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Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

(88) Date of publication of the international search report:
17 March 2016

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(54) Title: RECOMBINANT COLLAGEN IV SURROGATES AND USES THEREOF

(57) Abstract: The disclosure describes compositions that mimic certain structural and functional characteristics of collagen IV. Additionally provided are methods for the recombinant production of said compositions and particular methods of use.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 15/42687

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C07K 14/78; A61K 38/39 (2015.01)
CPC - C07K 14/78; A61L 27/24
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - C07K 14/78; A61K 38/39 (2015.01)
CPC - C07K 14/78; A61L 27/24

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC: C07K 14/78; A61L 27/24 (text search)
USPC: :530/356, 350; 514/801 (text search)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Electronic data bases: PatBase; Google Patents; Google Scholar; GenCore sequence search (AA)
Search terms: Collagen IV, structural and functional mimics, (non-collagenous) NC1 domain with arginine at residue 76, cysteine-rich sequence between NC1 domain and collagenous domain, formulation, heterotrimeric complex assembly as a function of halide co

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y ---- A	KOBAYASHI et al. Characterization of assembly of recombinant type IV collagen alpha3, alpha4, and alpha5 chains in transfected cell strains. Kidney Int December 2003 Vol 64 No 6 Pages 1986-1996. Especially pg 1986 col 2 para 2, pg 1998 col 2 para 2, pg 1990 figs 2 and 4, pg 1990 col 1 para 1.	1-3, 5 --------- 4
Y ---- A	BOUTAUD et al. Type IV collagen of the glomerular basement membrane. Evidence that the chain specificity of network assembly is encoded by the noncollagenous NC1 domains. J. Biol Chem. 29 September 2000 Vol 275 Vol 39 Pages 30716-30724. Especially pg 70717 col 1 para 1, pg 70717 col 2 para 5, pg 70717 fig 1 lower panel.	1-3, 5 --------- 4
Y	THAN et al. The 1.9-Angstrom crystal structure of the noncollagenous (NC1) domain of human placenta collagen IV shows stabilization via a novel type of covalent Met-Lys cross-link. Proc Nat Acad Sci 14 May 2002 Vol 99 No 10 Pages 6607-6612. Especially pg 6611 col 1 para 3.	2
A	UNIPROT W8YEY2. Uncharacterized Protein. [online] 14 May 2014 [retrieved 27 November 2015]. Available on the internet:<URL: http://www.uniprot.org/uniprot/W8YEY2.txt?version=1>. Especially pg 1.	4

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 November 2015 (27.11.2015)	19 JAN 2016
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

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Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

GenCore ver 6.4.1 SEQ ID NO: 1 was specifically examined as part of the International Search and Opinion.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 15/42687

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
-------Go to Extra Sheet for continuation-------

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Claims 1-5 limited to NC1 lacking arginine at position 76 and having a cysteine rich sequence, SEQ ID NO: 1 (Claims 1-5)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 15/42687

-----continuation of Box III (Lack of Unity of Invention)------

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I+: Claims 1-5, drawn to a composition comprising three recombinant proteins wherein each recombinant protein contains a C-terminal NC1 domain and a collagenous domain. The heterotrimeric complex will be searched to the extent that the NC1 domains do not contain an arginine residue at position 76 (claim 2), and does contain the cysteine-rich sequence of SEQ ID NO: 1 (claim 4). It is believed that claims 1-5 read on this first named invention and thus these claims will be searched without fee to the extent that they encompass a lack of arginine at position 76 in the NC1 domain and a cysteine-rich domain defined as SEQ ID NO: 1. Additional polypeptide compositions will be searched upon payment of additional fees. Applicant must specify the claims that encompass any additional elected amino acid residue(s) and/or cysteine-rich sequence(s). Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be: lack of a valine residue at position 27, and contains the cysteine rich sequence SEQ ID NO: 4 (claims 1-5).

Group II: Claims 6-13, drawn to a method involving administering the composition of claim 1 (claim 1 is generic to Groups I+ and II).

Group III: Claim 14-18, drawn to a method of administering an antibody that disrupts basement membrane function by binding to collagen IV NC1 domain.

The inventions listed as Groups I+, II and III do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features:

Group I+ has the special technical feature of expressing each of the three recombinant proteins separately in a mammalian cell line and assembling them into a heterotrimeric complex in vitro, not required by Groups II or III.

Group II has the special technical feature of a method involving administering a heterotrimeric complex similar to protomeric collagen IV to treat a disease, not required by Groups I+ or III.

Group III has the special technical feature of an antibody that binds to the NC1 domain of collagen IV, not required by Groups I+ or II.

Among the inventions listed as Groups I+ are the specific lack of amino acid residues in the NC1 domain recited therein. The inventions do not share a special technical feature, because the structural-activity relationship of each NC1 domain having specific amino acid deletion can not readily be ascertained among NC1 variants.

Among the inventions listed as Groups I+ are the specific cysteine-rich sequences recited therein. The inventions do not share a special technical feature, because no significant structural similarities can readily be ascertained among sequences.

Common Technical Features

1.Groups I+, II-III share the common technical feature of the NC1 domain of collagen IV.

2. Groups II-III share the common technical feature of treating a disorder related to collagen IV NC1 domains.

3. Claim 1 is generic to Groups I+ and II.

However, said common technical feature does not represent a contribution over the prior art, and is obvious over US 6,277,558 B1 (HUDSON), in view of the publication titled "Characterization of assembly of recombinant type IV collagen alpha3, alpha4, and alpha5 chains in transfected cell strains" by Kobayashi et al. (hereinafter "Kobayashi") [Kidney Int December 2003 Vol 64 No 6 Pages 1986-1996], in further view of the publication titled "Type IV collagen of the glomerular basement membrane. Evidence that the chain specificity of network assembly is encoded by the noncollagenous NC1 domains" by Boutaud et al. (hereinafter "Boutaud") [J. Biol Chem. 29 September 2000 Vol 275 Vol 39 Pages 30716-30724].

Concerning common technical features #1 and #2, Hudson teaches (claim 5; "A method of treating Goodpasture syndrome in a patient by neutralizing Goodpasture antibodies in the patient's blood or liquid fraction thereof by contacting the blood or liquid fraction thereof with an effective antibody neutralizing amount of an a(IV)NC1 polypeptide that contains a conformational epitope for Goodpasture antibodies, wherein the conformational epitope comprises, at least one amino acid sequence selected from the group consisting of TAIPSCPEGTVPLYS (SEQ ID NO. 1) and TDIPPCPHGWISLWK").

.--------continued on next sheet--------

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As to the common technical feature #3, claim 1 (generic to Groups I+ and II), a composition comprising three recombinant proteins, formulated in a pharmaceutically-acceptable carrier containing less than 30 mM halide ions, that assemble into a heterotrimeric complex with similarity to protomeric collagen IV, wherein:
a) each recombinant protein contains a C-terminal NCI domain and a collagenous domain;
b)each recombinant protein in the heterotrimeric complex is independently expressed in a mammalian cell line;
c)the heterotrimeric complex is assembled at a temperature below 37 degree C and in a solution containing less than 30 mM halide concentration; and
d) the heterotrimeric complex is capable of binding another heterotrimeric NCI containing complex via the NCI domain upon entering a solution with halide concentration above at least 30 mM.

Kobayashi teaches a) each recombinant protein contains a C-terminal NC1 domain and a collagenous domain (pg 1986 col 2 para 2; "Type IV collagen is a family of six distinct a chains, a1(IV), a2(IV), a3(IV), a4(IV), a5(IV), and a6(IV). Each chain consists of a short 7S domain at the amino terminus, a long collagenous domain of approximately 1400 residues, with Gly-X-Y repeats that are frequently interrupted by short noncollagenous sequences, and a noncollagenous (NC1) domain of about 230 residues at the carboxyl terminus"). Kobayashi further teaches b) each recombinant protein in the heterotrimeric complex is independently expressed in a mammalian cell line (pg 1998 col 2 para 2; "Recombinant production of a3(IV), a4(IV), and a5(IV): The cDNAs, including the entire open reading frames for the mouse a3(IV), a4(IV), and a5(IV) chains, were cloned into the eukaryotic expression vectors pcDNA3.1/Hygro, pcDNA6/V5-His A, and pCAGGS, respectively. These expression plasmids were transfected into HEK293 cells, and stable transfectants were established [.....] These results indicate that the transfected HEK293 cells produced the recombinant a3(IV), a4(IV), or a5(IV) chain, which was secreted into the culture medium. The stably transfected HEK293 cells were named a3, a4, and a5 cells, respectively"). Concerning claim limitation c) the heterotrimeric complex is assembled at a temperature below 37 degree C and in a solution containing less than 30 mM halide concentration, although Kobayashi teaches successful assembly of a3, a4 and a5 into heterotrimers when expressed together in a single cell line (pg 1990 fig 2; Fig. 2. Formation of a heterotrimer of a3(IV), a4(IV), and a5(IV) chains in a 345 cells"), Kobayashi does not teach successful assembly when secreted a3, a4 and a5 were mixed in vitro (pg 1990 col 1 para 1 and fig 4). However, based on Boutaud's teaching that it was necessary to have zero salt available when NC1 hexamers were disassembled and also keeping the temperature below 37C (Pg 30717 col 2 para 5; "The absence of salt from the buffer was necessary for complete hexamer dissociation. Reassembly of the dissociated NC1 domains was performed by changing the buffer to Tris-buffered saline (50 mM Tris, pH 7.4, 150 mM NaCl) by repeated dilution-concentration cycles in Centricon- 10 concentrators (Millipore Corp.). In some reassembly experiments, recombinant NC1 domains were also added to the reaction mixture. After incubating the NC1 domains at a concentration of about 1 mg/ml for 24 h at room temperature"), it would have been obvious for one of ordinary skill in the art to explore very low salt concentrations, as taught by Boutaud, or < 30 mM halide, to allow the three recombinant proteins to form heterotrimeric structures, but prevent them from forming hexamers, without undue effort. Consequently, it would have been obvious to have followed the teaching of Boutaud in meeting claim limitation d) the heterotrimeric complex is capable of binding another heterotrimeric NCI containing complex via the NCI domain upon entering a solution with halide concentration above at least 30 mM would be met by adding back higher salt/halide concentrations (pg 30717 col 2 para 5; "Reassembly of the dissociated NC1 domains was performed by changing the buffer to Tris-buffered saline (50 mM Tris, pH 7.4, 150 mM NaCl) by repeated dilution-concentration cycles in Centricon- 10 concentrators [.....] Hexamer assembly from purified a1-a5(IV) NC1 domains was carried out similarly).

None of Hudson, Kobayashi or Boutaud specifically teaches that the three recombinant proteins are formulated in a pharmaceutically-acceptable carrier. However, pharmaceutically acceptable carriers were well-known in the art of formulation science, and the specific salts and other elements could have easily been substituted into the heterotrimeric protein composition to allow its use within experimental animals or human subjects.

As the common technical feature was known in the art at the time of the invention, this cannot be considered a common special technical feature that would otherwise unify the groups. The inventions lack unity with one another.

Therefore, Groups I+, II and III lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Note concerning claim 3: Claim 3 includes the term, "collagenase domain", for which there is no antecedent precedent. For the purposes of the International Search and Opinion, "collagenase domain" is interpreted as "collagenous domain" as per antecedent precedent in claim 1.