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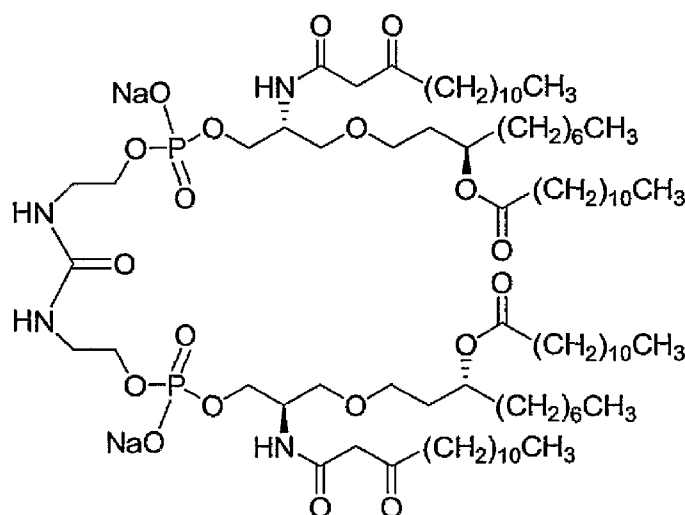
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(54) Title: IMMUNOLOGICAL ADJUVANT FORMULATIONS COMPRISING TLR4 AGONIST E6020

FIG. 1



(57) Abstract: Disclosed are immunologically active metabolizable lipid nanoparticle in water compositions comprising a non-microbial-lipopolysaccharide-derived TLR-4 receptor agonist in a metabolizable lipid nanoparticle in water composition and vaccine compositions made using said compositions for the prevention and treatment of disease. Methods of preparation, vaccine and treatment kits comprising the adjuvants, and uses of vaccines and vaccine kits comprising the adjuvants are also disclosed.

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IMMUNOLOGICAL ADJUVANT FORMULATIONS COMPRISING TLR4 AGONIST E6020

Technical Field

[0001] The present disclosure relates to immunologically active metabolizable lipid nanoparticle in water compositions comprising a non-microbial-lipopolysaccharide-derived
5 TLR-4 receptor agonist in a metabolizable lipid and immunoactive saponin nanoparticle, and kits of parts and vaccine compositions made using said compositions for the prevention and treatment of diseases.

Background

10 [0002] There exists a range of potential adjuvant formulations for the effective display of an antigen and stimulation of immune response, almost all of which are in the form of a sterile injectable liquid, but at the nanoscopic level vary greatly in precise structure. Perhaps the simplest adjuvant in wide use today is aluminium salts. However, aluminium salts alone may not compatible with all antigens. Binding to aluminium salts may cause steric alterations that
15 modify one or more epitopes, effectively removing the functionality of the antigen to sensitize an immune system to a target disease. Additionally, aluminium salts-induced immune responses alone may not always be sufficient to induce high levels of T-cell responses and/or may be unable to induce sufficient production of protective antibodies.

[0003] The incorporation of a TLR-4 receptor agonist into an adjuvant formulation for use in
20 vaccines is of great benefit to the efficacy of the resulting vaccine. TLR-4 receptor agonists enhance antibody and T-cell responses to an antigen when associated with some adjuvants (van Maele et al 2019, Leroux-Roels et al 2016).

[0004] Perhaps the most used TLR-4 receptor agonist to date is 3DMPL. However, 3DMPL is derived from *Salmonella* so must undergo stringent purification methods in an effort to
25 reduce the many other molecular species present. Advantageously, E6020 is a synthetic molecule, so presents a more attractive option for the vaccine community in terms of consistency of product, ease of use, quality control and consistency of results in the clinic. E6020 is a TLR-4 receptor agonist that is highly unusual in the art in that it is not a microbial lipopolysaccharide nor derived from a microbial lipopolysaccharide. E6020 has a far simpler
30 production process than microbial-derived TLR-4 receptor agonists (such as 3DMPL), which firstly involve the long and difficult process of growing suitable bacteria to high population levels without contamination by other organisms, then extracting and purifying to

pharmaceutical grade the TLR-4 agonist from a massive population of chemically related molecules. In contrast, the chemical synthetic route of production and quality control of E6020 is more easily scalable and therefore much more suitable for treating large populations at relatively short notice, such as during or in preparation for a large outbreak or pandemic.

5 Additionally, E6020 has distinct properties to other TLR-4 agonists, such as its solubility in both water and ethanol, permitting formulations and methods of formulating TLR-4 receptor agonist-containing adjuvants and vaccines that are inappropriate or suboptimal for other TLR-4 receptor agonists such as 3DMPL.

10 **[0005]** Saponins (including but not limited to QuilA or QS21) also have stimulatory immune-modulator effects, though the mechanism of action is quite different to that of TLR-4 receptor agonists. As such, it is an object of the current disclosure to provide adjuvants comprising both E6020 and one or more immune-stimulatory saponins to synergistically provide a substantial boost to the immune-stimulation effect of an adjuvant.

15 **Summary**

[0006] Compositions of the present application comprise immunologically active metabolizable lipid-based nanoparticle composition comprising 0.004 mg/mL - 0.1 mg/mL E6020, 0.04-200 mg/mL metabolizable lipid, 0.02 mg/mL – 0.4 mg/mL saponin and 0.02 mg/mL – 50 mg/mL sterol, wherein the ratio (wt/wt) of sterol to saponin is between 1:1 to 100:1
20 and the average (mean) nanoparticle diameter of the lipid-based nanoparticles is less than 200nm, and wherein said nanoparticles are present in water or an aqueous buffer solution. Average (mean) nanoparticle diameter may be determined, for example, by dynamic light scattering (“DLS”). The immunologically active metabolizable lipid-based nanoparticles described herein are able to activate immune responses to enhance the subsequent
25 acquisition of immunity to the condition associated with the antigen of interest. The current disclosure provides a range of sizes and chemical compositions for immunologically active metabolizable lipid-based nanoparticles comprising E6020, appropriate for incorporation of a range of antigens or antigen-carrying molecules of different sizes and chemical properties.

30 **Description of drawings and figures**

[0007] FIG. 1 shows the chemical structure of E6020.

[0008] FIG. 2 shows a pre-clinical evaluation of LEQ-adjuvanted influenza (SH5N1 + LEQ) immune responses, as measured by HAI titer, in comparison to unadjuvanted antigen (SH5N1 (2 µg HA) alone, to a squalene-in-water emulsion benchmark adjuvant (SH5N1 + O/W emulsion) and to LQ (SH5N1 + LQ).

[0009] FIG. 3A and FIG. 3B show a pre-clinical evaluation of the breadth of LEQ-adjuvanted influenza (SH5N1 + LEQ) immune activation 35 days after delivery (vaccination), as measured by HAI titer to the homologous strain (A/tk/Turkey/1/05 wt Clade 2.2.1, FIG. 3A) or to a heterologous strain (A/Anhui/1/05 Clade 2.3.4, FIG. 3B), in comparison to unadjuvanted antigen alone (SH5N1), to a squalene-in-water emulsion benchmark adjuvant (SH5N1 + O/W emulsion) and to LQ (SH5N1 + LQ).

[0010] FIG. 4A and FIG. 4B show a pre-clinical evaluation of immune CD4+ and CD8+ T cell responses to LEQ-adjuvanted split H5N1 vaccine (SH5N1+LEQ) in comparison to unadjuvanted antigen alone (SH5N1), to a benchmark squalene lipid-in-water emulsion (SH5N1+O/W emulsion and to LQ (SH5N1 + LQ). The results for immune CD4+ and CD8+ T cell responses are in FIG. 4A and FIG. 4B, respectively.

[0011] FIG. 5 shows a pre-clinical evaluation of antibody responses 3 weeks post LEQ-adjuvanted (Ag + LEQ) and SEQ-adjuvanted (Ag + SEQ) COVID-19 vaccine in comparison to unadjuvanted antigen alone (Ag + Excipient), to a benchmark aluminium salt (AlOH) adjuvanted (Ag + AlOH), to LQ adjuvanted (Ag + LQ) and to SQ adjuvanted (Ag + SQ) COVID-19 vaccine.

[0012] FIG. 6 shows a pre-clinical evaluation of immune CD4+ T cell responses to LEQ-adjuvanted (Ag + LEQ) and SEQ-adjuvanted (Ag + SEQ) COVID-19 vaccine in comparison to unadjuvanted antigen alone in PBS buffer (Ag + PBS), to a benchmark aluminium salts (AlOH) adjuvanted (Ag + AlOH), to LQ adjuvanted (Ag + LQ) and to SQ adjuvanted (Ag + SQ) COVID-19 vaccine.

[0013] FIG. 7 shows a pre-clinical evaluation of immune CD8+ T cell responses to LEQ-adjuvanted (Ag+LEQ) and SEQ-adjuvanted (Ag+SEQ) COVID-19 vaccine in comparison to unadjuvanted antigen alone in PBS buffer (Ag+PBS) and to a benchmark aluminium salts (AlOH) adjuvanted (Ag + AlOH), to LQ adjuvanted (Ag + LQ) and to SQ adjuvanted (Ag + SQ) COVID-19 vaccine.

[0014] FIG. 8 shows a pre-clinical evaluation of SEQ- and LEQ-adjuvanted influenza recombinant HA1 H3N2 (2 µg HA), measured by HAI titer, in comparison to unadjuvanted antigen rH3N2 (2 µg HA), or to antigen formulated with SQ adjuvant (rH3N2 + SQ) and to LQ adjuvant (rH3N2 + LQ).

[0015] FIG. 9A and FIG. 9B show a pre-clinical evaluation of immune CD4+ and CD8+ T cell

responses to SEQ- and LEQ-adjuvanted influenza recombinant HA1 H3N2 (2 µg HA) in comparison to unadjuvanted antigen rH3N2 (2 µg HA), or to antigen formulated with SQ adjuvant (rH3N2 + SQ) and to LQ adjuvant (rH3N2 + LQ). The results for immune CD4+ and CD8+ T cell responses are presented in FIG. 9A and FIG. 9B, respectively.

5 **[0016]** FIG. 10 shows a pre-clinical evaluation of SEQ-adjuvanted (Ag+SEQ) COVID-19 vaccine with antibody responses measured by surrogate virus neutralization, in comparison to unadjuvanted antigen alone in PBS buffer (Antigen + Excipient), to a benchmark squalene lipid-in-water emulsion (Antigen +O/W emulsion) and to SQ (Antigen +SQ).

10 **[0017]** FIG. 11A and FIG. 11B show a pre-clinical evaluation of immune CD4+ and CD8+ T cell responses to SEQ-adjuvanted COVID-19 vaccine (Antigen +SEQ) in comparison to unadjuvanted antigen alone in PBS buffer (Antigen + Excipient), to a benchmark squalene lipid-in-water emulsion (Antigen +O/W emulsion) and to SQ (Antigen +SQ). The results for immune CD4+ and CD8+ T cell responses are presented in FIG. 11A and FIG. 11B, respectively.

15 **[0018]** FIG. 12 shows a pre-clinical evaluation of LEQ-adjuvanted (Antigen +LEQ) COVID-19 vaccine with antibody responses measured by surrogate virus neutralization, in comparison to unadjuvanted antigen alone in PBS buffer (Antigen + Excipient), to LE (Antigen +LE) and to LQ (Antigen +LQ).

20 **[0019]** FIG. 13A and FIG. 13B show a pre-clinical evaluation of immune CD4+ and CD8+ T cell responses to LEQ-adjuvanted COVID-19 vaccine (Antigen +LEQ) in comparison to unadjuvanted antigen alone in PBS buffer (Antigen + Excipient), to LE (Antigen +LE) and to LQ (Antigen +LQ). The results for immune CD4+ and CD8+ T cell responses are presented in FIG. 13A and FIG. 13B, respectively.

Incorporation by reference

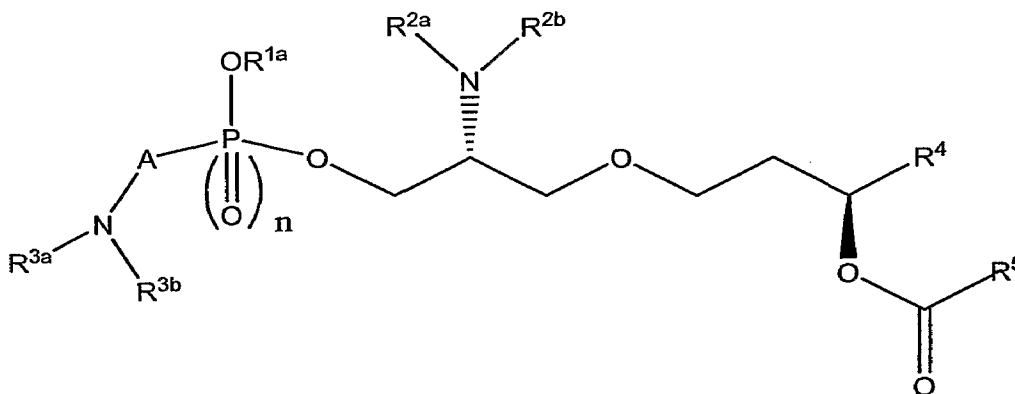
25 **[0020]** All publications, patents, and patent applications referred to herein (including, but not limited to WO2007005583A1) are incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. In the event of a conflict between a term herein and a term in an incorporated reference, the term herein prevails and controls.

30

Detailed Description

[0021] Immunologically active metabolizable lipid nanoparticle in water compositions and vaccine compositions.

[0022] Adjuvants or vaccine compositions described herein contain at least one TLR-4 receptor agonist of formula (I):



5

Formula (I)

wherein:

A is $-(CH_2)_x-O-$ or a covalent bond;

n is 0 or 1;

10 x is 1-6;

R^{1a} is hydrogen, a C_1 - C_6 alkyl group, a C_3 - C_6 alkenyl group, a C_3 - C_6 alkynyl group, or a phosphite oxygen protecting group or a phosphate oxygen protecting group;

one of R^{2a} and R^{2b} is H and the other is a monovalent nitrogen protecting group;

or R^{2a} and R^{2b} taken together are a divalent nitrogen protecting group;

15 when A is $-(CH_2)_x-O-$, one of R^{3a} and R^{3b} is H and the other is a monovalent nitrogen protecting group, or R^{3a} and R^{3b} taken together are a divalent nitrogen protecting group;

when A is a covalent bond, R^{3a} and R^{3b} are a C_1 - C_6 alkyl group, or R^{3a} and R^{3b} taken together are $-(CH_2)_4-$, $-(CH_2)S-$, or $-(CH_2)_2O(CH_2)_2-$;

R^4 is a C_5 - C_{12} alkyl group or a C_5 - C_{12} alkenyl group; and

R⁵ is a C₅-C₁₅ alkyl group or a C₅-C₁₅ alkenyl group;

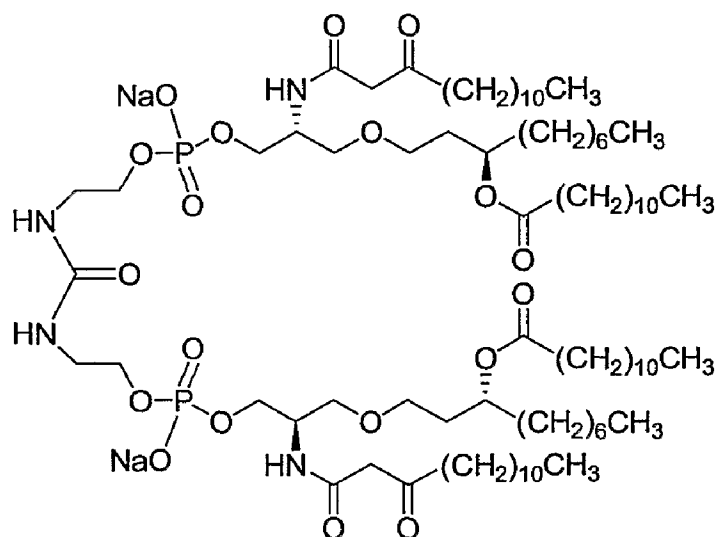
or a salt thereof.

[0023] For the purpose of the disclosure, an immunologically active metabolizable lipid nanoparticle in water composition is also referred to as an adjuvant or adjuvant composition.

[0024] TLR-4 agonists according to Formula 1 herein contain asymmetric carbon atoms and hence can exist as stereoisomers, both enantiomers and diastereomers. One of ordinary skill in the art will recognize that the inventive method may be adapted to the preparation of any of all possible stereoisomers of TLR-4 agonists according to Formula 1 herein. One of ordinary skill in the art will be able to adapt the methods for synthesizing TLR-4 agonists according to Formula 1 herein as taught in WO2007005583A1, which is incorporated herein in its entirety, to produce any particular stereoisomer species.

[0025] In some embodiments, the TLR-4 receptor agonist is E6020 or any salt thereof.

E6020 can be represented by the formula:



[0026] E6020 is a potent TLR-4 receptor agonist, helping to activate or enhance an immune response. Thus the compound is useful as an immunological adjuvant when co-administered with antigens such as vaccines for bacterial and viral diseases, or for immunotherapy treatment of cancer. For example, E6020 may be used in combination with any suitable antigen or vaccine component, e.g., an antigenic agent selected from the group consisting of

antigens from pathogenic and non-pathogenic organisms, viruses, and fungi. As a further example, E6020 may be used in combination with proteins, peptides, antigens and vaccines which are pharmacologically active for disease states and conditions such as a influenza, SARS-CoV-2, SARS-CoV-1, rhinovirus, malaria, tuberculosis, HIV/AIDS, leishmaniasis, Group A streptococcus, respiratory syncytial virus (RSV), Middle East Respiratory Syndrome virus (MERS), hepatitis B, varicella zoster, coronavirus or human papilloma virus infection, or any combination thereof. In certain embodiments, E6020 and the antigen are each present in an amount effective to elicit an immune response when administered to an animal, mammal, domesticated animal, farm animal, human, embryo, or ovum vaccinated therewith. In some aspects the E6020 may be present in the immunologically active metabolizable lipid nanoparticle in water composition in the range 0.001 mg/mL to 1.0 mg/mL, 0.002 mg/mL to 0.5 mg/mL, 0.004 mg/mL to 0.1 mg/mL, 0.01 mg/mL to 0.09 mg/mL, 0.05 mg/mL to 0.08 mg/mL, or 0.01 mg/mL to 0.08 mg/mL. In some aspects, the E6020 may be present in the immunologically active metabolizable lipid nanoparticle in water composition suitable for administration to humans at a concentration range of 0.004 to 0.04 mg/mL. In some aspects, the E6020 may be present in the immunologically active metabolizable lipid nanoparticle in water composition formulated with antigen suitable for administration to humans such that the E6020 is present in the range of 1 to 10 µg of E6020 per dose.

[0027] In other embodiments, the adjuvant or vaccine compositions may comprise a sodium salt, a lithium salt, a hydrochloride salt, a sulfate salt, an acetate salt, a potassium salt, a calcium salt, a citrate salt, a nitrate salt, an aluminium salt, a chloride salt or any mixture thereof of E6020.

[0028] For purposes of this application, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 75th Ed., inside cover, and specific functional groups are generally defined as described therein. Additionally, general principles of organic chemistry, as well as specific functional moieties and reactivity, are described in "Organic Chemistry", Thomas Sorrell, University Science Books, Sausalito: 1999, the entire contents of which are incorporated herein by reference. Furthermore, it will be appreciated by one of ordinary skill in the art that synthetic methods of preparation of E6020, as described in WO2007005583A1 incorporated in its entirety by reference herein, utilize a variety of protecting groups. The term "protecting group", as used herein, means that a particular functional moiety, e.g., O, S, P, or N₅ is temporarily blocked so that a reaction can be carried out selectively at another reactive site in a multifunctional compound. In some aspects, a protecting group reacts selectively in good yield to give a protected substrate that is stable to the projected reactions; the protecting group

must be selectively removed in good yield by readily available, preferably nontoxic reagents that do not attack the other functional groups; the protecting group forms an easily separable derivative (more preferably without the generation of new stereogenic centers); and the protecting group has a minimum of additional functionality to avoid further sites of reaction.

5 Oxygen, sulfur, nitrogen, phosphorous, and carbon protecting groups may be utilized.

[0029] For example, in some aspects of the current disclosure, certain exemplary oxygen protecting groups may be utilized, including, but not limited to methyl ethers, substituted methyl ethers (e.g., MOM (methoxymethyl ether), MTM (methylthiomethyl ether), BOM (benzyloxymethyl ether), PMBM (p-methoxybenzyloxymethyl ether), to name a few),
10 substituted ethyl ethers, substituted benzyl ethers, silyl ethers (e.g., TMS (trimethylsilyl ether), TES (triethylsilyl ether), TIPS (triisopropylsilyl ether), TBDMS (t-butyl dimethylsilyl ether), tribenzyl silyl ether, TBDPS (t-butyl diphenyl silyl ether)), esters (e.g., formate, acetate, benzoate (Bz), trifluoroacetate, dichloroacetate, to name a few), carbonates, cyclic acetals and ketals. Protecting groups for phosphite oxygens and phosphate oxygens include, for
15 example, alkyl phosphates/phosphites such as: methyl, ethyl; isopropyl; t-butyl; cyclohexyl; 1-adamantyl; and 2-trimethylsilylprop-2-enyl; alkenyl phosphates/phosphites such as ethenyl and allyl; 2-substituted ethyl phosphates/phosphites such as: 2-cyanoethyl, 2-cyano-1,1-dimethylethyl, 2-(trimethylsilyl)ethyl, 2-(4-nitrophenyl)ethyl, 2-(phenylsulfonyl)ethyl, and 2-(benzylsulfonyl)ethyl; haloethyl phosphates/phosphites such as: 2,2,2-trichloroethyl, 2,2,2-trichloro-1,1-dimethylethyl, 2,2,2-tribromoethyl, 2,3-dibromopropyl, benzyl
20 phosphates/phosphites such as: benzyl; 4-nitrobenzyl, 4-chlorobenzyl; 1-oxido-4-methoxy-2-picolyl, fluorenyl-9-methyl, 5-benzisoxazolylmethylene, $(C_6H_5)_2C=$; and phenyl phosphates/phosphites such as: phenyl; 4-nitrophenyl, and 4-chlorophenyl.; and silyl phosphates/phosphites such as: trimethylsilyl.

25 **[0030]** In some aspects of the current disclosure, nitrogen protecting groups are utilized. These nitrogen protecting groups may be monovalent or divalent protecting groups such as, but are not limited to, carbamates (including methyl, ethyl and substituted ethyl carbamates (e.g., Troc), to name a few) amides, cyclic imide derivatives, N-Alkyl and N-Aryl amines, imine derivatives, and enamine derivatives, to name a few. Amine protecting groups such as Cbz,
30 Boc, Fmoc, TROC, TMS-ethylcarbonyl, cyanoethylcarbonyl, allyloxycarbonyl or $(C_6H_5)_2C=$ (diphenylmethylene) may also be mentioned. Certain other exemplary protecting groups are detailed herein, however, it will be appreciated that the present disclosure is not intended to be limited to these protecting groups; rather, a variety of additional equivalent protecting groups can be readily identified using the above criteria and utilized in the present disclosure.
35 Additionally, a variety of protecting groups are described in "Protective Groups in Organic

Synthesis" Third Ed. Greene, T. W. and Wuts, P.G., Eds., John Wiley & Sons, New York: 1999, the entire contents of which are hereby incorporated by reference.

[0031] It is understood that the compounds, as described herein, may be substituted with any number of substituents or functional moieties, in general, the term "substituted" whether preceded by the term "optionally" or not, and substituents contained in formulas of this disclosure, refer to the replacement of hydrogen radicals in a given structure with the radical of a specified substituent. When more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the substituent may be either the same or different at every position. As used herein, the term "substituted" is contemplated to include all permissible substituents of organic compounds, hi a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and non-aromatic, carbon and heteroatom substituents of organic compounds. For purposes of this disclosure, heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valencies of the heteroatoms. Furthermore, this invention is not intended to be limited in any manner by the permissible substituents of organic compounds. Combinations of substituents and variables envisioned by this invention are preferably those that result in the formation of stable compounds useful in the treatment and prevention, for example of disorders, as described generally above. Examples of substituents include, but are not limited to, halo substituents, e.g. F; Cl; Br; or I; a hydroxyl group; a C₁-C₆ alkoxy group, e.g. -OCH₃, -OCH₂CH₃, or -OCH(CH₃)₂; a C_j-C₆ haloalkyl group, e.g., -CF₃; -CH₂CF₃; or -CHCl₂; C_j-C₆ alkylthio; amino; mono and dialkyl amino groups; -NO₂; -CN; a sulfate group, and the like.

[0032] Many of the compositions described herein can be assimilated as a dilute solution, and as such, the person skilled in the art will appreciate that when describing the concentration ranges of the components, the "% wt/wt" and "mg/mL" can be considered to have the following conversions: 1% (wt/wt) = 10 mg/mL; 1 mg/mL = 0.1% (wt/wt).

[0033] The term "stable", as used herein, preferably refers to compounds which possess stability sufficient to allow manufacture and which maintain the integrity of the compound for a sufficient period of time to be detected and preferably for a sufficient period of time to be useful for the purposes detailed herein.

[0034] As used herein, the term "alkyl" includes straight and branched alkyl groups. An analogous convention applies to other generic terms such as "alkenyl", "alkynyl" and the like. Furthermore, as used herein, the terms "alkyl", "alkenyl", "alkynyl" and the like encompass both substituted and unsubstituted groups. In certain embodiments, as used herein, "lower

alkyl" is used to indicate those alkyl groups (cyclic, acyclic, substituted, unsubstituted, branched or unbranched) having 1-6 carbon atoms. In other embodiments, C₁₋₄, C₂₋₄, C₁₋₃ or C₃₋₆ alkyl or alkenyl are used.

[0035] In certain embodiments, the alkyl, alkenyl and alkynyl groups employed in the disclosure contain 1-20 aliphatic carbon atoms for alkyl groups and 2-20 carbon atoms for alkenyl and alkynyl groups. In certain other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the disclosure contain 1-15 aliphatic carbon atoms. In yet other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the disclosure contain 1-8 aliphatic carbon atoms. In still other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the disclosure contain 1-6 aliphatic carbon atoms. In yet other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the disclosure contain 1-4 carbon atoms. Illustrative aliphatic groups thus include, but are not limited to, for example, methyl, ethyl, n-propyl, isopropyl, allyl, n-butyl, sec-butyl, isobutyl, tert-butyl, n-pentyl, sec-pentyl, isopentyl, tert-pentyl, n-hexyl, sec-hexyl, moieties and the like, which again, may bear one or more substituents. Alkenyl groups include, but are not limited to, for example, ethenyl, propenyl, butenyl, 1-methyl-2-buten-1-yl, and the like. Representative alkynyl groups include, but are not limited to, ethynyl, 2-propynyl (propargyl), 1-propynyl and the like.

[0036] The term "alicyclic", as used herein, refers to compounds which combine the properties of aliphatic and cyclic compounds and include but are not limited to cyclic, or polycyclic aliphatic hydrocarbons and bridged cycloalkyl compounds, which are optionally substituted with one or more functional groups. As will be appreciated by one of ordinary skill in the art, "alicyclic" is intended herein to include, but is not limited to, cycloalkyl, cycloalkenyl, and cycloalkynyl moieties, which are optionally substituted with one or more functional groups. Illustrative alicyclic groups thus include, but are not limited to, for example, cyclopropyl, -CH₂-cyclopropyl, cyclobutyl, -CH₂-cyclobutyl, cyclopentyl, -CH₂-cyclopentyl-n, cyclohexyl, -CH₂-cyclohexyl, cyclohexenylethyl, cyclohexanylethyl, norborbyl moieties and the like, which again, may bear one or more substituents.

[0037] The term "alkoxy" (or "alkyloxy"), or "thioalkyl" as used herein refers to an alkyl or cycloalkyl group, as previously defined, attached to the parent molecular moiety through an oxygen atom or through a sulfur atom. In certain embodiments, the alkyl or cycloalkyl group contains 1-20 aliphatic or alicyclic carbon atoms. In certain other embodiments, the alkyl or cycloalkyl group contains 1-10 aliphatic or alicyclic carbon atoms. In yet other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the disclosure contain 1-8 aliphatic or alicyclic carbon atoms. In still other embodiments, the alkyl group contains 1-6 aliphatic or alicyclic carbon atoms. In yet other embodiments, the alkyl group contains 1-4 aliphatic or

alicyclic carbon atoms. Examples of alkoxy, include but are not limited to, methoxy, ethoxy, propoxy, isopropoxy, n-butoxy, tert-butoxy, neopentoxy and n-hexoxy. Examples of thioalkyl include, but are not limited to, methylthio, ethylthio, propylthio, isopropylthio, n-butylthio, and the like.

5 **[0038]** The term "alkylamino" refers to a group having the structure -NHR' wherein R' is alkyl or cycloalkyl, as defined herein. The term "dialkylamino" refers to a group having the structure -N(R')₂, wherein each occurrence of R' is independently alkyl or cycloalkyl, as defined herein.

10 **[0039]** The term "aminoalkyl" refers to a group having the structure NH₂R', wherein R' is alkyl or cycloalkyl, as defined herein. In certain embodiments, the alkyl group contains 1-20 aliphatic or alicyclic carbon atoms. In certain other embodiments, the alkyl or cycloalkyl group contains 1-10 aliphatic or alicyclic carbon atoms. In yet other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the disclosure contain 1-8 aliphatic or alicyclic carbon atoms. In still other embodiments, the alkyl or cycloalkyl group contains 1-6 aliphatic or alicyclic carbon atoms. In yet other embodiments, the alkyl or cycloalkyl group contains 1-4 aliphatic or alicyclic carbon atoms. Examples of alkylamino include, but are not limited to, methylamino, ethylamino, iso-propylamino and the like.

15 **[0040]** In general, the terms "aryl" and "heteroaryl", as used herein, refer to stable mono- or polycyclic, heterocyclic, polycyclic, and polyheterocyclic unsaturated moieties having preferably 3-14 carbon atoms, each of which may be substituted or unsubstituted. It will also be appreciated that aryl and heteroaryl moieties, as defined herein may be attached via an alkyl or heteroalkyl moiety and thus also include -(alkyl)aryl, -(heteroalkyl)aryl, - (heteroalkyl)aryl, and - (heteroalkyl)heteroaryl moieties. Thus, as used herein, the phrases "aryl or heteroaryl" and "aryl, heteroaryl, -(alkyl)aryl, -(heteroalkyl)aryl, -(heteroalkyl)aryl, and - (heteroalkyl)heteroaryl" are interchangeable. Substituents include, but are not limited to, any of the previously mentioned substituents, i.e., the substituents recited for aliphatic moieties, or for other moieties as disclosed herein, resulting in the formation of a stable compound. In certain embodiments of the present disclosure, "aryl" refers to a mono- or bicyclic carbocyclic ring system having one or two aromatic rings including, but not limited to, phenyl, naphthyl, tetrahydronaphthyl, indanyl, indenyl and the like. In certain embodiments of the present disclosure, the term "heteroaryl", as used herein, refers to a cyclic aromatic radical having from five to ten ring atoms of which one ring atom is selected from S, O and N; zero, one or two ring atoms are additional heteroatoms independently selected from S, O and N; and the remaining ring atoms are carbon, the radical being joined to the rest of the molecule via any of the ring atoms, such as, for example, pyridyl, pyrazinyl, pyrimidinyl, pyrrolyl, pyrazolyl, imidazolyl, thiazolyl, oxazolyl, isooxazolyl, thiadiazolyl, oxadiazolyl, thiophenyl, furanyl,

quinolinyl, isoquinolinyl, and the like.

[0041] It will be appreciated that aryl and heteroaryl groups (including bicyclic aryl groups) can be unsubstituted or substituted, wherein substitution includes replacement of one or more of the hydrogen atoms thereon independently with any one or more of the substituents generally described above. Additional examples of generally applicable substituents are illustrated by the specific embodiments shown in the Examples that are described herein.

[0042] The term "cycloalkyl", as used herein, refers specifically to groups having three to seven, preferably three to ten carbon atoms. Suitable cycloalkyls include, but are not limited to cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl and the like, which, as in the case of other alicyclic, heteroalicyclic or heterocyclic moieties, may optionally be substituted with one or more of the substituents generally described above. An analogous convention applies to other generic terms such as "cycloalkenyl", "cycloalkynyl" and the like. Additionally, it will be appreciated that any of the alicyclic or heteroalicyclic moieties described above and herein may comprise an aryl or heteroaryl moiety fused thereto. Additional examples of generally applicable substituents are illustrated by the specific embodiments shown in the Examples that are described herein.

[0043] The term "heteroaliphatic", as used herein, refers to aliphatic moieties in which one or more carbon atoms in the main chain have been substituted with a heteroatom. Thus, a heteroaliphatic group refers to an aliphatic chain which contains one or more oxygen, sulfur, nitrogen, phosphorus or silicon atoms, e.g., in place of carbon atoms. Heteroaliphatic moieties may be branched or linear unbranched. In certain embodiments, heteroaliphatic moieties are substituted by independent replacement of one or more of the hydrogen atoms thereon with one or more of the substituents generally described above. Additional examples of generally applicable substituents are illustrated by the specific embodiments shown in the Examples that are described herein.

[0044] The term "heteroalicyclic", as used herein, refers to compounds which combine the properties of heteroaliphatic and cyclic compounds and include but are not limited to saturated and unsaturated mono- or polycyclic heterocycles such as morpholino, pyrrolidinyl, furanyl, thiofuranyl, pyrrolyl etc., which are optionally substituted with one or more functional groups, as defined herein.

[0045] Additionally, it will be appreciated that any of the alicyclic or heteroalicyclic moieties described above and herein may comprise an aryl or heteroaryl moiety fused thereto. Additional examples of generally applicable substituents are illustrated by the specific embodiments shown in the Examples that are described herein.

[0046] The terms "halo" and "halogen" as used herein refer to an atom selected from fluorine,

chlorine, bromine and iodine.

[0047] The term “haloalkyl” denotes an alkyl group, as defined above, having one, two, or three halogen atoms attached thereto and is exemplified by such groups as chloromethyl, bromoethyl, trifluoromethyl, and the like.

5 **[0048]** The term “heterocycloalkyl” or “heterocycle”, as used herein, refers to a non- aromatic 5-, 6- or 7- membered ring or a polycyclic group, including, but not limited to a bi- or tri-cyclic group comprising fused six-membered rings having between one and three heteroatoms independently selected from oxygen, sulfur and nitrogen, wherein (i) each 5-membered ring has 0 to 1 double bonds and each 6-membered ring has 0 to 2 double bonds, (ii) the nitrogen
10 and sulfur heteroatoms may be optionally be oxidized, (iii) the nitrogen heteroatom may optionally be quaternized, and (iv) any of the above heterocyclic rings may be fused to a substituted or unsubstituted aryl or heteroaryl ring. Representative heterocycles include, but are not limited to, pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, and
15 tetrahydrofuryl. In certain embodiments, a “substituted heterocycloalkyl or heterocycle” group is utilized and as used herein, refers to a heterocycloalkyl or heterocycle group, as defined above, substituted by the independent replacement of one or more of the hydrogen atoms thereon with one or more of the substituents generally described above. Additional examples or generally applicable substituents are illustrated by the specific embodiments shown in the
20 Examples that are described herein.

[0049] As used herein, the terms “aliphatic”, “heteroaliphatic”, “alkyl”, “alkenyl”, “alkynyl”, “heteroalkyl”, “heteroalkenyl”, “heteroalkynyl”, and the like encompass substituted and unsubstituted, saturated and unsaturated, and linear and branched groups. Similarly, the terms “alicyclic”, “heteroalicyclic”, “heterocycloalkyl”, “heterocycle” and the like encompass
25 substituted and unsubstituted, and saturated and unsaturated groups. Additionally, the terms “cycloalkyl”, “cycloalkenyl”, “cycloalkynyl”, “heterocycloalkyl”, “heterocycloalkenyl”, “heterocycloalkynyl”, “aryl”, “heteroaryl” and the like encompass both substituted and unsubstituted groups.

[0050] In some embodiments, the adjuvant or vaccine compositions further comprise
30 metabolizable lipid-based nanoparticles comprising a TLR-4 agonist.

[0051] The metabolizable lipid-based nanoparticles may have an average nanoparticle diameter of less than 200nm or from 50 to 180 nm. The average (mean) nanoparticle diameter of the metabolizable lipid-based nanoparticles can be determined with dynamic light scattering (DLS), which is a technique commonly used by those skilled in art (such as taught in Falke S.,
35 Betzel C. (2019) Dynamic Light Scattering (DLS). In: Pereira A., Tavares P., Limão-Vieira P.

(eds) Radiation in Bioanalysis. Bioanalysis (Advanced Materials, Methods, and Devices), vol 8. Springer, Cham.; Chu B, Laser light scattering: Basic principles and practice. Academic Press, Boston, Second Edition, 1991, 343 pp; and Brown W., ed. Dynamic light scattering, the method and some applications. Clarendon, Oxford, 1993. 735 pp.). The metabolizable

5 lipid-based nanoparticles comprising the TLR-4 agonist may be present in an aqueous solution. Thus the formulations of the adjuvants or vaccine compositions can comprise both hydrophilic and hydrophobic components.

[0052] The terms "hydrophilic" or "lipophobic" and their grammatical equivalents as used interchangeably herein refers to a molecule or portion of a molecule whose interactions with
10 water and other polar substances are more thermodynamically favorable than their interactions with lipid or other hydrophobic solvents. Hydrophilic molecules are typically charge-polarized and capable of hydrogen bonding, resulting in good solubility in polar solvents such as water and aqueous solutions derived therefrom.

[0053] The terms "lipophilic" or "hydrophobic" and their grammatical equivalents as used interchangeably herein refers to a molecule or portion of a molecule whose interactions with
15 lipids (such as an lipid) and other non-polar substances are more thermodynamically favorable than their interactions with lipids or other hydrophilic solvents. Hydrophobic molecules are typically non-charged, resulting in good solubility in non-polar solvents such as lipids. Examples of hydrophobic molecules include alkenes, and lipids such as oils and fats.

[0054] As used herein, the term "metabolizable lipid" in aspects of the current disclosure means that the one or more lipids of the lipid-based nanoparticles of the adjuvants or vaccine compositions are metabolizable in and biocompatible with the patient or subject into which the adjuvant or vaccine formulation will be introduced. As such, the term "metabolizable lipid" with
20 respect to lipid-based nanoparticle human vaccines refers to any lipid, including but not limited to fats, oils, phospholipids and mixtures thereof, that is capable of being metabolized or functions as a substrate, precursor, intermediate or product in a human metabolic process.

[0055] In some embodiments, the metabolizable lipid at least partly comprising the nanoparticle adjuvant compositions may be one or more metabolizable amphipathic lipids, metabolizable phospholipids, metabolizable glycerophospholipids or a mixture thereof.

[0056] In some embodiments, the metabolizable lipid at least partly comprising the nanoparticle adjuvant compositions may be one or more metabolizable oils. In some
30 embodiments, the adjuvants or vaccine compositions may include lipids produced from an animal, fish, vegetable, tree, and/or microbe (including recombinant bacteria, yeast or fungi engineered to produce such lipids). For example, nuts, beans, seeds and grains are typically lipid-rich sources, and give rise to such common lipids as safflower lipid, cottonseed lipid,
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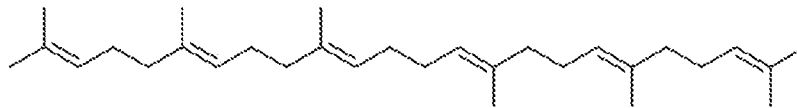
sunflower seed lipid, sesame seed lipid, peanut lipid, soybean lipid, coconut lipid, olive lipid, jojoba lipid, corn lipid, wheat lipid, oat lipid, rye lipid, rice lipid, teff lipid, triticale lipid and the like may also be used. Further non-limiting examples of metabolizable oils (metabolizable lipids) suitable for use in immunologically active metabolizable lipid nanoparticles of the current disclosure are provided in Table 1.

Table 1: Metabolizable oils (lipids) suitable for use in emulsions and liposomes of the current disclosure.

Castor oil
Coconut oil
Corn oil
Cottonseed oil
Evening primrose oil
Fish oil
Jojoba oil Lard oil
Linseed oil
Olive oil
Peanut oil
Safflower oil
Sesame oil
Soybean oil
Squalene
Sunflower oil
Wheatgerm oil
Mineral oil

[0057] Fats and oils from mammalian milk are metabolizable lipids and may therefore be used in the practice of aspects of this disclosure. The procedures for separation, purification, saponification and other means necessary for obtaining pure lipids from animal sources are well known in the art. Most fish, whales and sharks contain metabolizable lipids which may be readily recovered and suitable for use herein, including but not limited to cod liver oils, shark liver oils (such as squalene), and whale oils such as spermaceti. In some aspects of the current disclosure, terpenoids (branched chain oils composed of 5-carbon isoprene units), such as squalene, are the metabolizable oil. Squalene is a colourless triterpene with formula $(C_5H_8)_6$,

originally isolated from shark liver oil, though all plants and animals produce squalene as a biochemical intermediate, being a precursor of such materials as sterols and steroids.



Squalene.

- 5 **[0058]** Squalene and squalane are available from commercial sources at high purity levels suitable for pharmaceutical use. As a non-limiting example, one suitable method of preparing squalene by purification distillation is taught in US20160051670A1. Environmental concerns over shark hunting for squalene harvesting have motivated the production of squalene from recombinant microbes, non-limiting methods and biosynthetic pathways f which are taught in
 10 for example Spanova and Günther, 2011 ("Squalene – biochemistry, molecular biology, process biotechnology, and applications". European Journal of Lipid Science and Technology. 113 (11): 1299–1320. Doi:10.1002/ejlt.201100203) and Pan et al. 2015 ("Biosynthesis of Squalene from Farnesyl Diphosphate in Bacteria: Three Steps Catalysed by Three Enzymes". ACS Central Science. 1 (2): 77–82. Doi:10.1021/acscentsci.5b00115. PMID 26258173).
 15 Squalane, the saturated analog to squalene, can also be used.

- [0059]** In some embodiments, tocopherols are the metabolizable lipids, such as α -tocopherols and the isomer DL- α -tocopherol. The tocopherol may be used in several forms, such as but not limited to salts selected from the organic salts: acetate, nicotinate, and preferably succinate. It should be clear to one skilled in the art that synthetic derivatives of such lipids
 20 may also be suitable, such as the 6-10 carbon fatty acid esters of glycerol and 1,2-propanediol, which although they do not naturally occur in seed oils, may be prepared by hydrolysis, separation and esterification of the appropriate nut and seed oils. The immunologically active metabolizable lipid-based nanoparticle adjuvants and resulting formulated vaccines of the current disclosure may comprise a mixture or blend of the lipids described herein.
- 25 **[0060]** In some aspects the metabolizable lipid, such as a metabolizable oil, or metabolizable amphipathic lipid (such as a phospholipid or glycerophospholipid) may be present in the immunologically active metabolizable lipid nanoparticle in water composition in the range of 0.4 to 20% wt/wt (4 – 200 mg/mL), 0.5% to 20% wt/wt (5 – 200 mg/mL), 1% to 9% wt/wt (10 – 90 mg/mL), 2% to 8% wt/wt (20 – 80 mg/mL), 3% to 7% wt/wt (30 – 70 mg/mL), or 4% to 6%
 30 wt/wt (40 – 60 mg/mL). In some aspects the metabolizable lipid, such as a metabolizable oil, or metabolizable amphipathic lipid (such as a phospholipid or glycerophospholipid) may be

present in the immunologically active metabolizable lipid nanoparticle in water composition in the range of 0.004 – 1% wt/wt (0.04 – 10 mg/mL), or 0.1 – 0.8% wt/wt (1 - 8 mg/mL). In some aspects the metabolizable lipid is DOPC present in the immunologically active metabolizable lipid nanoparticle in water composition in the range of 0.004 – 1% wt/wt (0.04 – 10 mg/mL), or 0.1 – 0.8% wt/wt (1 - 8 mg/mL). In some aspects the metabolizable lipid, such as a metabolizable oil, or metabolizable amphipathic lipid (such as a phospholipid or glycerophospholipid) may be present in the immunologically active metabolizable lipid nanoparticle in water composition in the range of 0.05 – 5% wt/wt (0.5 – 50 mg/mL), or 2 – 4% wt/wt (20 - 40 mg/mL). In some aspects the metabolizable lipid is squalene present in the immunologically active metabolizable lipid nanoparticle in water composition in the range of 0.05 – 5% wt/wt (0.5 – 50 mg/mL), or 2 – 4% wt/wt (20 - 40 mg/mL).

[0061] As used herein, the term “metabolizable lipid nanoparticle in water”, means a lipid colloidal suspension in water or a water-based solution (typically an aqueous buffer) which is at least partly metabolizable and/or comprises one or more components that are metabolizable (i.e. metabolizable in and biocompatible with the patient or subject into which the adjuvant or vaccine formulation will be introduced). In aspects of the current disclosure, the aqueous component can be distilled water or can include further components e.g. solutes. In some embodiments, the water-based solution includes salts to form an aqueous buffer e.g. citrate or phosphate salts, such as sodium salts. Suitable aqueous solutions for use in the current disclosure include pharmaceutically acceptable buffers with a pH between 4 and 9 including, but not limited to phosphate, citrate, acetate, Tris, borate, histidine, lactate, tromethamine, gluconate, aspartate, tartrate, succinate, maleate, and/or fumarate buffers. In some embodiments, suitable pharmaceutically acceptable aqueous buffers include one or more of: a phosphate buffer (e.g., PBS buffer); a Tris buffer; a borate buffer; a succinate buffer; a histidine buffer; or a citrate buffer. In some embodiments, pharmaceutically acceptable aqueous buffer strengths are within the 5-20 mM range, the 8 to 17mM range or the 10 to 15mM range. In some embodiments, the one or more pharmaceutically acceptable aqueous buffer has a typical pH within the pH 4-9 range, the 5-8 range, or the 6-7 range. In some embodiments, the pH range of the aqueous buffer in the immunologically active metabolizable lipid nanoparticle in water composition is pH 5.5-7 range, pH 6-6.5 range or pH 5.5-6. The composition may include a preservative such as thiomersal or 2-phenoxyethanol.

[0062] In some embodiments, the immunologically active metabolizable lipid nanoparticle in water is a colloid suspension such as an emulsion, a micelle, a liposome, or any mixture thereof. In some embodiments, an emulsion is the immunologically active metabolizable lipid nanoparticle in water composition. In other embodiments, a liposome is the immunologically

active metabolizable lipid nanoparticle in water composition. Different antigens are best formulated with liposomes or in emulsions. In aspects of the current disclosure, the addition to the metabolizable lipid of surfactants co-surfactants and co-solvents before fluidization, microfluidization, shaking, stirring, homogenizing, or exposure to power ultrasound /
5 sonication, as described herein, will assist in the production of stable immunologically active metabolizable lipid nanoparticles.

[0063] As used herein, the term “emulsion” means a mixture of two or more liquids, at least two of which are immiscible owing to liquid-liquid phase separation. In some embodiments, the adjuvants or vaccine compositions are lipid in water emulsions, i.e. lipid/lipid phase
10 dispersed in an aqueous phase. In other embodiments, the emulsion comprises nanoparticles comprising one or more metabolizable lipids (such as squalene), a saponin adjuvant, a sterol (such as cholesterol) and E6020, distributed within an aqueous phase (such as a buffer solution). In some aspects the metabolizable lipid, such as a metabolizable oil, or metabolizable amphipathic lipid (such as a phospholipid or glycerophospholipid) may be
15 present in the immunologically active oil in water emulsion in the range of 0.05 – 5% wt/wt (0.5 – 50 mg/mL), or 2 – 4% wt/wt (20 - 40 mg/mL). In some aspects the metabolizable lipid is squalene present in the immunologically active oil in water emulsion in the range of 0.05 – 5% wt/wt (0.5 – 50 mg/mL), or 2 – 4% wt/wt (20 - 40 mg/mL).

[0064] As used herein, the term “micelle” means an immunologically active metabolizable lipid
20 nanoparticle comprising an aggregate of surfactant molecules suspended in an aqueous solution, such that the hydrophilic regions of the surfactant are in contact with the aqueous solution and the hydrophobic regions of the surfactant are sequestered (clustered) towards the center of the micelle. Micelles may be approximately spherical, cylindrical or ellipsoid in shape, with populations of micelles often comprising examples of each structure, but having
25 a distinct bias towards one type as a result of their precise composition and method of manufacture.

[0065] As used herein, the term “liposome” means an immunologically active metabolizable lipid nanoparticle comprising at least one lipid bilayer. In some aspects of the current disclosure, the liposomes may be unilamellar (comprised of one lipid bilayer), multilamellar
30 (comprised of several lamellar phase lipid bilayers), cochleate or mixtures of any of these. The liposomes may contain a metabolizable amphipathic lipid (such as an amphipathic surfactant) which is a neutral lipid, for example phosphatidylcholine, which is preferably non-crystalline at room temperature, for example egg yolk phosphatidylcholine, dioleoyl phosphatidylcholine or dilauryl phosphatidylcholine. The liposomes may also contain a charged lipid which increases
35 the stability of the liposome-saponin structure for liposomes composed of saturated lipids. In

these cases the amount of charged lipid is preferably 1-20% w/w, most preferably 5-10%. The ratio of sterol to phospholipid is 1-50% (mol mol), most preferably 20-25%. In some aspects the metabolizable lipid may be present in the immunologically active liposome in water composition in the range of 0.004 – 1% wt/wt (0.04 – 10 mg/mL), or 0.1 – 0.8% wt/wt (1 - 8 mg/mL). In some aspects the metabolizable lipid is DOPC present in the immunologically active liposome in water composition in the range of 0.004 – 1% wt/wt (0.04 – 10 mg/mL), or 0.1 – 0.8% wt/wt (1 - 8 mg/mL).

[0066] As used herein and as understood by a person skilled in the art, the term “surfactant” (a well-recognized abbreviation of “*surface-active agent*”) relates to compounds that are able to lower the surface tension (or interfacial tension) between two liquids, between a gas and a liquid, or between a liquid and a solid. This ability of surfactants makes them useful as emulsifiers, foaming agents, detergents, wetting agents and dispersants. Surfactants are typically able to do this by virtue of their amphiphilic nature, i.e. at least part of the surfactant molecule is hydrophilic, permitting interaction with polar solvents such as water, and at least part of the surfactant is hydrophobic, permitting interaction with non-polar solvents such as lipids. Surfactants suitable for use in the adjuvant or vaccine compositions are metabolizable in and biocompatible with the patient or subject into which the adjuvant or vaccine formulation will be introduced. Surfactants may be classified by their relative interactions with the hydrophilic and hydrophobic phases, which is described by those skilled in the art by the hydrophile/lipophile balance (“HLB”) value of the surfactant.

[0067] In some embodiments, the adjuvant or vaccine compositions are formulated as emulsions comprising metabolizable lipid droplets coated with at least one surfactant or a combination of surfactants.

[0068] As used herein, the term “hydrophilic surfactant” refers to an aqueous phase surfactant, including but not limited to those with an HLB value between 8 and 18. Hydrophilic surfactants suitable for use in some embodiments have an HLB of at least 10, preferably at least 15, and more preferably at least 16. Hydrophilic surfactants suitable for use in some embodiments include, but are not limited to, one or more of polyoxyethylene sorbitan esters (commonly referred to as the Tweens, alkyl sulfate (e.g., sodium lauryl sulfate), polyoxyethylene lauryl ether, polyoxyethylene monostearate, fatty acid polyethoxylate, ethoxylated cetyl/stearyl alcohol, polyoxyl lauryl ether, or blends comprising one or more thereof. Further examples of Tweens include polyoxyethylenesorbitan monooleate (e.g., polysorbate 80, TWEEN 80), polyoxyethylenesorbitan monolaurate (polysorbate 20, TWEEN 20). In some aspects the hydrophilic surfactant may be present in the immunologically active metabolizable lipid nanoparticle in water composition in the range of 0.05% to 10% wt/wt (0.5

– 100 mg/mL), 0.1% to 8.5% wt/wt (1 – 85 mg/mL), 0.5% to 7% wt/wt (5 – 70 mg/mL), 1% to 6% wt/wt (10 – 60 mg/mL), or 1.5% to 5% wt/wt (15 – 50 mg/mL).

[0069] As used herein, the term “hydrophobic surfactant” (otherwise known as lipophilic surfactants) refers to a lipid phase surfactant, including but not limited to those with an HLB value between 3 and 6. Nonionic surfactants are hydrophobic surfactants. Hydrophobic surfactants suitable for use in some embodiments of the adjuvant or vaccine compositions have an HLB of less than 6, preferably of less than 4, and more preferably less than 3. Hydrophobic surfactants suitable for use in some embodiments of the adjuvant or vaccine compositions are one or more sorbitan esters (commonly known as the SPANs), such as sorbitan trioleate (commonly known as Span 85), sorbitan monolaurate (e.g., Span 60), or blends comprising one or more thereof. Nonionic surfactants suitable for use in some embodiments of the adjuvant or vaccine compositions include ethylene oxide (EO), propylene oxide (PO), and/or butylene oxide (BO), sold under the DOWFAX™ tradename, such as linear or branched EO/PO block copolymers sold under the trade name TERGITOL™. Nonionic surfactants suitable for use in some embodiments of the adjuvant or vaccine compositions include octoxynols, which can vary in the number of repeating ethoxy (oxy-1,2-ethanediyl) groups, such as octoxynol-9 (Triton X-100, or t-octylphenoxypolyethoxyethanol). In some aspects the hydrophobic surfactant may be present in the immunologically active metabolizable lipid nanoparticle in water composition in the range of 0.05% to 10% wt/wt (0.5 – 100 mg/mL), 0.1% to 8.5% wt/wt (1 – 85 mg/mL), 0.5% to 7% wt/wt (5 – 70 mg/mL), 1% to 6% wt/wt (10 – 60 mg/mL), or 1.5% to 5% wt/wt (15 – 50 mg/mL).

[0070] In some embodiments, the metabolizable lipid may be a metabolizable amphipathic lipid that is an amphipathic surfactant. Amphipathic surfactants suitable for use in the current disclosure include phospholipids and glycerophospholipids, including but not limited to phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, and blends thereof such as commercially available lecithins, such as those derived from soybean lipid, sunflower, lipid, etc. Non-limiting examples of phospholipids suitable for use in immunologically active metabolizable lipid nanoparticles, such as emulsions or liposomes, are provided in Table 2.

Table 2: -Metabolizable phospholipids (metabolizable lipids, also metabolizable amphipathic surfactants) suitable for use in aspects of the current disclosure

Name	Short name	Fatty Acid
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		(chain length and saturation)
1,2-dilauroyl-sn-glycero-3-phosphatidylcholine	DLPC	12:0
1,2-dimyristoyl-sn-glycero-3-phosphatidylcholine	DMPC	14:0
1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine	DPPC	16:0
1,2-distearoyl-sn-glycero-3-phosphatidylcholine	DSPC	18:0
1,2-dioleoyl-sn-glycero-3-phosphatidylcholine	DOPC	18:1
1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine	DMPE	14:0
1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine	DPPE	16:0
1,2-dioleoyl-sn-glycero-3-phosphoethanolamine	DOPE	18:1
1,2-dimyristoyl-sn-glycero-3-phosphate (sodium salt)	DMPA•Na	14:0
1,2-dipalmitoyl-sn-glycero-3-phosphate (sodium salt)	DPPA•Na	16:0
1,2-dioleoyl-sn-glycero-3-phosphate (sodium salt)	DOPA•Na	18:1
1,2-dimyristoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (sodium salt)	DMPG•Na	14:0
1,2-dipalmitoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (sodium salt)	DPPG•Na	16:0
1,2-dioleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (sodium salt)	DOPG•Na	18:1
1,2-dimyristoyl-sn-glycero-3-phospho-L-serine (sodium salt)	DMPS•Na	14:0
1,2-dipalmitoyl-sn-glycero-3-phospho-L-serine (sodium salt)	DPPS•Na	16:0
1,2-dioleoyl-sn-glycero-3-phospho-L-serine (sodium salt)	DOPS•Na	18:1
1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(glutaryl) (sodium salt)	DOPE-Glutaryl•(Na) ₂	18:1
1',3'-bis[1,2-dimyristoyl-sn-glycero-3-phospho]-glycerol (ammonium salt)	Tetramyristoyl Cardiolipin•(Na) ₂	14:0

1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (ammonium salt)	DSPE-mPEG-2000•Na	18:0
1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-5000] (ammonium salt)	DSPE-mPEG-5000•Na	18:0
1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[maleimide(polyethylene glycol)-2000] (ammonium salt)	DSPE-Maleimide PEG-2000•Na	18:0
1,2-dioleoyl-3-trimethylammonium-propane (chloride salt)	DOTAP•Cl	18:1
<u>1,2-Diarachidoyl-sn-glycero-3-phosphocholine (DAPC)</u>	DAPC	20:4
1,2-Dierucoyl-sn-Glycero-3-Phosphatidylcholine	DEPC	22:1
<u>1,2-Diheptadecanoin-sn-Glycero-3-Phosphatidylserine Na salt</u>		17:0
<u>1,2-Dilinoleoyl-sn-Glycero-3-Phosphatidylcholine</u>		18:2
<u>1,2-Dinonadecanoyl-sn-Glycero-3-Phosphatidylcholine</u>		19:0
<u>1,2-Diphytanoyl-sn-glycero-3-PE (DpyPE)</u>		16:0
<u>1-Docosahexaenoyl-2-Stearoyl-sn-Glycero-3-Phosphatidylcholine</u>		22:6
1,2-Dilauroyl-sn-glycero-3-phosphorylethanolamine	DLPE-PEG-COOH	12:0
Phosphatidylinositol-4,5-diphosphate C-16	DPPI-4,5-P	16:0
Lyso-Phosphatidylcholine from egg	LPC	
Lyso-Phosphatidylcholine from soybean	LPC	
Lyso-Phosphatidylcholine from subflower	LPC	
Lyso-Phosphatidylcholine from rapeseed	LPC	
Lyso-Phosphatidylcholine from bovine liver	LPC	
Lyso-Phosphatidylethanolamine from egg, from bovine, from soybean	LPE	

Phosphatidylcholine from egg, bovine liver, rapeseed, soybean, sunflower	PC	
Phosphatidylethanolamine from egg, bovine brain, bovine heart, soybean	PE	

[0071] Mixtures of surfactants may be used in aspects of the current invention, including but not limited to Tween 80/Span 85 mixtures, or Tween 80/Triton-X100 mixtures. For example, in some aspects a combination of a polyoxyethylene sorbitan ester such as polyoxyethylene sorbitan monooleate (Tween 80) and an octoxynol such as t-octylphenoxypolyethoxyethanol (Triton X-100) is suitable. In another aspect, the surfactants include Tween 80 (polyoxyethylene sorbitan monooleate), Span 85 (sorbitan trioleate), lecithin and Triton X-100. In another aspect, a mixture of surfactants may comprise laureth 9 plus a polyoxyethylene sorbitan ester and/or an octoxynol. Possible amounts of surfactants (% by weight) are: polyoxyethylene sorbitan esters (such as Tween 80) 0.01 to 1%, in particular about 0.1%; octyl- or nonylphenoxy polyoxyethanols (such as Triton X-100, or other detergents in the Triton series) 0.001 to 0.1%, in particular 0.005 to 0.02%; polyoxyethylene ethers (such as laureth 9) 0.1 to 20%, preferably 0.1 to 10% and in particular 0.1 to 1% or about 0.5%.

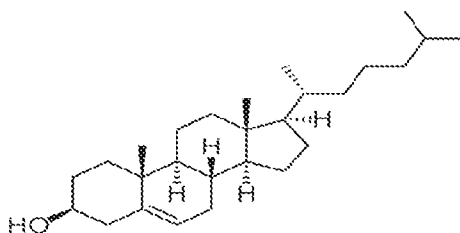
[0072] In some embodiments, the immunologically active metabolizable lipid nanoparticle in water compositions and vaccine compositions further comprise a sterol. In some aspects the sterol may be present in the immunologically active metabolizable lipid nanoparticle in water composition in the range of 0.02 mg/mL – 50 mg/mL, 0.05 mg/mL to 5 mg/mL, 0.08 mg/mL to 4 mg/mL, 0.1 mg/mL to 2 mg/mL, 0.2 mg/mL to 1 mg/mL, or 0.4 mg/mL to 1 mg/mL.

[0073] As used herein and commonly used by those skilled in the art, the term “sterol” refers to an organic alcohol. Sterols can be purified and isolated from most eukaryotes, including plants (phytosterols, such as campesterol, sitosterol, stigmasterol), animals (zoosterols, such as cholesterol), fungi (such as ergosterol), and some bacteria. Suitable sterols for use in the current disclosure include but are not limited to β -sitosterol, stigmasterol, ergosterol, ergocalciferol and cholesterol. Further non-limiting examples of sterols suitable for use in immunologically active metabolizable lipid nanoparticles of the current disclosure are provided in Table 3.

Table 3: Metabolizable sterols suitable for use in emulsions and liposomes of the current disclosure.

Name	Formula
Cholesterol	C ₂₇ H ₄₆ O
3-sitosterol	C ₂₉ H ₅₀ O
stigmasterol	C ₂₉ H ₄₈ O
Ergosterol (Provitamin D2)	C ₂₈ H ₄₄ O
Ergocalciferol (Vitamin D2)	C ₂₈ H ₄₄ O

[0074] Sterols are well known in the art, for example cholesterol is disclosed in the Merck Index, 11th Edn., page 341, as a naturally occurring sterol found in animal fat. In some aspects
5 of the current disclosure, cholesterol is the sterol. Cholesterol is the principal sterol synthesized by all animals and has formula C₂₇H₄₆O.



Cholesterol

[0075] In some embodiments, the immunologically active metabolizable lipid nanoparticle in
10 water compositions and vaccine compositions further comprise a saponin.

[0076] As used herein and commonly used by those skilled in the art, the term "saponin" refers to triterpene glycosides that have a foaming quality when agitated in water. Natural sources of saponins include the Saponaria genus of flowering plants and the soapbark tree *Quillaja Saponaria*. Possible saponins for use in the current disclosure are one or more
15 immunologically active saponins, such as immunologically active saponin fractions derived from the bark of *Quillaja Saponaria Molina* such. For example, QS21, also known as QS-21 and QA21, is a purified fraction from the *Quillaja Saponaria Molina* tree, one method of purification being disclosed (as QA21) in US patent No. 5,057,540. *Quillaia* saponin has also been disclosed as an adjuvant by Scott *et al*, Int. Archs. Allergy Appl. Immun., 1985, 77,
20 409. The immunologically active compositions may contain a immunologically active saponin fraction in substantially pure form. The immunologically active compositions may contain QS21 in substantially pure form, such that the QS21 is at least 90% pure, preferably at least

95% pure and most preferably at least 98% pure. Other non-limiting examples of immunologically active saponin fractions useful in the adjuvant or vaccine compositions include QS7, QS18 (also known as QA18) and QS17 (also known as QA17). In some embodiments, only partially purified saponins may be used, such as the Quil A extract of the bark of *Quillaja Saponaria*. In further embodiments, saponins produced from recombinant microbes may be used, and/or triterpene saponin analogues such as those disclosed in WO17079582 or WO18200645, the contents of which are incorporated herein. In some embodiments, saponin derivatives that are immune-stimulatory and surface active may be used. In other embodiments, blends of saponins or saponin fractions may be used, such as QS21 and QS17, QuilA supplemented with QS21, and so on. All saponins suitable for use in the compositions described herein are used in concentrations which are non-toxic to the patient or subject. In some aspects the saponin may be present in the immunologically active metabolizable lipid nanoparticle in water composition in the range of 0.02 mg/mL to 0.4 mg/mL, 0.04 mg/mL to 0.2 mg/mL, 0.06 mg/mL to 0.2 mg/mL or 0.08 mg/mL to 0.1 mg/mL.

[0077] The adjuvant or vaccine compositions described herein may contain a carrier (otherwise known as a “platform”) such as nanoparticles, keyhole limpet hemocyanin (KLH), CRM197 a genetically detoxified form of diphtheria toxin, rotavirus VP6 or combinations thereof, influenza virus derived virosomes, hepatitis B surface antigen virus like particles, or other platforms presenting a repetitive array of antigens on their surface.

[0078] The adjuvant or vaccine compositions described herein may contain antioxidant vitamins and/or minerals such as B carotene, vitamin A, vitamin C, vitamin D, vitamin E, co-enzyme Q10, selenium or combinations thereof.

[0079] The adjuvant or vaccine compositions described herein may additionally comprise excipients, including but not limited to one or more of, a sugar, such as sucrose, sorbitol, and/or proteins such as gelatin. In some embodiments an excipient such as gelatin, may be added to adjust the viscosity or stability, such as protection against temperature fluctuations during transport and/or storage, of the final adjuvant or vaccine.

[0080] The adjuvant or vaccine compositions described herein can be used to treat a patient or subject suffering from a variety of diseases.

[0081] The adjuvant or vaccine compositions described herein can be used to treat a patient or subject or subject suffering from a variety of diseases.

[0082] The term “disease” as used interchangeably with “medical indication” herein refers to a medical condition resulting from a pathogenic infection, a transmissible disease, an acquired disease, an infectious disease, a foodborne disease, an airborne disease, or an internal dysfunction such as a cancer. For the avoidance of doubt, “disease” includes predisease

states or subclinical conditions, such as pre-cancer identified by abnormal structure or function of cellular tissues. Non-limiting examples of target diseases in some aspects of the current disclosure include diseases such as influenza, SARS-CoV-2, SARS-CoV-1, rhinovirus, malaria, tuberculosis, HIV/AIDS, leishmaniasis, Group A streptococcus, respiratory syncytial virus (RSV), Middle East Respiratory Syndrome virus (MERS), hepatitis B, varicella zoster, coronavirus or human papilloma virus infection. In some aspects of the current disclosure, use of a vaccine comprising the adjuvant formulations disclosed herein may be performed as a prophylactic treatment, a vaccination program, as a means of reducing the ability of the patient or subject to act as a vector for spread of a transmissible disease, or as a means of reducing the severity of prognosis or symptoms of an existing disease.

[0083] The term “patient” or “subject” as used herein may refer to a target group (i.e. a plurality of patients or subjects) of vertebrates (animals, such as a domesticated animal, farm animal, mammal, primate, fowl or fish), species (such as a human, pig, cow, horse, dog, cat) and/or a specific vaccine recipient, patient, subject, population or sub-population or population cohort considered to be at high risk of contracting or transmitting a disease or having been diagnosed with having the disease or suffering from a high disease burden measured by mortality, morbidity, financial cost, days of work lost, and so on. In some aspects of the current disclosure, the patient or subject may be a human considered to be at risk of contracting a transmissible disease, such as influenza, SARS-CoV-2, SARS-CoV-1, rhinovirus, malaria, tuberculosis, HIV/AIDS, leishmaniasis, Group A streptococcus, respiratory syncytial virus (RSV), Middle East Respiratory Syndrome virus (MERS), hepatitis B, varicella zoster, coronavirus or human papilloma virus infection.

[0084] The adjuvant or vaccine compositions described herein may contain an antigen.

[0085] As used herein, the term “antigen” is used interchangeably with “molecule comprising an antigen”, and is recognized by one skilled in the art to mean a molecule or molecular structure, often natively present on the outside of a pathogen, that can be bound by an antigen-specific antibody, antigen-binding antibody fragment or B-cell antigen receptor. Antigens may be proteins, peptides and/or polysaccharides, of which a great many are known in the art, or virosomes. The presence of non-self (“foreign”) antigens in the body of a patient or subject vertebrate, such as a mammal or human, normally triggers an immune response.

[0086] The term “immune response” and “immunity” as used interchangeably herein refers to the adaptive immune system of vertebrates In some aspects of the current disclosure, formulations comprising nanoparticles of metabolizable lipid comprising antigens derived from a disease may be intentionally administered to a recipient patient or subject to induce the memory function of the adaptive immune system towards antigens derived from the disease.

Such artificial (man-mediated through the use of a vaccine formulation) active inducement of the adaptive immune system of a vertebrate patient or subject is frequently referred to as immunization, and if performed on a population of patient or subjects, as an immunization campaign or program. Immunization confers a degree of protection, seen as mild or inapparent disease symptoms when the immunized patient or subject comes into contact with pathogens causing the disease. This resistance to the disease following immunization is therefore a function of the immunological memory and is the goal of most vaccinations. With reference to the antigen being delivered and forming the basis of immune recognition (and therefore optimally immunity), there are general classifications of vaccines: inactivated vaccines, live attenuated vaccines, toxoids, a fourth group comprising subunit, recombinant, polysaccharide, and conjugate vaccines, and recombinant vector vaccines.

[0087] Inactivated vaccines are composed of micro-organisms that have been killed with chemicals and/or heat and are no longer infectious. Examples are vaccines against flu, cholera, plague, and hepatitis A. Most vaccines of this type are likely to require booster shots.

[0088] Live, attenuated vaccines are composed of micro-organisms that have been cultivated under conditions which disable their ability to induce disease. These responses are more durable, however, they may require booster shots. Examples include yellow fever, measles, rubella, and mumps.

[0089] Toxoids are inactivated toxic compounds from micro-organisms in cases where these (rather than the micro-organism itself) cause illness, used prior to an encounter with the toxin of the micro-organism. Examples of toxoid-based vaccines include tetanus and diphtheria.

[0090] Subunit, recombinant, polysaccharide, and conjugate vaccines are composed of small fragments or pieces from a pathogenic (disease-causing) organism. A characteristic example is the subunit vaccine against Hepatitis B virus.

[0091] Current work in this field is pioneering vaccination technologies such as mRNA/DNA vaccines are composed of nucleotides encoding protein antigens from the pathogen. These vaccines are inexpensive, relatively easy to make and generate a strong, long-term immunity. A related group of vaccines are recombinant vector vaccines (platform-based vaccines) that are harmless live viruses able to encode one or more antigens from a pathogenic organism. They are used widely in veterinary medicine.

[0092] The immunologically active metabolizable lipid nanoparticle in water compositions may be used to deliver an antigen in a vaccine composition, wherein the vaccine composition is an inactivated vaccine, a live attenuated vaccine, a toxoid, a subunit vaccine, a recombinant vaccine, a polysaccharide vaccine, a conjugate vaccine, or a recombinant vector vaccine. In other embodiments of the current disclosure, the immunologically active metabolizable lipid

nanoparticle in water compositions are a carrier for a subunit antigen, a toxoid antigen, a polysaccharide antigen, a soluble antigen or a recombinantly produced antigen.

[0093] As used herein, the term “immunologically active” refers to the ability of a molecule to activate or enhance an immune response. For example, an antigen or molecule comprising an antigen may be described herein as immunologically active because it may be recognized by a patient or subject immune system and activate an immune response (i.e. is “immunogenic”). Additionally, E6020 is a potent TLR-4 receptor agonist, helping or enhancing an immune response, so may also be described herein as “immunologically active”. Likewise, saponins (including but not limited to QuilA or QS21) also have stimulatory immune-modulator effects so may be described herein as “immunologically active”. It is an object of the current disclosure to provide adjuvants offering a substantial increase in immune response (and thus effectiveness of vaccination) when used to deliver an antigen in an inoculation. It should be noted that E6020 as TLR-4 agonist and saponin as immune-stimulators activate different pathways of immune-modulation and therefore have a synergistic rather than additive immune-stimulation effect. Therefore, possible embodiments of the immunologically active metabolizable lipid nanoparticle adjuvant or vaccine compositions comprise a metabolizable lipid, E6020, a saponin and a sterol, dispersed in an aqueous buffer solution.

[0094] The term “*in vivo*”, as used herein refers to within a living cell or organism, including, for example an animal, a mammal, a primate or a human.

[0095] The term “*in vitro*”, as used herein refers to outside a living cell or organism, including, without limitation, for example, in a microwell plate, a tube, a flask, a beaker, a tank, a reactor and the like.

[0096] The terms “substantially” or “approximately” or “about”, as used herein refers to a reasonable deviation around a value or parameter such that the value or parameter is not significantly changed. These terms of deviation from a value should be construed as including a deviation of the value where the deviation would not negate the meaning of the value deviated from. For example, in relation to a reference numerical value the terms of degree can include a range of values plus or minus 10% from that value. For example, deviation from a value can include a specified value plus or minus a certain percentage from that value, such as plus or minus 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% from the specified value.

[0097] The term “and/or” as used herein is intended to represent an inclusive “or”. The wording X and/or Y is meant to mean both X or Y and X and Y. Further the wording X, Y and/or Z is intended to mean X, Y and Z alone or any combination of X, Y, and Z.

[0098] The term “isolated” as used herein about a compound, refers to any compound, which by means of human intervention, has been put in a form or environment that differs from the

form or environment in which it is found in nature. Isolated compounds include but is no limited to compounds for which the ratio of the compounds relative to other constituents with which they are associated in nature is increased or decreased. In some embodiments, the amount of compound is increased relative to other constituents with which the compound is associated in nature. In other embodiments, the compound may be isolated in a pure or substantially pure ("purified") form. In this context a substantially pure compound means that the compound is separated from other extraneous or unwanted material present from the onset of producing the compound or generated in the manufacturing process. Such a substantially pure compound preparation contains less than 10%, such as less than 8%, such as less than 6%, such as less than 5%, such as less than 4%, such as less than 3%, such as less than 2%, such as less than 1 %, such as less than 0.5% by weight of other extraneous or unwanted material usually associated with the compound when expressed natively or recombinantly. In an embodiment the isolated compound is at least 90% pure, such as at least 91% pure, such as at least 92% pure, such as at least 93% pure, such as at least 94% pure, such as at least 95% pure, such as at least 96% pure, such as at least 97% pure, such as at least 98% pure, such as at least 99% pure, such as at least 99.5% pure, such as 100 % pure by weight.

[0099] The term "non-naturally occurring" as used herein about a substance, refers to any substance that is not normally found in nature or natural biological systems. In this context the term "found in nature or in natural biological systems" does not include the finding of a substance in nature resulting from releasing the substance to nature by deliberate or accidental human intervention. Non-naturally occurring substances may include substances completely or partially synthesized by human intervention and/or substances prepared by human modification of a natural substance.

[0100] The term "comprise" and "include" as used throughout the specification and the accompanying items as well as variations such as "comprises", "comprising", "includes" and "including" are to be interpreted inclusively. These words are intended to convey the possible inclusion of other elements or integers not specifically recited, where the context allows.

[0101] The articles "a" and "an" are used herein refers to one or to more than one (i.e. to one or at least one) of the grammatical object of the article. By way of example, "an element" may mean one element or more than one element.

[0102] Terms like "preferably", "commonly", "particularly", and "typically" are not utilized herein to limit the scope of the itemed disclosure or to imply that certain features are critical, essential, or even important to the structure or function of the itemed disclosure. Rather, these terms are merely intended to highlight alternative or additional features that can or cannot be utilized in a particular embodiment of the present disclosure.

[0103] All methods described herein can be performed in any suitable order of steps unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein is intended merely to better illuminate specific embodiments and does not pose a limitation on the scope of the application otherwise claimed. No language in the specification should be construed as indicating any non-claimed element essential to the practice of the material presented herein.

[0104] All percentages, ratios and proportions herein are by weight, unless otherwise specified. A weight percent (weight %, also as wt. %) of a component, unless specifically stated to the contrary, is based on the total weight of the composition in which the component is included (e.g., on the total amount of the reaction mixture).

[0105] Terms used herein may be preceded and/or followed by a single dash, "-", or a double dash, "=", to indicate the bond order of the bond between the named substituent and its parent moiety; a single dash indicates a single bond and a double dash indicates a double bond or a pair of single bonds in the case of a spiro-substituent. In the absence of a single or double dash it is understood that a single bond is formed between the substituent and its parent moiety; further, substituents are intended to be read "left to right" with reference to the chemical structure referred to unless a dash indicates otherwise. For example, arylalkyl, arylalkyl-, and alkylaryl indicate the same functionality.

[0106] For simplicity, chemical moieties are defined and referred to throughout primarily as univalent chemical moieties (e.g., alkyl, aryl, etc.). Nevertheless, such terms are also used to convey corresponding multivalent moieties under the appropriate structural circumstances clear to those skilled in the art. For example, while an "alkyl" moiety can refer to a monovalent radical (e.g. CH₃-CH₂-), in some circumstances a bivalent linking moiety can be "alkyl," in which case those skilled in the art will understand the alkyl to be a divalent radical (e.g., -CH₂-CH₂-), which is equivalent to the term "alkylene." (Similarly, in circumstances in which a divalent moiety is required and is stated as being "aryl," those skilled in the art will understand that the term "aryl" refers to the corresponding divalent moiety, arylene). All atoms are understood to have their normal number of valences for bond formation (i.e., 4 for carbon, 3 for N, 2 for O, and 2, 4, or 6 for S, depending on the oxidation state of the S). Nitrogens in the presently disclosed compounds can be hypervalent, e.g., an N-oxide or tetrasubstituted ammonium salt. On occasion a moiety may be defined, for example, as -B-(A)_a, wherein a is 0 or 1. In such instances, when a is 0 the moiety is -B and when a is 1 the moiety is -B-A.

[0107] As used herein, the term "alkyl" or "alkane" includes a saturated hydrocarbon having a designed number of carbon atoms, such as 1 to 40 carbons (i.e., inclusive of 1 and 40), 1 to 35 carbons, 1 to 25 carbons, 1 to 20 carbons, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15,

16, 17, or 18. Alkyl groups or alkanes may be straight or branched and depending on context, may be a monovalent radical or a divalent radical (i.e., an alkylene group). For example, the moiety “-(C1 C6 alkyl) O-” signifies connection of an oxygen through an alkylene bridge having from 1 to 6 carbons and C1-C3 alkyl represents methyl, ethyl, and propyl moieties. Examples of “alkyl” include, for example, methyl, ethyl, propyl, isopropyl, butyl, iso, sec and tert butyl, pentyl, and hexyl. Examples of “alkane” include, for example, methane, ethane, propane, isopropane, butane, isobutane, sec-butane, tert-butane, pentane, hexane, heptane, and octane.

[0108] The term “substituted,” when used to modify a specified group or radical, means that one or more hydrogen atoms of the specified group or radical are each, independently of one another, replaced with the same or different substituent groups as defined below, unless specified otherwise.

[0109] Specific protecting groups may be used to protect reactive functionalities of a starting material or intermediate to prepare a desired product. In general, the need for such protecting groups as well as the conditions necessary to attach and remove such groups will be apparent to those skilled in the art of organic synthesis. An authoritative account describing the many alternatives to the trained practitioner are J. F. W. McOmie, “Protective Groups in Organic Chemistry”, Plenum Press, London and New York 1973, in T. W. Greene and P. G. M. Wuts, “Protective Groups in Organic Synthesis”, Third edition, Wiley, New York 1999, in “The Peptides”; Volume 3 (editors: E. Gross and J. Meienhofer), Academic Press, London and New York 1981, in “Methoden der organischen Chemie”, Houben-Weyl, 4.sup.th edition, Vol. 15/I, Georg Thieme Verlag, Stuttgart 1974, in H.-D. Jakubke and H. Jescheit, “Aminosäuren, Peptide, Proteine”, Verlag Chemie, Weinheim, Deerfield Beach, and Basel 1982, and/or in Jochen Lehmann, “Chemie der Kohlenhydrate: Monosaccharide and Derivate”, Georg Thieme Verlag, Stuttgart 1974. The protecting groups may be removed at a convenient subsequent stage using methods known from the art.

Methods of producing the adjuvant or vaccine compositions.

[0110] In aspects of the current disclosure in which the TLR-4 receptor agonist of formula (I), such as E6020, in a metabolizable lipid and immunoactive saponin nanoparticle in water composition is present in a stable lipid-in-water emulsion, the lipid-in-water emulsion may be manufactured using a method comprising the steps of: (i) preparation through rapid agitation (optionally by homogenization and/or microfluidization) of a first emulsion, also known as a preliminary emulsion or a pre-emulsion, having a first average lipid droplet size (optionally less

than 5µm); (ii) further agitation (such as by fluidization, microfluidization, shaking, stirring, homogenizing, or exposure to power ultrasound / sonication) of the first emulsion to form a second emulsion having a second average lipid droplet size which is less than the first average lipid droplet size (preferably less than 220 nm); and (iii) nanofiltration, preferably by filter sterilization, of the second emulsion.

[0111] The pH of the compositions disclosed herein have a typical pH within the pH 4-9 range, generally between 5.0 and 8.1, and more typically between 6.0 and 8.0 e.g. between 6.5 and 7.5. Methods of producing compositions as disclosed herein may therefore include a step of adjusting the pH of the adjuvant or vaccine comprising adjuvant as disclosed herein, prior to packaging.

[0112] In some embodiments, incorporation of an antigen may be performed either during or immediately after production of the adjuvant formulation. In other embodiments of the invention, larger volumes of adjuvant formulation may be produced and stored, for subsequent incorporation of antigen into the formulation at a time of need, optionally followed by aliquoting into dosage volumes suitable for injection. In other embodiments, the adjuvant formulation may be stored in separate receptacles to the antigen, permitting supply of a kit of parts comprising adjuvant formulation and antigen, which may be extemporaneously mixed into the final injectable formulation, for example in a local medical clinic or at the location of a subject to be treated or vaccinated. In certain aspects, such a kit of parts permits transportation of vaccine components that would be less stable if already formulated as a single vaccine formulation.

[0113] The antigen can be added to the lipid-in-water emulsions extemporaneously (at the time of administration of the vaccine compositions) or directly after the formation of the lipid-in-water emulsions, thereby forming a pre-mixed vaccine composition.

[0114] In some embodiments, a vaccine composition may be prepared by combining antigen (present in some embodiments as a liquid solution or suspension of antigen in an aqueous buffer) with bulk adjuvant in a 4:1 to a 1:4 ration by volume. In some embodiments, a vaccine composition may be prepared by combining antigen (present in some embodiments as a liquid solution or suspension of antigen in buffer) with bulk adjuvant in 1:1 ratio by volume. For example, a typical human dose of 0.5mL may comprise 0.25mL adjuvant (often referred to as "bulk adjuvant") and 0.25mL antigen. Dosage volumes for small animals are typically 0.05mL, comprising 0.025mL adjuvant and 0.025mL antigen. The skilled person appreciates that whilst the smaller volumes must be used in pre-clinical testing, the larger dosages would likely be more appropriate for human subjects.

[0115] Various antigens can be used with lipid-in-water emulsions, including but not limited

to: viral antigens, such as viral surface proteins; bacterial antigens, such as protein and/or saccharide antigens; fungal antigens; parasite antigens; soluble antigens such as virosomes and tumor antigens. The disclosure is particularly useful for antigens derived from a coronavirus, a rhinovirus, an influenza virus, a *Plasmodium* parasite, a mycobacterium, *Leishmania* parasite, streptococcus bacteria, respiratory syncytial virus (RSV), a human papilloma virus, an human immunodeficiency (HIV) virus, hepatitis B virus, varicella zoster virus, or any combination thereof.

Packaging and kits comprising adjuvant or vaccine compositions

10 [0116] The adjuvant comprising the non- microbial-lipopolysaccharide-derived TLR-4 receptor agonist of formula (I), such as E6020, in a metabolizable lipid and immunoactive saponin nanoparticle in water composition may be diluted with a buffer prior to packaging into a vial or a syringe. Typical buffers include: a phosphate buffer; a Tris buffer; a borate buffer; a succinate buffer; a histidine buffer; or a citrate buffer. Dilution can reduce the concentration of the adjuvant's components while retaining their relative proportions e.g. to provide a "half-strength" adjuvant.

[0117] Vaccines are typically administered to human patients or subjects in a dosage volume of, for example, 0.2 mL (200 μ L), 0.25 mL, 0.3 mL, 0.5 mL, 1.0 mL, 2.0 mL, although a half dose (i.e. about 0.25 mL) may be administered to children. Containers enclosing compositions of the current application may therefore be marked to show a half-dose volume e.g. to facilitate delivery to children. For instance, a syringe containing a 0.5 mL dose may have a mark showing a 0.25 mL volume.

20 [0118] Where a glass container (e.g. a syringe or a vial) is used, the container can be made from a borosilicate glass or a soda lime glass. The composition may include material for a single immunization, or may include material for multiple immunizations (i.e. a 'multidose' kit). The composition may also include a preservative.

[0119] One aspect of the application provides kits and compositions prepared using the methods presented herein. The compositions prepared according to the methods of the application are suitable for administration to vertebrates, such as human subjects or patients, and the disclosure provides a method of raising an immune response in a subject or patient, comprising the step of administering such a composition to the subject or patient. The disclosure also provides these kits and compositions for use as medicaments.

30 [0120] The packing may enclose sufficient composition according to the current disclosure for a single immunization, or may include sufficient volume for multiple immunizations (i.e. a

'multidose' kit), and in such multidose arrangement aspects of the current disclosure, the composition can include a preservative (such as for example thiomersal or 2-phenoxyethanol).

[0121] Where antigen and adjuvant are presented as separate components within a kit, they are physically separate from each other within the kit, and this separation can be achieved in various ways. For instance, the components may be in separate containers, such as vials, the contents of which can then be mixed when needed e.g. by removing the contents of one vial and adding them to the other vial, or by separately removing the contents of both vials and mixing them in a third container.

[0122] In another arrangement, one of the kit components is in a syringe and the other is in a container such as a vial. The syringe can be used (e.g. with a needle) to insert its contents into the vial for mixing, and the mixture can then be withdrawn into the syringe. The mixed contents of the syringe can then be administered to a patient or subject, typically through a new sterile needle. Packing one component in a syringe eliminates the need for using a separate syringe for administration to the patient or subject.

[0123] In another possible arrangement, the two kit components are held together but separately in the same syringe e.g. a dual-chamber syringe, such that when the syringe is actuated (e.g. during administration to a patient) the contents of the two chambers are mixed. This arrangement avoids the need for a separate mixing step at time of use.

[0124] The contents of the various kit components will generally all be in liquid form. In some arrangements, a component (typically the antigen component rather than the TLR-4 receptor agonist of formula (I) (such as E6020) component in a metabolizable lipid and immunoactive saponin nanoparticle in water composition) is in dry form (e.g. in a lyophilized form), with the other component being in liquid form. The two components can be mixed in order to reactivate the dry component and give a liquid composition for administration to a patient or subject. A lyophilized component will typically be located within a vial rather than a syringe. Dried components may include stabilizers such as lactose, sucrose or mannitol, as well as mixtures thereof e.g. lactose/sucrose mixtures, sucrose/mannitol mixtures, etc. A non-limiting example of one possible arrangement is a liquid emulsion or liposome component in a pre-filled syringe and an antigen component in a vial or in a second compartment of the pre-filled syringe.

[0125] If vaccines contain components in addition to the TLR-4 receptor agonist of formula (I) (such as E6020) in a metabolizable lipid and immunoactive saponin nanoparticle in water composition and antigen then these further components may be included in one these two-kit components, or may be part of a third kit component.

[0126] Suitable containers for mixed vaccines, or for individual kit components, include vials and disposable syringes. These containers should be sterile.

[0127] Where a composition/component is located in a container, such as a vial, the container can be made of a glass or plastic material. The vial can also be sterilized before the composition is added to it. To avoid problems with latex-sensitive patients, vials can be sealed with a latex-free stopper and the vial can be packaged in the absence of latex packing materials. In one embodiment, a vial has a butyl rubber stopper.

[0128] The vial may include a single dose of vaccine/component, or it may include more than one dose (a 'multidose' vial) e.g. 5, 6, or 10 doses. For example, with a single dose volume of 0.5 mL, a vial may include 10x0.5 mL composition for 10 doses, or 2.5 mL for 5 doses, and the container may be marked to show a single dose volume. In certain aspects, a container may contain multiple dosage volume (e.g., a multiple dose vial). In some embodiments, the vaccines are to be diluted with sterile solution (e.g., sterile 0.9% sodium chloride Injection, USP) before administration to subjects or patients.

[0129] The vials can be made of glass, such as colorless glass. A vial can have a cap (e.g. a Luer lock) adapted such that a pre-filled syringe can be inserted into the cap, the contents of the syringe can be expelled into the vial (e.g. to reconstitute lyophilized material therein), and the contents of the vial can be removed back into the syringe. After removal of the syringe from the vial, a needle can then be attached and the composition can be administered to a patient or subject. The cap is preferably located inside a seal or cover, such that the seal or cover has to be removed before the cap can be accessed.

[0130] Where a composition/component is packaged into a syringe, the syringe will not normally have a needle attached to it, although a separate needle may be supplied with the syringe for assembly and use. Safety needles can be used. 1-inch 23-gauge, 1-inch 25-gauge and 5/8-inch 25-gauge needles are typical. Syringes may be provided with peel-off labels on which the lot number, influenza season and expiration date of the contents may be printed, to facilitate record keeping. The plunger in the syringe preferably has a stopper to prevent the plunger from being accidentally removed during aspiration. The syringes may have a latex rubber cap and/or plunger. Disposable syringes contain a single dose of vaccine. The syringe will generally have a tip cap to seal the tip prior to attachment of a needle, and the tip cap is preferably made of a butyl rubber. If the syringe and needle are packaged separately then the needle is preferably fitted with a butyl rubber shield.

Methods of use of the adjuvant and vaccine compositions, methods of vaccination and treatment

[0131] The disclosure also provides the use of: (i) an aqueous preparation of an antigen; and

(ii) an lipid-in-water emulsion comprising squalene prepared according to the disclosure, in the manufacture of a medicament for raising an immune response in a subject or patient.

[0132] In some aspects, the recipient of a metabolizable lipid and immunoactive saponin nanoparticle in water composition or a vaccine composition is a patient or subject undergoing therapeutic treatment. Non-limiting examples include cancer treatment, chronic hepatitis, cytomegalovirus (CMV) infection, or tuberculosis infection.

[0133] In some aspects, the recipient of a metabolizable lipid and immunoactive saponin nanoparticle in water composition or a vaccine composition is a patient or subject undergoing prophylactic intervention. Non-limiting examples include influenza, SARS-CoV-2, SARS-CoV-1, rhinovirus, malaria, tuberculosis, HIV/AIDS, leishmaniasis, Group A streptococcus, respiratory syncytial virus (RSV), Middle East Respiratory Syndrome virus (MERS), hepatitis B, varicella zoster, coronavirus or human papilloma virus infection.

[0134] Various antigens can be used with the TLR-4 receptor agonist of formula (I) (for example, E6020) in a metabolizable lipid and immunoactive saponin nanoparticle in water composition, including but not limited to: viral antigens, such as viral surface proteins; bacterial antigens, such as protein and/or saccharide antigens; fungal antigens; parasite antigens; and tumor antigens. The application is particularly useful for vaccines against, for example, influenza, SARS-CoV-2, SARS-CoV-1, rhinovirus, malaria, tuberculosis, HIV/AIDS, leishmaniasis, Group A streptococcus, respiratory syncytial virus (RSV), Middle East Respiratory Syndrome virus (MERS), hepatitis B, varicella zoster, coronavirus or human papilloma virus infection.

[0135] The compositions can be administered in various ways. Possible methods of administration (including typical methods of immunization) include by intramuscular injection or subcutaneous injection, though alternative methods include intranasal, oral, intradermal, transcutaneous, and transdermal administration. Most vaccines are given by hypodermic or intramuscular injection as they are not absorbed reliably through the gut, however, live attenuated polio and some typhoid and cholera vaccines are given orally in order to produce immunity based in the bowel. In many aspects of the current disclosure, the method of administration is intramuscular or subcutaneous injection.

[0136] Vaccines prepared according to the disclosure may be used to inoculate both children and adults. The patient or subject may be less than 1 year old, 1-5 years old, 5-15 years old, 15-55 years old, or at least 55 years old. The patient or subject may be elderly (e.g. at least 50 years old, preferably at least 65 years), the young (e.g. less than 5 years old), hospitalized patients, healthcare workers, armed service and military personnel, pregnant women, the chronically ill, immunodeficient patients and subjects, and people travelling abroad. The

vaccines are not suitable solely for these groups, however, and may be used more generally in a population.

[0137] Vaccines may be administered to patients or subjects at substantially the same time as (e.g., during the same medical consultation or visit to a healthcare professional) other vaccines.

Examples

Materials and methods

[0138] Chemicals used in the examples herein, e.g. for buffers and substrates, are commercial products of at least reagent grade. Water utilized in the examples was sterile water for injection (sterile nonpyrogenic solute-free preparation of distilled water suitable for injection). "Adjuvant" or "adjuvant composition" used herein refers to an immunologically active metabolizable lipid nanoparticle in water composition and is used interchangeably.

Example 1: preparation of the LQ and LEQ adjuvants

[0139] In this example, liposomes and E6020-loaded liposomes were produced by ethanol injection. DOPC (dioleoyl phosphatidylcholine) and cholesterol with or without E6020 were dissolved in ethanol, then injected rapidly under stirring in an aqueous phase made of phosphate buffered saline (12 mM total phosphate, 137 mM NaCl, 2.7 mM KCl, pH 6.3), resulting in an homogenous suspension of liposomes. The ethanol was then removed by tangential flow filtration using a membrane with a molecular cut-off of 100 kDa. The LQ and LEQ adjuvants were prepared by mixing a volume of neutral liposomes or E6020-loaded liposomes with a concentrated QS21 solution in PBS buffer pH 6.3 respectively. The resulting LQ and LEQ liposome suspensions were then diluted to 2 mg/mL DOPC, 0.5 mg/mL cholesterol and 0.2mg/mL QS21 for the LQ adjuvant or 2 mg/mL DOPC, 0.5 mg/mL cholesterol, 0.08 mg/mL E6020 and 0.2 mg/mL QS21 for the LEQ adjuvant and sterile filtered through a 0.22 μ m membrane under aseptic conditions. The sterile adjuvants were then stored in sterile polypropylene containers and kept at 2-8°C until use. Thus, the only difference between the resulting LQ and LEQ adjuvants is the inclusion of E6020 in LEQ. Various dose ranges of E6020 in the resulting LEQ were tested and observed to be effective using the assays described herein, for example, in the range of 0.004 mg/mL to 0.1 mg/mL, or from 0.01

mg/mL to 0.08 mg/mL.

Example 2: preparation of the SQ and SEQ adjuvants

5 [0140] In this example, squalene lipid in water emulsion (O/W emulsion) and E6020-loaded squalene emulsion were produced by microfluidization. Squalene, polyoxyethylene sorbitan monooleate, sorbitan trioleate, with or without cholesterol and E6020 and citrate buffer (10 mM total citrate, pH 6.5) were mixed by high-speed homogenization and the mixtures submitted to microfluidization at 20 000 psi to yield squalene lipid in water emulsion or E6020-
10 loaded squalene emulsion. The SQ and SEQ adjuvants were prepared by mixing a volume of squalene lipid in water emulsion or E6020-loaded squalene lipid in water emulsion with a concentrated QS21 solution in phosphate buffered saline (12 mM total phosphate, 137 mM NaCl, 2.7 mM KCl, pH 6.3) respectively. The resulting SQ and SEQ emulsions were then diluted to 42 mg/mL squalene, 1 mg/mL cholesterol, 5 mg/mL polyoxyethylene sorbitan
15 monooleate, 5 mg/mL sorbitan trioleate, and 0.2 mg/mL QS21 (SQ) or 42 mg/mL squalene, 1 mg/mL cholesterol, 5 mg/mL polyoxyethylene sorbitan monooleate, 5 mg/mL sorbitan trioleate, 0.08 mg/mL E6020 and 0.2 mg/mL QS21 (SEQ) and sterile filtered through a 0.22 µm membrane under aseptic conditions. The sterile adjuvants were then stored in sterile polypropylene containers and kept at 2-8°C until use. Thus, the only difference between the
20 resulting SQ and SEQ adjuvants is the inclusion of E6020 in SEQ. Various dose ranges of E6020 in the resulting SEQ were tested and observed to be effective using the assays described herein, such as in the range of 0.004 mg/mL to 0.1 mg/mL, or from 0.01 mg/mL to 0.08 mg/mL.

25 Example 3: pre-clinical evaluation of LEQ-adjuvanted influenza vaccine in mice

Example 3 a: duration

[0141] Prior to vaccination, compatibility of the split H5N1 antigen (SH5N1) with the LQ and
30 LEQ adjuvants, as prepared in Example 1, was confirmed in formulation studies, with the antigen integrity checked by Single Radial Immuno-Diffusion (hereinafter, "SRID"). C57BL/6J mice were vaccinated intramuscularly at day 0 and day 21 with 50µL split H5N1 (A/turkey/Turkey/1/2005 (SH5N1)) containing 2µg of hemagglutinin either (i) non adjuvanted or adjuvanted with a (ii) squalene lipid in water emulsion ("O/W emulsion"), (iii) LQ (comprising

5µg QS21) or (iv) LEQ adjuvant (comprising 2 µg E6020, 5 µg QS21) of Example 1. The squalene lipid in water emulsion adjuvant used in the examples herein comprised 3.9% (39 mg/mL) of squalene, 0.47% (4.7 mg/mL) Tween 80, 0.47% (4.7 mg/mL) Span 85, citrate buffer 10 mM pH 6.5. Mice were bled at various time points between 14 days and 149 days. Serum antibody titers against HA were determined using hemagglutination inhibition assay (HAI), performed according to the WHO manual for laboratory diagnosis and virological surveillance of influenza (World Health Organization, 2011). All serum samples were pre-treated overnight at 37 °C with receptor-destroying enzyme and further diluted in 96-well round-bottom plates to a final serum dilution of 1/10. Individual sera were serially 2-fold diluted in V-bottom microtiter plates and mixed with whole inactivated virus adjusted to 4 hemagglutination units for 30 min at room temperature. Chicken red blood cells (RBC) were then added. Once control RBCs were settled (30-40 min), the plate was tilted, and the assay imaged for further analysis. HAI titers were calculated as the reciprocal of the last serum dilution that contained non-agglutinated RBCs (tearing). Samples with a result below the lowest dilution (1:10) were assigned an HAI titer of 9.

[0142] The results are displayed in FIG. 2. HAI titers obtained with LEQ adjuvant remained substantially high during the full duration of the experiment and higher than HAI titers obtained with antigen alone, or antigen formulated with a squalene lipid in in water emulsion ("O/W emulsion") adjuvant or the LQ adjuvant. Furthermore, the average HAI titer obtained for the LEQ-adjuvanted formulation always remained well above the critical threshold titer of 40. Indeed, the average LEQ HAI titers at the final time point were greater than 80, i.e. more than twice the recognized threshold and at the longest measured time point for immune response. For the avoidance of doubt, LEQ was the most effective adjuvant tested.

Example 3 b: breadth of response

[0143] Prior to vaccination, compatibility of the split H5N1 antigen with the O/W emulsion, LQ and LEQ adjuvants was confirmed in formulation studies and antigen integrity confirmed by SRID. C57BL/6J mice were vaccinated intramuscularly on days 0 and 21 with 50µL comprising 2µg HA equivalent of split H5N1 (A/turkey/Turkey/1/2005 ("SH5N1")) either (i) non adjuvanted or adjuvanted with (ii) a squalene lipid in water emulsion adjuvant (O/W emulsion), (iii) the LQ adjuvant (comprising 5µg QS21saponin) and (iv) the LEQ adjuvant (comprising 2 µg E6020, 5 µg QS21) of Example 1. Mice were bled at day 35. Serum antibody titers against HA from a homologous strain (A/tk/Turkey/1/2005 wt Clade 2.2.1) shown in FIG. 3A and FIG. 3B for convenience or a heterologous strain (A/Anhui/1/05 Clade 2.3.4) were determined using

hemagglutination inhibition (HAI), performed according to the WHO manual for the laboratory diagnosis and virological surveillance of influenza (World Health Organization, 2011). All serum samples were pre-treated overnight at 37 °C with receptor-destroying enzyme and further diluted in 96-well round-bottom plates to a final serum dilution of 1/10. Individual sera were serially 2-fold diluted in V-bottom microtiter plates and mixed with whole inactivated virus (WIV) adjusted to 4 hemagglutination units for 30 min at room temperature. Chicken red blood cells were then added. Once control RBCs were settled (30-40 min), the plate was tilted, and the assay imaged for further analysis. HAI titers were calculated as the reciprocal of the last serum dilution that contained non-agglutinated RBCs (tearing). Samples with a result below the lowest dilution (1:10) were assigned an HAI titer of 8.

[0144] Results presented in FIG. 3A and FIG. 3B show that heterologous A/Anhui/1/05 clade 2.3.4 H5N1 HAI titers observed in all groups vaccinated with adjuvanted SH5N1 were higher than those induced by non-adjuvanted SH5N1 antigen. Furthermore, the LEQ adjuvanted SH5N1 formulation induced higher hemagglutination inhibition titers to both homologous ("A/tk/Tk/1/05 WT Clade 2.2.1") and heterologous ("A/Anhui/1/05" Clade 2.3.4") strains than the LQ adjuvant. Furthermore, samples from 100% of the animals that received LEQ-adjuvanted formulation achieved average HAI titers on or above the threshold value of 40, for both homologous and heterologous strains. On the other hand, only animals that received LQ and LEQ adjuvanted formulation displayed HAI titers above the 40 thresholds for the heterologous strain. For the avoidance of doubt, the homologous strain assay measures immune response to the specific antigen used in the vaccine formulation, whereas the heterologous strain assay measures whether antibodies induced by vaccination can recognise a heterologous strain where there is some degree of diversity in the haemagglutinin sequence (the antigen being tested), representative of potential mutations that could arise during a pandemic or outbreak. For example, influenza viruses are known to undergo antigenic drift, a change due to the gradual accumulation of mutations in the antigen sequence. As antigenic drift can lead to virus particles not being effectively inhibited by antibodies raised against a related virus or vaccine antigen, cross protective vaccine formulations are more effective at protecting populations from seasonal or pandemic influenza. Thus, the heterologous strain assay is considered a reliable bellwether for likely cross-protective vaccine formulations. SEQ performed particularly well in the heterologous strain assay.

[0145] To summarise, these data clearly demonstrate a considerable improvement in immune activation (correlated to immunity and therefore vaccine efficacy) of antigen delivered with immunologically active adjuvant compositions comprising E6020 in a metabolizable lipid nanoparticle in water composition of the current disclosure, when compared to antigen

delivered with a benchmark squalene lipid in water emulsion adjuvant, antigen delivered with a liposome adjuvant containing the QS21 saponin, and to non-adjuvanted antigen.

Example 3c:

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[0146] C57BL/6J mice were vaccinated intramuscularly on days 0 and 21 with 2 µg per dose (50µL) of (i) non adjuvanted SH5N1, (ii) SH5N1 adjuvanted with squalene lipid in water emulsion, (O/W emulsion) (iii) LQ (comprising 5 µg QS21) and (iv) LEQ (comprising 2 µg E6020, 5 µg QS21) of Example 1. On day 175, the mice were boosted with non-adjuvanted SH5N1 and spleens were collected on day 182. Splenocytes were isolated by crushing spleens through a 70 µm cell strainer, then washed with incomplete RPMI medium (1% penicillin/streptomycin, 20 mM Hepes + 1X Minimum Essential Medium Non Essential Amino Acids (MEM NEAA) followed by red blood cell removal using Lympholyte®-M cell separation medium. One million cells were stimulated in round-bottom 96-well plates in complete RPMI medium (incomplete RPMI + 6% fetal calf serum (FCS) + 50 µM β-mercaptoethanol) with 50 µL/well of whole inactivated H5N1 virus at a final concentration of 30 µg/mL total viral proteins and incubated for 2 h at 37°C. Cytokine secretion was blocked using GolgiPlug™ (available from BD Biosciences) protein transport inhibitor (50 µL/well, 1:1000 dilution) and cells were further incubated overnight at 37°C with 5% CO₂. Cells were put on ice for 30 minutes, then transferred to V-bottom 96-well plates and stained for 15 min at 4°C using the LIVE/DEAD™ Kit. Afterwards, cell surface staining was performed with anti-CD3-FITC (1:100) (eBioscience), anti-CD8a-PerCP-Cy5.5 (1:100) and anti-CD4-Pacific Blue (1:200) (both from BioLegend®) for 15 min on ice. After washing, fixation and permeabilization, cells were incubated for 30 min at 4°C with anti-IL-2-APC (BioLegend®) and anti-IFNγ-PE (BD Pharmingen™) for intracellular staining. Cells were washed 3 times with Perm/Wash buffer and were finally suspended in 300 µL PBS and filtered prior to acquisition on an Attune™ NxT flow cytometer (Invitrogen). Compensation was performed using the Arc™ Amine Reactive Compensation Bead Kit and AbC™ Anti-Rat/Hamster Bead kit (both from Invitrogen), and compensation matrices were calculated with the Attune™ NxT software (Invitrogen). FACS data were analyzed using FlowJo software (FlowJo LLC, USA). Results are shown as the mean of cumulated frequencies of cells expressing IL-2 only, IFNγ only or IL-2 and IFNγ-minus unstimulated background, as in FIG. 4A and FIG. 4B. While all adjuvanted H5N1 vaccinated groups had detectable CD4 T cells expressing IL-2 and or IFNγ, LEQ showed the highest detectable T cell responses. We again conclude from these data that the immunologically active adjuvant formulations of the current compositions comprising E6020 in a metabolizable lipid

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nanoparticle in water composition demonstrated in these assays considerable improvements in immune activation over the benchmark squalene lipid in water emulsion adjuvant and the LQ adjuvant.

5 Example 4: pre-clinical evaluation of LEQ-adjuvanted and SEQ-adjuvanted COVID-19 vaccine in mice

Example 4 a: antibody responses

10 **[0147]** C57BL/6J mice were vaccinated intramuscularly on days 0 and 21 with 50µL comprising 2 µg of the SARS-CoV-2 prefusion spike protein antigen, either (i) non adjuvanted or (ii) adjuvanted with alum, (iii) adjuvanted with LQ (comprising 5 µg QS21) of Example 1, (iv) adjuvanted with LEQ adjuvant (comprising 2 µg E6020, 5 µg QS21) of Example 1, (v) adjuvanted with SQ (comprising 5 µg QS21) of Example 2, or (vi) adjuvanted with SEQ
15 adjuvant (comprising 2 µg E6020, 5 µg QS21) of Example 2. Mice were bled on day 42 (3 weeks after the second injection). Functional activity of sera was analysed using the SARS-CoV-2 surrogate virus neutralization test (sVNT) kit from Genscript®. Correlation between conventional VNT and sVNT was confirmed. The sVNT assay uses purified receptor binding domain (RBD) from the SARS-CoV-2 viral spike (S) protein and the patient or subject cell
20 receptor ACE2, and is accepted by those skilled as a suitable assay because it mimics the virus-patient or subject interaction by the same direct protein-protein interaction in a test tube or an ELISA plate well. This highly specific interaction can then be neutralized, i.e., blocked by the neutralizing antibodies (Nabs) in patient or animal sera in the same manner as in a conventional VNT. As show in FIG. 5 at 3 weeks after the second injection, both LEQ adjuvant
25 of Example 1 and SEQ adjuvant of Example 2 were observed to induce higher neutralization titers compared to the non-adjuvanted formulation, to the aluminium salts-based benchmark adjuvant formulation and to the LQ and SQ adjuvanted formulations. We conclude from these data that the immunologically active adjuvant formulations of the current compositions comprising E6020 in a metabolizable lipid nanoparticle in water composition demonstrate
30 considerable improvements in immune activation, over benchmark adjuvant formulation and metabolizable lipid nanoparticle associated with QS21 as measured by functional antibody titer.

Example 4 b: cellular response

[0148] C57BL/6J mice were vaccinated intramuscularly on day 0 and day 21 with 2 µg per dose (50 µL) of SARS-CoV-2 prefusion spike protein either (i) non adjuvanted, (ii) adjuvanted with aluminium salts, (iii) adjuvanted with SQ adjuvant (comprising 5 µg QS21) of Example 2, (iv) adjuvanted with SEQ adjuvant (2 µg E6020, 5 µg QS21) of Example 2, (v) adjuvanted with LQ adjuvant (comprising 5 µg QS21) of Example 1, and LEQ adjuvant (comprising 2 µg E6020, 5 µg QS21) of Example 1. At day 28, spleens from mice were collected. Splenocytes were isolated by crushing spleens through a 70 µm cell strainer, then washed with incomplete RPMI medium (1% penicillin/streptomycin, 20 mM Hepes + 1X MEM NEAA) followed by red blood cell removal using Lympholyte®-M cell separation medium. One million cells were stimulated in round-bottom 96-well plates in complete RPMI medium (incomplete RPMI + 6% FCS + 50 µM β-mercaptoethanol) with 50 µL/well of peptide medium at a final concentration of 1.44 µg/mL and incubated for 2 h at 37°C, 5% CO₂. Cytokine secretion was blocked using GolgiPlug™ (available from BD Biosciences) protein transport inhibitor (50 µL/well, 1:1000 final dilution) and cells were further incubated overnight at 37°C, 5% CO₂. Cells were put on ice for 30 minutes then transferred to V-bottom 96-well plates and were stained for 15 minutes at 4°C using the LIVE/DEAD™ Kit. Afterwards, cell surface staining was performed with anti-CD3-PE-Cy7 and anti-CD8a-BV605 (BioLegend®) at 1:400 dilution and anti-CD4-PerCP-Cy5.5 (BioLegend®) at 1:800 dilution for 15 min on ice. After washing, fixation and permeabilization, cells were incubated for 30 min at 4°C with anti-IL-2-A488 (BioLegend®) and anti-IFNγ-PE (BD Pharmingen™) for intracellular staining. Cells were washed 3 times with Perm/Wash buffer and were finally suspended in 300 µL PBS and filtered prior to acquisition on an Attune™ NxT flow cytometer (Invitrogen). Compensation was performed using the Arc™ Amine Reactive Compensation Bead Kit and AbC™ Anti-Rat/Hamster Bead kit (both from Invitrogen), and compensation matrices were calculated with the Attune™ NxT software (Invitrogen). FACS data were analyzed using FlowJo software (FlowJo LLC, USA). Results are shown as the mean of cumulated frequencies of cells expressing IL-2 only, IFNγ only or IL-2 and IFNγ-minus unstimulated background.

[0149] Both LEQ and SEQ adjuvants induced high detectable T cell responses compared to the non-adjuvanted formulation and aluminium salts formulation for which the T cell responses (CD4⁺ and CD8⁺) were at the background level, as shown in FIG. 6 and FIG. 7. Furthermore, the SEQ and LEQ adjuvanted formulations induced higher CD4⁺ and CD8⁺ T cell responses than those of the SQ and LQ adjuvanted groups respectively. We again conclude from these data that the immunologically active adjuvant formulations of the current compositions comprising E6020 in a metabolizable lipid nanoparticle in water composition also

demonstrated in these assays considerable improvements in immune activation over the aluminium salts benchmark adjuvant formulation or the corresponding liposomal or emulsion adjuvants comprising QS21 saponin and not E6020.

5 **Example 5: pre-clinical evaluation of LEQ-adjuvanted and SEQ-adjuvanted influenza vaccine in mice**

Example 5 a: antibody responses

10 **[0150]** Prior to vaccination, compatibility of the recombinant HA1 subunit influenza antigen (H3N2) with the LEQ and SEQ adjuvants, as prepared in Example 1 and Example 2, was confirmed in formulation studies, with the antigen integrity checked by SRID and Bio-layer interferometry (hereinafter, "BLI"). C57BL/6J mice were vaccinated intramuscularly at day 0 and day 21 with 50µL comprising recombinant HA1 H3N2 (A/Texas/50/2012 (H3N2))
15 containing 2 µg of haemagglutinin either (i) non adjuvanted or adjuvanted with a (ii) squalene oil in water emulsion ("O/W emulsion"), (iii) SQ adjuvant (comprising 5 µg QS21) of Example 2, (iv) SEQ (comprising 2 µg E6020, 5 µg QS21) of Example 2, or (v) LQ adjuvant (comprising 5 µg QS21) of Example 1, (vi) SEQ (comprising 2 µg E6020, 5 µg QS21) of Example 1. The squalene oil in water emulsion adjuvant used in the examples herein comprised 3.9% of
20 squalene (39 mg/mL of squalene), 0.47% Tween 80 (4.7 mg/mL of Tween 80), 0.47% Span 85 (4.7 mg/mL of Span85), and citrate buffer 10 mM pH 6.5. Mice were bled at various time points between 14 days and 154 days. Serum antibody titers against HA were determined using hemagglutination inhibition assay (HAI), performed according to the WHO manual for laboratory diagnosis and virological surveillance of influenza (World Health Organization,
25 2011). All serum samples were pre-treated overnight at 37°C with receptor-destroying enzyme and further diluted in 96-well round-bottom plates to a final serum dilution of 1/10. Individual sera were serially 2-fold diluted in V-bottom microtiter plates and mixed with whole inactivated virus adjusted to 4 hemagglutination units for 30 min at room temperature. Washed Turkey red blood cells (RBC) were then added. Once control RBCs were settled (30-40 min), the plate
30 was tilted, and the assay imaged for further analysis. Homologous HAI titers were calculated as the reciprocal of the last serum dilution that contained non-agglutinated RBCs (tearing). Samples with a result below the lowest dilution (1:10) were assigned an HAI titer of 9.

[0151] The resulting homologous HAI titers are displayed in FIG. 8. Homologous HAI titers obtained with SEQ and LEQ adjuvant remained substantially high during the full duration of
35 the experiment and higher than homologous HAI titers obtained with antigen alone, or antigen

formulated with a squalene oil in water emulsion ("O/W emulsion") or with the SQ or LQ adjuvant. Furthermore, the average homologous HAI titer obtained for the SEQ- and LEQ- adjuvanted formulation always remained well above 40. Indeed, the average SEQ homologous HAI titers were higher than LEQ homologous HAI titers at the longest measured time point for immune response. For the avoidance of doubt, LEQ and SEQ were the most effective adjuvants tested.

Example 5 b: cellular response

10 [0152] C57BL/6J mice were vaccinated intramuscularly at day 0 and day 21 with 50µL comprising recombinant HA1 H3N2 (A/Texas/50/2012 (H3N2)) containing 2 µg of hemagglutinin either (i) non adjuvanted or adjuvanted with a (ii) squalene oil in water emulsion ("O/W emulsion"), (iii) SQ adjuvant (comprising 5 µg QS21) of Example 2, (iv) SEQ (comprising 2 µg E6020, 5 µg QS21) of Example 2, or (v) LQ adjuvant (comprising 5 µg QS21) of Example 1, (vi) LEQ (comprising 2 µg E6020, 5 µg QS21) of Example 1. At day 28, spleens from mice were collected. Splenocytes were isolated by crushing spleens through a 70 µm cell strainer, then washed with incomplete RPMI medium (1% penicillin/streptomycin, 20 mM Hepes + 1X MEM NEAA) followed by red blood cell removal using Lympholyte®-M cell separation medium. One million cells were stimulated in round-bottom 96-well plates in complete RPMI medium (incomplete RPMI + 6% FCS + 50 µM β-mercaptoethanol) with 50 µL/well of peptide medium at a final concentration of 1.44 µg/mL and incubated for 2 h at 37°C, 5% CO₂. Cytokine secretion was blocked using GolgiPlug™ (available from BD Biosciences) protein transport inhibitor (50 µL/well, 1:1000 dilution) and cells were further incubated overnight at 37°C, 5% CO₂. Cells were put on ice for 30 minutes then transferred to V-bottom 96-well plates and were stained for 15 minutes at 4°C using the LIVE/DEAD™ Kit. Afterwards, cell surface staining was performed with anti-CD3-FITC (1:100) (eBioscience), anti-CD8a-PerCP-Cy5.5 (1:100) and anti-CD4-Pacific Blue (1:200) (both from (BioLegend®) for 15 min on ice. After washing, fixation and permeabilization, cells were incubated for 30 min at 4°C with anti-IL-2-APC and anti-IFNγ-PE (BD Pharmingen™) for intracellular staining. Cells were washed 3 times with Perm/Wash buffer and were finally suspended in 300 µL PBS and filtered prior to acquisition on an Attune™ NxT flow cytometer (Invitrogen). Compensation was performed using the Arc™ Amine Reactive Compensation Bead Kit and AbCTM Anti-Rat/Hamster Bead kit (both from Invitrogen) and compensation matrices were calculated with the Attune™ NxT software (Invitrogen). FACS data were analyzed using FlowJo software

(FlowJo LLC, USA). Results are shown as the mean of cumulated frequencies of cells expressing IL-2 only, IFN γ only or IL-2 and IFN γ -minus unstimulated background.

[0153] Results presented in FIG. 9A and FIG. 9B show that both LEQ and SEQ adjuvants induced high T cell responses compared to the non-adjuvanted formulation for which the T cell responses (CD4+ and CD8+) were at the background level, as shown in FIG. 6 and FIG. 7. Furthermore, the SEQ and LEQ adjuvanted formulations induced higher CD4+ and CD8+ T cell responses than those of the SQ and LQ adjuvanted groups respectively. We again conclude from these data that the immunologically active adjuvant formulations of the current compositions comprising E6020 in a metabolizable lipid nanoparticle in water composition also demonstrated in these assays considerable improvements in immune activation over the corresponding liposomal or emulsion adjuvants comprising QS21 saponin and not E6020.

[0154] In summary, these data clearly demonstrate a considerable improvement in immune activation (correlated to immunity and therefore vaccine efficacy) of antigen delivered with immunologically active adjuvant compositions comprising E6020 in a metabolizable lipid nanoparticle in water composition of the current disclosure, when compared to antigen delivered with a benchmark squalene oil-in-water emulsion adjuvant, antigen delivered with a liposome adjuvant containing the QS21 saponin, and to non-adjuvanted antigen.

Example 6: pre-clinical evaluation of SEQ-adjuvanted COVID-19 vaccine in mice

Example 6 a: antibody responses

[0155] C57BL/6J mice were vaccinated intramuscularly on days 0 and 21 with 50 μ L comprising 2 μ g of the SARS-CoV-2 prefusion spike protein antigen, either (i) non adjuvanted or (ii) squalene oil in water emulsion ("O/W emulsion"), (iii) adjuvanted with SQ (comprising 5 μ g QS21) of Example 2, or (iv) adjuvanted with SEQ adjuvant (comprising 2 μ g E6020, 5 μ g QS21) of Example 2. Mice were bled on day 35 (2 weeks after the second injection). Functional activity of sera was analyzed using the SARS-CoV-2 surrogate virus neutralization test (sVNT) kit from Genscript®. Correlation between conventional VNT and sVNT was confirmed. The sVNT assay uses purified receptor binding domain (RBD) from the SARS-CoV-2 viral spike (S) protein and the patient or subject cell receptor ACE2 and is accepted by those skilled in this art as a suitable assay because it mimics the virus-patient or subject interaction by the same direct protein-protein interaction. This highly specific interaction can then be neutralized, i.e., blocked by the neutralizing antibodies (Nabs) in patient or animal sera in the same manner as in a conventional VNT. As shown in FIG. 10 at 2 weeks after the second injection, SEQ adjuvant of Example 2, was observed to induce higher neutralization titres compared to the

non-adjuvanted formulation, to the O/W emulsion adjuvanted formulations, and to the SQ adjuvanted formulations. We conclude from these data that immunologically active metabolizable lipid nanoparticle in water compositions comprising E6020 demonstrate considerable improvements in immune activation, as measured by functional antibody titre, over benchmark adjuvant formulations and metabolizable lipid nanoparticle in water compositions comprising QS21 and not E6020.

Example 6 b: cellular response

[0156] C57BL/6J mice were vaccinated intramuscularly on day 0 and day 21 with 2 µg per dose (50 µL) of SARS-CoV-2 prefusion spike protein antigen either (i) non adjuvanted or (ii) squalene oil in water emulsion ("O/W emulsion"), (iii) adjuvanted with SQ (comprising 5 µg QS21) of Example 2, or (iv) adjuvanted with SEQ adjuvant (comprising 2 µg E6020, 5 µg QS21) of Example 2. At day 35, spleens from mice were collected. Splenocytes were isolated by crushing spleens through a 70 µm cell strainer, then washed with incomplete RPMI medium (1% penicillin/streptomycin, 20 mM Hepes + 1X MEM NEAA) followed by red blood cell removal using Lympholyte®-M cell separation medium. One million cells were stimulated in round-bottom 96-well plates in complete RPMI medium (incomplete RPMI + 6% FCS + 50 µM β-mercaptoethanol) with 50 µL/well of peptide medium at a final concentration of 1.44 µg/mL and incubated for 2 h at 37°C, 5% CO₂. Cytokine secretion was blocked using GolgiPlug™ (available from BD Biosciences) protein transport inhibitor (50 µL/well, 1:1000 final dilution) and cells were further incubated overnight at 37°C, 5% CO₂. Cells were put on ice for 30 minutes then transferred to V-bottom 96-well plates and were stained for 15 minutes at 4°C using the LIVE/DEAD™ Kit. Afterwards, cell surface staining was performed with anti-CD3-FITC (1:100) (eBioscience), anti-CD8a-PerCP-Cy5.5 (1:100) and anti-CD4-Pacific Blue (1:200) (both from (BioLegend®) for 15 min on ice. After washing, fixation and permeabilization, cells were incubated for 30 min at 4°C with anti-IL-2-APC and anti-IFNγ-PE (BD Pharmingen™) for intracellular staining. Cells were washed 3 times with Perm/Wash buffer and were finally suspended in 300 µL PBS and filtered prior to acquisition on an Attune™ NxT flow cytometer (Invitrogen). Compensation was performed using the Arc™ Amine Reactive Compensation Bead Kit and AbC™ Anti-Rat/Hamster Bead kit (both from Invitrogen) and compensation matrices were calculated with the Attune™ NxT software (Invitrogen). FACS data were analyzed using FlowJo software (FlowJo LLC, USA). Results are shown as the mean of cumulated frequencies of cells expressing IL-2 only, IFNγ only or IL-2 and IFNγ-minus unstimulated background.

[0157] Results presented in FIG. 11A and FIG. 11B show that SEQ adjuvant induced high detectable T cell responses compared to the non-adjuvanted formulation, and the O/W emulsion adjuvanted formulations, for which the T cell response (CD8+) were at the background level, as shown in FIG. 11B. Furthermore, the SEQ adjuvanted formulation induced higher CD4+ and CD8+ T cell responses than SQ adjuvanted groups, respectively. We again conclude from these data that immunologically active metabolizable lipid nanoparticle in water compositions comprising E6020 also demonstrated in these assays considerable improvements in immune activation over the corresponding emulsion adjuvants comprising QS21 saponin and not E6020.

[0158] To summarise, these data clearly demonstrate a considerable improvement in immune activation (correlated to immunity and therefore vaccine efficacy) of antigen delivered with immunologically active metabolizable lipid nanoparticle in water compositions comprising E6020 of the current disclosure, when compared to antigen delivered with a benchmark squalene oil-in-water emulsion adjuvant containing the QS21 saponin without E6020, and to non-adjuvanted antigen.

Example 7: pre-clinical evaluation of LEQ-adjuvanted COVID-19 vaccine in mice

Example 7 a: antibody responses

[0159] C57BL/6J mice were vaccinated intramuscularly on days 0 and 21 with 50µL comprising 2 µg of the SARS-CoV-2 prefusion spike protein antigen, either (i) non adjuvanted or (ii) adjuvanted with LE (comprising 2 µg E6020), (iii) adjuvanted with LQ adjuvant (comprising 5 µg QS21), or (iii) adjuvanted with LEQ (comprising 2 µg E6020, 5 µg QS21) of Example 1. Mice were bled on day 35 (2 weeks after the second injection). Functional activity of sera was analyzed using the SARS-CoV-2 surrogate virus neutralization test (sVNT) kit from Genscript®. Correlation between conventional VNT and sVNT was confirmed. The sVNT assay uses purified receptor binding domain (RBD) from the SARS-CoV-2 viral spike (S) protein and the patient or subject cell receptor ACE2, and is accepted by those skilled in this art as a suitable assay because it mimics the virus-patient or subject interaction by the same direct protein-protein interaction. This highly specific interaction can then be neutralized, i.e., blocked, by the neutralizing antibodies (Nabs) in patient or animal sera in the same manner as in a conventional VNT. Results presented in FIG. 12 show that 2 weeks after the second injection, the LEQ adjuvant of Example 1 was observed to induce higher neutralization titres compared to the non-adjuvanted formulation, LE and LQ adjuvanted formulations. We

conclude from these data that the combination of immunologically active adjuvant formulations of the current compositions comprising E6020 and QS21 in a metabolizable lipid nanoparticle demonstrate considerable improvements in immune activation, over an adjuvant associated with QS21 or E6020 alone, as measured by functional antibody titer.

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Example 7 b: cellular response

[0160] C57BL/6J mice were vaccinated intramuscularly on day 0 and day 21 with 2 µg per dose (50 µL) of SARS-CoV-2 prefusion spike protein antigen either (i) non adjuvanted or (ii) 10 adjuvanted with LE (comprising 2 µg E6020), (iii) adjuvanted with LQ adjuvant (comprising 5 µg QS21), (iii) adjuvanted with LEQ (comprising 2 µg E6020, 5 µg QS21) of Example 1. At day 35, spleens from mice were collected. Splenocytes were isolated by crushing spleens through a 70 µm cell strainer, then washed with incomplete RPMI medium (1% penicillin/streptomycin, 20 mM Hepes + 1X MEM NEAA) followed by red blood cell removal 15 using Lympholyte®-M cell separation medium. One million cells were stimulated in round-bottom 96-well plates in complete RPMI medium (incomplete RPMI + 6% FCS + 50 µM β-mercaptoethanol) with 50 µL/well of peptide medium at a final concentration of 1.44 µg/mL and incubated for 2 h at 37°C, 5% CO₂. Cytokine secretion was blocked using GolgiPlug™ (available from BD Biosciences) protein transport inhibitor (50 µL/well, 1:1000 final dilution) 20 and cells were further incubated overnight at 37°C, 5% CO₂. Cells were put on ice for 30 minutes then transferred to V-bottom 96-well plates and were stained for 15 minutes at 4°C using the LIVE/DEAD™ Kit. Afterwards, cell surface staining was performed with anti-CD3-FITC (1:100) (eBioscience), anti-CD8a-PerCP-Cy5.5 (1:100) and anti-CD4-Pacific Blue (1:200) (both from (BioLegend®) for 15 min on ice. After washing, fixation and 25 permeabilization, cells were incubated for 30 min at 4°C with anti-IL-2-A488 (BioLegend®) and anti-IFNγ-PE (BD Pharmingen™) for intracellular staining. Cells were washed 3 times with Perm/Wash buffer and were finally suspended in 300 µL PBS and filtered prior to acquisition on an Attune™ NxT flow cytometer (Invitrogen). Compensation was performed using the Arc™ Amine Reactive Compensation Bead Kit and AbC™ Anti-Rat/Hasmter Bead kit (both from 30 Invitrogen) and compensation matrices were calculated with the Attune™ NxT software (Invitrogen). FACS data were analyzed using FlowJo software (FlowJo LLC, USA). Results are shown as the mean of cumulated frequencies of cells expressing IL-2 only, IFNγ only or IL-2 and IFNγ-minus unstimulated background.

[0161] Results presented in FIG. 13A and FIG. 13B show that LEQ adjuvant induced high

detectable T cell responses compared to the non-adjuvanted formulation. Furthermore, the LEQ adjuvanted formulation induced higher CD4+ and CD8+ T cell responses than LE and LQ adjuvanted groups, respectively. We again conclude from these data that the immunologically active metabolizable lipid nanoparticle in water compositions comprising E6020 and QS21 of the current disclosure demonstrate considerable improvements in immune activation over the corresponding emulsion adjuvants comprising either QS21 saponin or E6020.

[0162] To summarise, these data clearly demonstrate a considerable improvement in immune activation (correlated to immunity and therefore vaccine efficacy) of antigen delivered with immunologically active adjuvant compositions comprising E6020 and QS21 in a metabolizable lipid nanoparticle of the current disclosure, when compared to antigen delivered with a benchmark antigen delivered with a liposome adjuvant containing the E6020 or QS21 saponin alone, and to non-adjuvanted antigen.

Embodiments

Item 1. An immunologically active metabolizable lipid nanoparticle in water composition comprising:

- a) 0.004 – 0.1 mg/mL E6020,
- b) 0.04 – 200 mg/mL metabolizable lipid,
- c) 0.02– 0.4 mg/mL saponin, and
- d) 0.02– 50 mg/mL sterol,

wherein the ratio (wt/wt) of sterol to saponin is between 1:1 to 100:1 and the average nanoparticle diameter of the metabolizable lipid nanoparticles is less than 200nm.

Item 2. The immunologically active metabolizable lipid nanoparticle in water composition of item 1, wherein immunologically active metabolizable lipid nanoparticle is present as an emulsion.

Item 3. The composition of item 1 or 2, comprising:

- a) 0.004 – 0.1 mg/mL E6020,
- b) 0.04 – 200 mg/mL metabolizable lipid,
- c) 0.02– 0.4 mg/mL saponin,

- d) 0.5 – 100 mg/mL hydrophilic surfactant,
 - e) 0.5 – 100 mg/mL hydrophobic surfactant,
 - f) 0.02 – 50 mg/mL sterol, and
 - g) pharmaceutically acceptable aqueous buffer pH 4-9,
- 5 wherein the ratio (wt/wt) of sterol to saponin is between 1:1 to 100:1.

Item 4. The composition of any preceding item, comprising:

- a) 0.004 mg/mL – 0.04 mg/mL E6020,
 - b) 5 – 100 mg/mL squalene,
 - 10 c) 0.02 mg/mL – 0.4 mg/mL QS-21,
 - d) 0.5 – 100 mg/mL polysorbate,
 - e) 0.5 – 100 mg/mL sorbitan ester,
 - f) 0.02 mg/mL – 50 mg/mL cholesterol, and
 - g) aqueous buffer 10 mM isotonic with NaCl pH 5.5-7,
- 15 wherein the ratio (wt/wt) of squalene to each of the surfactants polysorbate and sorbitan ester are 1:1,
- the ratio (wt/wt) of cholesterol to sorbitan ester is 1:1, and the ratio (wt/wt) of cholesterol to QS-21 is between 1:1 and 100:1.

20 Item 5. The composition of any preceding item, comprising:

- a) 0.040 mg/mL E6020,
 - b) 39 mg/mL squalene,
 - c) 0.2 mg/mL QS-21,
 - d) 0.47 mg/mL polysorbate,
 - 25 e) 0.47 mg/mL sorbitan ester,
 - f) 1 mg/mL cholesterol, and
 - g) aqueous citrate buffer 10 mM isotonic with NaCl pH 6-6.5,
- wherein the composition is an emulsion with an average nanoparticle diameter less than 200nm.

30 Item 6. The composition of item 1, comprising:

- a) 0.004 mg/mL – 0.1 mg/mL E6020,
- b) 0.02 mg/mL – 0.4 mg/mL QS21,
- c) 0.04 mg/mL – 32 mg/mL dioleoyl phosphatidylcholine (DOPC),
- 35 d) 0.02 mg/mL – 4 mg/mL cholesterol,

e) pharmaceutically acceptable aqueous buffer of pH 4-9,
wherein the ratio (wt/wt) of cholesterol to QS-21 is from 1:1 to 100:1, the ratio (wt/wt) of
DOPC to cholesterol is from 2:1 to 8:1, and the ratio (wt/wt) of QS21 to DOPC is from 1:1
to 1:100, from 1:5 to 1:15 or 1:10.

5 Item 7. The composition of item 6, wherein the ratio (wt/wt) of E6020 to cholesterol is from
1:1 to 1:20, from 1:1 to 1:10, from 1:5 to 1:10, or 1:6.25.

Item 8. The composition of item 6, wherein the ratio (wt/wt) of E6020 to DOPC is from 1:10
to 1:80, from 1:20 to 1:50, or 1:25.

10

Item 9. The composition of any of items 6 - 8, comprising:

- a) 0.08 mg/mL E6020,
- b) 0.2 mg/mL QS21,
- c) 2 mg/mL dioleoyl phosphatidylcholine (DOPC),
- 15 d) 0.5 mg/mL cholesterol,
- e) pharmaceutically acceptable aqueous buffer of pH 4-9.

15

Item 10. A vaccine composition comprising at least one antigen in aqueous buffer mixed
with the immunologically active metabolizable lipid nanoparticle in water composition of any
preceding item.

20

Item 11. The vaccine composition of item 10, wherein at least one antigen in aqueous
buffer is mixed with the immunologically active metabolizable lipid nanoparticle in a ratio of
between 4:1 and 1:4 vol/vol.

25

Item 12. The composition of item 1, 10 or 11, wherein the immunologically active
metabolizable lipid nanoparticle is a liposome.

Item 13. The composition of item 12, wherein the liposome is spheroid, rod or disc shaped.

30

Item 14. The vaccine composition of any one of items 11-13, wherein the at least one
antigen is derived from a coronavirus, a rhinovirus, an influenza virus, a *Plasmodium* parasite,
a mycobacterium, *Leishmania* parasite, streptococcus bacteria, respiratory syncytial virus
(RSV), a human papilloma virus, an human immunodeficiency (HIV) virus, hepatitis B virus,
35 varicella zoster virus, or any combination thereof.

35

Item 15. The vaccine composition of item 14, wherein the at least one antigen is derived from an influenza virus, or SARS-COV-2, HIV, hepatitis B virus, respiratory syncytial virus, Middle East Respiratory Syndrome virus (MERS), Group A Streptococcus, *Plasmodium sp.* Or
5 *Mycobacterium tuberculosis*.

Item 16. The composition of any one of items 11-15, wherein the composition comprises 0.1 to 50 µg of E6020 per 500 µL dose of the vaccine composition.

10 Item 17. The vaccine composition of any of items 11-16, wherein the composition comprises 2 to 25 µg of E6020 and 5 to 100 µg of saponin per 500 µL dose of the vaccine composition.

Item 18. The vaccine composition of any of items 11-17, wherein the composition
15 comprises 1 to 10 µg of E6020 per 500 µL dose of the vaccine composition.

Item 19. The composition of any of items 11-18, wherein the composition comprises 5 to 100 µg of QS21 per 500 µL dose of the vaccine composition.

20 Item 20. The vaccine composition of any of claims 11-19, further comprising at least one of a carrier, an agent, an additive, an additional adjuvant, an excipient, a solubiliser, an antioxidant, a stabilizer or any mixture thereof.

Item 21. A method of eliciting an immune response in a subject or patient, comprising
25 administering an immunologically active metabolizable lipid nanoparticle in water composition of any of items 1 to 6 or a vaccine composition of any of claims 11 to 20 to a subject or patient in need thereof.

Item 22. The method according to item 21, wherein the subject or patient is at risk of
30 developing an influenza, SARS-CoV-2, SARS-CoV-1, rhinovirus, malaria, tuberculosis, HIV/AIDS, leishmaniasis, Group A streptococcus, respiratory syncytial virus (RSV), Middle East Respiratory Syndrome virus (MERS), hepatitis B, varicella zoster, coronavirus or human papilloma virus infection.

35 Item 23. The method according to item 21 or 22, further comprising administering at least

one booster dose of said vaccine composition after administration of a first dose of the vaccine composition to the patient or subject in need thereof.

Item 24. Use of the vaccine composition of any of items 12-20, in the preparation of a medicament or vaccine for the prevention, treatment, or reduction in symptoms of an influenza virus infection, a SARS-CoV-2 infection, a SARS-CoV-1 infections, a rhinovirus infection, a *Plasmodium sp.* Infection, a *Mycobacterium tuberculosis* infection, an HIV/AIDS infection, a *Leishmania* infection, a Group A *Streptococcus* infection, a respiratory syncytial virus (RSV) infection, a Middle East Respiratory Syndrome virus (MERS) infection, a hepatitis B infection, a *Varicella zoster* infection, coronavirus or a human papilloma virus infection.

Item 25. A method for preparing an immunologically active metabolizable lipid nanoparticle in water composition, wherein the method comprises:

- (a) homogenizing a mixture of E6020, squalene, cholesterol, polyoxyethylene sorbitan monooleate, sorbitan trioleate to generate a mixture,
- (b) adding the mixture of step (a) into an aqueous buffer comprising QS21 and agitating to produce an emulsion,
- (c) optionally diluting the emulsion of step (b) to obtain a diluted emulsion,
- (d) sterile filtering the emulsion of step (b) or the diluted emulsion of step (c) under aseptic conditions through a filter having a pore size of 0.22 μm or less to obtain the immunologically active metabolizable lipid nanoparticle in water composition.

Item 26. A method for preparing an immunologically active metabolizable lipid nanoparticle in water composition, wherein the method comprises:

- (a) dissolving DOPC (dioleoyl phosphatidylcholine), cholesterol and E6020 into ethanol to generate a mixture,
- (b) injecting the mixture of step (a) rapidly under agitation into an aqueous buffer,
- (c) removing ethanol by tangential flow filtration using a membrane with a molecular cut-off of 100 kDa to produce liposomes,
- (d) mixing a volume of the liposomes of step (c) with a solution of QS21 in aqueous buffer to generate a QS-liposome composition,
- (e) optionally diluting the QS-liposome composition of step (d) to generate diluted QS-liposome composition,
- (f) sterile filtering the QS-liposome composition of step (d) or diluted QS-liposome composition of step (e) under aseptic conditions through a filter having a pore size of

0.22 μm or less to obtain the immunologically active metabolizable lipid nanoparticle in water composition.

5 Item 27. The method of item 25 or 26, wherein the method further comprises storing the immunologically active metabolizable lipid nanoparticle in water composition at 4°C before administration of the composition to a patient or subject in need thereof.

10 Item 28. The method according to item 27, wherein at least one antigen is added to the immunologically active metabolizable lipid nanoparticle in water composition before storing the compositions at 4°C.

15 Item 29. The method according to item 28, wherein at least one antigen is added to the immunologically active metabolizable lipid nanoparticle in water composition extemporaneously before administration of the composition to a patient or subject in need thereof.

Item 30. A kit of parts comprising:

- 20 a) an immunologically active metabolizable lipid nanoparticle in water composition of any of items 1 to 11 enclosed in a first receptacle, and
b) a second composition comprising one or more antigens enclosed in a second receptacle.

Item 31. The kit of parts of item 30, wherein the first receptacle is a syringe.

25 Item 32. The kit of parts of item 30 or 31, wherein the first and second receptacles are present within the same apparatus.

30 Item 33. A method of performing a vaccination to a patient or subject in need thereof, comprising using the kit of any of items 30 to 32, wherein (a) the immunologically active metabolizable lipid nanoparticle in water composition is mixed with (b) the second composition comprising one or more antigens, and the resulting mixture is administered subcutaneously, intradermally, mucosally, intravenously or intramuscularly.

* * *

Claims

1. An immunologically active metabolizable lipid nanoparticle in water composition comprising:

- 5 a) 0.004 – 0.1 mg/mL E6020,
- b) 0.04 – 200 mg/mL metabolizable lipid,
- c) 0.02– 0.4 mg/mL saponin, and
- d) 0.02– 50 mg/mL sterol,

10 wherein the ratio (wt/wt) of sterol to saponin is between 1:1 to 100:1 and the average nanoparticle diameter of the metabolizable lipid nanoparticles is less than 200nm.

2. The immunologically active metabolizable lipid nanoparticle in water composition of claim 1, wherein immunologically active metabolizable lipid nanoparticle is present as an emulsion.

3. The composition of claim 1 or 2, comprising:

- 15 a) 0.004 – 0.1 mg/mL E6020,
 - b) 0.04 – 200 mg/mL metabolizable lipid,
 - c) 0.02– 0.4 mg/mL saponin,
 - d) 0.5 – 100 mg/mL hydrophilic surfactant,
 - e) 0.5 – 100 mg/mL hydrophobic surfactant,
 - 20 f) 0.02 – 50 mg/mL sterol, and
 - g) pharmaceutically acceptable aqueous buffer pH 4-9,
- wherein the ratio (wt/wt) of sterol to saponin is between 1:1 to 100:1.

4. The composition of any preceding claim, comprising:

- 25 a) 0.004 mg/mL – 0.04 mg/mL E6020,
 - b) 5 – 100 mg/mL squalene,
 - c) 0.02 mg/mL – 0.4 mg/mL QS-21,
 - d) 0.5 – 100 mg/mL polysorbate,
 - e) 0.5 – 100 mg/mL sorbitan ester,
 - f) 0.02 mg/mL – 50 mg/mL cholesterol, and
 - 30 g) aqueous buffer 10 mM isotonic with NaCl pH 5.5-7,
- wherein the ratio (wt/wt) of squalene to each of the surfactants polysorbate and sorbitan ester are 1:1, the ratio (wt/wt) of cholesterol to sorbitan ester is 1:1, and the ratio (wt/wt) of cholesterol to QS-21 is between 1:1 and 100:1.

5. The composition of any preceding claim, comprising:

- 35 a) 0.040 mg/mL E6020,

- b) 39 mg/mL squalene,
- c) 0.2 mg/mL QS-21,
- d) 0.47 mg/mL polysorbate,
- e) 0.47 mg/mL sorbitan ester,
- 5 f) 1 mg/mL cholesterol, and
- g) aqueous citrate buffer 10 mM isotonic with NaCl pH 6-6.5,

wherein the composition is an emulsion with an average nanoparticle diameter less than 200nm.

6. The composition of claim 1, comprising:

- 10 a) 0.004 mg/mL – 0.1 mg/mL E6020,
- b) 0.02 mg/mL – 0.4 mg/mL QS21,
- c) 0.04 mg/mL – 32 mg/mL dioleoyl phosphatidylcholine (DOPC),
- d) 0.02 mg/mL – 4 mg/mL cholesterol,
- e) pharmaceutically acceptable aqueous buffer of pH 4-9,

15 wherein the ratio (wt/wt) of cholesterol to QS-21 is from 1:1 to 100:1, the ratio (wt/wt) of DOPC to cholesterol is from 2:1 to 8:1, and the ratio (wt/wt) of QS21 to DOPC is from 1:1 to 1:100, from 1:5 to 1:15 or 1:10.

7. The composition of claim 6, wherein the ratio (wt/wt) of E6020 to cholesterol is from 1:1 to 1:20, from 1:1 to 1:10, from 1:5 to 1:10, or 1:6.25.

20 8. The composition of claim 6, wherein the ratio (wt/wt) of E6020 to DOPC is from 1:10 to 1:80, from 1:20 to 1:50, or 1:25.

9. The composition of any of claims 6 - 8, comprising:

- a) 0.08 mg/mL E6020,
- b) 0.2 mg/mL QS21,
- 25 c) 2 mg/mL dioleoyl phosphatidylcholine (DOPC),
- d) 0.5 mg/mL cholesterol,
- e) pharmaceutically acceptable aqueous buffer of pH 4-9.

10. A vaccine composition comprising at least one antigen in aqueous buffer mixed with the immunologically active metabolizable lipid nanoparticle in water composition of any preceding claim.

11. The vaccine composition of claim 10, wherein at least one antigen in aqueous buffer is mixed with the immunologically active metabolizable lipid nanoparticle in a ratio of between 4:1 and 1:4 vol/vol.

12. The composition of claim 1, 10 or 11, wherein the immunologically active metabolizable lipid nanoparticle is a liposome.

13. The composition of claim 12, wherein the liposome is spheroid, rod or disc shaped.

14. The vaccine composition of any one of claims 11-13, wherein the at least one antigen is derived from a coronavirus, a rhinovirus, an influenza virus, a *Plasmodium* parasite, a mycobacterium, *Leishmania* parasite, streptococcus bacteria, respiratory syncytial virus (RSV), a human papilloma virus, an human immunodeficiency (HIV) virus, hepatitis B virus, varicella zoster virus, or any combination thereof.

15. The vaccine composition of claim 14, wherein the at least one antigen is derived from an influenza virus, or SARS-COV-2, HIV, hepatitis B virus, respiratory syncytial virus, Middle East Respiratory Syndrome virus (MERS), Group A Streptococcus, *Plasmodium sp.* Or *Mycobacterium tuberculosis*.

16. The composition of any one of claims 11-15, wherein the composition comprises 0.1 to 50 µg of E6020 per 500 µL dose of the vaccine composition.

17. The vaccine composition of any of claims 11-16, wherein the composition comprises 2 to 25 µg of E6020 and 5 to 100 µg of saponin per 500 µL dose of the vaccine composition.

18. The vaccine composition of any of claims 11-17, wherein the composition comprises 1 to 10 µg of E6020 per 500 µL dose of the vaccine composition.

19. The composition of any of claims 11-18, wherein the composition comprises 5 to 100 µg of QS21 per 500 µL dose of the vaccine composition.

20. The vaccine composition of any of claims 11-19, further comprising at least one of a carrier, an agent, an additive, an additional adjuvant, an excipient, a solubiliser, an antioxidant, a stabilizer or any mixture thereof.

21. A method of eliciting an immune response in a subject or patient, comprising administering an immunologically active metabolizable lipid nanoparticle in water composition of any of claims 1 to 6 or a vaccine composition of any of claims 11 to 20 to a subject or patient in need thereof.

22. The method according to claim 21, wherein the subject or patient is at risk of developing an influenza, SARS-CoV-2, SARS-CoV-1, rhinovirus, malaria, tuberculosis, HIV/AIDS, leishmaniasis, Group A streptococcus, respiratory syncytial virus (RSV), Middle East Respiratory Syndrome virus (MERS), hepatitis B, varicella zoster, coronavirus or human papilloma virus infection.

23. The method according to claim 21 or 22, further comprising administering at least one booster dose of said vaccine composition after administration of a first dose of the vaccine composition to the patient or subject in need thereof.

24. Use of the vaccine composition of any of claims 12-20, in the preparation of a medicament or vaccine for the prevention, treatment, or reduction in symptoms of an influenza

virus infection, a SARS-CoV-2 infection, a SARS-CoV-1 infections, a rhinovirus infection, a *Plasmodium sp.* Infection, a *Mycobacterium tuberculosis* infection, an HIV/AIDS infection, a *Leishmania* infection, a Group A *Streptococcus* infection, a respiratory syncytial virus (RSV) infection, a Middle East Respiratory Syndrome virus (MERS) infection, a hepatitis B infection, a *Varicella zoster* infection, coronavirus or a human papilloma virus infection.

25. A method for preparing an immunologically active metabolizable lipid nanoparticle in water composition, wherein the method comprises:

(a) homogenizing a mixture of E6020, squalene, cholesterol, polyoxyethylene sorbitan monooleate, sorbitan trioleate to generate a mixture,

(b) adding the mixture of step (a) into an aqueous buffer comprising QS21 and agitating to produce an emulsion,

(c) optionally diluting the emulsion of step (b) to obtain a diluted emulsion,

(d) sterile filtering the emulsion of step (b) or the diluted emulsion of step (c) under aseptic conditions through a filter having a pore size of 0.22 μm or less to obtain the immunologically active metabolizable lipid nanoparticle in water composition.

26. A method for preparing an immunologically active metabolizable lipid nanoparticle in water composition, wherein the method comprises:

(a) dissolving DOPC (dioleoyl phosphatidylcholine), cholesterol and E6020 into ethanol to generate a mixture,

(b) injecting the mixture of step (a) rapidly under agitation into an aqueous buffer,

(c) removing ethanol by tangential flow filtration using a membrane with a molecular cut-off of 100 kDa to produce liposomes,

(d) mixing a volume of the liposomes of step (c) with a solution of QS21 in aqueous buffer to generate a QS-liposome composition,

(e) optionally diluting the QS-liposome composition of step (d) to generate diluted QS-liposome composition,

(f) sterile filtering the QS-liposome composition of step (d) or diluted QS-liposome composition of step (e) under aseptic conditions through a filter having a pore size of 0.22 μm or less to obtain the immunologically active metabolizable lipid nanoparticle in water composition.

27. The method of claim 25 or 26, wherein the method further comprises storing the immunologically active metabolizable lipid nanoparticle in water composition at 4°C before administration of the composition to a patient or subject in need thereof.

28. The method according to claim 27, wherein at least one antigen is added to the immunologically active metabolizable lipid nanoparticle in water composition before storing

the compositions at 4°C.

29. The method according to claim 28, wherein at least one antigen is added to the immunologically active metabolizable lipid nanoparticle in water composition extemporaneously before administration of the composition to a patient or subject in need thereof.

30. A kit of parts comprising:

- a) an immunologically active metabolizable lipid nanoparticle in water composition of any of claims 1 to 11 enclosed in a first receptacle, and
- b) a second composition comprising one or more antigens enclosed in a second receptacle.

31. The kit of parts of claim 30, wherein the first receptacle is a syringe.

32. The kit of parts of claim 30 or 31, wherein the first and second receptacles are present within the same apparatus.

33. A method of performing a vaccination to a patient or subject in need thereof, comprising using the kit of any of claims 30 to 32, wherein (a) the immunologically active metabolizable lipid nanoparticle in water composition is mixed with (b) the second composition comprising one or more antigens, and the resulting mixture is administered subcutaneously, intradermally, mucosally, intravenously or intramuscularly.

* * *

FIG. 1

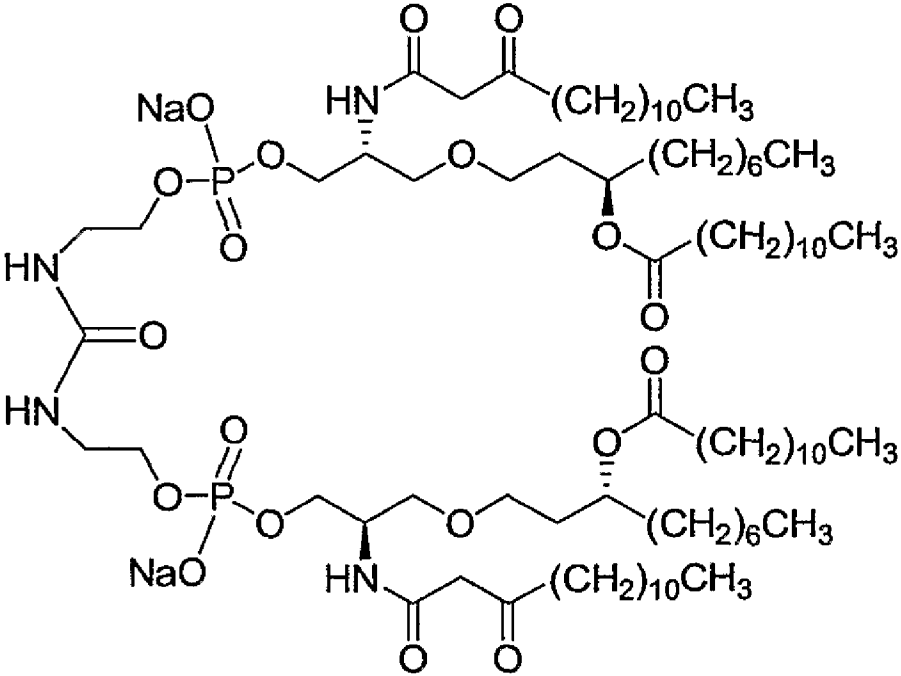


FIG. 2

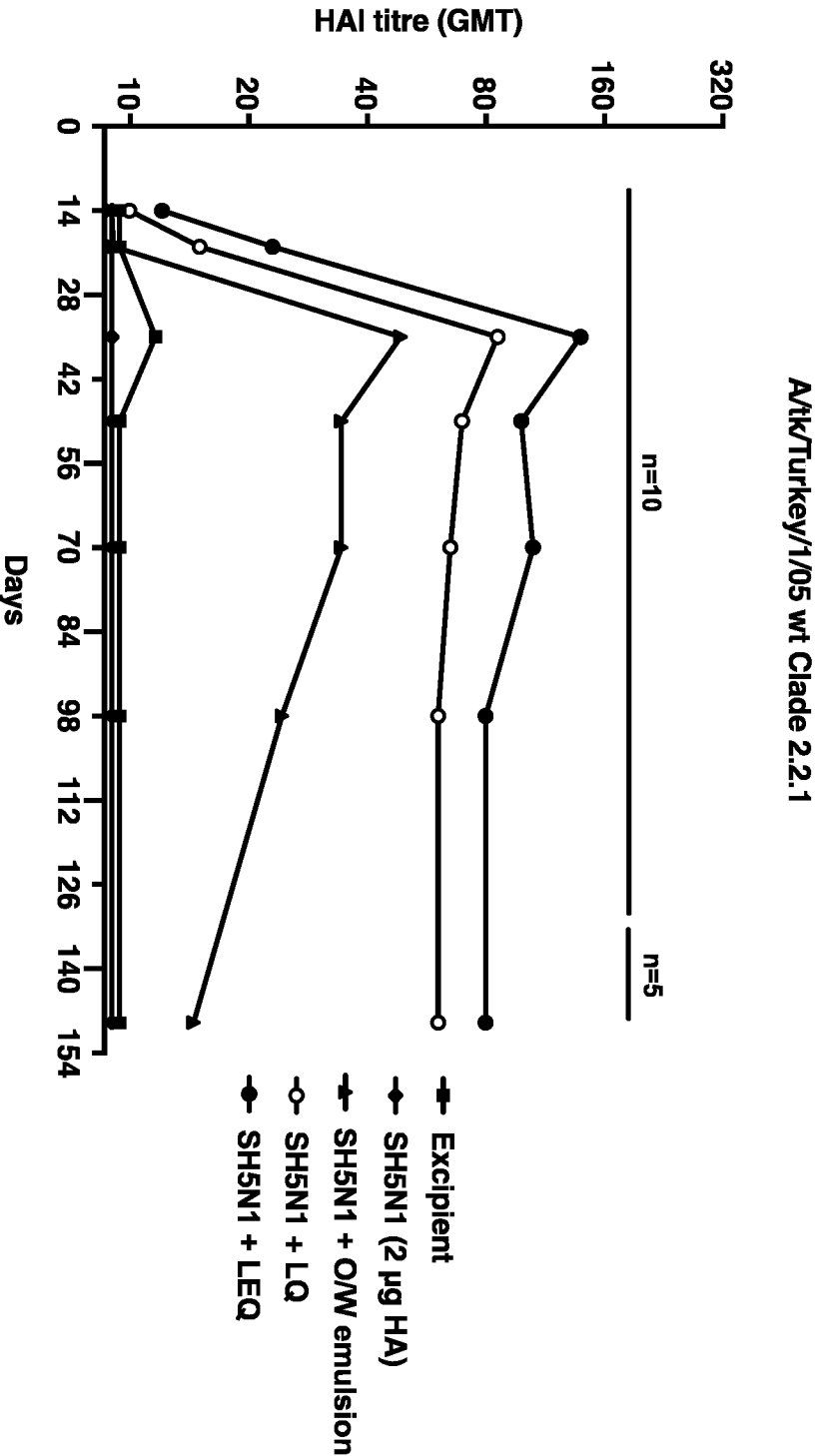


FIG. 3A

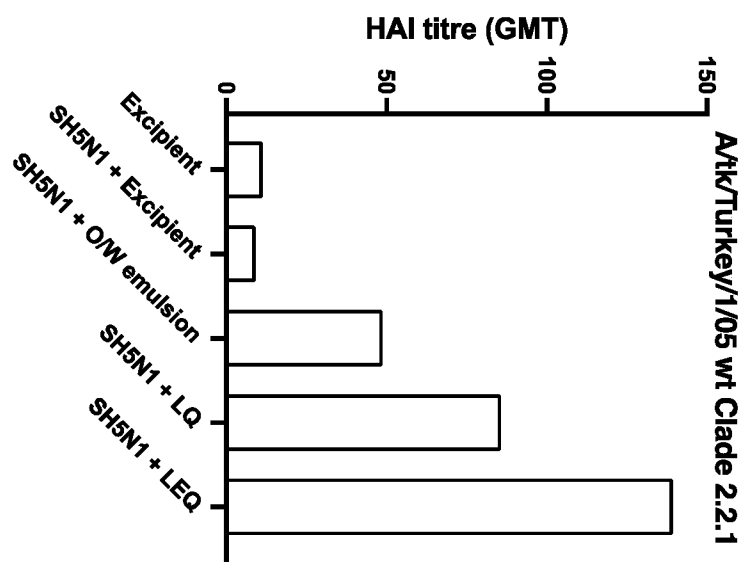


FIG. 3B

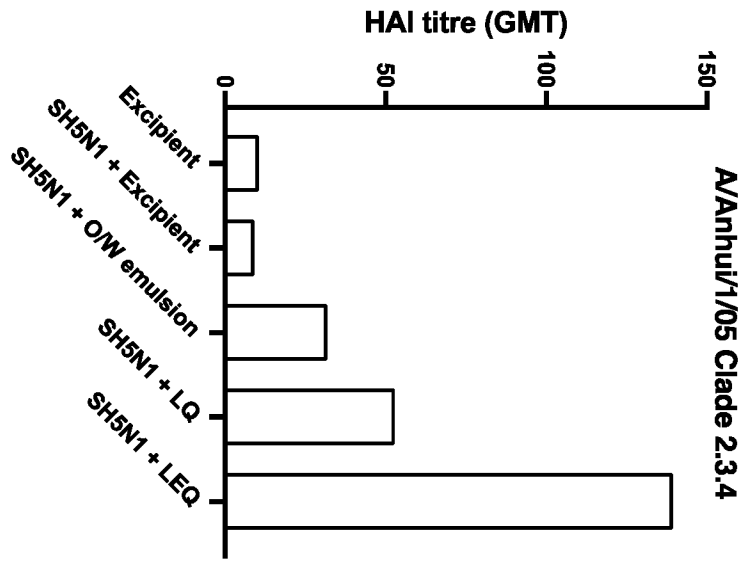


FIG. 4A

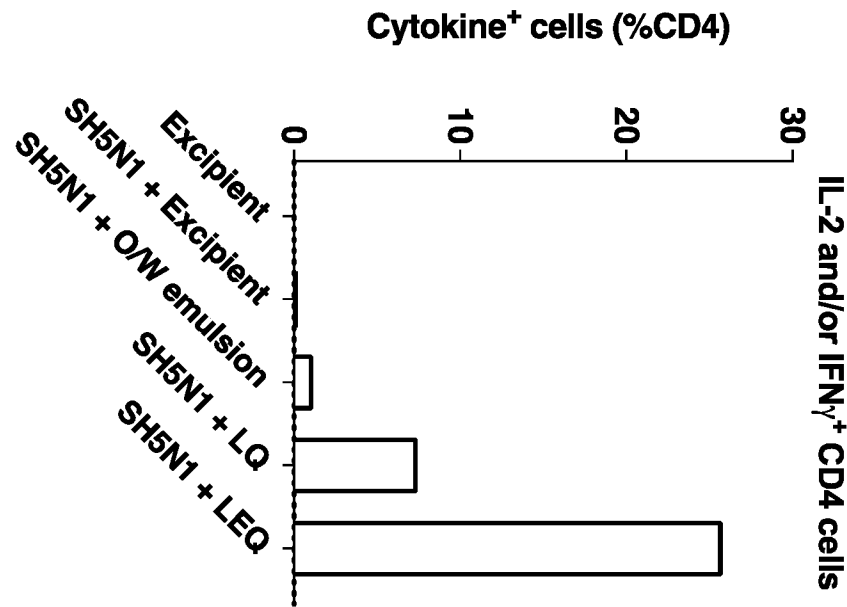


FIG. 4B

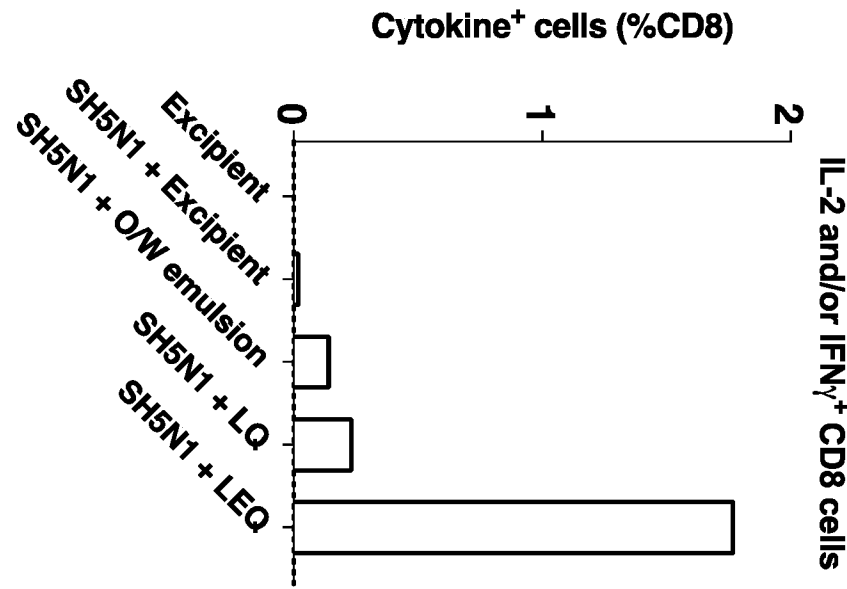


FIG. 5

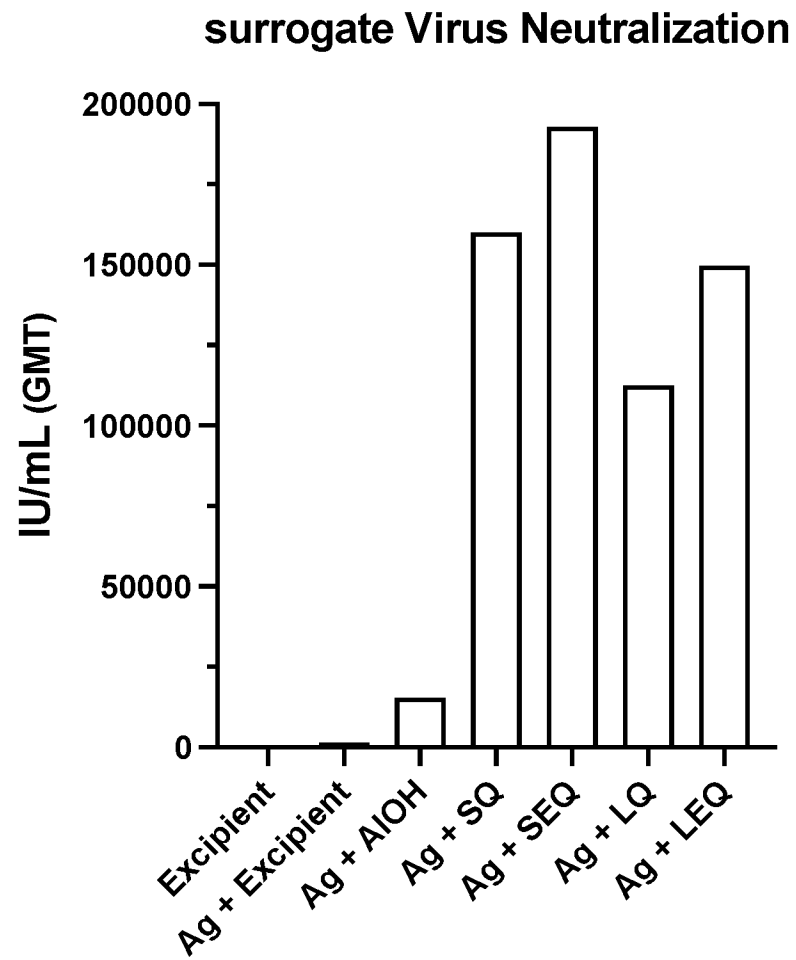


FIG. 6

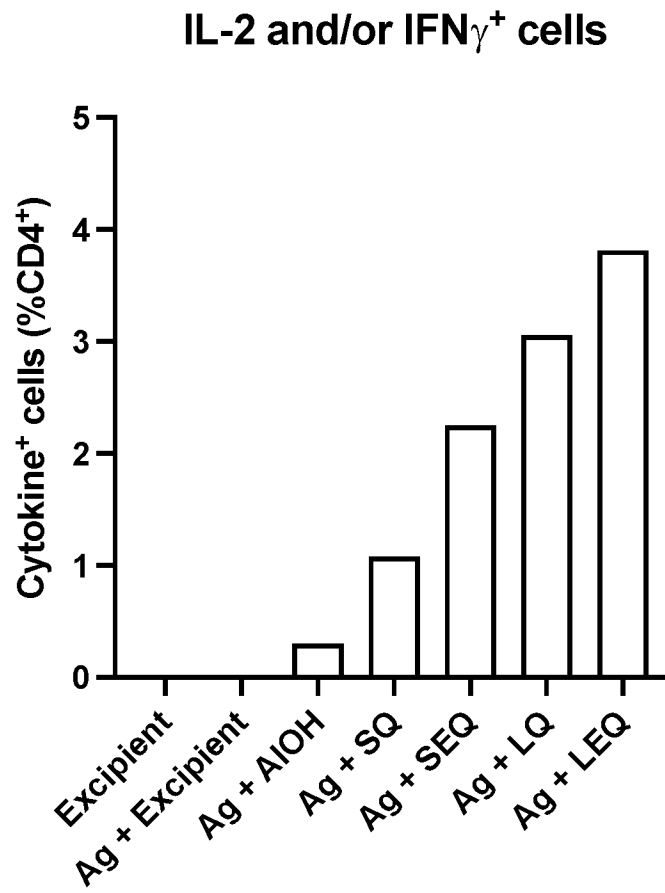


FIG. 7

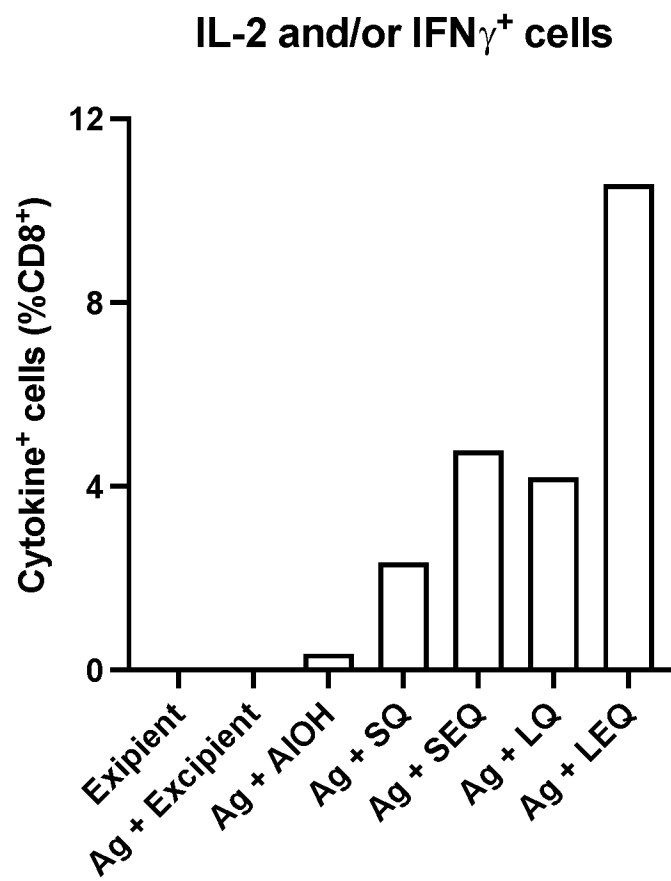


FIG. 8

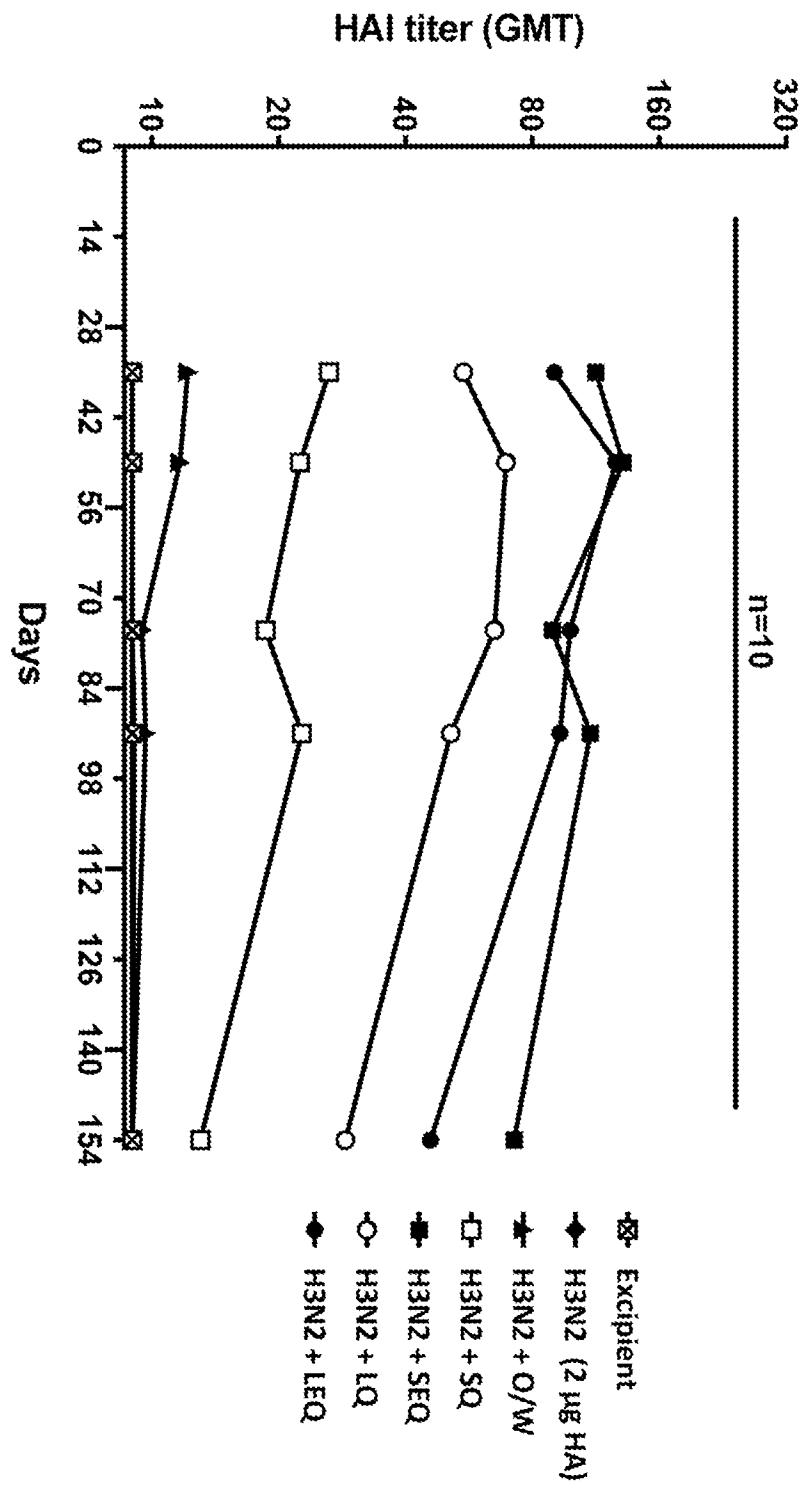


FIG. 9A

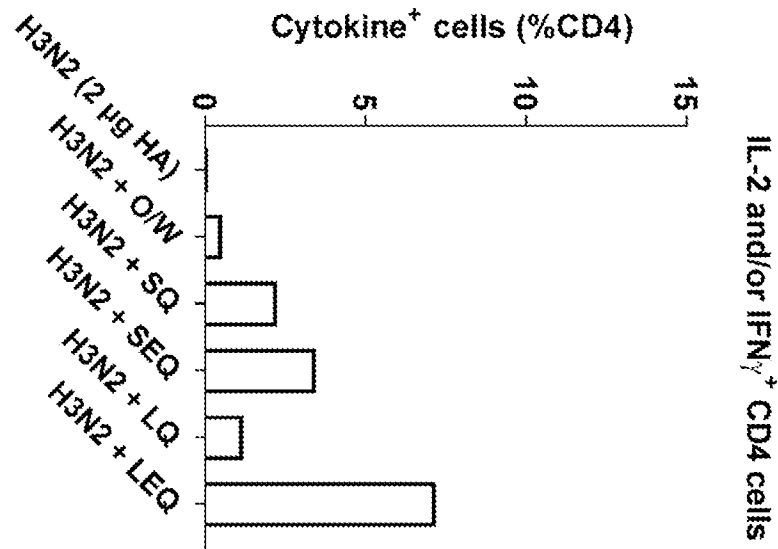


FIG. 9B

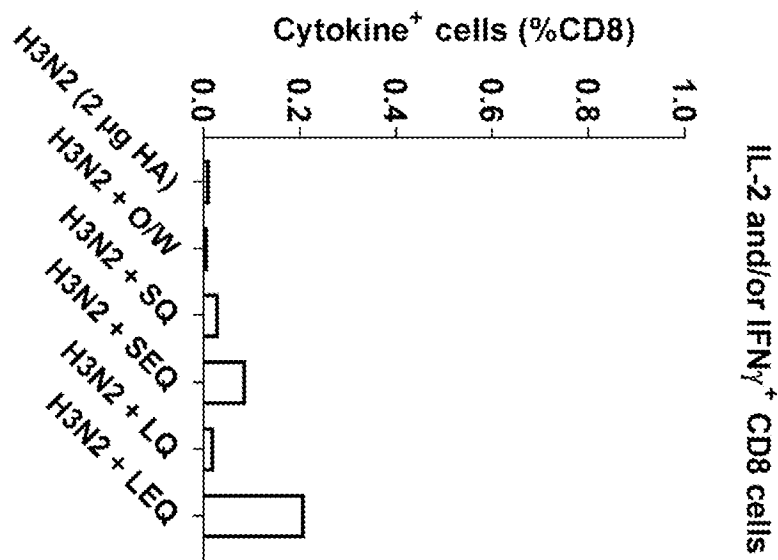


FIG. 10

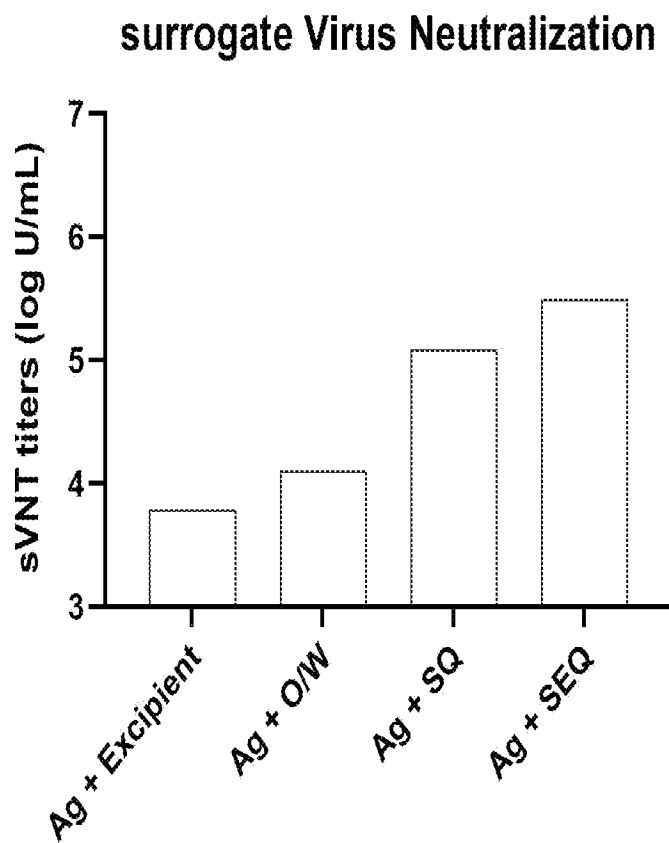


FIG. 11A

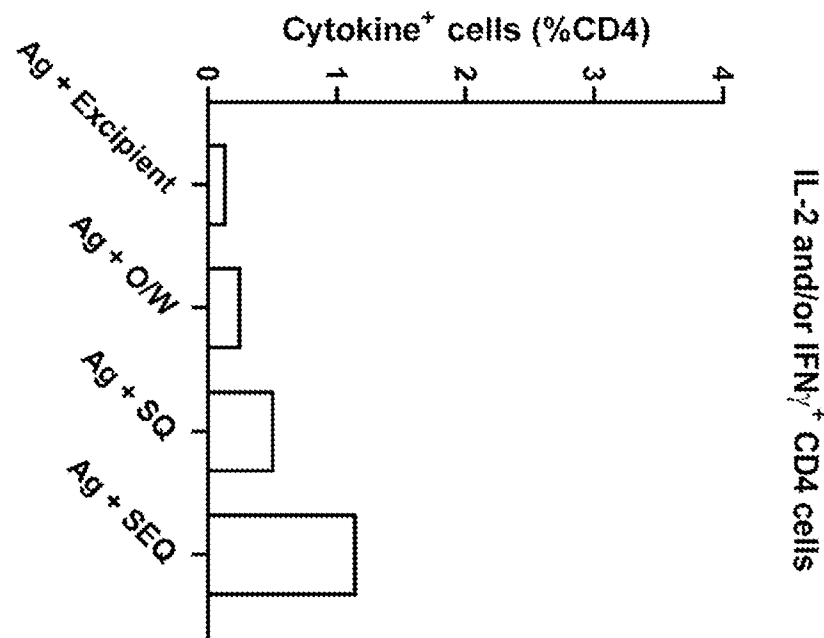


FIG. 11B

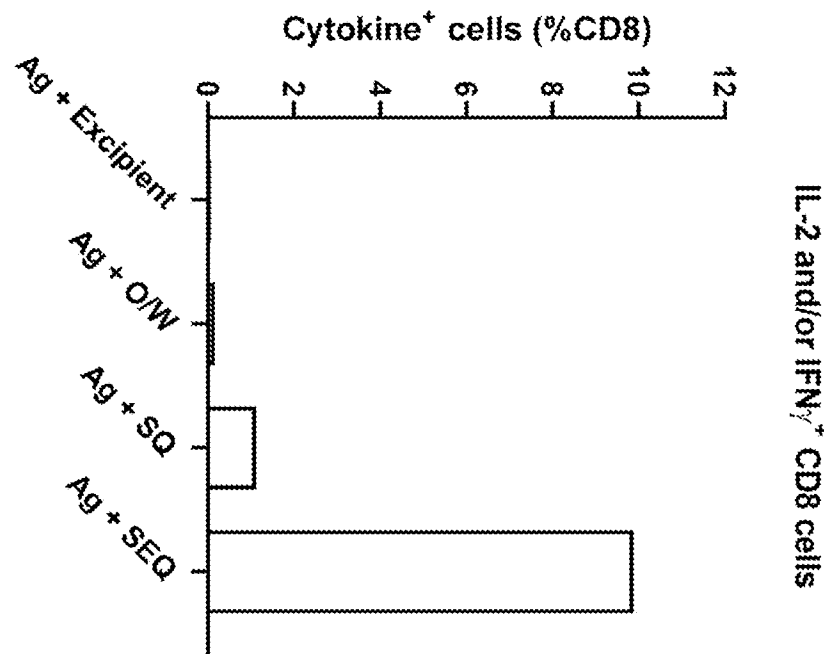


FIG. 12

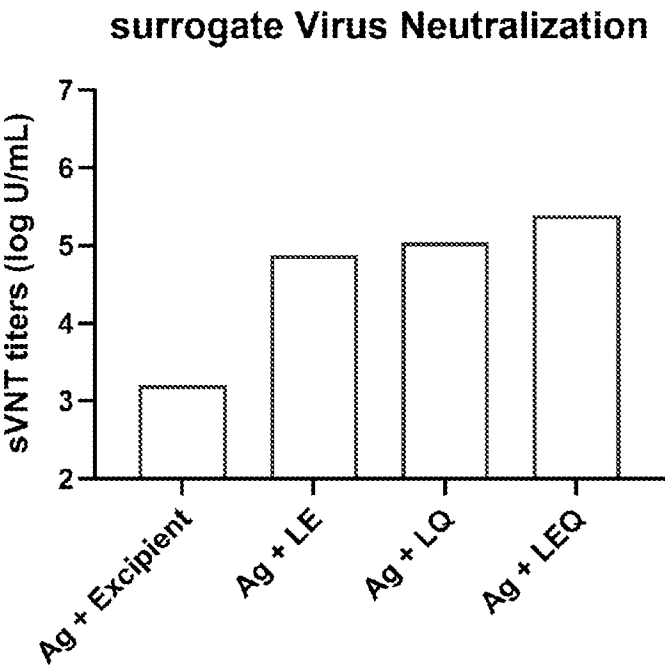


FIG. 13A

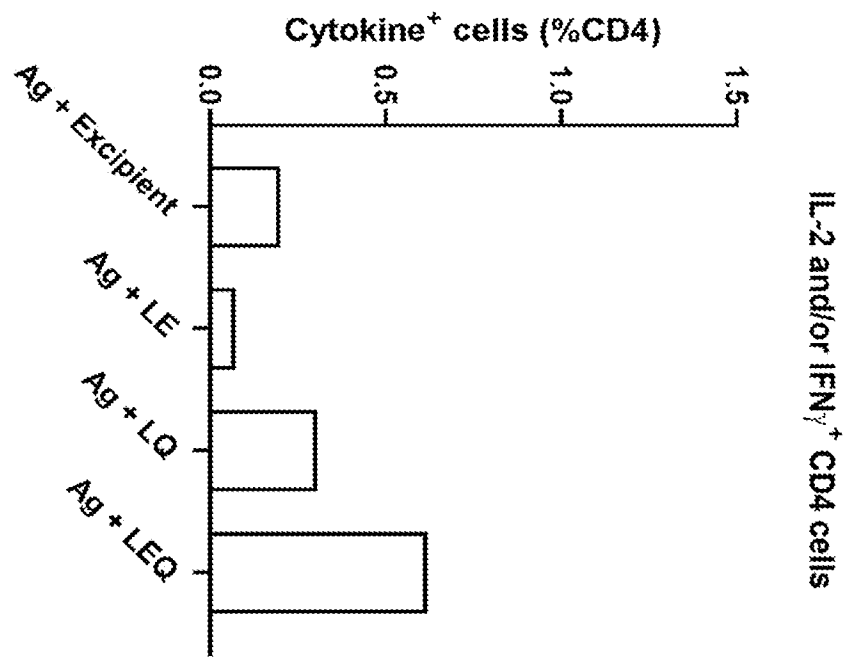
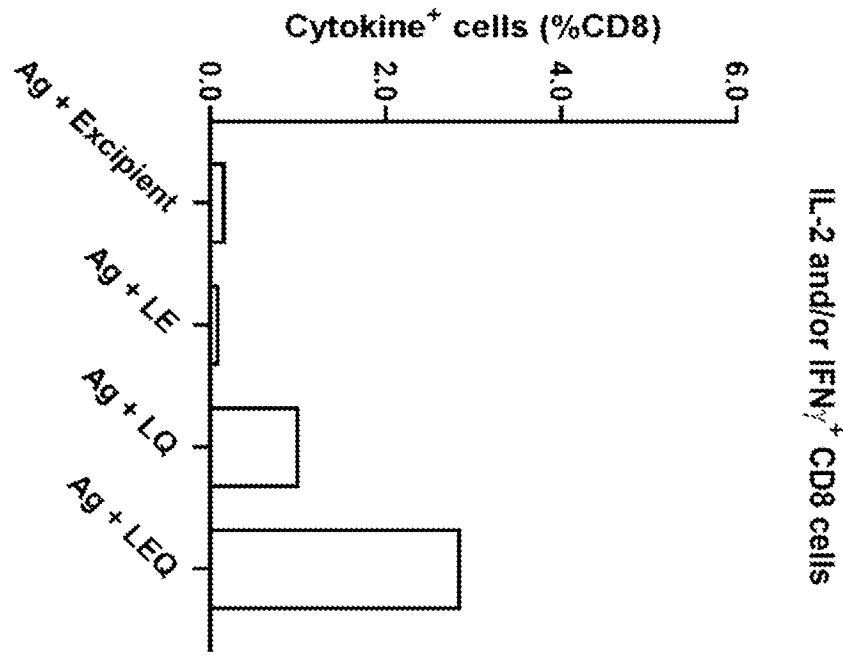


FIG. 13B



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2022/045529

A. CLASSIFICATION OF SUBJECT MATTER INV. A61K39/12 A61P31/14 A61K9/00 A61K9/127 A61P31/16 ADD.		
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) A61K A61P C12N		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, WPI Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2020/055503 A1 (MASSACHUSETTS INST TECHNOLOGY [US] ET AL.) 19 March 2020 (2020-03-19) whole document, in particular pages 3, 13, 22, 36, 47; example section -----	1-33
Y	WO 2007/005583 A1 (EISAI CO LTD [JP]; FANG FRANCIS G [US] ET AL.) 11 January 2007 (2007-01-11) cited in the application p. [0038] - [0039] ----- --/--	1-33
☒ 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 "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 2022		04/01/2023
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Bernhardt, Wiebke

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2022/045529

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HAENSLER JEAN ET AL: "Design and preclinical characterization of a novel vaccine adjuvant formulation consisting of a synthetic TLR4 agonist in a thermoreversible squalene emulsion", INTERNATIONAL JOURNAL OF PHARMACEUTICS, ELSEVIER, NL, vol. 486, no. 1, 17 March 2015 (2015-03-17), pages 99-111, XP029217766, ISSN: 0378-5173, DOI: 10.1016/J.IJPHARM.2015.03.028 abstract, page 100, right-hand col., figures -----	1-33
Y	BARBARA C BAUDNER ET AL: "MF59 Emulsion Is an Effective Delivery System for a Synthetic TLR4 Agonist (E6020)", PHARMACEUTICAL RESEARCH, KLUWER ACADEMIC PUBLISHERS-PLENUM PUBLISHERS, NL, vol. 26, no. 6, 3 March 2009 (2009-03-03), pages 1477-1485, XP019686195, ISSN: 1573-904X, DOI: 10.1007/S11095-009-9859-5 abstract, page 1478, right-hand col., figures -----	1-5, 10-33
Y	NDWANDWE DUDUZILE ET AL: "COVID-19 vaccines", CURRENT OPINION IN IMMUNOLOGY, ELSEVIER, OXFORD, GB, vol. 71, 12 July 2021 (2021-07-12), pages 111-116, XP086767217, ISSN: 0952-7915, DOI: 10.1016/J.COI.2021.07.003 [retrieved on 2021-07-12] table A1 -----	1-33
Y	WO 2018/219521 A1 (GLAXOSMITHKLINE BIOLOGICALS SA [BE]) 6 December 2018 (2018-12-06) pages 5-7, in particular page 7, lines 5-8 -----	25-29
Y	WO 2020/225769 A1 (UNIV DO MINHO [PT]) 12 November 2020 (2020-11-12) paragraph [0027], claims ----- -/--	25-29

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2022/045529

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Anonymous: "Liposomes and Lipid Nanoparticles as Delivery Vehicles for Personalized Medicine", , 16 November 2018 (2018-11-16), pages 1-5, XP093009345, Retrieved from the Internet: URL: https://www.exeleadbiopharma.com/news/liposomes-and-lipid-nanoparticles-as-delivery-vehicles-for-personalized-medicine [retrieved on 2022-12-19] figure on page 2 -----	1-33
Y	ISHIZAKA SALLY T ET AL: "E6020: a synthetic Toll-like receptor 4 agonist as a vaccine adjuvant", EXPERT REVIEW OF VACCINES, FUTURE DRUGS, LONDON, GB, vol. 6, no. 5, 1 October 2007 (2007-10-01) , pages 773-784, XP008101056, ISSN: 1476-0584, DOI: 10.1586/14760584.6.5.773 abstract, figures 1-6, tables 1-4 -----	1-33

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2022/045529

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2020055503 A1	19-03-2020	EP 3849521 A1	21-07-2021
		US 2020085756 A1	19-03-2020
		WO 2020055503 A1	19-03-2020
WO 2007005583 A1	11-01-2007	AU 2006265986 A1	11-01-2007
		CA 2611721 A1	11-01-2007
		CN 101213199 A	02-07-2008
		CN 103012169 A	03-04-2013
		CN 103012304 A	03-04-2013
		EP 1910387 A1	16-04-2008
		EP 2292628 A2	09-03-2011
		EP 3138844 A1	08-03-2017
		HK 1183863 A1	10-01-2014
		IL 187819 A	30-04-2012
		IL 213174 A	31-10-2012
		JP 5214446 B2	19-06-2013
		JP 5923373 B2	24-05-2016
		JP 2009502742 A	29-01-2009
		JP 2012162562 A	30-08-2012
		JP 2015051978 A	19-03-2015
		KR 20080031350 A	08-04-2008
		US 2007082875 A1	12-04-2007
		US 2010197951 A1	05-08-2010
		US 2012232298 A1	13-09-2012
		US 2014012011 A1	09-01-2014
		US 2014323739 A1	30-10-2014
		WO 2007005583 A1	11-01-2007
WO 2018219521 A1	06-12-2018	BR 112019025193 A2	23-06-2020
		CA 3063954 A1	06-12-2018
		CN 111032080 A	17-04-2020
		DE 112018002827 T5	09-04-2020
		EP 3630176 A1	08-04-2020
		FR 3066920 A1	07-12-2018
		GB 2577232 A	18-03-2020
		GB 2600652 A	04-05-2022
		GB 2600653 A	04-05-2022
		GB 2600654 A	04-05-2022
		JP 2020522485 A	30-07-2020
		US 2021162042 A1	03-06-2021
		WO 2018219521 A1	06-12-2018
WO 2020225769 A1	12-11-2020	AU 2020269626 A1	28-10-2021
		CA 3137789 A1	12-11-2020
		CN 113825497 A	21-12-2021
		EP 3965738 A1	16-03-2022
		JP 2022531610 A	07-07-2022
		KR 20220005446 A	13-01-2022
		US 2022241203 A1	04-08-2022
		WO 2020225769 A1	12-11-2020