTRVTNDNASCRLKQSRCLCMVRPC (SEQ ID NO: 2, from CCN2);
 CIEQTTEWTACSKSCGMGFSTRVTNRNRQCEMLKQTRLCMVRPC (SEQ ID NO: 3, from CCN3);
 CIAYTSPWSPCSTSCGLGVSTRISNVNAQCWPEQESRLCNLRPC (SEQ ID NO: 4, from CCN4);
 5 CPEWSTAWGPCSTTCGLGMATRVSNQNRFCRLETQRRCLCLSRPC (SEQ ID NO: 5, from CCN5); or
 CLVQATKWTPCSRTC GMGISNRVTNENSNCCEMRKEKRLCYIQPC (SEQ ID NO: 6 from CCN6);

- or may have at least 70% identity thereto, e.g. at least 75% identity, at least 80%, 85%, 90% or 95% identity to one of those sequences. In practice, this means they may have a maximum of 12 differences from one of those sequences, e.g. a maximum of 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 difference from one of those sequences. As will be apparent from the foregoing discussion, the 6 cysteine residues will typically be conserved, and preferably also the spacing between them. To maintain cysteine spacing, any differences will typically be substitutions.

- 15 SEQ ID NO: 3, from CCN3, may be particularly preferred.

The active agent may comprise or consist of one of the following human dIII sequences:

- CIVQTTWSQCSKTCGTGISTRVTNDNPECRLVKETRICEVRPCGQPVSLSLKKGKK (SEQ ID NO: 7, from CCN1);
 20 CLVQTTEWSACSKTCGMGISTRVTNDNASCRLKQSRCLCMVRPCEADLEENIKKGKK (SEQ ID NO: 8, from CCN2);
 CIEQTTEWTACSKSCGMGFSTRVTNRNRQCEMLKQTRLCMVRPCEQEPEQPTDKKGKK (SEQ ID NO: 9, from CCN3);
 25 CIAYTSPWSPCSTSCGLGVSTRISNVNAQCWPEQESRLCNLRPCDVIDHTLIKAGKK (SEQ ID NO: 10, from CCN4);
 CPEWSTAWGPCSTTCGLGMATRVSNQNRFCRLETQRRCLCLSRPCPPSRGRSPQNSAF (SEQ ID NO: 11, from CCN5); or
 CLVQATKWTPCSRTC GMGISNRVTNENSNCCEMRKEKRLCYIQPCDSNLIKTIKIPKGKT (SEQ ID NO: 12, from CCN6);
 30

- or may have at least 70% identity thereto, e.g. at least 75% identity, at least 80%, 85%, 90% or 95% identity to one of those sequences. As will be apparent from the foregoing discussion, the 6 cysteine residues will typically be conserved, and preferably also the spacing between them. In practice, they will typically have a maximum of 12 differences from one of those sequences between the first and sixth cysteine residues, e.g. a maximum of 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 difference from one of those sequences between those residues. To maintain cysteine spacing, any differences will typically be substitutions.

SEQ ID NO: 9, from CCN3, may be particularly preferred.

It has been found that substitution of the residue immediately following the first cysteine residue (as compared to the wild type residue at that position) may improve stability of the dIII sequence in vivo, e.g. towards proteolytic degradation. Thus, in any of the above embodiments, it may be desirable that the residue immediately following the first cysteine residue (residue 2 of SEQ ID NOs: 1 to 12, corresponding to residue A1 of Formula II) is not I (e.g. for CCN1, CCN3 or CCN4), L (e.g. for CCN2 or CCN6) or P (e.g. for CCN5). For example, it may be alanine, although other alternatives may also be beneficial, including non-proteinogenic residues such as Aib (2-aminoisobutyric acid).

10

Thus, for example, the dIII domain sequence (or fragment thereof) may comprise or consist of

CAEQTTEWTACSKSCGMGFSTRVTNRRQCEMLKQTRLCMVRPC (SEQ ID NO: 13; 44 amino acid fragment of CCN3 dIII with I2A substitution); or

15

CAEQTTEWTACSKSCGMGFSTRVTNRRQCEMLKQTRLCMVRPCEQEPEQPTDKKGKK (SEQ ID NO: 14; full-length CCN3 dIII with I2A substitution).

The dIII domain is the only CCN domain present in the active agent. Thus, the active agent does not contain any of a CCN dI domain sequence, a CCN dII domain sequence, or a CCN dIV domain sequence.

20

Thus, for example, it does not contain the sequence of human CCN3 domain I:

TQRCPPQCPGRCPATPPTCAPGVRAVLGDCSCCLVCARQRGESCDLEPCDESSGLYCDRSADPSNQ
TGICTAV (SEQ ID NO: 23; human CCN3 dI);

25

the sequence of human CCN3 domain II:

DNCVFDGVIYRSGEKFQPSCKFQCTCRDGGIGCVPRCQLDVLLPEPNCPAPRKVEVPGECCCKWICG
(SEQ ID NO: 24; human CCN3 dII);

or the sequence of human CCN3 domain IV:

CLRTKKSLLKAIHLQFKNCTSLHTYKPRFCGVCSDGRCCPHNTKTIQAEFQCSPGQIVKKPVMVIGTCTC
HTNCP (SEQ ID NO: 25; human CCN3 dIV);

30

or a sequence having 70% identity or more thereto, e.g. a sequence having 75%, 80%, 85%, 90%, 95% identity or more, to any one of those sequences.

The active agent also does not contain sequences corresponding to non-TSP type-1 domains from other CCN proteins.

5

Thus, for example, the active agent does not contain the sequence of human CCN1 domain I:

TCPAACHCPLKAPKCAPGVGLVRDGCCKVCAQLNEDCSKTQPCDHTKGLECNFGASSTALKGICR
AQ (SEQ ID NO: 26; human CCN1 dI);

the sequence of human CCN1 domain II:

10 RPCEYNSRIYQNGESFQPNCKHQCTCIDGAVGCIPLCPQELSLPNLGCPNPRLVKVTGQCCEEWVCD
(SEQ ID NO: 27; human CCN1 dII);

or the sequence of human CCN1 domain IV:

CSKTKKSPEPVRFTYAGCLSVKKYRPKYCGSCVDGRCCTPQLTRTVKMRFRCEGETFSKNVMMIQSC
KCNYNCP (SEQ ID NO: 28; human CCN1 dIV);

15 or a sequence having 70% identity or more thereto, e.g. a sequence having 75%, 80%, 85%, 90%, 95%
identity or more, to any one of those sequences.

The active agent does not contain the sequence of human CCN2 domain I:

QNCSGPCRCPDEPAPRCAPAGVSLVDGCGCCRVCAQLGELCTERDPCDPHKGLFCHFGSPANRKIGV
20 CTAK (SEQ ID NO: 29; human CCN2 dI);

the sequence of human CCN2 domain II:

APCIFGGTVYRSGESFQSSCKYQCTCLDGAVGCMPLCSMDVRLPSPDCPFPRRVKLPGKCCEEWVCD
(SEQ ID NO: 30; human CCN2 dII);

or the sequence of human CCN2 domain IV:

25 CIRTPKISKPIKFELSGCTSMKTYRAKFCGVCTDGRCTPHRTTTLPEVFKCPDGEVMKKNMFIKTCAC
HYNCP (SEQ ID NO: 31; human CCN2 dIV);

or a sequence having 70% identity or more thereto, e.g. a sequence having 75%, 80%, 85%, 90%, 95%
identity or more, to any one of those sequences.

30 The active agent does not contain the sequence of human CCN4 domain I:

RPQFCKWPCECPPSPRCPLGVSLITDGCECCKMCAQQLGDNCTEAAICDPHRGLYCDYSGDRPRYAI
GVCAQV (SEQ ID NO: 32; human CCN4 dI);

the sequence of human CCN4 domain II:

VGCVLDGVRNNGQSFQPNCKYNCTCIDGAVGCTPLCLRVRPPRLWCPHPRRSIPGHCCEQWVCE
(SEQ ID NO: 33; human CCN4 dII);

or the sequence of human CCN4 domain IV:

- 5 CLAVYQPEASMNFTLAGCISTRSYQPKYCGVCMDNRCCIPYKSKTIDVSFQCPDGLGFSRQVLWINACF
CNLSCR (SEQ ID NO: 34; human CCN4 dIV);

or a sequence having 70% identity or more thereto, e.g. a sequence having 75%, 80%, 85%, 90%, 95% identity or more, to any one of those sequences.

- 10 The active agent does not contain the sequence of human CCN5 domain I:

QLCPTPCTCPWPPRCPLGVPLVLDGCGCCRVCCARRLGEPDQLHVCDASQGLVCQPGAGPGGRGAL
CLLAE (SEQ ID NO: 35; human CCN5 dI); or

the sequence of human CCN5 domain II:

- SSCEVNGRLYREGETFQPHCSIRCRCEGGFTCVPLCSEDVRLPSWDCPHPRRVEVLGKCCPEWVCG
15 (SEQ ID NO: 36; human CCN5 dII);

or a sequence having 70% identity or more thereto, e.g. a sequence having 75%, 80%, 85%, 90%, 95% identity or more, to any one of those sequences.

The active agent does not contain the sequence of human CCN6 domain I:

- 20 RKQFCHWPCKCPQQKPRCPPGVSLVRDGC GCKICAKQPGEICNEADLCDPHKGLYCDYSVDRPRYET
GVCAYL (SEQ ID NO: 37; human CCN6 dI);

the sequence of human CCN6 domain II:

VGCEFNQVHYHNGQVFQPNPLFSCLCVSGAIGCTPLFIPKLAGSHCSGAKGGKSDQSNCS (SEQ ID
NO: 38; human CCN6 dII);

- 25 or the sequence of human CCN6 domain IV:

CQPTFQLSKAEKFVFSGCSSTQSYKPTFCGICLDKRCCIPNKS KMITIQFDCPNEGSFKWKMLWITSCVC
QRNCR (SEQ ID NO: 39; human CCN6 dIV);

or a sequence having 70% identity or more thereto, e.g. a sequence having 75%, 80%, 85%, 90%, 95% identity or more, to any one of those sequences.

30

The active agent, and specifically the dIII component of the active agent, possesses dIII activity.

In this context, "dIII activity" may be the ability to inhibit weight loss or promote weight gain in an individual, and/or to inhibit development of, or to reduce, white adipose tissue in an individual. Such activity may be exerted in an experimental model, such as an obese mouse model, e.g. a female db/db mouse model, e.g. under the conditions of Example 1 below.

5

Additionally or alternatively, "dIII activity" may be the ability to treat polycystic kidney disease in an individual, e.g. to inhibit cyst growth or reduce the volume of existing cysts in an individual. Such activity may be exerted in an experimental model, such as a mouse model of polycystic kidney disease, e.g. a *Pkd1*^{RCRC} 129s6 mouse model, e.g. under the conditions of Example 2 below.

10

It will be understood that these are provided for the purposes of experimental testing, and that the active agents are able to inhibit weight loss or promote weight gain, or to treat polycystic kidney disease, in a therapeutic context, especially in a human subject in need thereof, as defined elsewhere in this specification.

15

Heterologous moiety

The active agent may comprise a heterologous moiety. In some embodiments, the heterologous moiety increases the stability of the active agent. In some embodiments, the heterologous moiety increases the serum half-life of the active agent as compared to a similar agent in the absence of the heterologous moiety.

20

The heterologous moiety may be peptidic in nature or may be non-peptide. Non-peptide heterologous moieties may be or may comprise lipids, carbohydrates, polymers etc..

25 Where the heterologous moiety is peptidic (i.e. is a polypeptide or peptide) it is a polypeptide or peptide which is not present in combination (i.e., not linked to, directly or indirectly) with CCN dIII domain sequence (or fragment thereof) in nature. In some embodiments, the peptide heterologous moiety is a non-CCN dIII peptide sequence, e.g. a non-CCN sequence. Typically a peptide is considered to be up to 50 amino acids in length, while a polypeptide is greater than 50 amino acids in length. However, the term
30 "peptide" or "peptidic" may be used to indicate the nature of the moiety, i.e. that it is formed from amino acids linked by peptide bonds.

The heterologous moiety may be a peptide or polypeptide. In such embodiments, the heterologous moiety may form a fusion protein with said CCN dIII domain sequence or fragment thereof, i.e. the CCN
35 dIII domain sequence or fragment thereof, and the heterologous moiety, are part of the same peptide or polypeptide chain. The heterologous moiety may be present at the N-terminus of the CCN dIII domain sequence or fragment thereof. Alternatively, the heterologous moiety may present at the C-terminus of the CCN dIII domain sequence or fragment thereof. In some embodiments, the peptide or polypeptide is

covalently attached to the CCN dIII polypeptide or peptide. In some embodiments, the peptide or polypeptide is non-covalently attached to the CCN dIII polypeptide or peptide.

- In some embodiments, the heterologous moiety is at least 20, 50, 100, 200, 300, 400 or 500 amino acids long, or may for example be a maximum of 20, 50, 100, 200, 300, 400 or 500 amino acids in length. In some embodiments, the heterologous moiety is selected from an Fc fragment, serum albumin (e.g., human serum albumin (HSA)), a serum albumin binding moiety, fibrinogen, glutathione S-transferase, transferrin, or streptavidin.
- 10 In some embodiments, the heterologous moiety is a monomeric Fc. In some embodiments, the heterologous moiety is dimeric Fc. The Fc fragment may be an Fc fragment of any subclass or chimera of any subclasses. In some embodiments, the Fc fragment is a IgG1, IgG2, or IgG4 Fc fragment. IgG1, IgG2 and IgG4 are often preferred to IgG3 due to their longer half-lives.
- 15 A serum albumin-binding moiety in this context is a protein or peptide which binds to serum albumin. (As noted below, other non-peptide heterologous moieties which bind to serum albumin are also known, and are typically lipid moieties.) It may comprise an antigen-binding portion or fragment of an antibody or antibody-like molecule. Preferably the serum albumin-binding moiety comprises the necessary antigen-binding elements of the antibody or antibody-like molecule in a single peptide chain. Such molecules
- 20 include Fab-like molecules (Better et al (1988) Science 240, 1041); Fv molecules (Skerra et al (1988) Science 240, 1038); single-chain Fv (ScFv) molecules where the VH and VL partner domains are linked via a flexible oligopeptide (Bird et al (1988) Science 242, 423; Huston et al (1988) Proc. Natl. Acad. Sci. USA 85, 5879), single domain antibodies (dAbs) comprising isolated V domains (Ward et al (1989) Nature 341, 544), VHH domain (variable domain of heavy chain of heavy chain antibody, or "nanobody",
- 25 e.g. derived from a camelid antibody), and VNAR (variable domain of new antigen receptor, e.g. derived from immunoglobulins from cartilaginous fishes). A general review of the techniques involved in the synthesis of antibody fragments which retain their specific binding sites is to be found in Winter & Milstein (1991) Nature 349, 293- 299.
- 30 As a specific example, the heterologous moiety may comprise or consist of amino acids 25-609 of human serum albumin (HSA) having the sequence:

DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVADES
AE
NCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNP
NLPRLVLRPEV
35 DVMCTAFHDNEETFLKKYLYEIARRHPYFYAPPELLFFAKRYKAAFT
ECCQAADKAACLLP
KLDELRDEGKASSAKQRLKCASLQKFGERAFAKAWAVARLSQRFPAEFAEVS
KLVTDLT
K
VHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLEKSHCIAE
VENDEMPA
DLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLRLAKTY
ETTLEKC
CAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNAL
LVRYTKKVPQVST
40 PTLVEVSRNLGKVGSKCKHPEAKRMPCAEDYLSVVLNQLC
VLHEKTPVSDRVTKCCTES

LVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKERQIKKQIALVELVKHKPKAT
KEQLKAVMDDFAAFVEKCKADDKETCFAEEGKKLVAASQAALGL (SEQ ID NO: 15)

- or may have at least 70% identity thereto, e.g. at least 75% identity, at least 80%, 85%, 90%, 91%, 92%,
5 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to that sequence.

- A number of modifications of the serum albumin sequence have been identified as being potentially beneficial. These include modification of the residues designated K573, E505Q and T527M in US 10,781,245 B2 (K597, E529 and T551 in SEQ ID NO: 15 above, indicated by underlining). Preferred
10 modifications at these positions are K573P (or K597P) E505Q (E529Q) and T527M (T551M) although other substituents may also be beneficial. Indeed it is believed that all proteinogenic residues other than K at position 573 (597) improve Fc receptor binding and so increase half life. The triple mutant E505Q/T527M/K573P (designated QMP) may be particularly preferred.

15

In alternative embodiments, the heterologous moiety may be a carbohydrate molecule. Examples, include dextran, glycosylation, polysialylation, hydroxyethylation (HESylation), heparosanylation (HEPylation), and hyaluronic acid polysaccharide (HAllylation).

- 20 In some embodiments, the heterologous moiety is a polymer. Polymers may include synthetic polymers or polypeptides. For example, synthetic polymer may include PEG. In some embodiments, the heterologous moiety is a chemical modification. The chemical modification may include PEGylation. Any suitable chemical modification known in the art for use in increasing stability of therapeutic proteins may be used. Example polypeptides polymers include XTEN (protein polymer developed by Amunix),
25 PASylation (proline-alanine-serine polymer), ELPylation (elastin-like polypeptides), HAPylation (repeated sequence of a glycine rich – Gly₄Ser)_n polypeptide, gelatin-like protein (GLK – has a (Gly-XY)_n structure).

- In some embodiments, the heterologous moiety is a lipid. Lipidation involves the transfer of a lipid group to a protein and can be used to increase the half-life of therapeutic proteins, reduce immunogenicity, and
30 increase cell membrane permeability (Menacho-Melgar, 2019., J Control Release (295) 1-12, Kurtzhals P et al., Nat Rev Drug Discov. 2023 Jan;22(1):59-80.). Without wishing to be bound by theory, it is believed that lipidation improves drug half-life by enabling the binding of serum albumin that is present in the blood of a subject. Example lipid modifications include, laureate, myristate, palmitate or fatty diacids with or without linkers (e.g. such as L-γ-glutamoyl) and/or spacers, (e.g. such as 3,8-dioxa-aminooctanoic acid).

35

Any heterologous moiety known in the art for use with therapeutic proteins may be used.

The heterologous moiety may be located N-terminal or C-terminal of the CCN dIII domain. However, it may be preferred that the heterologous moiety is located C-terminal of the CCN dIII domain.

40

Linker

The active agent may further comprise a linker between the CCN dIII domain sequence (or fragment thereof) and the heterologous moiety. Especially when the active agent is a fusion protein, the linker may be a peptide linker.

5

Thus, the fusion protein may comprise, from N- to C-terminus, CCN dIII domain sequence (or fragment thereof) – linker – heterologous moiety, or heterologous moiety – linker – CCN dIII domain sequence (or fragment thereof). The former may be preferred.

- 10 In some embodiments, the linker comprises no more than 50 amino acids, e.g. no more than 25 amino acids, e.g. no more than 10 amino acids. The linker may be 1-50, 1-45, 1-40, 1-30, 1-25, 1-20, 1-15, 1-12, 1-10, e.g. 1-8, 1-6, 1-5, or 1-4, amino acids long.

- Any peptide linker may be used (as long as it is not a CCN protein sequence), many of which are known and described in the art. The linker may be a flexible linker sequence (which may include repeats of a flexible linker sequence motif). Typical linkers known in the art are rich in small non-polar (e.g. glycine) or polar (e.g. serine or threonine) residues, and commonly consist of stretches of glycine and serine residues (GS) or other amino acid residues such alanine, lysine and/or glutamate (A, K, and/or E), or indeed any amino acids. A commonly used linker has the sequence GGGGS (SEQ ID NO: 40), which
 15 and described in the art. The linker may be a flexible linker sequence (which may include repeats of a flexible linker sequence motif). Typical linkers known in the art are rich in small non-polar (e.g. glycine) or polar (e.g. serine or threonine) residues, and commonly consist of stretches of glycine and serine residues (GS) or other amino acid residues such alanine, lysine and/or glutamate (A, K, and/or E), or indeed any amino acids. A commonly used linker has the sequence GGGGS (SEQ ID NO: 40), which
 20 may be provided as a repeating unit in a linker (as (GGGGS)_n, where the copy number of n may be adjusted, e.g. from 1-10, 1-6, 1-4 etc.).

- US 20180273603 describes α-helical linkers comprising repetitions of the sequence A(EAAAK)_nA (SEQ ID No. 41). Furthermore, US2018/0127478 discloses the use of an amino acid linker consisting of one to
 25 three repetitions of the sequence EAAAK (SEQ ID NO: 42).

Thus, the linker may comprise one or more iterations of the amino acid sequence EAAAK, e.g. it may comprise or consist of the sequence EAAAK.

- 30 In certain embodiments, the active agent may have the mature sequence:

CAEQTTTEWTACSKSCGMGFSTRVTNRNRQCEMLKQTRLKCMVRPCEAAAKDAHKSEVAHRFKDLGEENFKA
LVLIAFAQYLOQCPFEDHVKLVNEVTEFAKTCVADESAENCCKSLHTLFGDKLCTVATLRETYGEMADCC
AKQEPERNECFLOHKDDNPPLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIAARRHPYFYAPELLFFAKRY

KAAFTECCQAADKAACLLPKLDELRLDEGKASSAKQRLKASLQKFGERAFAKAWAVARLSQRFPKAEFAEV
 SKLVTDLTKVHTECCCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPAD
 LPSLAADFVESKDVCKNYAEAKDVFLLGMFLYEYARRHPDYSVVLRLAKTYETTLEKCCAAADPHECYA
 KVFDEFKPLVEEPQNLIKQNCLEFQLGLEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCKHP
 5 EAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHA
 DICTLSEKERQIKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCKADDKETCFAEEGKKLVAAASQA
 ALGI (SEQ ID NO: 16);

being composed of (i) the human CCN3 dIII domain (underlined) with an I2A substitution ; (ii) a short
 10 linker sequence (*italics*); and (iii) amino acids 25-609 of human serum albumin (HSA) (double underlined).

Therapeutic uses

According to the invention, the active agents described are useful, *inter alia*, in promotion of weight loss,
 and inhibition or reduction of weight gain. They may therefore provide an attractive treatment option for,
 15 *inter alia*, obesity and metabolic diseases caused, characterised by, or associated with, excess body
 weight.

Thus, the compounds may be used in a method of treating, inhibiting or reducing weight gain, promoting
 weight loss, and/or reducing body weight, e.g. excess body weight. In particular, the agents may be used
 20 in a method of treatment or prophylaxis of obesity or a disorder caused by obesity.

The compounds may be used in a method of treating obesity.

The compounds may also be used in methods of treating diseases, disorders and health conditions
 25 associated with obesity or excess body weight, particularly those in which the symptoms or pathogenesis
 may be beneficially influenced or modulated via an effect on body weight. It will be understood that the
 benefit provided is not necessarily limited to reducing symptoms or pathogenesis of a given condition, but
 that prophylaxis or inhibiting progression (as compared to the absence of treatment), e.g. by inhibiting
 further weight gain, may also be considered beneficial. Thus, it will be understood that these conditions
 30 may occur (and may be treated) in individuals who are obese. The effect of the active agents on these
 conditions may be mediated via an effect on body weight, or may be independent thereof. In some
 embodiments, the condition to be treated is caused by obesity.

The following are examples of relevant conditions.

For example, the agent may be used in a method of treatment or prophylaxis of obesity-linked inflammation and/or obesity-linked gallbladder disease. In some embodiments, the obesity-linked inflammation or obesity-linked gallbladder disease are caused by obesity.

- 5 The agent may be used in a method of treatment or prophylaxis of diabetes, particularly type 2 diabetes, and associated conditions such as pre-diabetes, insulin resistance syndrome, impaired glucose tolerance (IGT), metabolic syndrome, hyperglycemia and diabetic heart disease (see Kivimäki et al. (2022); Nguyen et al. (2011)).
- 10 The agent may also find use in diseases of insulin resistance and/or impaired glucose intolerance, e.g. like diabetes or pre-diabetes, in the presence of obesity. They may therefore provide an attractive treatment option for, *inter alia*, diabetes and metabolic diseases caused, characterised by, or associated with, insulin resistance and/or impaired glucose intolerance in obese subjects.
- 15 Where the condition is a condition associated with insulin resistance and/or impaired glucose tolerance, for example, insulin resistance syndrome, impaired glucose tolerance (IGT), pre-diabetes, diabetes, metabolic syndrome, hyperglycemia or diabetic heart disease, the subject may also be obese. Therefore, the condition associated with insulin resistance and/or glucose tolerance may be caused by obesity. In a given subject, obesity may be accompanied with a condition associated with insulin resistance and/or
- 20 glucose tolerance.

Conditions related to insulin resistance and/or impaired glucose tolerance may be assessed by measuring the levels of glycated hemoglobin (HbA1c) in a given subject. The non-enzymatic attachment of glucose to the N-terminal valine of β -chain of hemoglobin is termed hemoglobin A_{1c} (A1C) (Sacks et al., Diabetes Care 2024). Glycated hemoglobin is quantified by separating the glycated from the nonglycated protein and measuring the amount of each form (Sacks et al., *supra*). The HbA1c level in a given subject may be measured in a blood sample.

Without wishing to be bound by theory, it is considered that individuals (humans) with HbA1c $\geq 5.7\%$, but $\leq 6.4\%$ are considered to be pre-diabetic, i.e. they are considered to have an abnormal carbohydrate metabolism resulting in elevated blood glucose levels (American Diabetes Association Professional Practice Committee, Diabetes Care, 2025). Individuals with HbA1c $\geq 6.5\%$ (≥ 48 mmol/mol) in two independent measurements are considered to have diabetes (American Diabetes Association Professional Practice Committee, Diabetes Care, 2025).

35 In some embodiments, the agent described herein is capable of reducing the level of HbA1c in a subject. The level of HbA1c may be reduced to at least $\leq 6.4\%$. In some embodiments, the level of HbA1c is reduced to at least $\leq 8.0\%$, $\leq 7.5\%$, $\leq 7.0\%$, $\leq 6.5\%$, $\leq 6.4\%$, $\leq 6.3\%$, $\leq 6.2\%$, $\leq 6.1\%$, $\leq 6.0\%$, $\leq 5.9\%$, $\leq 5.8\%$, $\leq 5.7\%$, $\leq 5.6\%$ or $\leq 5.5\%$. The level of HbA1c in a given subject may be $\leq 6.0\%$.

Optionally, the level of HbA1c in a given subject may be reduced to $\leq 5.6\%$. The agent may reduce the level of HbA1c but does not induce hypoglycemia in a given patient.

- In some embodiments, the agent reduces the levels of HbA1c by at least 2.5 %, 2.0 %, 1.5 %, 1.0 % or 0.5 % compared to the starting % HbA1c level in a given patient. In other embodiments, the agent reduces the levels of HbA1c by $\leq 2.5\%$, $\leq 2.0\%$, $\leq 1.5\%$, $\leq 1.0\%$ or $\leq 0.5\%$, but by more than 0.1%, compared to the starting % HbA1c level in a given patient. The agent may reduce the levels of HbA1c by 0.5 % to 2.5%, by 0.5 % to 2.0 %, by 0.5 % to 1.5 %, by 0.5 % to 1.0 %, by 1.0 % to 2.5 %, by 1.0 % to 2.0 %, by 1.0 % to 1.5 %, by 1.5 % to 2.5%, by 1.5 % to 2.0 %, or by 2.0 % to 2.5% compared to the starting % HbA1c level in a given patient.

The agent may be used in a method of treatment or prophylaxis of hypertension (see Kivimäki et al. (2022); Kotchen et al. (2008)).

- 15 The agent may be used in a method of treatment or prophylaxis of cardiovascular disease, including ischemic heart disease and arrhythmia (Kivimäki et al. (2022)), heart failure and myocardial infarction (Kivimäki et al. (2022); Wilson et al. (2002)), coronary heart disease (Nordestgaard et al. (2012); Flegal et al. (2007); Wilson et al. (2002)) and cerebrovascular disease such as stroke (Lincoff et al. (2023)).

- 20 The agent may be used in a method of treatment or prophylaxis of atherogenic dyslipidemia, hepatic steatosis ("fatty liver"), including non-alcoholic fatty liver disease (NAFLD, also known as metabolic dysfunction associated steatotic liver disease (MASLD)), non-alcoholic steatohepatitis (NASH, also known as metabolic dysfunction associated steatohepatitis (MASH)), and metabolic dysfunction and alcohol associated steatotic liver disease (MetALD) (Loomis et al. (2016)),

25

The agent may be used in a method of treatment or prophylaxis of kidney disease and/or kidney failure (Kivimäki et al. (2022); Wang et al. (2008); Hsu et al. (2006)).

The agent may be used in a method of treatment or prophylaxis of arteriosclerosis (e.g. atherosclerosis).

30

The agent may be used in a method of treatment or prophylaxis of osteoarthritis (Kivimäki et al. (2022); Reyes et al., (2016)).

- 35 The agent may be used in a method of treatment or prophylaxis of gout (Kivimäki et al. (2022); Nguyen et al. (2017));

The agent may be used in a method of treatment or prophylaxis of reproductive disorders, including polycystic ovary syndrome (PCOS) (Yildiz et al. (2008); Barber et al. (2019));

The agent may be used in a method of treatment or prophylaxis of respiratory disorders, including obstructive sleep apnea (or obesity-induced sleep apnea) (Li et al. (2010)) and asthma (Kivimäki et al. (2022)).

5

Metabolic syndrome is characterized by a group of metabolic risk factors in one person. They include abdominal obesity (excessive fat tissue around the abdominal internal organs), atherogenic dyslipidemia (blood fat disorders including high triglycerides, low HDL cholesterol and/or high LDL cholesterol, which foster plaque buildup in artery walls), elevated blood pressure (hypertension), insulin resistance and

10 glucose intolerance, prothrombotic state (e.g. high fibrinogen or plasminogen activator inhibitor-1 in the blood), and proinflammatory state (e.g., elevated C-reactive protein in the blood). Individuals with metabolic syndrome are at increased risk of coronary heart disease and other diseases related to other manifestations of arteriosclerosis (e.g. stroke and peripheral vascular disease). The dominant underlying risk factor for this syndrome appears to be abdominal obesity.

15

The active agents also find use in the prophylaxis or treatment of polycystic kidney disease (PKD). Examples of polycystic kidney diseases are autosomal dominant polycystic kidney disease (ADPKD) and autosomal recessive polycystic kidney disease (ARPKD). ADPKD is more common and typically involves a mutation in one of the genes PKD1, PKD2 or GANAB (PKD3). ARPKD is less common, and typically

20 involves a mutation in the gene PKHD1. Both types of PKD appear to result from defects in the primary cilium in cells of the nephron.

25

The terms "treatment", "treat", or "treating" are used herein to refer to the reduction in severity of a disease or condition, the reduction in the duration of a disease; the amelioration or elimination of one or

more symptoms associated with a disease or condition, or the provision of beneficial effect to a subject with a disease or condition. The term also encompasses prophylaxis of a disease or condition or its symptoms thereof. "Prophylaxis" is known in the art to mean decreasing or reducing the occurrence or severity of a particular disease outcome.

30

In some embodiments, the agent of the invention is administered to a subject. Depending on the agents described herein may be administered in a suitable format/route, including, but not limited to oral, by inhalation, intravenous, subcutaneous, intradermal, intraperitoneal, intrapleural, intraocular, intraarticular, intrathecal, intratumorally, locally into organ the target organ(s) or administered as part of a medical device, e.g., medical or aesthetic implants.

35

Administration is preferably in a "prophylactically effective amount" or a "therapeutically effective amount", this being sufficient to show benefit to the individual. The actual amount administered, and rate and time-course of administration, may depend on the individual subject and the nature and severity of their condition.

40

As used herein, the term "subject" refers to a human or any non-human animal (e.g., mouse, rat, rabbit, dog, cat, cattle, swine, sheep, horse or primate). In many embodiments, a subject is a human being. A subject can be a patient, which refers to a human presenting to a medical provider for diagnosis or treatment of a disease. The term "subject" is used herein interchangeably with "individual" or "patient." In some embodiments, the subject is human. A subject can be afflicted with or is susceptible to a disease or disorder but may or may not display symptoms of the disease or disorder.

Expression vectors and recombinant protein expression

Molecular biology techniques suitable for producing agents according to the invention in cells are well known in the art, such as those set out in Sambrook et al., Molecular Cloning: A Laboratory Manual, New York: Cold Spring Harbor Press, 1989

Thus, the active agent (when entirely peptidic), or the CCN dIII component thereof, may be expressed recombinantly from a nucleotide sequence. It will be understood that this is only possible when the relevant active agent (CCN dIII domain plus any heterologous moiety and linker moiety) are composed entirely of proteinogenic amino acids, i.e. amino acids that can be encoded by the standard genetic code.

The nucleotide sequence may be contained in a vector present in a host cell, or may be incorporated into the genome of a host cell.

A "vector" as used herein is an oligonucleotide molecule (DNA or RNA) used as a vehicle to transfer foreign genetic material into a cell. The vector may be an expression vector for expression of the foreign genetic material in the cell. Such vectors may include a promoter sequence operably linked to the nucleotide sequence encoding the gene sequence to be expressed. A vector may also include a termination codon and expression enhancers. Any suitable vectors, promoters, enhancers and termination codons known in the art may be used. Suitable vectors include plasmids, binary vectors, viral vectors and artificial chromosomes (e.g. yeast artificial chromosomes), and may be linear or circular.

In this specification the term "operably linked" may include the situation where a selected nucleotide sequence and regulatory nucleotide sequence (e.g. promoter and/or enhancer) are covalently linked in such a way as to place the expression of the nucleotide sequence under the influence or control of the regulatory sequence (thereby forming an expression cassette). Thus a regulatory sequence is operably linked to the selected nucleotide sequence if the regulatory sequence is capable of effecting transcription of the nucleotide sequence. Where appropriate, the resulting transcript may then be translated into a desired protein or polypeptide.

Any cell suitable for the expression of polypeptides may be used for producing fusion proteins according to the invention. The cell may be a prokaryote or eukaryote. Preferably the cell is a eukaryotic cell such as a yeast cell, a plant cell, insect cell or a mammalian cell. In some cases the cell is not a prokaryotic cell because some prokaryotic cells do not allow for the same post-translational modifications as eukaryotes. In addition, very high expression levels are possible in eukaryotes and proteins can be

easier to purify from eukaryotes using appropriate tags. Specific plasmids may also be utilised which enhance secretion of the protein into the media.

- Typically the protein (or peptide) product is intended to be secreted after expression by the host cell, e.g. for purification purposes. Thus it may be desirable for the nucleic acid to encode a suitable signal peptide, typically at the N-terminus of the fusion protein. Typically the signal peptide will be cleaved from the expression product (i.e. the active agent or CCN dIII domain) as part of the expression process, resulting in the expression product in the culture medium lacking the signal peptide.
- 10 Methods of producing a protein or peptide of interest may involve culture or fermentation of a eukaryotic cell modified to express the desired product. The culture or fermentation may be performed in a bioreactor provided with an appropriate supply of nutrients, air/oxygen and/or growth factors. Secreted products can be collected by partitioning culture media/fermentation broth from the cells, extracting the protein content, and separating individual proteins. Culture, fermentation and separation techniques are well known to those of skill in the art.

- Once the product of interest has been isolated from culture it may be necessary to concentrate the product. A number of methods for concentrating a protein or peptide of interest are known in the art, such as ultrafiltration or lyophilisation. Depending on the identity of the recombinantly expressed product, further downstream modification (e.g. addition of non-peptide heterologous moieties) may be desired.

Gene therapy

- The active agents may be delivered in the form of a therapeutic nucleic acid (e.g. DNA or RNA) sequence encoding the active agent. The nucleic acid sequence is capable of being taken up by a suitable target cell type such that the active agent is expressed by the target cell. Thus the nucleic acid is typically an expression vector. Typically, the active agent will also be secreted from the target cell. Methods which employ delivery of nucleic acid or an expression vector to achieve therapeutic expression of an active agent within a target cell may be described as "gene therapy".
- 30 For gene therapy to be practical, it is desirable to employ a nucleic acid transfer method that: (1) directs the therapeutic sequence into a desired target cell type, (2) is highly efficient in mediating uptake of the therapeutic polynucleotide into the target cell population, and (3) is suited for use in vivo for therapeutic application.
- 35 The presently available methodologies include transfection with a viral vector; fusion with a lipid; and cationic supported DNA introduction. Each of these techniques has advantages and disadvantages, so that the selection of which technique to use depends upon the particular situation and its demands.

- Any suitable type of viral vector may be employed as a gene delivery vehicle. These include adenovirus, adeno-associated virus (AAV), retrovirus (especially lentivirus) and herpesvirus vectors. Adenovirus and

lentivirus may be particularly preferred as they have the capacity to achieve expression of the gene(s) delivered in cells which are not actively dividing.

5 Adenoviral vectors have also been described for use in human gene therapy (Rosenfeld et al, Cell 68:143 (1992)). Advantages of adenovirus vectors include their potential to carry larger insert polynucleotide sequences than retroviral vectors, very high viral titres, ability to infect non-replicating cells, and suitability for infecting tissues in situ, especially in the lung. Disadvantages include the inclusion of adenovirus genes in the vectors which encode viral proteins that can be immunogenic or result in other adverse effects, and potential instability of gene expression due to unstable integration into chromosomal DNA.

10

Alternatively, adenoassociated viruses (AAVs) can also be used, as can other viral systems depending on the target site, or natural/engineered tissue tropism. In this regard, a further viral vector that has advantage for use in the present invention is hepatitis virus. Hepatitis virus may be preferred when liver is the target tissue.

15

The viral vector typically comprises viral structural proteins and a nucleic acid payload which comprises the desired expression construct in a form functional to express the gene in the target cell or tissue. Thus the gene is typically operably linked to a promoter and other appropriate transcriptional regulatory signals.

20

In adenoviral vectors, the viral nucleic acid is typically a double stranded DNA (dsDNA) molecule. In retroviral vectors, it is typically single stranded RNA.

The viral nucleic acid typically contains further elements required for it to be packaged into the gene delivery vehicle and appropriately processed in the target cell or tissue.

25

For adenoviral vectors, these may include adenoviral inverted terminal repeat (ITR) sequences and an appropriate packaging signal.

30 For retroviral vectors, these include characteristic terminal sequences (so-called "R-U5" and "U3-R" sequences) and a packaging signal. The terminal sequences enable the generation of direct repeat sequences ("long terminal repeats" or "LTRs") at either end of the provirus which results from reverse transcription, which then facilitate integration of the provirus into the host cell genome and direct subsequent expression.

35 The viral nucleic acid may also contain a selectable marker, i.e. a gene encoding a product which allows ready detection of transduced cells. Examples include genes for fluorescent proteins (e.g. GFP), enzymes which produce a visible reaction product (e.g. beta-galactosidase, luciferase) and antibiotic resistance genes.

40 The viral vector is typically not replication-competent. That is to say, the viral nucleic acid delivered by the vector to the target cell does not contain all of the viral genes (and other genetic elements) necessary

for viral replication. The viral vector will nevertheless contain all of the structural proteins and enzyme activities required for introduction of the nucleic acid into the host cell and for appropriate processing such that the encoded agent can be expressed. Where these structural proteins are not encoded by the vector itself, they will typically be supplied by a packaging cell line. The skilled person will be well aware of
5 suitable cell lines which can be used to generate appropriate viral delivery vehicles.

Thus, for an adenoviral vector, the viral nucleic acid typically lacks one or more functional adenoviral genes from the E1, E2, E3 or E4 regions. These genes may be deleted or otherwise inactivated, e.g. by
10 insertion of a transcription unit comprising the heterologous gene or a selective marker.

In some embodiments, the nucleic acid contains no functional viral genes. Thus, for an adenoviral vector, the only viral components present may be the ITRs and packaging signal.

Nucleic acids having no functional viral genes may be preferred, as they reduce the risk of a host immune
15 response developing against the transduced target cell or tissue as a result of viral protein synthesis.

Viral vectors may be engineered so that they possess modified surface proteins capable of binding to markers on the target cell, thus increasing the chance that the desired target cell will be transduced and reducing the chance of non-specific transduction of other cell or tissue types. This approach is
20 sometimes referred to as pseudotyping.

Another gene transfer method suitable for use in humans is physical transfer of nucleic acid such as plasmid DNA in liposomes directly into cells in situ. Unlike viral vectors that must be propagated in cultured cells, plasmid DNA can be purified to homogeneity thereby reducing the potential for pathogenic
25 contamination. It may not be necessary for the exogenous DNA to stably integrate into the transduced cell. Liposome-mediated DNA transfer has been described by various investigators (Wang and Huang, Biochem. Biophys. Res. Commun. 147:980 (1987); Wang and Huang, Biochemistry 28:9508 (1989); Litzinger and Huang, Biochem. Biophys. Acta 1113:201 (1992); Gao and Huang, Biochem. Biophys. Res. Commun. 179:280 (1991); Felgner, WO 91/17424; WO 91/16024). Liposomal compositions, however,
30 may not possess the specificity necessary to deliver the exogenous DNA to all target cell types and non-physiological pH conditions may be necessary to effect fusion.

Immunoliposomes have also been described as carriers of exogenous polynucleotides (Wang and Huang, Proc. Natl. Acad. Sci. USA 84:7851 (1987); Trubetskoy et al, Biochem. Biophys. Acta 1131:311
35 (1992)). Immunoliposomes can be expected to have improved cell type specificity as compared to liposomes due to the inclusion of specific antibodies that bind to surface antigens on target cell types. Since antibodies can be cross-reactive and bind to a variety of proteins bearing cross-reactive epitopes, it may be disadvantageous to use antibodies raised against a cell surface antigen that is a member of a conserved gene family or that contains a conserved sequence present in many other cell surface
40 proteins. Further, immunoglobulins that bind cell surface proteins may be inefficiently endocytosed and/or may cause premature disruption of the immunoliposome upon binding antigen thereby releasing the

exogenous DNA from the immunoliposome prior to fusion (Ho and Huang, J. Immunol. 134:4035 (1985)). In addition, immunoliposome-DNA preparations can be relatively inefficient for transfection.

Behr et al, Proc. Natl. Acad. Sci. USA 86:6982 (1989) reported using lipopolyamine as a reagent to
5 mediate transfection, without the necessity of any additional phospholipid to form liposomes. Lipopolyamines, however, do not impart a predetermined targeting specificity to the exogenous DNA; cells can be transfected indiscriminately.

Low molecular weight polylysine ("PL") and other polycations are carriers that can be used to effect DNA-
10 mediated transfection into cells. Zhou et al, Biochem. Biophys. Acta 1065:8 (1991) have reported synthesis of a polylysine-phospholipid conjugate, a lipopolylysine comprising PL linked to N-glutarylphosphatidylethanolamine, which reportedly increases the transfection efficiency of DNA as compared to lipofectin, a commercially used transfection reagent. A lipopolylysine may not, however, provide satisfactory cell type specificity and transformation efficiency.

15 Liposome mediated transfection is highly efficient and generally not cell type specific, and lipid:DNA complexes rapidly associate with cells of the reticuloendothelial system (Mannino and Gould-Fogerite, BioTech 6:682 (1988)). Receptor-mediated transfection should allow any size DNA or RNA to be transfected, however, efficiency can be affected by lysosomal degradation of nucleic acid. Accordingly,
20 the use of inhibitors of lysosomal degradation (lysosomotropic agents), which are usually administered essentially contemporaneously, may be advantageous. Cytotoxicity of many of these agents (like chloroquine) may, however, limit the universal employment of receptor mediated transfection (Dean et al, Biochem. J. 217:27 (1984)).

25 Essentially, any suitable nucleic acid delivery method can be used in the context of the present invention, although direct physical application of naked DNA comprising the expression construct/transgene to the target-cell population may be preferred.

30 The nucleic acid-containing compositions of the invention can be stored and administered in a sterile physiologically acceptable carrier, where the nucleic acid is dispersed in conjunction with any agents which aid in the introduction of the nucleic acid (e.g. DNA) into cells.

Various sterile solutions may be used for administration of the composition, including water, PBS, ethanol, lipids, etc. The concentration of the nucleic acid will be sufficient to provide a therapeutic dose,
35 which will depend on the efficiency of transport into the cells.

Actual delivery of the gene sequence, formulated as described above, can be carried out by a variety of techniques including direct injection, instillation of lung and other epithelial surfaces, intravenous injection and other physical methods (including microprojectiles to target visible and accessible regions of tissue
40 (e.g. with naked DNA, lithotriptic shock can be used to focus on specific body regions and render them

receptive to DNA uptake). Administration may be by syringe needle, trocar, cannula, catheter, etc, as a bolus, a plurality of doses or extended infusion, etc.

Naked DNA has been shown to be taken up relatively efficiently in certain tissues (especially muscle).

- 5 Direct injection of viruses or liposomes can thus be used alone or in combination with systemic delivery.

- 10 Whatever the route of administration, the nucleic acid typically comprises an expression construct, comprising a nucleic acid sequence encoding the agent to be expressed, operably linked to appropriate regulatory sequences to direct expression. The regulatory sequences may be selected depending on the target cell, but will typically include an appropriate promoter and optionally an enhancer which direct transcription by RNA polymerase II, as well as a transcriptional terminator (normally including a polyadenylation signal).

- 15 The promoter may be a tissue-specific promoter, which drives transcription preferentially or exclusively in the target cell or tissue as compared to other cell or tissue types.

Pharmaceutical compositions

The active agents, nucleic acids, vectors and host cells described herein can be formulated in pharmaceutical compositions.

- 20 Pharmaceutical compositions may comprise, in addition to one of the above substances, a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other material well known to those skilled in the art. Such substances should be non-toxic and should not interfere with the efficacy of the active ingredient. In some embodiments, the composition further comprises a pharmaceutically acceptable excipient.

Various sterile solutions may be used for administration of the composition, including water, PBS, ethanol, lipids, etc. The concentration of the agent will be sufficient to provide a therapeutic dose, which will depend on the efficiency of transport into the cells.

- 30 Pharmaceutical compositions may be prepared using a pharmaceutically acceptable "carrier" composed of materials that are considered safe and effective. The term "carrier" refers to diluents, binders, lubricants and disintegrants. Those with skill in the art are familiar with such pharmaceutical carriers and methods of compounding pharmaceutical compositions using such carriers. "Pharmaceutically acceptable" refers to molecular entities and compositions that are "generally regarded as safe", e.g., that are physiologically tolerable and do not typically produce an allergic or similar untoward reaction, such as gastric upset and the like, when administered to a human. In some embodiments, this term refers to molecular entities and compositions approved by a regulatory agency of the US federal or a state government, as the GRAS list under section 204(s) and 409 of the Federal Food, Drug and Cosmetic Act,

that is subject to premarket review and approval by the FDA or similar lists, the U.S. Pharmacopeia or another generally recognised pharmacopeia for use in animals, and more particularly in humans.

- The pharmaceutical compositions provided herein may include one or more excipients, e.g., solvents, solubility enhancers, suspending agents, buffering agents, isotonicity agents, antioxidants or antimicrobial preservatives. When used, the excipients of the compositions will not adversely affect the stability, bioavailability, safety, and/or efficacy of the active ingredients. Thus, the skilled person will appreciate that compositions are provided wherein there is no incompatibility between any of the components of the dosage form. Excipients may be selected from the group consisting of buffering agents, solubilizing agents, tonicity agents, chelating agents, antioxidants, antimicrobial agents, and preservatives.

Examples

MATERIALS AND METHODS

15 Production of TRX-45 fusion protein

A fusion protein was constructed containing (from N- to C-terminus) the dIII domain of human CCN3, a linker peptide EAAAK, and the mature sequence of murine serum albumin. The endogenous isoleucine at the second residue of the CCN3 TSP-1 domain was substituted with alanine.

- 20 A signal peptide from human serum albumin was used.

The protein was expressed in mammalian cells and purified as described in more detail below. The mature protein is designated TRX-45.

- 25 DNA sequence encoding the signal peptide of human serum albumin (amino acids 1-18 of UniProt P02768 (release no. 2023_03/2023_03, 28 June 2023)) was fused in-frame to a DNA sequence encoding (i) CCN3 domain III (amino acids 206-249 of human CCN3; UniProt P48745 (release no. 2023_03/2023_03, 28 June 2023)) with the substitution A207I; (ii) a peptide linker sequence EAAAK; and (iii) mature mouse serum albumin (amino acids 25 – 608 of UniProt P07724 (release no. 30 2023_03/2023_03, 28 June 2023)) was constructed.

- The DNA sequence encoding the fusion protein was codon optimized for protein expression in hamster cells (by the algorithm of the commercial supplier), a KOZAK sequence for efficient translation was appended at the 5' end, a STOP-codon was introduced at the 3' end (SEQ ID NO.: 12) and the sequence 35 was synthesized in the form of an Entry vector compatible with Gateway cloning and sequence verified by the manufacturer (ThermoFisherScientific). The Entry vector was further recombined with a destination vector using LR gateway recombinase. The destination vector used was pUCOE-DHFR-DEST, as described by Kaasbøll et al., (J. Biol. Chem., 293(46):17953-17970, 2018) to generate an expression vector.

The nucleotide sequence encoding the fusion protein, with Kozak sequence and stop codon was as follows:

GCCACCATGAAATGGGTACCTTTATCTCCCTGCTGTTCTCTCCGCTACTCTTGTGCCGAGC
5 AGACCACAGAGTGGACCGCTGCTCTAAGTCTTGCGCATGGGCTTCTCCACCAGAGTGACCAACCGGAA
CAGACAGTGCAGATGCTGAAGCAGACCCGGCTGTGTATGGTTCGACCTTGCGAGGCCGCTGCCAAAGAG
GCTCACAAGTCTGAGATCGCCACCGGTACAACGATCTGGGCGAGCAGCACTTCAAAGCCTGGTGTCTGA
TCGCCTTCAGCCAGTACCTGCAGAAGTGCTCCTACGACGAGCAGCCAAAGCTGGTGCAAGAAGTGACCGA
CTTCGCCAAGACCTGCGTGGCCGATGAGTCTGCCGCCAACTGCGATAAGTCTCTGCACACCCTGTTTCGGC
10 GACAAGCTGTGCGCTATCCCCAACCTGAGAGAGAACTACGGCGAGCTGGCCGACTGCTGCACAAAGCAAG
AGCCCGAGAGAAAAGAGTGCTTCTGCAGCACAAGGACGACAACCCAGCCTGCCTCCATTGAGAGGCC
TGAGGCTGAGGCCATGTGTACCAGCTTCAAAGAGAACCCACCACCTTCATGGGCCACTACCTGCATGAG
GTGGCCAGACGGCACCCCTACTTTTATGCCCCGTGAGTGTCTGTACTACGCCGAGCAGTACAACGAGATCC
TGACACAGTGTGCGCCGAGGCCGACAAAGAGTCTTGTCTGACCCCTAAGCTGGACGGCGTGAAAGAAAA
15 GGCCCTGGTGTCTATCCGTGCGGCAGAGAATGAAGTGACGCTCCATGCAGAAGTTCGGCGAGAGAGCCTTT
AAGGCCTGGGCCGTGCTAGACTGTCCCAGACCTTTCCTAACGCCGATTTCGCCGAGATCACCAAGCTGG
CCACCGACCTGACCAAAGTGAACAAAGAGTGCTGCCACGGCGACCTGTGGAATGCGCTGATGATAGAGC
CGAGCTGGCTAAGTACATGTGCGAGAACCAGGCCACCATCTCCTCCAAGCTGCAGACCTGCTGTGACAAG
CCCCGTGCTGAAGAAGGCCCACTGCCTGTCTGAGGTGGAACACGATACCATGCCTGCCGACCTGCCTGCTA
20 TCGCCGCTGACTTCGTGGAAGATCAAGAAGTGTGCAAGAACTACGCAGAGGCCAAGGACGTGTTCTGGG
CACCTTCTGTACGAGTACTCTCGGAGACACCCCGACTACTCCGTGTCTCTGCTGCTGAGACTGGCCAAG
AAGTACGAGGCTACCCTGGAAGAGTGCTGTGCTGAGGCTAACCTCCTGCCTGTTATGGCACTGTGCTGG
CCGAGTTTCAGCCCCCTGGTGAAGAACCCAAGAACCTGGTCAAGACCAACTGTGATCTGTACGAGAAGCT
GGGCGAGTACGGCTTCCAGAACGCTATCCTCGTGCGGTACACCCAGAAAGCCCCCTCAGGTGTCCACACCT
25 ACACTGGTCGAGGCCGCCAGAAATCTGGGCAGAGTGGGCACCAAGTGTGCACCTTGCCTGAGGATCAGA
GACTGCCTTGTGTGGAAGATTACCTGTCCGCCATCCTGAACAGAGTGTGCCTGCTGCACGAAAAGACCCC
TGTGTCTGAGCACGTGACCAAGTGTGCTCCGGCTCTCTGGTCGAGAGAAGGCCTTGTTTCTCTGCCCTG
ACCGTGACGAGACATACGTGCCCCAAAGAGTTCAAGGCCGAGACATTACCTTCCACTCCGACATCTGTA
CCCTGCCTGAGAAAGAGAAGCAGATTAAGAAGCAGACAGCCCTGGCTGAGCTGGTCAAGCACAAAGCCTAA
30 GGCTACCGCTGAGCAGCTGAAAACCGTGATGGATGACTTCGCCCAGTTCCTGGATACCTGCTGCAAGGCC
GCCGACAAGGACACCTGTTTCTCTACCGAGGGACCCAACCTCGTGACCAGATGCAAGGACGCCCTGGCTT
AA (SEQ ID NO: 43)

The sequence of the fusion protein, including signal sequence, is as follows:

35 MKWVTFISLLFLFSSAYS**CAEQ**TTTEWTACSKSCGMGFSTRVTNRRNQCEMLKQTRL~~CMVRPCEAAAKEAH~~
KSEIAHRYNDLGEQHFGLVLIAFSQYLQKCSYDEHAKLVQEVTDFAKTCVADESAANDKSLHTLFGDK
LCAIPNLRENYGELADCCTKQEPERNECF LQHKDDNPSLPFERPEAEAMCTSFKENPTTFMGHYLHEVA
RRHPYFYAPELLYYAEQYNEILTQCCAEADKESCLTPKLDGVKEKALVSSVRQRMKCSSMQKFEGERAFKA
WAVARLSQTFPNADFAEITKLATDLTKVNKECCHGDLLECADDRAELAKYMCENQATISSKLQTCCKPL

LKKAHCLSEVEHDTMPADLPAIAADFVEDQEVCKNYAEAKDVFLGTFLYEYSRRHPDYSVSLLLRLAKKY
EATLEKCCAEANPPACYGTVLAEFQPLVEEPKNLVKTNCDLYEKLGEYGFQNAILVRYTQKAPQVSTPTL
VEAARNLGRVGTKCCTLPEDQRLPCVEDYLSAILNRVCLLHEKTPVSEHVTKCCSGSLVERRPCFSALTV
DETYVPKEFKAETFTFHSDICTLPEKEKQIKQTALAEVLVHKPKATAEQLKTVMDDFQAQFLDTCCKAAD

5 KDTCFSTEGPNLVTRCKDALA (SEQ ID NO: 44)

[CCN3 dIII-derived sequence underlined; L to A substitution bold; linker peptide in italics; mouse serum albumin double underlined]

Following transfection of competent *E. coli*, mutated to allow for efficient propagation of plasmids (One Shot Top10™ cells), the expression vector was isolated with standard plasmid isolation techniques using a QIAGEN™ Plasmid Plus Maxi Kit. The resulting expression vector was verified by standard restriction enzyme digestion and DNA gel electrophoresis according to standard techniques well known to the skilled person. The resulting expression vector was then transferred into ExpiCHO suspension culture adapted CHO cells according to the "Creation and Scale up of a Stable Cell Line Using ExpiCHO™ Products" protocol supplied by the manufacturer of the ExpiCHO™ Stable Production Medium (Gibco Cat.#: A3711001). The cells were maintained in vented Erlenmeyer flasks in cell culture incubators kept at 37°C with 8% CO₂ on a shaker platform (as described in Kaasbøll et al., supra). The transfected cells were kept overnight in ExpiCHO™ Expression medium before being transferred to ExpiCHO™ expression medium supplemented with 0.1µM methotrexate. The cells were then sub-cultured until the viability again approached 80%, at which point the medium was supplemented with 1µM methotrexate. The cells were again sub-cultured until the viability exceeded 30% at which point the cells were transferred to ExpiCHO™ Stable Production Medium supplemented with 1µM methotrexate and further sub-cultured until the viability exceeded 95% and the doubling-time decreased to less than 20 hours, at which point the cell pool was considered stably transfected.

25 Once the stable cell pool was established the cell culture volume was expanded to allow for the seeding of stably transfected cells for production at a density of 1*10⁶ cells/mL, which was supplemented daily with 5% (v/v of the starting volume) with 2X EfficientFeed™ C+ supplement. The cell culture was harvested 10 days after seeding by centrifugation at 4000g.

30 The expressed fusion protein, designated TRX-45, was subsequently purified from the harvested cell culture supernatant by affinity (Albupure™, Astrea Bioseparations, UK) and size exclusion chromatographic techniques.

35 Briefly, the harvested cell culture supernatant was treated aseptically and pH adjusted to 5.7 with NaH₂PO₄ before sterile filtration and loading onto a column packed with Albupure™ (Astrea bioseparations, UK) chromatography media and pre-equilibrated with buffer A (100mM Na_{1/2}H_{1/2}PO₄, 100mM L-Arginine, pH 5.7) connected to a FPLC chromatography system (BioRad NGC). The column was washed with buffer A and eluted with buffer B (1M NaCl, 50mM Na_{1/2}H_{1/2}PO₄, 100mM L-Arginine, pH 40 7.5). Fractions were analysed by RP-HPLC and fractions containing the CCN3 dIII comprising fusion

proteins were pooled to generate the sample for the size exclusion step. The pooled fractions were loaded onto a HiScale 26-40 Superdex200Inc size exclusion column (GE HealthCare) pre-equilibrated with buffer A2 (100mM Na_{1/2}H_{1/2}PO₄, 100mM L-arginine, pH 6.5), the column was eluted with buffer A2, the eluted fractions were analysed by RP-HPLC, the main peak on the chromatogram was confirmed to contain the CCN3 dIII comprising fusion protein and thereafter pooled, sterile-filtered and analyzed for endotoxin contents (ThermoFisherScientific, Catalog number: A39552).

The concentration of the purified TRX-45 fusion protein was determined with a micro BCA™ protein assay kit (ThermoFisherScientific) according to manufacturer's instructions.

10

Effect of TRX-45 fusion protein in an obese, diabetic mouse model

Female db/db (BKS.Cg-Dock7m +/- Leprdb/J, Charles River, Italy) mice at 5-6 weeks of age were group housed with free access to tap water and regular chow diet (Altromin 1324 from Brogaarden, Hørsholm, Denmark) for 2 weeks prior to single-dose administration (intravenous) of a bolus of a renin-encoding adeno-associated virus (reninAAV, 3¹⁰ genome copies per animal) (Vector Biolabs, Malvern, PA) for induction of hypertension as described in Harlan et al., 2015 and Østergaard et al., 2021. One week later, uni-nephrectomy was performed in all animals and after 3 weeks of recovery, animals were stratified according to body weight and non-fasting blood glucose and randomized into 4 treatment groups. Drug treatment was initiated 1 week later at 13-14 weeks of age.

20

Administration schedule of TRX-45 fusion protein

Chronic treatment with TRX-45 was initiated on study day 1. Either TRX-45 or vehicle were administered intraperitoneal every other day for 12 weeks. TRX-45 was administered at 3 doses (0.5, 3.0, and 20 mg/kg), while a control group received vehicle.

25

Assessments of body weight and subcutaneous white adipose tissue

During the drug treatment phase, body weight was recorded once daily. After 12 weeks, animals were euthanized by cardiac puncture and cervical dislocation under isoflurane anesthesia. After opening the abdomen, inguinal white adipose tissue was identified, dissected, and weighed.

30

Assessments of glycated hemoglobin A1c (HbA1c)

On the day of termination (week 12) tail vein blood samples were collected into heparinized glass capillary tubes and immediately suspended in Hemolyzing Reagent (Roche Diagnostics) and stored at -70°C until analysis. HbA1c was measured using commercial kits (Roche Diagnostics,) on the Cobas c501 autoanalyzer.

35

Data analysis

For HbA1c, terminal body and inguinal WAT weights, the vehicle and TRX-45 (20 mg/kg) groups were compared with t-test. P<0.05 was considered significant.

40

Effect of TRX-45 fusion protein in a mouse model of polycystic kidney disease

Female and male *Pkd1*^{RCRC} 129s6 mice were from the animal facility of the Mayo Clinic (Rochester, MN, USA) (Arroyo et al., 2021). When mice reached ~3 weeks of age, a baseline magnetic resonance imaging (MRI) scan was conducted using a Bruker Advance 700 Mhz vertical bore nuclear magnetic resonance spectrometer, to assess baseline disease severity as measured by total kidney volume. Subsequently, mice were stratified based on total kidney volume and randomized into 4 treatment groups. Drug treatment was initiated ~2 week later. For the duration of the study, animals were housed in groups with free access to drinking water and milled chow diet (LabDiet 0006939, St. Louis, MO, USA).

10 Administration schedule of CCN3 dIII comprising fusion protein

Chronic drug treatment with TRX-45 fusion protein was initiated on study day 1. Either TRX-45 or vehicle were administered intraperitoneally every other day for 12 weeks, while Tolvaptan was administered mixed into powdered chow (LabDiet 0006939, St. Louis, MO, USA). The Tolvaptan-treated group was also dosed with vehicle intraperitoneally every other day. TRX-45 was administered at 2 doses (2.0 and 20 mg/kg), while Tolvaptan was administered at 0.2% w/w in chow.

Assessments of body weight adjusted kidney growth by MRI

During the drug treatment phase, body weight was recorded every other day during the first 4 weeks of the study and after that every 4th day until study termination. After 11 weeks of drug treatment, a second MRI scan was conducted to assess total kidney volume and subsequently calculate body weight adjusted total kidney volume growth from baseline to week 11.

Data analysis

For single-timepoint continuous data, t-test was used to compare individual treatment groups to the Vehicle control within each sex. P<0.05 was considered significant.

EXAMPLE 1: Effect of TRX-45 fusion protein on body weight and white adipose tissue

After 12 weeks of drug treatment, TRX-45 demonstrated dose-dependent attenuation of absolute and relative body weight in obese mice (TRX-45 (20 mg/kg) vs. Vehicle: p=0.0526 and p=0.0109) (Figure 1, (A) and (B) respectively). Similarly, after 12 weeks of drug dosing, TRX-45 dose-dependently reduced the weight of the inguinal white adipose tissue (WAT) in obese mice (TRX-45 (20 mg/kg) vs. Vehicle: p=0.0287) (Figure 2).

EXAMPLE 2: Effect of TRX-45 fusion protein on cyst growth in polycystic kidney disease

In vivo magnetic resonance imaging (MRI)-based measurements of kidney volume growth corrected for body weight growth from baseline until 11 weeks of drug treatment demonstrated that TRX-45 reduced kidney growth in male mice with polycystic kidney disease (TRX-45, 20 mg/kg vs. Vehicle: *p=0.0415) (Figure 3).

EXAMPLE 3: Effect of TRX-45 fusion protein on glycated hemoglobin A1c (HbA1c)

Measurement of HbA1c after 12 weeks of drug treatment with TRX-45 demonstrated a significant reduction in HbA1c. (TRX-45 (20 mg/kg) vs. Vehicle: $p=0.0427$) (Figure 4). HbA1c reflects a “weighted” average blood glucose during the red blood cell lifespan (American Diabetes Association Professional Practice Committee, Diabetes Care, 2025), which is reported to be in the range of 50-60 days in mice (Goodman and Smit, Am J Phys, 1961). Therefore, reduced HbA1c levels at termination in mice administered TRX-45 reflect reduced blood glucose exposure over a prolonged time span prior to the termination time point.

References

A number of publications are cited above in order to more fully describe and disclose the invention and the state of the art to which the invention pertains. Full citations for these references are provided below. The entirety of each of these references is incorporated herein.

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For standard molecular biology techniques, see Sambrook, J., Russel, D.W. *Molecular Cloning, A Laboratory Manual*. 3 ed. 2001, Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press

The features disclosed in the foregoing description, or in the following claims, or in the accompanying drawings, expressed in their specific forms or in terms of a means for performing the disclosed function, or a method or process for obtaining the disclosed results, as appropriate, may, separately, or in any combination of such features, be utilised for realising the invention in diverse forms thereof.

- 5 While the invention has been described in conjunction with the exemplary embodiments described above, many equivalent modifications and variations will be apparent to those skilled in the art when given this disclosure. Accordingly, the exemplary embodiments of the invention set forth above are considered to be illustrative and not limiting. Various changes to the described embodiments may be made without departing from the spirit and scope of the invention.

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For the avoidance of any doubt, any theoretical explanations provided herein are provided for the purposes of improving the understanding of a reader. The inventors do not wish to be bound by any of these theoretical explanations.

- 15 Any section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

Throughout this specification, including the claims which follow, unless the context requires otherwise, the word "comprise" and "include", and variations such as "comprises", "comprising", and "including" will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

20

It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Ranges may be expressed herein as from "about" one particular value, and/or to "about" another particular value. When such a range is expressed, another embodiment includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by the use of the antecedent "about," it will be understood that the particular value forms another embodiment. The term "about" in relation to a numerical value is optional and means for example +/- 10%.

25

For standard molecular biology techniques, see Sambrook, J., Russel, D.W. *Molecular Cloning, A Laboratory Manual*. 3 ed. 2001, Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press

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Claims

1. An active agent for use in the prophylaxis or treatment of obesity, wherein the active agent comprises a CCN dIII domain sequence or a functionally active fragment thereof, and lacks a CCN dI domain sequence, a CCN dII domain sequence and a CCN dIV domain sequence.
- 5
2. An active agent for use in the prophylaxis or treatment of a condition associated with insulin resistance or glucose intolerance in a subject, wherein the subject is obese, and wherein the active agent comprises a CCN dIII domain sequence or a functionally active fragment thereof, and lacks a CCN dI domain sequence, a CCN dII domain sequence and a CCN dIV domain sequence.
- 10
3. An active agent for use according to claim 2, wherein the condition is selected from diabetes, pre-diabetes, insulin resistance syndrome, impaired glucose tolerance (IGT), metabolic syndrome, hyperglycemia, diabetic heart disease.
- 15
4. An active agent for use in prophylaxis or treatment of a condition associated with or caused by obesity or excess body weight, wherein the active agent comprises a CCN dIII domain sequence or a functionally active fragment thereof, and lacks a CCN dI domain sequence, a CCN dII domain sequence and a CCN dIV domain sequence.
- 20
5. An active agent for use according to claim 4 wherein the condition is selected from obesity-linked inflammation, obesity-linked gallbladder disease, diabetes, pre-diabetes, insulin resistance syndrome, impaired glucose tolerance (IGT), metabolic syndrome, hyperglycemia, diabetic heart disease, hypertension, cardiovascular disease, heart failure, myocardial infarction, coronary heart disease, stroke, atherogenic dyslipidemia, hepatic steatosis, kidney disease, kidney failure, arteriosclerosis, osteoarthritis,
- 25
- gout, reproductive disorders and respiratory disorders.
6. An active agent for use in the treatment of polycystic kidney disease (PKD), wherein the active agent comprises a CCN dIII domain sequence or a functionally active fragment thereof, and lacks a CCN dI domain sequence, a CCN dII domain sequence and a CCN dIV domain sequence.
- 30
7. An active agent for use according to claim 6, wherein the polycystic kidney disease is autosomal dominant polycystic kidney disease (ADPKD) or autosomal recessive polycystic kidney disease (ARPKD).

8. An active agent for use according to any of the preceding claims wherein the CCN dIII domain comprises or consists of the sequence:

- 5 CIVQTTWSQCSKTCGTGISTRVTNDNPECRLVKETRICEVRPC (SEQ ID NO: 1, from CCN1);
 CLVQTTEWSACSKTCGMGISTRVTNDNASCRLKQSRCLCMVRPC (SEQ ID NO: 2, from CCN2);
 CIEQTTEWTACSKSCGMGFSTRVTNRNRQCEMLKQTRLCMVRPC (SEQ ID NO: 3, from CCN3);
 CIAYTSPWSPCSTSCGLGVSTRISNVNAQCWPEQESRLCNLRPC (SEQ ID NO: 4, from CCN4);
 CPEWSTAWGPCSTTCGLGMATRVSNQNRFCRLETQRRCLCLSRPC (SEQ ID NO: 5, from CCN5); or
 CLVQATKWTPCSRTC GMGISNRVTNENSNCCEMRKEKRLCYIQPC (SEQ ID NO: 6 from CCN6);
 10 or has at least 70% identity to one of those sequences.

9. An active agent for use according to any one of the preceding claims, wherein the CCN dIII domain comprises or consists of the sequence:

- 15 CIVQTTWSQCSKTCGTGISTRVTNDNPECRLVKETRICEVRPCGQPVSLLKKGKK (SEQ ID NO: 7, from CCN1);
 CLVQTTEWSACSKTCGMGISTRVTNDNASCRLKQSRCLCMVRPCEADLEENIKKGKK (SEQ ID NO: 8, from CCN2);
 20 CIEQTTEWTACSKSCGMGFSTRVTNRNRQCEMLKQTRLCMVRPCEQEPEQPTDKKGKK (SEQ ID NO: 9, from CCN3);
 CIAYTSPWSPCSTSCGLGVSTRISNVNAQCWPEQESRLCNLRPCDVIHTLIKAGKK (SEQ ID NO: 10, from CCN4);
 CPEWSTAWGPCSTTCGLGMATRVSNQNRFCRLETQRRCLCLSRPCPPSRGRSPQNSAF (SEQ ID NO:
 25 11, from CCN5); or
 CLVQATKWTPCSRTC GMGISNRVTNENSNCCEMRKEKRLCYIQPCDSNLIKTIKIPKGKT (SEQ ID NO: 12, from CCN6);

or has at least 70% identity to one of those sequences.

30

10. An active agent for use according to claim 8 or claim 9 wherein the 6 cysteine residues are conserved.

11. An active agent for use according to claim 10 wherein the spacing between the 6 cysteine
 35 residues is conserved.

12. An active agent for use according to any one of claims 8 to 11 wherein the residue immediately following the first cysteine residue is not I, L or P.

13. An active agent for use according to claim 12 wherein the residue immediately following the first
5 cysteine residue is alanine or Aib.

14. An active agent for use according to claim 13 wherein the CCN dIII domain comprises or consists of the sequence:

CAEQTTTEWTACSKSCGMGFSTRVTNRNRQCEMLKQTRLCMVRPC (SEQ ID NO: 13); or

10 CAEQTTTEWTACSKSCGMGFSTRVTNRNRQCEMLKQTRLCMVRPCEQEPEQPTDKKGKK (SEQ ID NO: 14).

15. An active agent for use according to any one of the preceding claims wherein the active comprises a heterologous moiety.

15

16. An active agent for use according to claim 15 wherein the heterologous moiety increases the stability of the active agent, e.g. the heterologous moiety increases the serum half-life of the active agent as compared to a similar agent in the absence of the heterologous moiety.

20 17. An active agent for use according to claim 16 wherein the heterologous moiety is an Fc fragment, serum albumin, fibrinogen, glutathione S-transferase, transferrin or streptavidin.

18. An active agent for use according to claim 17 wherein the heterologous moiety comprises or consists of amino acids 25-609 of human serum albumin (HSA) having the sequence:

25

DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVADES
NCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLRLVRPEV
DVMCTAFHDNEETFLKKYLYEIAARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAACLLP
KLDELRLDEGKASSAKQRLKCASLQKFGERAFAKAWAVARLSQRFPKAEFAEVSKLVTDLT
30 VHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLEKSHCIAEVENDEMPA
DLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLRLAKTYETTLEKC
CAAADPHECYAKVFDEFKPLVEEPQNLIKQNCLEFELGQYKFNALLVRYTKKVPQVST
PTLVEVSRNLGKVGSKCKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTES
LVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKAT
35 KEQLKAVMDDFAAFVEKCKADDKETCFAEEGKKLVAASQAALGL (SEQ ID NO: 15)

or has at least 70% identity thereto.

19. An active agent for use according to any one of claims 15 to 18, further comprising a linker between the CCN dIII domain sequence or fragment thereof and the heterologous moiety.

5 20. An active agent for use according to claim 19 wherein the is a peptide linker which comprises or consists of the sequence EAAAK.

21. An active agent for use according to any one of the preceding claims wherein the active agent has the mature sequence:

10 CAEQTTTEWTACSKSCGMGFSTRVTNRNRQCEMLKQTRLCMVRPCEAAAKDAHKSEVAHREFKDLGEENFKA
LVLIIFAQYLYQQCPFEDHVKLVNEVTEFAKTCVADESAENCCKSLHTLFGDKLCTVATLRETYGEMADCC
AKQEPERNECFLOHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELFFAKRY
KAAFTTECCQAADKAACLLPKLDELRLDEGKASSAKQRLKASLQKFGERAFAKAWAVARLSQRFPAEFAEV
SKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLEKSHCIAEVENDEMPAD
15 LPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLLRLLAKTYETTLEKCCAAADPHECYA
KVFDEFKPLVEEPQNLIKQNCLEFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCKHP
EAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHA
DICTLSEKERQIKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCKADDKETCFAEEGKKLVAASQA
ALGL (SEQ ID NO: 16).

20

22. Use of an active agent in inhibiting weight gain or promoting weight loss, wherein the active agent comprises a CCN dIII domain sequence or a functionally active fragment thereof, and lacks a CCN dI domain sequence, a CCN dII domain sequence and a CCN dIV domain sequence.