(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 December 2024 (12.12.2024)

WIPO | PCT

(10) International Publication Number
WO 2024/254518 A3

(51) International Patent Classification:
A61K 9/14 (2006.01) *A61K 47/42* (2017.01)
A61P 39/00 (2006.01) *A61K 47/69* (2017.01)

(21) International Application Number:
PCT/US2024/033099

(22) International Filing Date:
07 June 2024 (07.06.2024)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/471,691 07 June 2023 (07.06.2023) US

(71) Applicant: **NVELOP THERAPEUTICS, INC.** [US/US]; 100 Technology Square, Floor 5, Cambridge, Massachusetts 02139 (US).

(72) Inventors: **PAJEROWSKI, Dave**; 100 Technology Square, Floor 5, Cambridge, Massachusetts 02139 (US). **CABECEIRAS, Peter**; 100 Technology Square, Floor 5, Cambridge, Massachusetts 02139 (US). **LEE, Jeffrey L.**; 100 Technology Square, Floor 5, Cambridge, Massachusetts 02139 (US). **YU, Yi**; 100 Technology Square, Floor 5, Cambridge, Massachusetts 02139 (US). **BUDZIK, Karol**; 100 Technology Square, Floor 5, Cambridge, Massachusetts 02139 (US).

(74) Agent: **BOBOILA, Shuobo** et al.; Wilson Sonsini Goodrich & Rosati, 650 Page Mill Road, Palo Alto, California 94304 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT,

(54) Title: COMPOSITIONS OF LIPID DELIVERY PARTICLES AND METHOD OF USE THEREOF

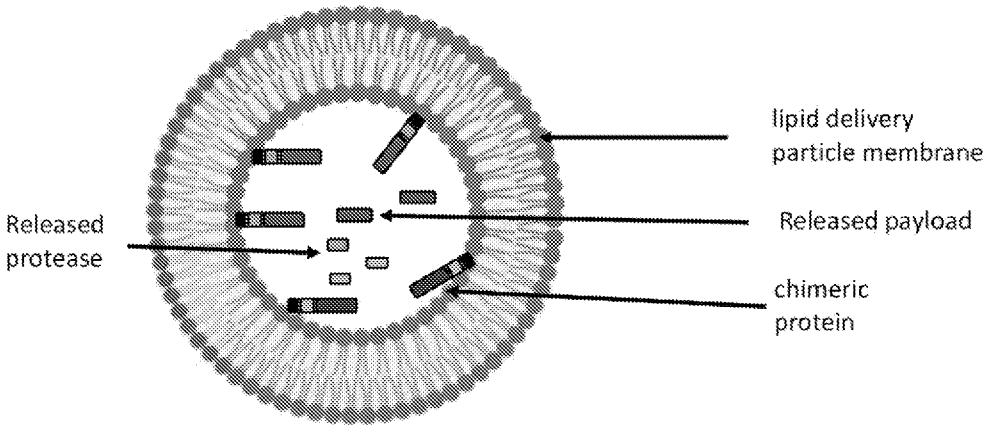

FIG. 1A

(57) Abstract: The present disclosure relates to lipid delivery particles for delivery of payloads across cell membrane into the target cell. The present disclosure relates to lipid delivery particles comprising a lipid membrane on the external side and a chimeric protein in the core comprising a transmembrane domain, a heterologous payload, and a cleavable linker cleavable by protease expressed in the target cells. The systems and methods described herein provide for: the ability to produce the lipid delivery particles and the ability to delivery payloads such as proteins and small molecules into the target cells.

WO 2024/254518 A3

[Continued on next page]

LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
— *of inventorship (Rule 4.17(iv))*

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))*

(88) Date of publication of the international search report:
06 March 2025 (06.03.2025)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/033099

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***A61K 9/14*** (2024.01); *A61P 39/00* (2024.01); *A61K 47/42* (2024.01); *A61K 47/69* (2024.01)

CPC: ***A61K 9/145;*** *A61K 47/42; A61K 47/6925; A61P 39/00*

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)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2020/0308234 A1 (TRUSTEES OF BOSTON UNIVERSITY) 01 October 2020 (01.10.2020) entire document	1-3, 134
A	US 2022/0259617 A1 (THE GENERAL HOSPITAL CORPORATION) 18 August 2022 (18.08.2022) entire document	1-3, 134
A	US 2022/0193223 A1 (MODERNATX INC.) 23 June 2022 (23.06.2022) entire document	1-3, 134
A	WO 2021/113772 A1 (SCRIBE THERAPEUTICS INC.) 10 June 2021 (10.06.2021) entire document	1-3, 134
A	US 2007/0196386 A1 (VU et al.) 23 August 2007 (23.08.2007) entire document	1-3, 134

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"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
20 December 2024 (20.12.2024)	**03 February 2025 (03.02.2025)**

Name and mailing address of the ISA/US

COMMISSIONER FOR PATENTS
MAIL STOP PCT, ATTN: ISA/US
P.O. Box 1450
Alexandria, VA 22313-1450
UNITED STATES OF AMERICA

Facsimile No. **571-273-8300**

Authorized officer

TAINA MATOS

Telephone No. **571-272-4300**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/033099

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.
   b. ☑ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)),
      ☑ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/033099

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.: **12-33, 54-133, 138-174**
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:

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 need to be paid.

Group I+: claims 1-11, 34-53, and 134-137 are drawn to chimeric proteins and lipid delivery particles comprising the same.

The first invention of Group I+ is restricted to a chimeric protein comprising a plasma membrane recruitment element, a heterologous payload, a cleavable linker, and a protease, where the plasma membrane recruitment element is selected to be SEQ ID NO:1; the cleavable linker is selected to be SEQ ID NO:126; and the protease is selected to be SEQ ID NO:105, and lipid delivery particles comprising the same. The first named invention has been selected based on the guidance set forth in section 10.54 of the PCT International Search and Preliminary Examination Guidelines. Specifically, the first named invention was selected based on the plasma membrane recruitment element sequences listed in instant claim 22, the cleavable linker sequences listed in instant claim 30, and the proteases listed in Table 7, of instant claim 3. It is believed that claims 1-3, and 134 read on this first named invention and thus these claims will be searched without fee to the extent that they read on SEQ ID NOs 1, 105 and 126.

Applicant is invited to elect additional chimeric proteins comprising plasma membrane recruitment elements, heterologous payloads, cleavable linkers, proteases, and their respective, corresponding, SEQ ID NOs to be searched in a specific combination by paying additional fee for each set of election. An exemplary election would be a chimeric protein comprising a plasma membrane recruitment element, a heterologous payload, a cleavable linker, and a protease, where the plasma membrane recruitment element is selected to be SEQ ID NO:2; the cleavable linker is selected to be SEQ ID NO:127; and the protease is selected to be SEQ ID NO:106, and lipid delivery particles comprising the same. Additional chimeric proteins comprising plasma membrane recruitment elements, heterologous payloads, cleavable linkers, proteases, and their respective, corresponding, SEQ ID NOs will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. 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.

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

The inventions listed in Groups I+ 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:

The Groups I+ formulas do not share a significant structural element responsible for delivering therapeutic payloads, requiring the selection of alternative chimeric proteins where "A lipid delivery particle, comprising: a lipid membrane on the external side; and a chimeric protein in the lipid delivery particle comprising (i) a plasma membrane recruitment element; (ii) a heterologous payload; (iii) one or more cleavable linkers; and (iv) a protease, wherein the one or more cleavable linkers are cleavable by the protease, wherein the one or more cleavable linkers are positioned such that cleavage by the protease releases the heterologous payload from the chimeric protein," and where "the viral protease is selected from Table 7."

Additionally, even if Groups I+ were considered to share the technical features of a lipid delivery particle, comprising: a lipid membrane on the external side; and a chimeric protein in the lipid delivery particle comprising (i) a plasma membrane recruitment element; (ii) a heterologous payload; (iii) one or more cleavable linkers; and (iv) a protease, wherein the one or more cleavable linkers are cleavable by the protease, wherein the one or more cleavable linkers are positioned such that cleavage by the protease releases the heterologous payload from the chimeric protein; a lipid delivery particle, comprising: a lipid membrane on the external side; a first chimeric protein in the lipid delivery particle comprising (i) a first plasma membrane recruitment element; (ii) a heterologous payload; and (iii) a cleavable linker; and a second chimeric protein in the lipid delivery particle comprising (i) a second plasma membrane recruitment element and (ii) a protease, wherein the cleavable linker is cleavable by the protease, wherein the cleavable linkers is positioned such that cleavage by the protease releases the heterologous payload from the first chimeric protein, a lipid delivery particle, comprising: a lipid membrane on the external side; a first chimeric protein in the lipid delivery particle comprising (i) a first plasma membrane recruitment element; (ii) a heterologous payload; and (iii) a cleavable linker; and a second chimeric protein in the lipid delivery particle comprising (i) a second plasma membrane recruitment element and (ii) a protease, wherein the second chimeric protein lacks a viral polymerase, wherein the cleavable linker is cleavable by the protease, wherein the cleavable linker is positioned such that cleavage by the protease releases the heterologous payload from the first chimeric protein; a lipid delivery particle, comprising: a lipid membrane on the external side; a first chimeric protein in the lipid delivery particle comprising (i) a first plasma membrane recruitment element; (ii) a heterologous payload; and (iii) a cleavable linker; and a second chimeric protein in the lipid delivery particle comprising (i) a second plasma membrane recruitment element and (ii) a first protease; a chimeric protein for delivering a heterologous payload to a target cell, comprising: (i) a plasma membrane recruitment element; (ii) a heterologous payload; (iii) one or more protease cleavable linkers; and (iv) a first protease, wherein the one or more cleavable linkers are cleavable by the protease, wherein the one or more cleavable linkers are positioned such that cleavage by the protease releases the heterologous payload from the chimeric protein construct. However, these shared technical features do not represent a contribution over the prior art.

Specifically, US 2022/0259617 A1 to The General Hospital Corporation (hereinafter, "General Hospital") discloses a lipid delivery particle ([h]uman-derived virus-like particles (heVLPs), comprising a membrane comprising a phospholipid bilayer …on the external side …for delivery of the cargo molecule to cells, Abstract), comprising: a lipid membrane on the external side (engineered heVLPs, comprising a membrane comprising a phospholipid bilayer …on the external side, Para. [0011]); and a chimeric protein in the lipid delivery particle comprising (i) a plasma membrane recruitment element; (ii) a heterologous payload (and a human endogenous GAG protein, other plasma membrane recruitment domain, and/or biomolecule/chemical cargo disposed in the core of the heVLP on the inside of the membrane …wherein the biomolecule cargo may or may not be fused to a human-endogenous GAG or other plasma membrane recruitment domain, Para. [0008]); (iii) one or more linkers (the linker between protein-based cargo and the human-endogenous GAG phospholipid bilayer recruitment domain is a polypeptide linker, Para. [0187]); a lipid delivery particle ([h]uman-derived virus-like particles (heVLPs), comprising a membrane comprising a phospholipid bilayer …on the external side …for delivery of the cargo molecule to cells, Abstract), comprising: a lipid membrane on the external side (engineered heVLPs, comprising a membrane comprising a phospholipid bilayer …on the external side, Para. [0011]); a first chimeric protein in the lipid delivery particle comprising (i) a first plasma membrane recruitment element (and a human endogenous GAG protein, other plasma membrane recruitment domain, and/or biomolecule/chemical cargo disposed in the core of the heVLP on the inside of the membrane …wherein the biomolecule cargo may or may not be fused to a human-endogenous GAG or

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

other plasma membrane recruitment domain, Para. [0008]); (ii) a heterologous payload; and a second chimeric protein in the lipid delivery particle comprising (i) a second plasma membrane recruitment element (heVLPs that contain cargo are produced and isolated can be loaded with additional biomolecule or chemical molecule cargo, Para. [0016]; and a human endogenous GAG protein, other plasma membrane recruitment domain, and/or biomolecule/chemical cargo disposed in the core of the heVLP on the inside of the membrane …wherein the biomolecule cargo may or may not be fused to a human-endogenous GAG or other plasma membrane recruitment domain, Para. [0008]); wherein the second chimeric protein lacks a viral polymerase (heVLPs that contain cargo are produced and isolated can be loaded with additional biomolecule or chemical molecule cargo, Para. [0016]; and a human endogenous GAG protein, other plasma membrane recruitment domain, and/or biomolecule/chemical cargo disposed in the core of the heVLP on the inside of the membrane …wherein the biomolecule cargo may or may not be fused to a human-endogenous GAG or other plasma membrane recruitment domain, Para. [0008]); a first chimeric protein in the lipid delivery particle comprising (i) a first plasma membrane recruitment element (and a human endogenous GAG protein, other plasma membrane recruitment domain, and/or biomolecule/chemical cargo disposed in the core of the heVLP on the inside of the membrane …wherein the biomolecule cargo may or may not be fused to a human-endogenous GAG or other plasma membrane recruitment domain, Para. [0008]); (ii) a heterologous payload (and a human endogenous GAG protein, other plasma membrane recruitment domain, and/or biomolecule/chemical cargo disposed in the core of the heVLP on the inside of the membrane …wherein the biomolecule cargo may or may not be fused to a human-endogenous GAG or other plasma membrane recruitment domain, Para. [0008]); and a second chimeric protein in the lipid delivery particle comprising (i) a second plasma membrane recruitment element (heVLPs that contain cargo are produced and isolated can be loaded with additional biomolecule or chemical molecule cargo, Para. [0016]; and a human endogenous GAG protein, other plasma membrane recruitment domain, and/or biomolecule/chemical cargo disposed in the core of the heVLP on the inside of the membrane …wherein the biomolecule cargo may or may not be fused to a human-endogenous GAG or other plasma membrane recruitment domain, Para. [0008]); a lipid delivery particle ([h]uman-derived virus-like particles (heVLPs), comprising a membrane comprising a phospholipid bilayer …on the external side …for delivery of the cargo molecule to cells, Abstract), comprising: a lipid membrane on the external side (engineered heVLPs, comprising a membrane comprising a phospholipid bilayer …on the external side, Para. [0011]); a first chimeric protein in the lipid delivery particle comprising (i) a first plasma membrane recruitment element; (ii) a heterologous payload; a lipid delivery particle ([h]uman-derived virus-like particles (heVLPs), comprising a membrane comprising a phospholipid bilayer …on the external side …for delivery of the cargo molecule to cells, Abstract), comprising: a lipid membrane on the external side; in the lipid delivery particle ([h]uman-derived virus-like particles (heVLPs), comprising a membrane comprising a phospholipid bilayer …on the external side …for delivery of the cargo molecule to cells, Abstract) comprising (i) a second plasma membrane recruitment element; comprising: (i) a plasma membrane recruitment element; (ii) a heterologous payload (heVLPs that contain cargo are produced and isolated can be loaded with additional biomolecule or chemical molecule cargo , Para. [0016]; and a human endogenous GAG protein, other plasma membrane recruitment domain, and/or biomolecule/chemical cargo disposed in the core of the heVLP on the inside of the membrane …wherein the biomolecule cargo may or may not be fused to a human-endogenous GAG or other plasma membrane recruitment domain, Para. [0008]). General Hospital fails to explicitly disclose one or more cleavable linkers, and (iv) a protease, wherein the one or more cleavable linkers are cleavable by the protease, wherein the one or more cleavable linkers are positioned such that cleavage by the protease releases the heterologous payload from the chimeric protein; and (iii) a cleavable linker; and (ii) a protease, wherein the cleavable linker is cleavable by the protease, wherein the cleavable linkers is positioned such that cleavage by the protease releases the heterologous payload from the first chimeric protein; and (iii) a cleavable linker; and (ii) a protease, wherein the second chimeric protein lacks a viral polymerase, wherein the cleavable linker is cleavable by the protease, wherein the cleavable linker is positioned such that cleavage by the protease releases the heterologous payload from the first chimeric protein; and (iii) a cleavable linker; and a second chimeric protein and (ii) a first protease; a chimeric protein for delivering a heterologous payload to a target cell, (iii) one or more protease cleavable linkers; and (iv) a first protease, wherein the one or more cleavable linkers are cleavable by the protease, wherein the one or more cleavable linkers are positioned such that cleavage by the protease releases the heterologous payload from the chimeric protein construct.

WO 2021/113772 A1 to Scribe Therapeutics Inc. (hereinafter, "Scribe") is in the field of particle delivery systems (Title) and teaches one or more cleavable linkers (the Gag polyprotein, the therapeutic payload, and a protease cleavage site, the order (5' to 3') may be Gag-cleavage site-therapeutic payload or it may be therapeutic payload-cleavage site-gag, Para. [0315]; a reference or CasX variant fusion protein can include

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

a CasX protein that is linked to an internally inserted heterologous amino acid or heterologous polypeptide (a heterologous amino acid sequence) via a linker polypeptide (e.g., one or more linker polypeptides, Para. [0240]), and (iv) a protease (heterologous protease capable of cleaving the cleavage sites, and a gag-transframe region-pol protease polyprotein, Para. [0017]), wherein the one or more cleavable linkers are cleavable by the protease (the Gag polyprotein, the therapeutic payload, and a protease cleavage site, the order (5' to 3') may be Gag-cleavage site-therapeutic payload or it may be therapeutic payload-cleavage site-gag, Para. [0315]; a reference or CasX variant fusion protein can include a CasX protein that is linked to an internally inserted heterologous amino acid or heterologous polypeptide (a heterologous amino acid sequence) via a linker polypeptide (e.g., one or more linker polypeptides, Para. [0240]), wherein the one or more cleavable linkers are positioned such that cleavage by the protease releases the heterologous payload from the chimeric protein (the Gag polyprotein, the therapeutic payload, and a protease cleavage site, the order (5' to 3') may be Gag-cleavage site-therapeutic payload or it may be therapeutic payload-cleavage site-gag, Para. [0315]; a reference or CasX variant fusion protein can include a CasX protein that is linked to an internally inserted heterologous amino acid or heterologous polypeptide (a heterologous amino acid sequence) via a linker polypeptide (e.g., one or more linker polypeptides, Para. [0240]; heterologous protease capable of cleaving the cleavage sites, and a gag-transframe region-pol protease polyprotein, Para. [0017]); and (iii) a cleavable linker; and (ii) a protease, wherein the cleavable linker is cleavable by the protease, wherein the cleavable linkers is positioned such that cleavage by the protease releases the heterologous payload from the first chimeric protein (the Gag polyprotein, the therapeutic payload, and a protease cleavage site, the order (5' to 3') may be Gag-cleavage site-therapeutic payload or it may be therapeutic payload-cleavage site-gag, Para. [0315]; a reference or CasX variant fusion protein can include a CasX protein that is linked to an internally inserted heterologous amino acid or heterologous polypeptide (a heterologous amino acid sequence) via a linker polypeptide (e.g., one or more linker polypeptides, Para. [0240]; heterologous protease capable of cleaving the cleavage sites, and a gag-transframe region-pol protease polyprotein, Para. [0017]); and (iii) a cleavable linker; and (ii) a protease, wherein the cleavable linker is cleavable by the protease (the Gag polyprotein, the therapeutic payload, and a protease cleavage site, the order (5' to 3') may be Gag-cleavage site-therapeutic payload or it may be therapeutic payload-cleavage site-gag, Para. [0315]; a reference or CasX variant fusion protein can include a CasX protein that is linked to an internally inserted heterologous amino acid or heterologous polypeptide (a heterologous amino acid sequence) via a linker polypeptide (e.g., one or more linker polypeptides, Para. [0240]; heterologous protease capable of cleaving the cleavage sites, and a gag-transframe region-pol protease polyprotein, Para. [0017]), wherein the cleavable linker is positioned such that cleavage by the protease releases the heterologous payload from the first chimeric protein (the Gag polyprotein, the therapeutic payload, and a protease cleavage site, the order (5' to 3') may be Gag-cleavage site-therapeutic payload or it may be therapeutic payload-cleavage site-gag, Para. [0315]; a reference or CasX variant fusion protein can include a CasX protein that is linked to an internally inserted heterologous amino acid or heterologous polypeptide (a heterologous amino acid sequence) via a linker polypeptide (e.g., one or more linker polypeptides, Para. [0240]; heterologous protease capable of cleaving the cleavage sites, and a gag-transframe region-pol protease polyprotein, Para. [0017]); and (iii) a cleavable linker; and a second chimeric protein and (ii) a first protease (the Gag polyprotein, the therapeutic payload, and a protease cleavage site, the order (5' to 3') may be Gag-cleavage site-therapeutic payload or it may be therapeutic payload-cleavage site-gag, Para. [0315]; a reference or CasX variant fusion protein can include a CasX protein that is linked to an internally inserted heterologous amino acid or heterologous polypeptide (a heterologous amino acid sequence) via a linker polypeptide (e.g., one or more linker polypeptides, Para. [0240]; heterologous protease capable of cleaving the cleavage sites, and a gag-transframe region-pol protease polyprotein, Para. [0017]); a chimeric protein for delivering a heterologous payload to a target cell, (iii) one or more protease cleavable linkers (the Gag polyprotein, the therapeutic payload, and a protease cleavage site, the order (5' to 3') may be Gag-cleavage site-therapeutic payload or it may be therapeutic payload-cleavage site-gag, Para. [0315]; a reference or CasX variant fusion protein can include a CasX protein that is linked to an internally inserted heterologous amino acid or heterologous polypeptide (a heterologous amino acid sequence) via a linker polypeptide (e.g., one or more linker polypeptides, Para. [0240]; heterologous protease capable of cleaving the cleavage sites, and a gag-transframe region-pol protease polyprotein, Para. [0017]); and (iv) a first protease, wherein the one or more cleavable linkers are cleavable by the protease, wherein the one or more cleavable linkers are positioned such that cleavage by the protease releases the heterologous payload from the chimeric protein construct (the Gag polyprotein, the therapeutic payload, and a protease cleavage site, the order (5' to 3') may be Gag-cleavage site-therapeutic payload or it may be therapeutic payload-cleavage site-gag, Para. [0315]; a reference or CasX variant fusion protein can include a CasX protein that is linked to an internally inserted heterologous amino acid or heterologous polypeptide (a heterologous amino acid sequence) via a linker polypeptide (e.g., one or

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

more linker polypeptides, Para. [0240]; heterologous protease capable of cleaving the cleavage sites, and a gag-transframe region-pol protease polyprotein, Para. [0017]).

It would have been obvious to one of ordinary skill in the art before the priority date to modify General Hospital with the teaching of Scribe for the purpose of targeted delivery of therapeutic cargoes with reduced off-target toxicity (Scribe, Para. [0003]).

The inventions listed in Groups I+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical features.

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.: **1-3, 134**

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.