

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

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PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43bis.1)

Date of mailing
(day/month/year)

30 OCT 2018

Applicant's or agent's file reference
32724-40714/WO

FOR FURTHER ACTION

See paragraph 2 below

International application No.

PCT/US2018/048026

International filing date (day/month/year)

24 August 2018

Priority date (day/month/year)

25 August 2017

International Patent Classification (IPC) or both national classification and IPC

IPC(8) - A61K 9/127; A61K 38/00; C07K 1/22; C07K 1/36; C07K 19/00; C12N 15/62 (2018.01)

CPC - A61K 9/127; A61K 9/5068; A61K 38/00; C07K 1/22; C07K 1/36; C07K 19/00; C12N 15/62; C12N 15/86; C12N 15/90 (2018.08)

Applicant **CODIAK BIOSCIENCES, INC.**

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450, Alexandria, VA 22313-1450
Facsimile No. 571-273-8300

Date of completion of this opinion

09 October 2018

Authorized officer

Blaine R. Copenheaver

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

**WRITTEN OPINION OF THE
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Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:
 - ☒ the international application in the language in which it was filed.
 - ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).
2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a)).
3. ☒ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established 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).
4. ☐ 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.

5. Additional comments:

SEQ ID NO: 33 was searched.

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Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

- ☐ the entire international application.
- ☒ claims Nos. 10-27, 32-49, 52, 64-66, 72-77

because:

- ☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search (*specify*):

- ☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 10-27, 32-49, 52, 64-66, 72-77 are so unclear that no meaningful opinion could be formed (*specify*):

Claims 10-27, 32-49, 52, 64-66, and 72-77 are multiple dependent claims not drafted in accordance with the second and third sentences of Rule 6.4(a).

- ☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed (*specify*):

- ☒ no international search report has been established for said claims Nos. 10-27, 32-49, 52, 64-66, 72-77

- ☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

- ☐ furnish a sequence listing in the form of an Annex C/ST.25 text file, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.
- ☐ furnish a sequence listing on paper or in the form of an image file complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13^{ter}.1(a) or (b).

- ☐ See Supplemental Box for further details.

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Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	5-9, 28-31, 50, 51, 53-63, 67-71	YES
	Claims	1-4	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-9, 28-31, 50, 51, 53-63, 67-71	NO
Industrial applicability (IA)	Claims	1-9, 28-31, 50, 51, 53-63, 67-71	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-4 lack novelty under PCT Article 33(2) as being anticipated by Caris Science, Inc. (hereinafter Caris).

Regarding Claim 1, Caris discloses a method of isolating an exosome (Para. [0041], Various techniques can be used to isolate the one or more microvesicle in whole or in part), comprising the steps of: providing a sample comprising the exosome (Para. [0089], Vesicles were isolated from human plasma samples using a procedure comprising thromboplastin-D treatment and ExoQuick isolation); contacting the sample with a binding agent having affinity to a target protein (Para. [0041], Various techniques can be used to isolate the one or more microvesicle in whole or in part. For example, the one or more microvesicle can be subjected to...immunoabsorbent capture, affinity purification, affinity capture), wherein the target protein comprises PTGFRN, BSG, IGSF2, IGSF3, IGSF8, ITGB1, ITGA4, SLC3A2, ATP transporter or a fragment or a variant thereof (Para. [0047], the invention provides an aptamer that comprises a first binding region to a first target, a second binding region to a second target, and a linker region between the first binding region and the second binding region. [0048], The first target may comprise a cancer or cell-of-origin specific protein marker. The first target can include a microvesicle surface antigen. In some embodiments, the first target is selected from any of Table 3, Table 4 or Table 5 herein. For example, the first target can be selected from the group consisting of...PTGFRN...SLC3A2; Table 5, BSG, IGSF3, IGSF8, ITGB1, ITGA4), and isolating the exosome based on a binding between the target protein and the agent (Para. [0011], contacting each of the one or more aliquots of retentate with one or more binding agent specific to a molecule of at least one microvesicle in the microvesicle population; [0039], The one or more binding agent can be used to capture and/or detect the one or more microvesicle).

Regarding Claim 2, Caris discloses the method of claim 1, wherein said sample is obtained from a cell grown in vitro, optionally wherein the cell is an HEK293 cell (Para. [0045], The methods of the invention can be performed in vitro, e.g., using an in vitro biological sample or a cell culture sample).

Regarding Claim 3, Caris discloses the method of claim 1, wherein said sample is obtained from a body fluid of a subject (Para. [0074], The plasma samples were from subjects with prostate cancer (PrC) or normal controls (normal); [0089], Vesicles were isolated from human plasma samples using a procedure comprising thromboplastin-D treatment and ExoQuick isolation).

Regarding Claim 4, Caris discloses the method of claim 2, wherein the cell is genetically modified to express the target protein (Para. [0213], The polypeptides may be naturally occurring, processed forms of naturally occurring polypeptides (such as by enzymatic digestion), chemically synthesized or recombinantly expressed; [0250], The biomarker can be present or absent, underexpressed or overexpressed, mutated, or modified in or on a vesicle and used in characterizing a condition).

Claims 5 and 6 lack an inventive step under PCT Article 33(3) as being obvious over Caris Science, Inc. (hereinafter Caris) in view of WO 2016/077639 A2 to VL27, Inc. (hereinafter VL27).

Regarding Claim 5, Caris discloses the method of claim 2. Caris fails to explicitly disclose wherein the cell comprises an expression plasmid encoding the target protein.

VL27 teaches a cell comprises an expression plasmid encoding the target protein (Para. [0019], In some embodiments, the producer cell comprises one or more surface molecules, such as, e.g., CD47, CD55, CD40, CD63, CD9, CD133 and CD59. In some embodiments, the producer cell naturally comprises the surface molecule. In some embodiments, the producer cell is modified to comprise the surface molecule; [0040], The levels of any desired surface marker may be modified directly on the nanomembrane delivery complex, e.g. by contacting the complex with recombinantly produced polypeptides to bring about insertion in or conjugation to the membrane of the complex. Alternatively or in addition, the levels of any desired surface marker may be modified directly on the producer cell, e.g. by contacting the complex with recombinantly produced polypeptides to bring about insertion in or conjugation to the membrane of the cell. Alternatively, the producer cell may be modified by transducing an exogenous nucleic acid into the producer cell to express a desired surface marker; [0181], A plasmid vector may be introduced into a producer cell or a nanomembrane delivery complex; [0297], includes a plasmid or viral chromosome into whose genome a fragment of recombinant DNA is inserted and used to introduce recombinant DNA, or a transgene, into a nanomembrane delivery complex).

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caris with the teaching of VL27 for the purpose of expressing a desired level of a desired surface marker as taught by VL27.

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Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:

Regarding Claim 6, Caris discloses the method of claim 4, wherein the cell is genetically modified to comprise an exogenous sequence expressing a tag having affinity to the binding agent (Para. [0039], The one or more binding agent can be used to capture and/or detect the one or more microvesicle; [0041], Various techniques can be used to isolate the one or more microvesicle in whole or in part. For example, the one or more microvesicle can be subjected to...immunoabsorbent capture, affinity purification, affinity capture (inherently employing an affinity tag); [0213], The polypeptides may be naturally occurring, processed forms of naturally occurring polypeptides (such as by enzymatic digestion), chemically synthesized or recombinantly expressed; [0250], The biomarker can be present or absent, underexpressed or overexpressed, mutated, or modified in or on a vesicle and used in characterizing a condition).

Caris fails to explicitly disclose wherein the exogenous sequence is inserted into a genome of the cell.

VL27 teaches an exogenous sequence encoding an affinity tag is inserted into a genome of a cell to affinity purify an exosome (Para. [0040], The levels of any desired surface marker may be modified directly on the nanomembrane delivery complex, e.g. by contacting the complex with recombinantly produced polypeptides to bring about insertion in or conjugation to the membrane of the complex.

Alternatively or in addition, the levels of any desired surface marker may be modified directly on the producer cell, e.g. by contacting the complex with recombinantly produced polypeptides to bring about insertion in or conjugation to the membrane of the cell. Alternatively, the producer cell may be modified by transducing an exogenous nucleic acid into the producer cell to express a desired surface marker; [0202], isolation and enrichment can be done in a more specific and selective manner (e.g., using producer cell-specific surface markers). For example, specific surface markers may be used in immunoprecipitation, FACS sorting, bead-bound ligands for magnetic separation etc; [0203], the producer cell derived nanomembrane delivery complexes can be separated from non-producer cell-derived nanomembrane delivery complexes by immunosorbent capture using an antigen antibody specific for the producer cell; [0297], which transfers and/or replicates an inserted nucleic acid molecule, such as a transgene or exogenous nucleic acid into and/or between host cells. It includes a plasmid or viral chromosome into whose genome a fragment of recombinant DNA is inserted and used to introduce recombinant DNA, or a transgene, into a nanomembrane delivery complex; [0300], The gene of interest is cloned into the multiple cloning site of lentivirus vector (an integrating vector)).

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caris with the teaching of VL27 for the purpose of expressing a desired level of a desired surface marker, such as for providing an affinity purification tag, as taught by VL27.

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Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:

Claims 7-9 lack an inventive step under PCT Article 33(3) as being obvious over Caris Science, Inc. (hereinafter Caris) in view of WO 2016/077639 A2 to VL27, Inc. (hereinafter VL27), Bond et al. (hereinafter Bond), and Massachusetts Institute of Technology et al. (hereinafter MIT).

Regarding Claim 7, modified Caris discloses the method of claim 6. Caris further discloses an endogenous sequence encoding PTGFRN, BSG, IGSF2, IGSF3, ITGB1, ITGA4, SLC3A2 or ATP transporter (Para. [0048]. The first target may comprise a cancer or cell-of-origin specific protein marker. The first target can include a microvesicle surface antigen. In some embodiments, the first target is selected from any of Table 3, Table 4 or Table 5 herein. For example, the first target can be selected from the group consisting of...PTGFRN...SLC3A2; Table 5, BSG, IGSF3, IGSF8, ITGB1, ITGA4).

Caris fails to explicitly disclose wherein the exogenous sequence is inserted in a genomic site located at 3' or 5' end of an endogenous sequence.

Bond teaches an affinity tag is inserted at a 3' end, within the coding region, of an endogenous gene, thereby encoding a fusion protein expressed on the surface of an exosome (Para. [0001]. The present application relates to methods of producing exosomes, and exosome targeted expression of fusion proteins with predefined sequences of interest for the therapeutic and diagnostic uses; [0004]. Nef is a protein expressed by primate lentiviruses, such as HIV and Sly. Nef is known to be secreted in association with exosomes and has been also shown to be present on the surface of HIV-infected cells. Nef-expressing cells have a dramatically altered subcellular morphology and have been shown to induce the intracellular accumulation of multivesicular bodies and the extracellular accumulation of exosomes; [0006]. The method comprises the steps of culturing an exosome-producing cell expressing a Nef-fusion protein comprising a Nef-derived peptide fused to a protein of interest; isolating exosomes from the exosome-producing cell culture; and purifying the protein of interest from the isolated exosomes; [0025]. In some embodiments, the Nef-fusion protein further comprises one or more additional amino acid sequences encoding one or more functional domains. Exemplary functional domains include, but are not limited to, affinity tags, protease cleavage sites, targeting domains, reporters, enzymes, or combination thereof; [0026]. In certain embodiments, an affinity tag may be included to facilitate purification of the Nef-fusion protein and/or protein of interest by affinity chromatography; [0081]. The green fluorescent protein (GFP) gene was cloned downstream of the HIV-1 Nef sequences such that a Nef-GFP fusion protein would be expressed. Nef sequences were able to drive secretion of GFP into the extracellular supernatant in vesicles).

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caris with the teaching of Bond for the purpose of providing a cell surface affinity tag on an exosome that may be used for exosome isolation as taught by Bond.

MIT teaches exogenous sequences may be inserted at selected endogenous sites within the genome of a target cell, at either end or within the coding region of a target gene (Abstract, methods for selecting specific cells by introducing precise mutations utilizing the CRISPR/Cas system; Para. [0017]. In some embodiments, said cleavage comprises cleaving one or two strands at the location of the target sequence by said CRISPR enzyme. In some embodiments, said cleavage results in decreased transcription of a target gene. In some embodiments, the method further comprises repairing said cleaved target polynucleotide by homologous recombination with an exogenous template polynucleotide, wherein said repair results in a mutation comprising an insertion, deletion, or substitution of one or more nucleotides of said target polynucleotide. In some embodiments, said mutation results in one or more amino acid changes in a protein expressed from a gene comprising the target sequence; [0126]. A target sequence may comprise any polynucleotide, such as DNA or RNA polynucleotides. In some embodiments, a target sequence is located in the nucleus or cytoplasm of a cell. In some embodiments, the target sequence may be within an organelle of a eukaryotic cell, for example, mitochondrion or chloroplast).

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caris with the teaching of MIT for the purpose of providing precise genome editing as taught by MIT (Para. [0085], a repair template in the form of a plasmid or single-stranded oligodeoxynucleotides (ssODN) can be supplied to leverage the homology-directed repair (HDR) pathway, which allows high fidelity and precise editing).

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Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:

Regarding Claim 8, modified Caris discloses the method of claim 6. Caris further discloses an endogenous sequence encoding PTGFRN, BSG, IGSF2, IGSF3, ITGB1, ITGA4, SLC3A2 or ATP transporter (Para. [0048], The first target may comprise a cancer or cell-of-origin specific protein marker. The first target can include a microvesicle surface antigen. In some embodiments, the first target is selected from any of Table 3, Table 4 or Table 5 herein. For example, the first target can be selected from the group consisting of...PTGFRN...SLC3A2; Table 5, BSG, IGSF3, IGSF8, ITGB1, IGTA4).

Caris fails to explicitly disclose wherein the exogenous sequence is inserted in a genomic site located within an endogenous coding sequence.

Bond teaches an affinity tag is inserted at the 3' end, within the coding region, of an endogenous gene, thereby encoding a fusion protein expressed on the surface of an exosome (Para. [0001], The present application relates to methods of producing exosomes, and exosome targeted expression of fusion proteins with predefined sequences of interest for the therapeutic and diagnostic uses; [0004], Nef is a protein expressed by primate lentiviruses, such as HIV and Sly. Nef is known to be secreted in association with exosomes and has been also shown to be present on the surface of HIV-infected cells. Nef-expressing cells have a dramatically altered subcellular morphology and have been shown to induce the intracellular accumulation of multivesicular bodies and the extracellular accumulation of exosomes; [0006], The method comprises the steps of culturing an exosome-producing cell expressing a Nef-fusion protein comprising a Nef-derived peptide fused to a protein of interest; isolating exosomes from the exosome-producing cell culture; and purifying the protein of interest from the isolated exosomes; [0025], In some embodiments, the Nef-fusion protein further comprises one or more additional amino acid sequences encoding one or more functional domains. Exemplary functional domains include, but are not limited to, affinity tags, protease cleavage sites, targeting domains, reporters, enzymes, or combination thereof; [0026], In certain embodiments, an affinity tag may be included to facilitate purification of the Nef-fusion protein and/or protein of interest by affinity chromatography; [0081], The green fluorescent protein (GFP) gene was cloned downstream of the HIV-1 Nef sequences such that a Nef-GFP fusion protein would be expressed. Nef sequences were able to drive secretion of GFP into the extracellular supernatant in vesicles).

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caris with the teaching of Bond for the purpose of providing a cell surface affinity tag on an exosome that may be used for exosome isolation as taught by Bond.

MIT teaches exogenous sequences may be inserted at selected endogenous sites within the genome of a target cell, at either end or within the coding region of a target gene (Abstract, methods for selecting specific cells by introducing precise mutations utilizing the CRISPR/Cas system; Para. [0017], In some embodiments, said cleavage comprises cleaving one or two strands at the location of the target sequence by said CRISPR enzyme. In some embodiments, said cleavage results in decreased transcription of a target gene. In some embodiments, the method further comprises repairing said cleaved target polynucleotide by homologous recombination with an exogenous template polynucleotide, wherein said repair results in a mutation comprising an insertion, deletion, or substitution of one or more nucleotides of said target polynucleotide. In some embodiments, said mutation results in one or more amino acid changes in a protein expressed from a gene comprising the target sequence; [0126], A target sequence may comprise any polynucleotide, such as DNA or RNA polynucleotides. In some embodiments, a target sequence is located in the nucleus or cytoplasm of a cell. In some embodiments, the target sequence may be within an organelle of a eukaryotic cell, for example, mitochondrion or chloroplast).

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caris with the teaching of MIT for the purpose of providing precise genome editing as taught by MIT (Para. [0085], a repair template in the form of a plasmid or single-stranded oligodeoxynucleotides (ssODN) can be supplied to leverage the homology-directed repair (HDR) pathway, which allows high fidelity and precise editing).

Regarding Claim 9, modified Caris discloses the method of claim 7. Caris further discloses the target protein is selected from PTGFRN, BSG, IGSF3, IGSF2, ITGB1, ITGA4, SLC3A2, ATP transporter or a fragment or a variant thereof (Para. [0047], the invention provides an aptamer that comprises a first binding region to a first target, a second binding region to a second target, and a linker region between the first binding region and the second binding region. [0048], The first target may comprise a cancer or cell-of-origin specific protein marker. The first target can include a microvesicle surface antigen. In some embodiments, the first target is selected from any of Table 3, Table 4 or Table 5 herein. For example, the first target can be selected from the group consisting of...PTGFRN...SLC3A2; Table 5, BSG, IGSF3, IGSF8, ITGB1, IGTA4), and is expressed on the exosome surface such that it can be used as a ligand for affinity purification of the exosomes (Para. [0011], contacting each of the one or more aliquots of retentate with one or more binding agent specific to a molecule of at least one microvesicle in the microvesicle population; [0039], The one or more binding agent can be used to capture and/or detect the one or more microvesicle; [0041], Various techniques can be used to isolate the one or more microvesicle in whole or in part. For example, the one or more microvesicle can be subjected to...immunoabsorbent capture, affinity purification, affinity capture).

Caris fails to explicitly disclose wherein the target protein is a fusion protein.

Bond teaches a fusion protein comprising an affinity tag and an exosomal surface protein, useful for affinity purification of the exosomes (Para. [0001], The present application relates to methods of producing exosomes, and exosome targeted expression of fusion proteins with predefined sequences of interest for the therapeutic and diagnostic uses; [0004], Nef is a protein expressed by primate lentiviruses, such as HIV and Sly. Nef is known to be secreted in association with exosomes and has been also shown to be present on the surface of HIV-infected cells. Nef-expressing cells have a dramatically altered subcellular morphology and have been shown to induce the intracellular accumulation of multivesicular bodies and the extracellular accumulation of exosomes; [0006], The method comprises the steps of culturing an exosome-producing cell expressing a Nef-fusion protein comprising a Nef-derived peptide fused to a protein of interest; isolating exosomes from the exosome-producing cell culture; and purifying the protein of interest from the isolated exosomes; [0025], In some embodiments, the Nef-fusion protein further comprises one or more additional amino acid sequences encoding one or more functional domains. Exemplary functional domains include, but are not limited to, affinity tags, protease cleavage sites, targeting domains, reporters, enzymes, or combination thereof; [0026], In certain embodiments, an affinity tag may be included to facilitate purification of the Nef-fusion protein and/or protein of interest by affinity chromatography; [0081], The green fluorescent protein (GFP) gene was cloned downstream of the HIV-1 Nef sequences such that a Nef-GFP fusion protein would be expressed. Nef sequences were able to drive secretion of GFP into the extracellular supernatant in vesicles).

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caris with the teaching of Bond for the purpose of providing a cell surface affinity tag on an exosome that may be used for exosome isolation as taught by Bond.

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Continuation of:

Claims 28, 50, and 51 lack an inventive step under PCT Article 33(3) as being obvious over Caris Science, Inc. (hereinafter Caris) in view of Bond et al. (hereinafter Bond).

Regarding Claim 28, Caris discloses an exosome comprising a target protein, the target protein comprising PTGFRN, BSG, IGSF3, IGSF8, IGSF2, ITGB1, ITGA4, SLC3A2, ATP transporter or a fragment or a variant thereof (Para. [0039]. The one or more binding agent can be used to capture and/or detect the one or more microvesicle; [0047]. the invention provides an aptamer that comprises a first binding region to a first target, a second binding region to a second target, and a linker region between the first binding region and the second binding region. [0048]. The first target may comprise a cancer or cell-of-origin specific protein marker. The first target can include a microvesicle surface antigen. In some embodiments, the first target is selected from any of Table 3, Table 4 or Table 5 herein. For example, the first target can be selected from the group consisting of...PTGFRN...SLC3A2; Table 5, BSG, IGSF3, IGSF8, ITGB1, ITGA4).

Caris fails to explicitly disclose wherein at least a part of the target protein is expressed from an exogenous sequence.

Bond teaches a target protein expressed on the surface of an exosome wherein at least a part of the target protein is expressed from an exogenous sequence (Para. [0006]. The method comprises the steps of culturing an exosome-producing cell expressing a Nef-fusion protein comprising a Nef-derived peptide fused to a protein of interest; isolating exosomes from the exosome-producing cell culture; and purifying the protein of interest from the isolated exosomes; [0025]. In some embodiments, the Nef-fusion protein further comprises one or more additional amino acid sequences encoding one or more functional domains. Exemplary functional domains include, but are not limited to, affinity tags, protease cleavage sites, targeting domains, reporters, enzymes, or combination thereof; [0026]. In certain embodiments, an affinity tag may be included to facilitate purification of the Nef-fusion protein and/or protein of interest by affinity chromatography; [0081]. The green fluorescent protein (GFP) gene was cloned downstream of the HIV-1 Nef sequences such that a Nef-GFP fusion protein would be expressed. Nef sequences were able to drive secretion of GFP into the extracellular supernatant in vesicles).

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caris with the teaching of Bond for the purpose of providing a cell surface affinity tag on an exosome that may be used for exosome isolation as taught by Bond.

Regarding Claim 50, modified Caris discloses the exosome of claim 28. Caris further discloses a pharmaceutical composition comprising the exosome and an excipient (Para. [0038]. The pharmaceutical compositions can optionally comprise one or more additional excipients or additives to provide a dosage form suitable for administration to an animal).

Regarding Claim 51, modified Caris discloses the pharmaceutical composition of claim 50. Caris further discloses the pharmaceutical composition is substantially free of macromolecules, wherein the macromolecules are selected from nucleic acids, contaminant proteins, lipids, carbohydrates, metabolites, and a combination thereof (Para. [0560]. contaminants are removed from a biological sample and the remaining vesicles are assessed for surface content and/or payload. For example, a column can be constructed comprising binding agents to contaminating proteins, vesicles, or other entities in the biological sample. The flow through will thereby be enriched in the circulating biomarkers or circulating microvesicles of interest).

Claims 29 and 30 lack an inventive step under PCT Article 33(3) as being obvious over Caris Science, Inc. (hereinafter Caris) in view of Bond et al. (hereinafter Bond) and WO 2016/077639 A2 to VL27, Inc. (hereinafter VL27).

Regarding Claim 29, modified Caris discloses the exosome of claim 28. Caris fails to explicitly disclose wherein the target protein is present on the surface of the exosome at a higher density than a different target protein of a different exosome, wherein the different target protein comprises a conventional exosome protein or a variant thereof.

VL27 teaches a target protein is present on the surface of an exosome at a higher density than a different target protein of a different exosome, wherein the different target protein comprises a conventional exosome protein or a variant thereof (Para. [0040]. the producer cell may be modified by transducing an exogenous nucleic acid into the producer cell to express a desired surface marker. The surface marker may already be naturally present on the producer cell, in which case the exogenous construct may lead to overexpression of the marker and increased concentration of the marker in or on the producer cell).

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caris with the teaching of VL27 for the purpose of expressing a desired level of a desired surface marker as taught by VL27 (Para. [0040]. the levels of any desired surface marker may be modified directly on the producer cell, e.g. by contacting the complex with recombinantly produced polypeptides to bring about insertion in or conjugation to the membrane of the cell. Alternatively, the producer cell may be modified by transducing an exogenous nucleic acid into the producer cell to express a desired surface marker).

Regarding Claim 30, modified Caris discloses the exosome of claim 29. Caris further discloses the conventional exosome protein is selected from the group consisting of CD9, CD63, CD81, PDGFR, GPI anchor proteins, lactadherin, LAMP2, LAMP2B, and a fragment thereof (Para. [0480]. the one or more microvesicle may be detected using multiple pairs of capture and detector agents...In some embodiments, the detector agent recognizes one or more of a tetraspanin, CD9, CD63, CD81, CD63, CD9, CD81, CD82, CD37, CD53, Rab-Sb, Annexin V, MFG-ES, or a protein in Table 3).

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Claim 31 lacks an inventive step under PCT Article 33(3) as being obvious over Caris Science, Inc. (hereinafter Caris) in view of Bond et al. (hereinafter Bond) and Al-Mahmood et al. (hereinafter Al-Mahmood).

Regarding Claim 31, modified Caris discloses the exosome of claim 28. Caris further discloses the target protein comprises PDGFRN (Para. [0025]. In any of the various aspects of the invention, the method comprises detecting one or more microvesicle antigens associated with the one or more microvesicles...The one or more microvesicle-associated antigen can include a protein selected from the group consisting of...PDGFRN).

Caris fails to explicitly disclose a polypeptide of SEQ ID NO: 33.

Al-Mahmood teaches PDGFRN comprising a polypeptide of SEQ ID NO: 33 (Para. [0011], Angiopoietin is homologous with the negative regulator of the prostaglandin F2 receptor (PTGFRN), (access no. XM_040709, nucleic sequence: 5975 bp, protein sequence: 560 aa); [0026], an antibody that has an affinity for one or more of the polypeptide sequences identified by...SEQ ID No. 31 (100% identical to SEQ ID NO: 33 of the instant application)).

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caris with the teaching of Al-Mahmood for the purpose of providing the amino acid sequence of proteins related to angiogenesis, as taught by Al-Mahmood (Para. [0024]. These genes are identified by their nucleotide sequences in the attached sequence listing. The present invention also pertains to the polypeptide sequences of the factors coded by said genes, which find their application in the clinical study of the angiogenesis process, the prognosis, diagnosis and treatment of pathologies linked to this process, as well as in the implementation of pharmacological, pharmacogenomic and drug identification trials).

Claims 53-59, 61, and 62 lack an inventive step under PCT Article 33(3) as being obvious over Caris Science, Inc. (hereinafter Caris) in view of WO 2016/077639 A2 to VL27, Inc. (hereinafter VL27) and Bond et al. (hereinafter Bond).

Regarding Claim 53, Caris discloses a cell line for producing exosomes (Para. [0087], Fig. 30M shows titration of sample input using purified microvesicles from cell line DU145; [1207], KRAS RNA was isolated from vesicles derived from CRC cell lines and sequenced), comprising genes encoding PTGFRN, BSG, IGSF3, IGSF2, ITGB1, ITGA4, SLC3A2, or ATP transporter expressed on the surface of exosomes (Para. [0011], contacting each of the one or more aliquots of retentate with one or more binding agent specific to a molecule of at least one microvesicle in the microvesicle population; [0039], The one or more binding agent can be used to capture and/or detect the one or more microvesicle; [0047], the invention provides an aptamer that comprises a first binding region to a first target, a second binding region to a second target, and a linker region between the first binding region and the second binding region; [0048], The first target may comprise a cancer or cell-of-origin specific protein marker. The first target can include a microvesicle surface antigen. In some embodiments, the first target is selected from any of Table 3, Table 4 or Table 5 herein. For example, the first target can be selected from the group consisting of...PTGFRN...SLC3A2; Table 5, BSG, IGSF3, IGSF8, ITGB1, ITGA4).

Caris fails to explicitly disclose an exogenous sequence inserted into a genomic sequence wherein the exogenous sequence and the genomic sequence encodes a fusion protein.

VL27 teaches an exogenous sequence encoding an affinity tag is inserted into a genome of the cell to affinity purify an exosome (Para. [0040]. The levels of any desired surface marker may be modified directly on the nanomembrane delivery complex, e.g. by contacting the complex with recombinantly produced polypeptides to bring about insertion in or conjugation to the membrane of the complex.

Alternatively or in addition, the levels of any desired surface marker may be modified directly on the producer cell, e.g. by contacting the complex with recombinantly produced polypeptides to bring about insertion in or conjugation to the membrane of the cell. Alternatively, the producer cell may be modified by transducing an exogenous nucleic acid into the producer cell to express a desired surface marker; [0202], isolation and enrichment can be done in a more specific and selective manner (e.g., using producer cell-specific surface markers). For example, specific surface markers may be used in immunoprecipitation, FACS sorting, bead-bound ligands for magnetic separation etc; [0203], the producer cell derived nanomembrane delivery complexes can be separated from non-producer cell-derived nanomembrane delivery complexes by immunosorbent capture using an antigen antibody specific for the producer cell; [0297], which transfers and/or replicates an inserted nucleic acid molecule, such as a transgene or exogenous nucleic acid into and/or between host cells. It includes a plasmid or viral chromosome into whose genome a fragment of recombinant DNA is inserted and used to introduce recombinant DNA, or a transgene, into a nanomembrane delivery complex; [0300], The gene of interest is cloned into the multiple cloning site of lentivirus vector (an integrating vector)).

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caris with the teaching of VL27 for the purpose of expressing a desired level of a desired surface marker, such as for providing an affinity purification tag, as taught by VL27.

Bond teaches an affinity tag is inserted within the coding region of an endogenous gene, thereby encoding a fusion protein expressed on the surface of an exosome (Para. [0001]. The present application relates to methods of producing exosomes, and exosome targeted expression of fusion proteins with predefined sequences of interest for the therapeutic and diagnostic uses; [0004], Nef is a protein expressed by primate lentiviruses, such as HIV and Sly. Nef is known to be secreted in association with exosomes and has been also shown to be present on the surface of HIV-infected cells. Nef-expressing cells have a dramatically altered subcellular morphology and have been shown to induce the intracellular accumulation of multivesicular bodies and the extracellular accumulation of exosomes; [0006], The method comprises the steps of culturing an exosome-producing cell expressing a Nef-fusion protein comprising a Nef-derived peptide fused to a protein of interest; isolating exosomes from the exosome-producing cell culture; and purifying the protein of interest from the isolated exosomes; [0025], In some embodiments, the Nef-fusion protein further comprises one or more additional amino acid sequences encoding one or more functional domains. Exemplary functional domains include, but are not limited to, affinity tags, protease cleavage sites, targeting domains, reporters, enzymes, or combination thereof; [0026], In certain embodiments, an affinity tag may be included to facilitate purification of the Nef-fusion protein and/or protein of interest by affinity chromatography; [0081], The green fluorescent protein (GFP) gene was cloned downstream of the HIV-1 Nef sequences such that a Nef-GFP fusion protein would be expressed. Nef sequences were able to drive secretion of GFP into the extracellular supernatant in vesicles).

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caris with the teaching of Bond for the purpose of providing a cell surface affinity tag on an exosome that may be used for exosome isolation as taught by Bond.

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Regarding Claim 54, modified Caris discloses the cell line of claim 53. Caris further discloses the exogenous sequence encodes an affinity tag (Para. [0039], The one or more binding agent can be used to capture and/or detect the one or more microvesicle; [0041], Various techniques can be used to isolate the one or more microvesicle in whole or in part. For example, the one or more microvesicle can be subjected to...immunoabsorbent capture, affinity purification, affinity capture (inherently employing an affinity tag); [0213], The polypeptides may be naturally occurring, processed forms of naturally occurring polypeptides (such as by enzymatic digestion), chemically synthesized or recombinantly expressed; [0250], The biomarker can be present or absent, underexpressed or overexpressed, mutated, or modified in or on a vesicle and used in characterizing a condition).

Regarding Claim 55, modified Caris discloses the cell line of claim 53. Caris fails to explicitly disclose wherein the exogenous sequence encodes a therapeutic peptide.

VL27 teaches an exogenous sequence encodes a therapeutic peptide (Para. [0035], In some embodiments, the nanomembrane delivery complex delivers the payload (therapeutic agent) to a target. The payload is a therapeutic agent that acts on a target (e.g. a target cell) that is contacted with the nanomembrane delivery complex. Contacting may occur, e.g. in vitro or in a subject. Payloads that may be introduced into a nanomembrane delivery complex and/or a producer cell include therapeutic agents such as, nucleotides...nucleic acids (e.g. DNA or mRNA molecules that encode a polypeptide such as an enzyme, or RNA molecules that have regulatory function such as miRNA, dsDNA, lncRNA, siRNA), amino acids (e.g. amino acids comprising a detectable moiety or a toxin or that disrupt translation)). It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caris with the teaching of VL27 for the purpose of delivering therapy to a target cell as taught by VL27 (Para. [0035], the nanomembrane delivery complex delivers the payload (therapeutic agent) to a target).

Regarding Claim 56, modified Caris discloses the cell line of claim 55. Caris fails to explicitly disclose wherein the therapeutic peptide is selected from the group consisting of a natural peptide, a recombinant peptide, a synthetic peptide, or a linker to a therapeutic compound.

VL27 teaches a therapeutic peptide is a natural peptide, a recombinant peptide, or a synthetic peptide (Para. [0035], In some embodiments, the nanomembrane delivery complex delivers the payload (therapeutic agent) to a target. The payload is a therapeutic agent that acts on a target (e.g. a target cell) that is contacted with the nanomembrane delivery complex. Contacting may occur, e.g. in vitro or in a subject. Payloads that may be introduced into a nanomembrane delivery complex and/or a producer cell include therapeutic agents such as...amino acids (e.g. amino acids comprising a detectable moiety or a toxin or that disrupt translation), polypeptides (e.g. enzymes), lipids, carbohydrates, and small molecules (e.g. small molecule drugs and toxins). The payload may comprise nucleotides, e.g. nucleotides that are labeled with a detectable or cytotoxic moiety (e.g. a radiolabel).

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caris with the teaching of VL27 for the purpose of delivering therapy to a target cell as taught by VL27 (Para. [0035], the nanomembrane delivery complex delivers the payload (therapeutic agent) to a target).

Regarding Claim 57, modified Caris discloses the cell line of claim 55. Caris fails to explicitly disclose wherein the therapeutic compound is selected from the group consisting of nucleotides, amino acids, lipids, carbohydrates, and small molecules.

VL27 teaches a therapeutic compound is nucleotides, amino acids, lipids, carbohydrates, or small molecules (Para. [0035], In some embodiments, the nanomembrane delivery complex delivers the payload (therapeutic agent) to a target. The payload is a therapeutic agent that acts on a target (e.g. a target cell) that is contacted with the nanomembrane delivery complex. Contacting may occur, e.g. in vitro or in a subject. Payloads that may be introduced into a nanomembrane delivery complex and/or a producer cell include therapeutic agents such as...amino acids (e.g. amino acids comprising a detectable moiety or a toxin or that disrupt translation), polypeptides (e.g. enzymes), lipids, carbohydrates, and small molecules (e.g. small molecule drugs and toxins). The payload may comprise nucleotides, e.g. nucleotides that are labeled with a detectable or cytotoxic moiety (e.g. a radiolabel).

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caris with the teaching of VL27 for the purpose of delivering therapy to a target cell as taught by VL27 (Para. [0035], the nanomembrane delivery complex delivers the payload (therapeutic agent) to a target).

Regarding Claim 58, modified Caris discloses the cell line of claim 55. Caris fails to explicitly disclose wherein the therapeutic peptide is an antibody or a fragment or a variant thereof.

VL27 teaches a therapeutic peptide is an antibody or a fragment or a variant thereof (Para. [0044], the payload that may be delivered to a target by a nanomembrane delivery complex includes, but is not limited to, RNA, DNA, siRNA, mRNA, lncRNA, iRNA, polypeptides, enzymes, cytokines, antibodies, antibody fragments; [0098], In some embodiments, the receiver polypeptide is associated with the membrane of the nanomembrane delivery complex. In other embodiments, the receiver polypeptide is not associated with the membrane of the nanomembrane delivery complex.; [0099], In one embodiment, the receiver comprises a polypeptide that comprises an amino acid sequence derived from an antibody. The antibody receiver may be expressed as a full-length protein or a fragment thereof. In one embodiment, the receiver comprises an antibody amino acid sequence that is specific for a desired target).

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caris with the teaching of VL27 for the purpose of delivering therapy to a target cell as taught by VL27 (Para. [0035], the nanomembrane delivery complex delivers the payload (therapeutic agent) to a target; [0099], the receiver comprises an antibody amino acid sequence that is specific for a desired target).

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Regarding Claim 59, modified Caris discloses the cell line of claim 55. Caris fails to explicitly disclose wherein the therapeutic peptide is an enzyme, a ligand, a receptor, or a fragment or a variant thereof.

VL27 teaches a therapeutic peptide is an enzyme (Para. [0035], In some embodiments, the nanomembrane delivery complex delivers the payload (therapeutic agent) to a target. The payload is a therapeutic agent that acts on a target (e.g. a target cell) that is contacted with the nanomembrane delivery complex. Contacting may occur, e.g. in vitro or in a subject. Payloads that may be introduced into a nanomembrane delivery complex and/or a producer cell include therapeutic agents such as... polypeptides (e.g. enzymes), lipids, carbohydrates, and small molecules).

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caris with the teaching of VL27 for the purpose of delivering therapy to a target cell as taught by VL27 (Para. [0035], the nanomembrane delivery complex delivers the payload (therapeutic agent) to a target).

Regarding Claim 61, modified Caris discloses the cell line of claim 53. Caris fails to explicitly disclose wherein the exogenous sequence encodes a targeting moiety.

Bond teaches an exogenous sequence encodes a targeting moiety (Para. [0025], The Nef-fusion protein comprises a Nef-derived peptide fused to a protein of interest. In some embodiments, the Nef-fusion protein further comprises one or more additional amino acid sequences encoding one or more functional domains. Exemplary functional domains include, but are not limited to, affinity tags, protease cleavage sites, targeting domains, reporters, enzymes, or combination thereof).

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caris with the teaching of Bond for the purpose of targeting exosomes to a particular cell type as taught by Bond (Para. [0028], The targeting domain may comprise amino acid sequences conferring cell-type specific or cell differentiation specific targeting. The targeting domain may be incorporated into the Nef-fusion protein or it can be fused to a coexpressed membrane-bound exosomal marker protein. Preferably the targeting domain is fused to an extracellular domain in the membrane-bound protein).

Regarding Claim 62, modified Caris discloses the cell line of claim 61. Caris fails to explicitly disclose wherein the targeting moiety is specific to an organ, a tissue, or a cell.

Bond teaches a targeting moiety is specific to an organ, a tissue, or a cell (Para. [0025], The Nef-fusion protein comprises a Nef-derived peptide fused to a protein of interest. In some embodiments, the Nef-fusion protein further comprises one or more additional amino acid sequences encoding one or more functional domains. Exemplary functional domains include, but are not limited to, affinity tags, protease cleavage sites, targeting domains, reporters, enzymes, or combination thereof).

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caris with the teaching of Bond for the purpose of targeting exosomes to a particular cell type as taught by Bond (Para. [0028], The targeting domain may comprise amino acid sequences conferring cell-type specific or cell differentiation specific targeting. The targeting domain may be incorporated into the Nef-fusion protein or it can be fused to a coexpressed membrane-bound exosomal marker protein. Preferably the targeting domain is fused to an extracellular domain in the membrane-bound protein).

Claim 60 lacks an inventive step under PCT Article 33(3) as being obvious over Caris Science, Inc. (hereinafter Caris) in view of WO 2016/077639 A2 to VL27, Inc. (hereinafter VL27), Bond et al. (hereinafter Bond), and Bouliskas.

Regarding Claim 60, modified Caris discloses the cell line of claim 55. Caris fails to explicitly disclose wherein the therapeutic peptide is an antimicrobial peptide or a fragment or a variant thereof.

Bouliskas teaches liposomes comprising antimicrobial peptides (Pg. 35, Lns. 15-35, A number of studies suggest that stabilization of an alpha-helical secondary structure of the peptide after insertion in lipid bilayers in membranes of cells or liposomes is responsible for the membrane fusion properties of peptides. Zn²⁺, enhances the fusogenic activity of peptides because it stabilizes the alpha-helical structure. For example, the HEXXH (SEQ ID NO:11) domain of the salivary antimicrobial peptide, located in the C-terminal functional domain of histatin-5, a recognized zinc-binding motif is in a helical conformation).

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caris with the teaching of Bouliskas for the purpose of promoting membrane fusion as taught by Bouliskas (Pg. 3, Lns. 15-20, Various strategies to enhance efficiency of gene transfer have been tested such as fusogenic peptides in combination with liposomes or polymers to enhance the release of plasmid DNA from endosomes; Pg. 35, Lns. 15-35, A number of studies suggest that stabilization of an alpha-helical secondary structure of the peptide after insertion in lipid bilayers in membranes of cells or liposomes is responsible for the membrane fusion properties of peptides).

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Claim 63 lacks an inventive step under PCT Article 33(3) as being obvious over Caris Science, Inc. (hereinafter Caris) in view of WO 2016/077639 A2 to VL27, Inc. (hereinafter VL27), Bond et al. (hereinafter Bond), and Moss et al. (hereinafter Moss).

Regarding Claim 63, modified Caris discloses the cell line of claim 62. Caris fails to explicitly disclose wherein the cell line is genetically modified to have a reduced expression of ADAM 10.

Moss teaches a cell line is genetically modified to have a reduced expression of ADAM 10, wherein ADAM 10 mediates shedding of membrane bound proteins (Pg. 151, Summary, Many membrane proteins have extracellular domains that are subject to proteolytic release, a process known as 'shedding'... ADAM 10 is implicated in the shedding of Notch, APP, ephrin-A2 and HB-EGF), and wherein cells genetically modified to have a reduced expression of ADAM 10 have a reduced level of membrane protein shedding (Pg.147, Para. 2, Like APP, Notch is processed by different proteases at three different positions. The first cleavage reaction is performed by a furin-like protease, while the second processing event depends on kuz (in Drosophila) or ADAM 10 and/or TACE (in mammals)...The first two cleavages are required to generate functional Notch, while the third cleavage occurs upon activation of Notch by its ligands, causing release of the cytosolic domain into the cytoplasm and subsequent translocation to the nucleus. The phenotype of the ADAM 10 knockout mice resembles the phenotype of the Notch knockout, supporting the idea that the second cleavage depends on ADAM 10 rather than TACE; Pg. 148, Para. 2, Transfection of HEK 293 cells with ADAM 10 increases the ability of the cells to produce sAPP, providing further evidence that it is an γ -secretase for APP processing. In addition, a construct of ADAM 10 carrying a point mutation in the catalytic domain, which renders it inactive, acts as a dominant negative factor, precluding release of sAPP from transfected HEK 293 cells).

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Caris with the teaching of Moss for the purpose of reducing shedding of membrane proteins as taught by Moss.

Claims 67-71 lack an inventive step under PCT Article 33(3) as being obvious over Bond et al. (hereinafter Bond) in view of Caris Science, Inc. (hereinafter Caris).

Regarding Claim 67, Bond discloses a method of isolating a non-exosomal material (Abstract, a method for preparing a protein composition comprising culturing an exosome-producing cell expressing a Nef-fusion protein comprising a Nef-derived peptide fused to a protein of interest; isolating exosomes from the exosome-producing cell culture; and purifying the protein of interest from the isolated exosomes), comprising the steps of: providing a sample comprising an exosome and the non-exosome material (Para. [0006], culturing an exosome-producing cell expressing a Nef-fusion protein comprising a Nef-derived peptide fused to a protein of interest; isolating exosomes from the exosome-producing cell culture); contacting the sample with a binding agent having affinity to a target protein, thereby inducing the exosome to bind to the binding agent (Para. [0026], In certain embodiments, an affinity tag may be included to facilitate purification of the Nef-fusion protein and/or protein of interest by affinity chromatography); and isolating the non-exosome material (Para. [0047], the isolated exosomes are treated to release the protein of interest from the Nef-derived peptide. The protein of interest is then purified from the treated exosomes using conventional protein purification methodologies).

Bond fails to explicitly disclose wherein the target protein comprises PTGFRN, BSG, IGSF2, IGSF3, IGSF8, ITGB 1, ITGA4, SLC3A2, ATP transporter or a fragment or a variant thereof.

Caris teaches affinity isolation of exosomes based on binding to a target protein, wherein the target protein is PTGFRN, BSG, IGSF2, IGSF3, IGSF8, ITGB1, ITGA4, or SLC3A2 (Para. [0039], The one or more binding agent can be used to capture and/or detect the one or more microvesicle; [0041], Various techniques can be used to isolate the one or more microvesicle in whole or in part. For example, the one or more microvesicle can be subjected to...immunoabsorbent capture, affinity purification, affinity capture; [0047], the invention provides an aptamer that comprises a first binding region to a first target, a second binding region to a second target, and a linker region between the first binding region and the second binding region. [0048], The first target may comprise a cancer or cell-of-origin specific protein marker. The first target can include a microvesicle surface antigen. In some embodiments, the first target is selected from any of Table 3, Table 4 or Table 5 herein. For example, the first target can be selected from the group consisting of...PTGFRN...SLC3A2; Table 5, BSG, IGSF3, IGSF8, ITGB1, ITGA4).

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Bond with the teaching of Caris for the purpose of Immunoaffinity purification of a particular population of exosomes as taught by Caris.

Regarding Claim 68, modified Bond discloses the method of claim 67. Bond further discloses the non-exosomal material is virus or a protein (Para. [0047], the isolated exosomes are treated to release the protein of interest from the Nef-derived peptide. The protein of interest is then purified from the treated exosomes using conventional protein purification methodologies).

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Regarding Claim 69, modified Bond discloses the method of claim 68. Bond further discloses the non-exosomal material is derived from lentivirus, retrovirus, adeno-associated virus, or other enveloped or non-enveloped virus (Para. [0043]). In some embodiments, the Nef-fusion proteins are delivered from viral-derived expression vectors. Exemplary viral vectors may include or be derived from adenovirus, adeno-associated virus, herpesvirus, vaccinia virus, poliovirus, poxvirus, HIV virus, lentivirus, retrovirus, Sindbis and other RNA viruses, and the like; [0052]. In another embodiment, the Nef-fusion protein comprises a Nef-derived fragment fused to an immunogenic protein from a bacterium, virus, fungus, or protozoan).

Bond fails to explicitly disclose isolation of viral particles.

Caris teaches viral particles may be isolated or enriched from exosomal preparations (Para. [0114]). Viral proteins, such as HIV or HCV-like particles can be assessed in a vesicle, to characterize a viral condition; [0135], the biological sample of the invention is understood to comprise a sample containing a separated, depleted, enriched, isolated, or otherwise processed derivative of another biological sample. As a nonlimiting example, a component of a patient sample or a cell culture can be isolated from the patient sample or the cell culture and resuspended in a buffer for further analysis; [0560], a column can be constructed comprising binding agents to contaminating proteins, vesicles, or other entities in the biological sample. The flow through will thereby be enriched in the circulating biomarkers or circulating microvesicles of interest. In a non-limiting example, a column is constructed to remove microvesicles derived from blood cells...the flow through that has been depleted in vesicles from blood cells can then be analyzed via a positive selection for vesicles of interest using affinity techniques or the like).

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Bond with the teaching of Caris for the purpose of enriching for an entity of interest for further analysis as taught by Caris (Para. [0560]), the flow through that has been depleted in vesicles from blood cells can then be analyzed via a positive selection for vesicles of interest using affinity techniques or the like).

Regarding Claim 70, modified Bond discloses the method of claim 68. Bond further discloses the non-exosomal material is a recombinant protein (Para. [0005]). The present invention provides compositions and methods for exosomal expression of recombinant proteins; [0006], isolating exosomes from the exosome-producing cell culture; and purifying the protein of interest from the isolated exosomes).

Regarding Claim 71, modified Bond discloses the method of claim 67. Bond further discloses the isolated non-exosomal material is substantially free of exosomes (Para. [0005]). The present invention provides compositions and methods for exosomal expression of recombinant proteins; [0006], isolating exosomes from the exosome-producing cell culture; and purifying the protein of interest from (i.e. free from) the isolated exosomes).

Claims 1-9, 28-31, 50, 51, 53-63, and 67-71 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.