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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
20 December 2018 (20.12.2018)

WIPO | PCT

(10) International Publication Number
WO 2018/232357 A1

(51) International Patent Classification:
A61K 9/51 (2006.01) *A61K 47/28* (2006.01)
A61K 31/7105 (2006.01) *A61K 47/54* (2017.01)
A61K 31/7088 (2006.01) *A61K 48/00* (2006.01)
A61K 47/14 (2017.01) *C12N 15/88* (2006.01)
A61K 47/24 (2006.01)

(21) International Application Number:
PCT/US2018/037922

(22) International Filing Date:
15 June 2018 (15.06.2018)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/520,530 15 June 2017 (15.06.2017) US
62/590,200 22 November 2017 (22.11.2017) US

(71) Applicant: **MODERNATX, INC.** [US/US]; 200 Technology Square, Cambridge, MA 02139 (US).

(72) Inventors: **HOGE, Stephen**; 320 Bent Street, Cambridge, MA 02141 (US). **SCHARITER, Joseph**; 207 Hammond Street, Waltham, MA 02451 (US). **BOWERMAN, Charles**; 745 South St, Waltham, MA 02453 (US). **SMITH, Michael, H.**; 25 Whiting Way, Needham, MA 02492 (US). **XIA, Yan**; 140 Lyman St., Apt. 5, Waltham, MA 02452 (US).

(74) Agent: **LOCKHART, Helen, C.** et al.; Wolf, Greenfield & Sacks, P.C., 600 Atlantic Avenue, Boston, MA 02210-2206 (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, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, 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, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, 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, LU, LV,

(54) Title: RNA FORMULATIONS

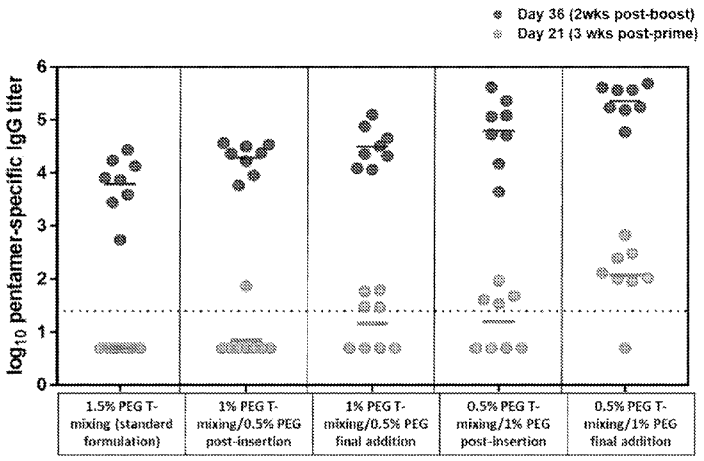

FIG. 15

(57) Abstract: This disclosure provides improved lipid-based compositions, including lipid nanoparticle compositions, and methods of use thereof for delivering agents in vivo including nucleic acids and proteins.

MC, 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).

Published:

— *with international search report (Art. 21(3))*
— *with sequence listing part of description (Rule 5.2(a))*

RNA FORMULATIONS

RELATED APPLICATIONS

This application claims the benefit under 35 U.S.C. § 119(e) of U.S. provisional application number 62/520,530, filed June 15, 2017 and U.S. provisional application number 62/590,200,, filed November 22, 2017, which are both incorporated by reference herein in their entirety.

FIELD OF INVENTION

The present embodiments relate generally to lipid nanoparticles, and more specifically, to lipid nanoparticles having a certain distribution of one or more components.

BACKGROUND

It is of great interest in the fields of therapeutics, diagnostics, reagents, and for biological assays to be able to control protein expression. Most methods rely upon regulation at the transcriptional level (e.g., from DNA to mRNA), but not at the translational level (e.g., from mRNA to protein). Although attempts have been made to control protein expression on the translational level, the low levels of translation, the immunogenicity, and other delivery issues have hampered the development of mRNA as a therapeutic.

There remains a need in the art to be able to design, synthesize, and deliver a nucleic acid, e.g., a ribonucleic acid (RNA) such as a messenger RNA (mRNA) encoding a peptide or polypeptide of interest inside a cell, whether in vitro, in vivo, in-situ, or ex vivo, so as to effect physiologic outcomes which are beneficial to the cell, tissue or organ and ultimately to an organism.

SUMMARY

Lipid nanoparticles having a certain distribution of one or more components, related compositions, and methods associated therewith are provided. The present disclosure is based, in part, on the discovery that the distribution of certain components within the lipid nanoparticles can influence and/or dictate physical (e.g., stability) and/or biological (e.g., efficacy, intracellular delivery, immunogenicity) properties of the lipid nanoparticles. Inventive lipid nanoparticles having a certain distribution of one or more components may

not suffer from one or more limitations of conventional particulate carriers, even though the inventive lipid nanoparticles may contain the same or similar molecules (e.g., at the molar ratios, at the same weight percentages) as the conventional particulate carrier. Compositions comprising inventive lipid nanoparticles may have advantageous biological and physical properties.

Methods for controlling the distribution of components capable of imparting beneficial properties to the lipid nanoparticle have also been discovered. In some cases, these methods may be readily applied to the formulation process using relatively simple techniques.

In one set of embodiments, compositions are provided. In one embodiment, a composition comprises lipid nanoparticles (LNPs) that comprise an ionizable lipid, a PEG lipid, and inaccessible mRNA, and a relatively small amount of accessible mRNA. In such cases, no more than about 50% (e.g., no more than about 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, 5%, 3%, or 1%) of mRNA in the composition is accessible mRNA and the half-life time of the PEG lipid in serum is relatively short, e.g., less than or equal to about 3.0 hours (e.g, less than or equal to about 2.75, 2.5, 2.25, 2.0, 175, 1.5, 1.25, 1.0, 0.75, 0.5, or 0.25 hours). In some cases, no more than 30% of mRNA in the composition is accessible mRNA. In certain cases, no more than 5% of mRNA in the composition is accessible mRNA. In some cases, the quantitative value of the amount of accessible mRNA is generated using an ion-exchange chromatography (IEX) assay and/or is not generated using a Ribogreen assay. In some embodiments, the lipid nanoparticles may also comprise a structural lipid and/or a neutral lipid. In some cases, the ionizable lipid is an ionizable amino lipid.

In another embodiment, a composition comprises lipid nanoparticles (LNPs) comprising an ionizable lipid, a PEG lipid, and mRNA and having an exterior region and one or more interior regions. The majority of the mRNA is positioned in the one or more interior regions and the majority of the PEG lipid is positioned within the exterior region. For instance, at least about 60% (e.g., at least about 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%) of the mRNA is positioned within the one or more interior regions and at least about 60% (e.g., at least about 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%) of the PEG lipid is positioned within the exterior region. In some embodiments, the lipid nanoparticles may also comprise a structural lipid and/or a neutral lipid. In some cases, the ionizable lipid

is an ionizable amino lipid. In some embodiments, the composition further comprises a continuous phase. In some such cases, the exterior region is in direct contact with the continuous phase.

In one embodiment, a composition comprises lipid nanoparticles (LNPs) comprising an ionizable lipid, a PEG lipid, and mRNA. The majority of the PEG lipid is surface accessible and the majority of the mRNA in the composition is inaccessible. For instance, at least about 50% (e.g., at least about 55%, 60% 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%) of the PEG lipid in the lipid nanoparticles is surface accessible and no more than about 50% of mRNA (e.g., no more than about 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, 5%, 3%, 1%, or 0%) in the composition is accessible mRNA. In some embodiments, the lipid nanoparticles may also comprise a structural lipid and/or a neutral lipid. In some cases, the ionizable lipid is an ionizable amino lipid.

In another embodiment, a composition comprises lipid nanoparticles (LNPs) comprising an ionizable lipid, a PEG lipid, and mRNA. The surface polarity of the lipid nanoparticles is relatively low (e.g., lower than a threshold) and the half-life time of the PEG lipid is relatively short. For instance, the half-life time of the PEG lipid in serum is less than or equal to about 3.0 hours (e.g., less than or equal to about 2.75, 2.5, 2.25, 2.0, 175, 1.5, 1.25, 1.0, 0.75, 0.5, or 0.25 hours) and the normalized general polarization of laurdan in the lipid nanoparticles is greater than or equal to about 0.5 (e.g., greater than or equal to about 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, or 0.85). In some cases, the normalized general polarization of laurdan in the lipid nanoparticles is greater than or equal to about 0.5 and less than or equal to about 0.9. In some embodiments, the lipid nanoparticles may also comprise a structural lipid and/or a neutral lipid. In some cases, the ionizable lipid is an ionizable amino lipid.

In one embodiment, a composition comprises lipid nanoparticles (LNPs) comprising an ionizable lipid, a PEG lipid, and mRNA. The surface polarity of the lipid nanoparticles is less than a threshold and the half-life time of the PEG lipid is relatively short. For instance, the half-life time of the PEG lipid in serum is less than or equal to about 3.0 hours (e.g., less than or equal to about 2.75, 2.5, 2.25, 2.0, 175, 1.5, 1.25, 1.0, 0.75, 0.5, or 0.25 hours). In some cases, the surface polarity of the lipid nanoparticles is less than that of a comparative lipid nanoparticle. In some cases, the comparative lipid nanoparticles formed via a nanoprecipitation reaction, wherein the comparative lipid nanoparticles comprise the same ionizable lipid, PEG lipid, and mRNA as the lipid nanoparticles, and wherein greater than

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.