VARICELLA ZOSTER

INCORPORATION BY REFERENCE OF RELATED APPLICATIONS

This application is based upon and claims priority under 35 U.S.C. § 119(e) to U.S. provisional application U.S. Ser. No. 62/930,727 filed November 5, 2019, the entire contents of all of which are incorporated herein by reference in their entirety.

FIELD OF THE INVENTION

The present application relates to compositions capable of inducing an immune response against Varicella zoster virus, methods of administering such compositions, and methods of producing such compositions.

STATEMENT

To comply with 37 C.F.R. § 1.821, this application contains sequence listings included in an ASCII text file submitted via EFS-WEB. The ASCII text file has the following attributes: (1) Name: VZV Sequences PCT_ST25.txt, (2) Date of creation: October 26, 2020, (3) File size in bytes: 48,422. Pursuant to MPEP § 2422.03(a), Applicant hereby incorporates by reference the foregoing ASCII text file and all material disclosed therein into this application.

BACKGROUND

Varicella zoster virus (VZV) is a human virus belonging to the α-herpesvirus family. VZV is present worldwide and is highly infectious. Primary infection leads to acute varicella or “chickenpox,” usually from exposure either through direct contact with a skin lesion or through airborne spread from respiratory droplets. (Sawyer MH, Chamberlin CJ, Wu YN, Aintablian N, Wallace MR, *Detection of varicella-zoster virus DNA in air samples from hospital rooms*, 169 J Infect Dis. 91-4 (1994).) After initial infection, VZV establishes lifelong latency in cranial nerve and dorsal root ganglia, and can reactivate years to decades later as herpes zoster (HZ) or “shingles.” (Gilden DH,

Kleinschmidt-DeMasters BK, LaGuardia JJ, Mahalingam R, Cohrs RJ, *Neurologic complications of the reactivation of varicella-zoster virus*, 342 N Engl J Med. 635–645 (2000).) More than 90% of adults in the United States acquired the disease in childhood, while the majority of children and young adults have been vaccinated with the live virus vaccine. (Marin M, Guris D, Chaves SS, Schmid S, Seward JF, *Prevention of varicella: recommendations of the Advisory Committee on Immunization Practices (ACIP)*, MMWR Recomm Rep. 2007; 56:1–40.)

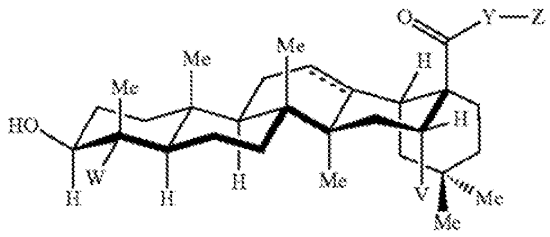
For adults who were not exposed to varicella during childhood, and occasionally to individuals who are immunocompromised, VZV can be life-threatening. Similarly, a VZV infection can be life-threatening to neonates, as the virus is capable of crossing the placenta. With direct contact, VZV is known to be a highly transmissible infectious disease.

Several vaccines capable of inducing an immune response against VZV are commercially available, including VARIVAX and PROQUAD for the prevention of varicella (chickenpox) and SHINGRIX and ZOSTAVAX for the prevention of herpes zoster (shingles). These vaccines have varied efficacy, and there remains a need for improved vaccines against varicella, herpes zoster, and related disorders such as post herpetic neuralgia (PHN).

SUMMARY

The present application provides compositions which induce an immune response against Varicella zoster virus, and thus are capable of vaccinating against varicella, herpes zoster, and related disorders such as post herpetic neuralgia (PHN), methods of administering such compositions, and methods of producing such compositions.

In an embodiment of the present application, a composition which induces an immune response against Varicella zoster virus comprises an optionally truncated VZV glycoprotein or a fragment thereof in combination with a triterpene glycoside saponin-derived adjuvant. The optionally truncated VZV glycoprotein may be VZV glycoprotein E or a fragment thereof, and may have the sequence of SEQ ID No. 1. The saponin-derived adjuvant may be a compound according to Formula I:



(I)

or a pharmaceutically acceptable salt thereof, wherein

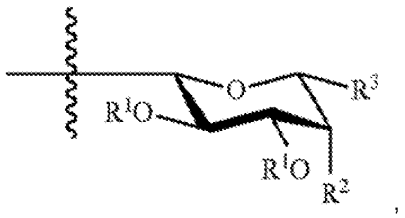
⎓ is a single or double bond;

W is —CHO;

V is —OH;

Y is —O—;

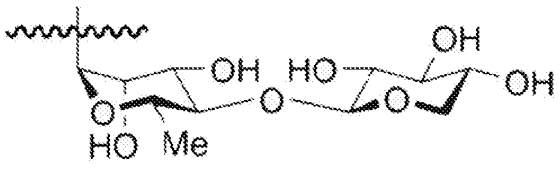
wherein Z is a carbohydrate domain having the structure:



,

wherein:

R^1 is independently H or

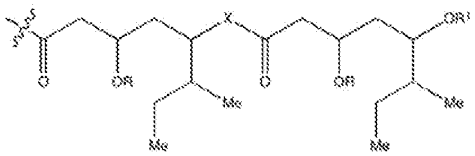


;

R^2 is NHR^4;

R^3 is CH_2OH; and

R^4 is -T-R^z, -C(O)-T-R^z, -NH-T-R^z, -O-T-R^z, -S-T-R^z, -C(O)NH-T-R^z, C(O)O-T-R^z, C(O)S-T-R^z, C(O)NH-T-O-T-R^z, -O-T-R^z, -T-O-T-R^z, -T-S-T-R^z, or



wherein:

X is —O—, —NR—, or T-R^z;

T is a covalent bond or a bivalent C_{1-26} saturated or unsaturated, straight or branched, aliphatic or heteroaliphatic chain;

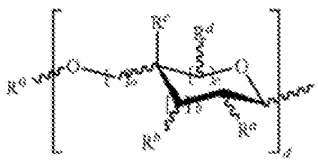
R^z is hydrogen, halogen, —OR, —OR^x, —$OR^{1'}$, —SR, NR_2, —C(O)OR, —C(O)R, -NHC(O)R, -NHC(O)OR, NC(O)OR, or an optionally substituted group selected from acyl, arylalkyl, heteroarylalkyl, C_{1-6} aliphatic, 6-10-membered aryl, 5-10-membered heteroaryl having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 4-7-membered heterocyclyl having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur;

R^x is independently hydrogen or an oxygen protecting group selected from the group consisting of alkyl ethers, benzyl ethers, silyl ethers, acetals, ketals, esters, carbamates, and carbonates; and

R is independently hydrogen, an optionally substituted group selected from acyl, arylalkyl, 6-10-membered aryl, C_{1-6} aliphatic, or C_{1-6} heteroaliphatic having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur, or:

two R on the same nitrogen atom are taken with the nitrogen atom to form a 4-7-membered heterocyclic ring having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur;

$R^{1'}$ is R^x or a carbohydrate domain having the structure:



wherein:

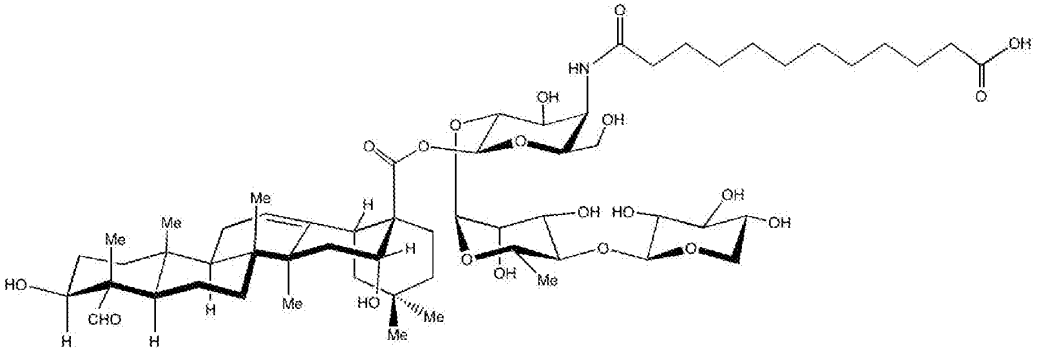
each occurrence of a, b, and c is independently 0, 1, or 2;

d is an integer from 1-5, wherein each d bracketed structure may be the same or different; with the proviso that the d bracketed structure represents a furanose or a pyranose moiety, and the sum of b and c is 1 or 2;

R^0 is hydrogen; an oxygen protecting group selected from the group consisting of alkyl ethers, benzyl ethers, silyl ethers, acetals, ketals, esters, carbamates, and carbonates; or an optionally substituted moiety selected from the group consisting of acyl, C_{1-10} aliphatic, C_{1-6} heteroaliphatic, 6-10-membered aryl, arylalkyl, 5-10 membered heteroaryl having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 4-7 membered heterocyclyl having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur;

each occurrence of R^a, R^b, R^c, and R^d is independently hydrogen, halogen, OH, OR, OR^x, NR_2, NHCOR, or an optionally substituted group selected from acyl, C_{1-10} aliphatic, C_{1-6} heteroaliphatic, 6-10-membered aryl, arylalkyl, 5-10-membered heteroaryl having 1-4 heteroatoms independently selected from nitrogen, oxygen, sulfur; 4-7-membered heterocyclyl having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur.

In a preferred embodiment of the present application, a composition which induces an immune response against Varicella zoster virus comprises a VZV glycoprotein E truncated to remove the carboxy terminal anchor region, which gE is not in the form of a fusion protein, in combination with an adjuvant comprising a compound of Formula II (or a pharmaceutically acceptable salt thereof), a TLR4 agonist, and a liposome-forming compound, such as DOPC, DMPC, DMPG, cholesterol, and/or combinations of the foregoing.



(II)

In preferred embodiments, the compound of Formula I or Formula II is in free acid form or choline salt form. In preferred embodiments, the TLR4 agonist is PHAD, MPL, or MPLA or variants thereof.

In some of the preferred embodiments, the VZV glycoprotein contains a non-native signal peptide on the N-terminus to improve cleavage. In the context of the present application, such non-native signal peptides together with truncated or untruncated glycoproteins are not considered fusion proteins.

In another preferred embodiment, the present application utilizes an emulsion-based technology instead of or in conjunction with a liposome-forming compound. In some embodiments, the present application provides a combination having liposomes containing a TLR4 agonist and an emulsion containing the compound of Formula I or Formula II (or a pharmaceutically acceptable salt thereof). In some embodiments, the present application provides liposomes containing a TLR4 agonist and the compound of Formula I or Formula II (or a pharmaceutically acceptable salt thereof). In some embodiments, the present application provides an emulsion containing a TLR4 agonist and the compound of Formula I or Formula II (or a pharmaceutically acceptable salt thereof).

The present application also relates to methods of using a composition which induces an immune response against Varicella zoster virus, in the preparation of a medicament for the prevention or amelioration of varicella, herpes zoster, and/or post herpetic neuralgia.

The present application also relates to a method for the prevention or amelioration of varicella, herpes zoster, and/or post herpetic neuralgia, the method comprising administering to a human in need thereof an immunogenic composition or

vaccine comprising a composition which induces an immune response against Varicella zoster virus as described above.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts the sequence of a truncated VZV gE.
FIG. 2 depicts the sequence of a truncated VZV gE.
FIG. 3 depicts the sequence of a VZV gE.
FIG. 4 depicts the sequence of a VZV gE.
FIG. 5 depicts the sequence of a VZV gB.
FIG. 6 depicts the sequence of a VZV gH.
FIG. 7 depicts the sequence of a VZV gI.
FIG. 8 depicts the sequence of a VZV gC.
FIG. 9 depicts the sequence of a truncated VZV gE.
FIG. 10 depicts gE-specific total IgG titer data as explained in Example 1.
FIG. 11 depicts gE-specific total IgG titer data as explained in Example 1.
FIG. 12 depicts gE-specific total IgG1 titer data as explained in Example 1.
FIG. 13 depicts gE-specific total IgG1 titer data as explained in Example 1.
FIG. 14 depicts gE-specific total IgG2c titer data as explained in Example 1.
FIG. 15 depicts gE-specific total IgG2c titer data as explained in Example 1.
FIG. 16 depicts gE-specific total IgG titer data as explained in Example 2.
FIGs. 17A-E depict gE-specific total IgG titer data as explained in Example 2.
FIG. 18 depicts gE-specific total IgG titer data as explained in Example 2.
FIGs. 19A-E depict gE-specific total IgG titer data as explained in Example 2.
FIG. 20 depicts gE-specific total IgG titer data as explained in Example 3.
FIG. 21 depicts gE-specific total IgG titer data as explained in Example 3.

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS

In its broadest aspect the present application relates to compositions and regimes for provoking an immune response to VZV. In one aspect the immune response generated by exposure to such compositions is higher and statistically

significant when compared to that obtained in individuals who have received no exposure to the compositions of the present application. The immune response may be assessed by analysis of any one or more aspects of CMI response and/or antibody responses using any of the techniques outlined below or those familiar to a person of ordinary skill in the art.

In another aspect, the present application discloses methods for preventing and/or decreasing the severity of varicella, herpes zoster, and post herpetic neuralgia (PHN). Thus, in one aspect, the present application discloses methods of prevention of the incidence of varicella. Where varicella does occur, the severity is reduced compared with an unvaccinated control (amelioration of varicella). In another aspect, the present application discloses methods of prevention of the incidence of herpes zoster. Where zoster does occur, the severity of the reactivation of zoster is reduced compared with an unvaccinated control (amelioration of zoster). In a further aspect, where zoster does occur, the present application discloses methods of prevention of the incidence of PHN. In a further aspect where PHN does occur then the severity of the PHN is suitably reduced compared with an unvaccinated control (amelioration of PHN). Reduction in severity can suitably be assessed by a reduction in the pain caused by varicella, herpes zoster, or PHN, for example, using known measures of burden of pain (e.g. Coplan et al J Pain 2004; 5 (6) 344-56). Reduction in severity can also be assessed by other criteria such as duration of varicella, herpes zoster, or PHN, proportion of body area affected by varicella, herpes zoster, or PHN, or the site of varicella, herpes zoster, or PHN.

The present application provides pharmaceutical compositions comprising the compounds of the present application together with an immunologically effective amount of an antigen associated with Varicella zoster virus (VZV). The application also includes methods of vaccinating a human patient comprising administering an immunologically effective amount of a pharmaceutical compositions or of the compounds of the present application. The application also includes methods for increasing the immune response to a vaccine comprising administering an immunologically effective amount of a pharmaceutical compositions or of the compounds of the present application.

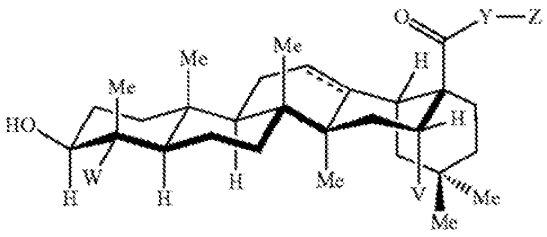
Adjuvant Compounds

Adjuvant compounds of this application include those described generally above, and are further illustrated by the classes, subclasses, and species disclosed herein. In some embodiments, provided compounds are analogs of naturally occurring triterpene glycoside saponins and intermediates thereto. 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. Additionally, general principles of organic chemistry are described in Organic Chemistry, Thomas Sorrell, University Science Books, Sausalito: 1999, and March's Advanced Organic Chemistry, 5th Ed., Ed.: Smith, M. B. and March, J., John Wiley & Sons, New York: 2001, the entire contents of which are hereby incorporated by reference.

Description of Exemplary Adjuvant Compounds

In some embodiments, provided adjuvant compounds are analogs of *Quillaja saponins*. In some embodiments, provided adjuvant compounds are prosapogenins. In certain embodiments, provided adjuvant compounds are analogs of QS-7 and QS-21 and possess potent adjuvant activity.

In one aspect, the present application provides adjuvant compounds of Formula I:



(I)

or a pharmaceutically acceptable salt thereof, wherein

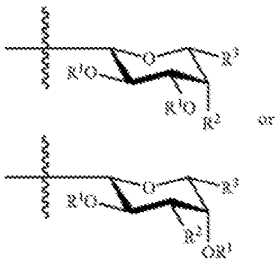
⎓ is a single or double bond;

W is —CHO;

V is hydrogen or OR^x;

Y is CH_2, —O—, —NR-, or —NH—;

Z is hydrogen; a cyclic or acyclic, optionally substituted moiety selected from the group consisting of acyl, aliphatic, heteroaliphatic, aryl, arylalkyl, heteroacyl, and heteroaryl; or a carbohydrate domain having the structure:



wherein each occurrence of R^1 is R^x or a carbohydrate domain having the structure:



wherein:

each occurrence of a, b, and c is independently 0, 1, or 2;

d is an integer from 1-5, wherein each d bracketed structure may be the same or different; with the proviso that the d bracketed structure represents a furanose or a pyranose moiety, and the sum of b and c is 1 or 2;

R^0 is hydrogen; an oxygen protecting group selected from the group consisting of alkyl ethers, benzyl ethers, silyl ethers, acetals, ketals, esters, carbamates, and carbonates; or an optionally substituted moiety selected from the group consisting of acyl, C_{1-10} aliphatic, C_{1-6} heteroaliphatic, 6-10-membered aryl, arylalkyl, 5-10 membered heteroaryl having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 4-7 membered heterocyclyl having 1-2

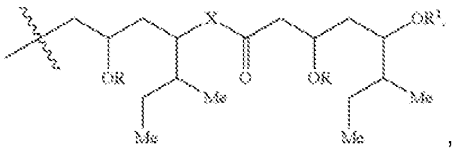
heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur;

each occurrence of R^a, R^b, R^c, and R^d is independently hydrogen, halogen, OH, OR, OR^x, NR_2, NHCOR, or an optionally substituted group selected from acyl, C_{1-10} aliphatic, C_{1-6} heteroaliphatic, 6-10-membered aryl, arylalkyl, 5-10-membered heteroaryl having 1-4 heteroatoms independently selected from nitrogen, oxygen, sulfur; 4-7-membered heterocyclyl having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur;

R^2 is hydrogen, halogen, OH, OR, $OC(O)R^4$, $OC(O)OR^4$, $OC(O)NHR^4$, $OC(O)NRR^4$, $OC(O)SR^4$, $NHC(O)R^4$, $NRC(O)R^4$, $NHC(O)OR^4$, $NHC(O)NHR^4$, $NHC(O)NRR^4$, NHR^4, $N(R^4)_2$, NHR^4, NRR^4, N_3, or an optionally substituted group selected from C_{1-10} aliphatic, C_{1-6} heteroaliphatic, 6-10-membered aryl, arylalkyl, 5-10 membered heteroaryl having 1-4 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur, 4-7-membered heterocyclyl having 1–2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur;

R^3 is hydrogen, halogen, CH_2OR^1, or an optionally substituted group selected from the group consisting of acyl, C_{1-10} aliphatic, C_{1-6} heteroaliphatic, 6-10-membered aryl, arylalkyl, 5-10-membered heteroaryl having 1-4 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur, 4-7-membered heterocyclyl having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur,

R^4 is $-T-R^z$, $-C(O)-T-R^z$, $-NH-T-R^z$, $-O-T-R^z$, $-S-T-R^z$, $-C(O)NH-T-R^z$, $C(O)O-T-R^z$, $C(O)S-T-R^z$, $C(O)NH-T-O-T-R^z$, $-O-T-R^z$, $-T-O-T-R^z$, $-T-S-T-R^z$, or



,

wherein

X is —O—, —NR—, or T-R^z;

T is a covalent bond or a bivalent C_{1-26} saturated or unsaturated, straight or branched, aliphatic or heteroaliphatic chain; and

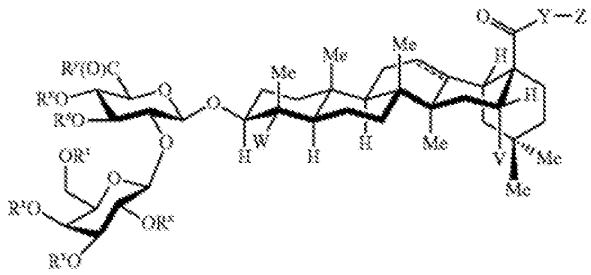
R^z is hydrogen, halogen, —OR, —OR^x, —OR^1, —SR, NR_2, —C(O)OR, —C(O)R, -NHC(O)R, -NHC(O)OR, NC(O)OR, or an optionally substituted group selected from acyl, arylalkyl, heteroarylalkyl, C_{1-6} aliphatic, 6-10-membered aryl, 5-10-membered heteroaryl having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 4-7-membered heterocyclyl having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur;

each occurrence of R^x is independently hydrogen or an oxygen protecting group selected from the group consisting of alkyl ethers, benzyl ethers, silyl ethers, acetals, ketals, esters, carbamates, and carbonates;

each occurrence of R is independently hydrogen, an optionally substituted group selected from acyl, arylalkyl, 6-10-membered aryl, C_{1-6} aliphatic, or C_{1-6} heteroaliphatic having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur, or:

two R on the same nitrogen atom are taken with the nitrogen atom to form a 4-7-membered heterocyclic ring having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur.

In one aspect, the present application provides compounds of Formula II:

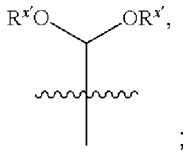


(II)

or a pharmaceutically acceptable salt thereof, wherein

=== is a single or double bond;

W is ME, —CHO, or

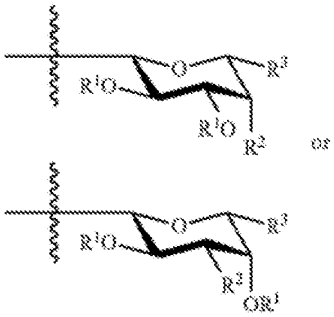


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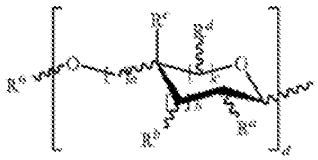
V is hydrogen or OR^x;

Y is CH_2, —O—, —NR-, or —NH—;

Z is hydrogen; a cyclic or acyclic, optionally substituted moiety selected from the group consisting of acyl, aliphatic, heteroaliphatic, aryl, arylalkyl, heteroacyl, and heteroaryl; or a carbohydrate domain having the structure:



wherein each occurrence of R^1 is R^x or a carbohydrate domain having the structure:



wherein:

each occurrence of a, b, and c is independently 0, 1, or 2;

d is an integer from 1-5, wherein each d bracketed structure may be the same or different; with the proviso that the d bracketed structure represents a furanose or a pyranose moiety, and the sum of b and c is 1 or 2;

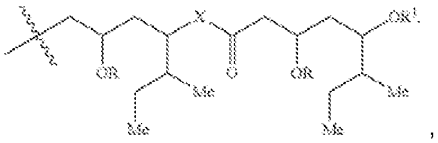
R^0 is hydrogen; an oxygen protecting group selected from the group consisting of alkyl ethers, benzyl ethers, silyl ethers, acetals, ketals, esters, carbamates, and carbonates; or an optionally substituted moiety selected from the group consisting of acyl, C_{1-10} aliphatic, C_{1-6} heteroaliphatic, 6-10-membered aryl, arylalkyl, 5-10 membered heteroaryl having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 4-7 membered heterocyclyl having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur;

each occurrence of R^a, R^b, R^c, and R^d is independently hydrogen, halogen, OH, OR, OR^x, NR_2, NHCOR, or an optionally substituted group selected from acyl, C_{1-10} aliphatic, C_{1-6} heteroaliphatic, 6-10-membered aryl, arylalkyl, 5-10-membered heteroaryl having 1-4 heteroatoms independently selected from nitrogen, oxygen, sulfur; 4-7-membered heterocyclyl having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur;

R^2 is hydrogen, halogen, OH, OR, $OC(O)R^4$, $OC(O)OR^4$, $OC(O)NHR^4$, $OC(O)NRR^4$, $OC(O)SR^4$, $NHC(O)R^4$, $NRC(O)R^4$, $NHC(O)OR^4$, $NHC(O)NHR^4$, $NHC(O)NRR^4$, NHR^4, $N(R^4)_2$, NHR^4, NRR^4, N_3, or an optionally substituted group selected from C_{1-10} aliphatic, C_{1-6} heteroaliphatic, 6-10-membered aryl, arylalkyl, 5-10 membered heteroaryl having 1-4 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur, 4-7-membered heterocyclyl having 1–2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur;

R^3 is hydrogen, halogen, CH_2OR^1, or an optionally substituted group selected from the group consisting of acyl, C_{1-10} aliphatic, C_{1-6} heteroaliphatic, 6-10-membered aryl, arylalkyl, 5-10-membered heteroaryl having 1-4 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur, 4-7-membered heterocyclyl having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur,

R^4 is $-T-R^z$, $-C(O)-T-R^z$, $-NH-T-R^z$, $-O-T-R^z$, $-S-T-R^z$, $-C(O)NH-T-R^z$, $C(O)O-T-R^z$, $C(O)S-T-R^z$, $C(O)NH-T-O-T-R^z$, $-O-T-R^z$, $-T-O-T-R^z$, $-T-S-T-R^z$, or



wherein

X is —O—, —NR—, or T-R^z;

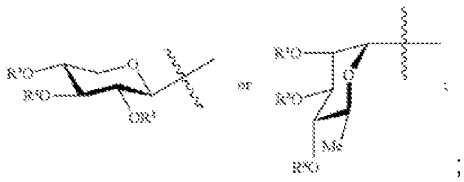
T is a covalent bond or a bivalent C_{1-26} saturated or unsaturated, straight or branched, aliphatic or heteroaliphatic chain; and

R^z is hydrogen, halogen, —OR, —OR^x, —OR^1, —SR, NR_2, —C(O)OR, —C(O)R, -NHC(O)R, -NHC(O)OR, NC(O)OR, or an optionally substituted group selected from acyl, arylalkyl, heteroarylalkyl, C_{1-6} aliphatic, 6-10-membered aryl, 5-10-membered heteroaryl having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 4-7-membered heterocyclyl having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur;

each occurrence of R^x is independently hydrogen or an oxygen protecting group selected from the group consisting of alkyl ethers, benzyl ethers, silyl ethers, acetals, ketals, esters, carbamates, and carbonates;

R^y is —OH, —OR, or a carboxyl protecting group selected from the group consisting of ester, amides, and hydrazides;

R^s is



;

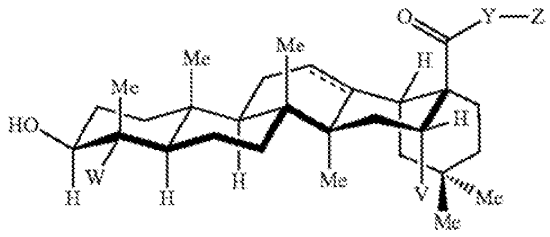
each occurrence of $R^{x'}$ is independently an optionally substituted group selected from 6-10-membered aryl, C_{1-6} aliphatic, or C_{1-6} heteroaliphatic having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur; or:

two $R^{x'}$ are taken together to form a 5-7-membered heterocyclic ring having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur;

each occurrence of R is independently hydrogen, an optionally substituted group selected from acyl, arylalkyl, 6-10-membered aryl, C_{1-6} aliphatic, or C_{1-6} heteroaliphatic having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur, or:

two R on the same nitrogen atom are taken with the nitrogen atom to form a 4-7-membered heterocyclic ring having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur.

In one aspect, the present application provides compounds of Formula I:



(I)

or a pharmaceutically acceptable salt thereof, wherein

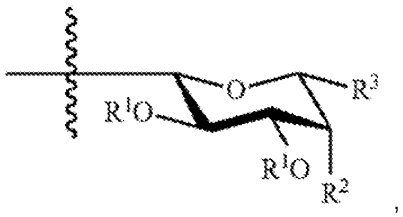
--- is a single or double bond;

W is —CHO;

V is —OH;

Y is —O—;

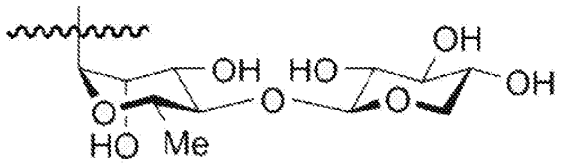
wherein Z is a carbohydrate domain having the structure:



,

wherein:

R^1 is independently H or

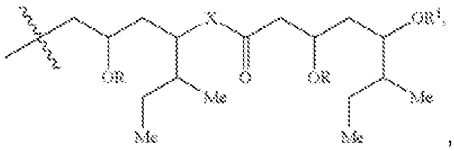


;

R^2 is NHR^4;

R^3 is CH_2OH; and

R^4 is -T-R^z, -C(O)-T-R^z, -NH-T-R^z, -O-T-R^z, -S-T-R^z, -C(O)NH-T-R^z, C(O)O-T-R^z, C(O)S-T-R^z, C(O)NH-T-O-T-R^z, -O-T-R^z, -T-O-T-R^z, -T-S-T-R^z, or



,

wherein:

X is —O—, —NR—, or T-R^z;

T is a covalent bond or a bivalent C_{1-26} saturated or unsaturated, straight or branched, aliphatic or heteroaliphatic chain; and

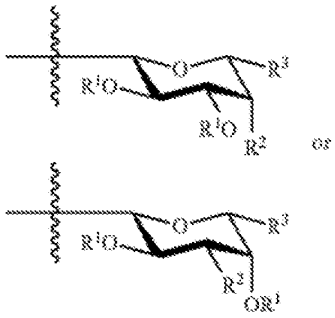
R^z is hydrogen, halogen, —OR, —OR^x, —OR^1, —SR, NR_2, —C(O)OR, —C(O)R, -NHC(O)R, -NHC(O)OR, NC(O)OR, or an optionally substituted group selected from acyl, arylalkyl, heteroarylalkyl, C_{1-6} aliphatic, 6-10-membered aryl, 5-10-membered heteroaryl having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 4-7-membered heterocyclyl having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur.

It will be appreciated by one of ordinary skill in the art that the compounds of the present application include but are not necessarily limited to those compounds encompassed in the genus definitions set forth as part of the present section. The compounds encompassed by this application include at least all of the compounds disclosed in the entire specification as a whole, including all individual species within each genus.

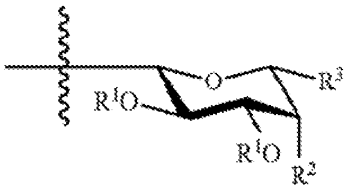
In certain embodiments, V is OR^x. In certain embodiments V is OH. In certain embodiments, V is H.

In certain embodiments, Y is –O-. In certain embodiments, Y is –NH-. In certain embodiments, Y is –NR-. In certain embodiments, Y is CH_2.

In certain embodiments, Z is hydrogen. In certain embodiments, Z is a cyclic or acyclic, optionally substituted moiety. In certain embodiments, Z is an acyl. In certain embodiments, Z is an aliphatic. In certain embodiments, Z is a heteroaliphatic. In certain embodiments, Z is aryl. In certain embodiments Z is arylalkyl. In certain embodiments, Z is heteroacyl. In certain embodiments, Z is heteroaryl. In certain embodiments, Z is a carbohydrate domain having the structure:

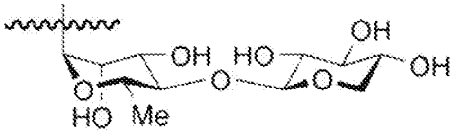


In some embodiments Z is a carbohydrate domain having the structure:



wherein:

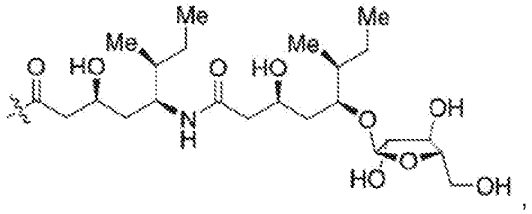
R^1 is independently H or



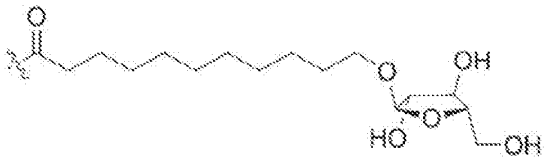
R^2 is NHR^4,

R^3 is CH_2OH, and

R^4 is selected from:


Me
Me
Me
Me
O
HO
O
HO
N
H
O
OH
HO
O
OH

,


O
O
OH
HO
O
OH

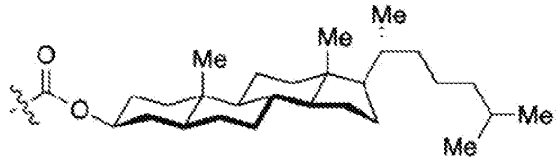
,


O

,


O
OH
O

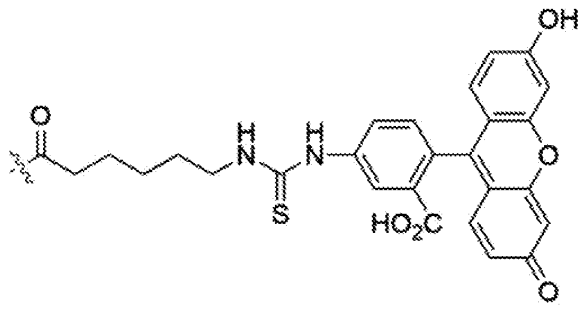
,


Me
Me
Me
O
O
Me
Me

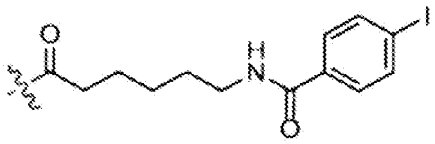
,


O
NH3+

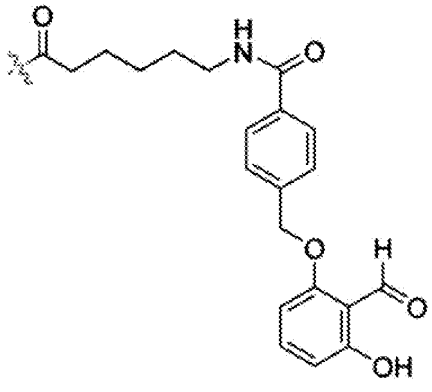
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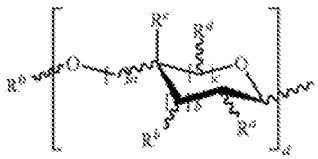


,



.

In some embodiments, R^1 is R^x. In other embodiments, R^1 a carbohydrate domain having the structure:



In some aspects, each occurrence of a, b, and c is independently 0, 1, or 2. In some embodiments, d is an integer from 1-5. In some embodiments, each d bracketed

structure may be the same. In some embodiments, each d bracketed structure may be different. In some embodiments, the d bracketed structure represents a furanose or a pyranose moiety. In some embodiments, and the sum of b and c is 1 or 2.

In some embodiments, R^0 is hydrogen. In some embodiments, R^0 is an oxygen protecting group selected from the group. In some embodiments, R^0 is an alkyl ether. In some embodiments, R^0 is a benzyl ether. In some embodiments, R^0 is a silyl ether. In some embodiments, R^0 is an acetal. In some embodiments, R^0 is ketal. In some embodiments, R^0 is an ester. In some embodiments, R^0 is a carbamate. In some embodiments, R^0 is a carbonate. In some embodiments, R^0 is an optionally substituted moiety. In some embodiments, R^0 is an acyl. In some embodiments, R^0 is a C_{1-10} aliphatic. In some embodiments, R^0 is a C_{1-6} heteroaliphatic. In some embodiments, R^0 is a 6-10-membered aryl. In some embodiments, R^0 is an arylalkyl. In some embodiments, R^0 is a 5-10 membered heteroaryl having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R^0 is a 4-7 membered heterocyclyl having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur.

In some embodiments, R^a is hydrogen. In some embodiments, R^a is a halogen. In some embodiments, R^a is OH. In some embodiments, R^a is OR. In some embodiments, R^a is OR^x. In some embodiments, R^a is NR_2. In some embodiments, R^a is NHCOR. In some embodiments, R^a an acyl. In some embodiments, R^a is C_{1-10} aliphatic. In some embodiments, R^a is C_{1-6} heteroaliphatic. In some embodiments, R^a is 6-10-membered aryl. In some embodiments, R^a is arylalkyl. In some embodiments, R^a is 5-10-membered heteroaryl having 1-4 heteroatoms independently selected from nitrogen, oxygen, sulfur. In some embodiments, R^a is 4-7-membered heterocyclyl having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur.

In some embodiments, R^b is hydrogen. In some embodiments, R^b is a halogen. In some embodiments, R^b is OH. In some embodiments, R^b is OR. In some embodiments, R^b is OR^x. In some embodiments, R^b is NR_2. In some embodiments, R^b is NHCOR. In some embodiments, R^b an acyl. In some embodiments, R^b is C_{1-10} aliphatic. In some embodiments, R^b is C_{1-6} heteroaliphatic. In some embodiments, R^b is 6-10-membered aryl. In some embodiments, R^b is arylalkyl. In some embodiments, R^b is 5-10-membered heteroaryl having 1-4 heteroatoms independently selected from nitrogen, oxygen, sulfur. In some embodiments, R^b is 4-7-membered heterocyclyl

having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur.

In some embodiments, R^b is hydrogen. In some embodiments, R^b is a halogen. In some embodiments, R^b is OH. In some embodiments, R^b is OR. In some embodiments, R^b is OR^x. In some embodiments, R^b is NR_2. In some embodiments, R^b is NHCOR. In some embodiments, R^b an acyl. In some embodiments, R^b is C_{1-10} aliphatic. In some embodiments, R^b is C_{1-6} heteroaliphatic. In some embodiments, R^b is 6-10-membered aryl. In some embodiments, R^b is arylalkyl. In some embodiments, R^b is 5-10-membered heteroaryl having 1-4 heteroatoms independently selected from nitrogen, oxygen, sulfur. In some embodiments, R^b is 4-7-membered heterocyclyl having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur.

In some embodiments, R^c is hydrogen. In some embodiments, R^c is a halogen. In some embodiments, R^c is OH. In some embodiments, R^c is OR. In some embodiments, R^c is OR^x. In some embodiments, R^c is NR_2. In some embodiments, R^c is NHCOR. In some embodiments, R^c an acyl. In some embodiments, R^c is C_{1-10} aliphatic. In some embodiments, R^c is C_{1-6} heteroaliphatic. In some embodiments, R^c is 6-10-membered aryl. In some embodiments, R^c is arylalkyl. In some embodiments, R^c is 5-10-membered heteroaryl having 1-4 heteroatoms independently selected from nitrogen, oxygen, sulfur. In some embodiments, R^c is 4-7-membered heterocyclyl having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur.

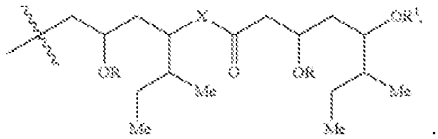
In some embodiments, R^d is hydrogen. In some embodiments, R^d is a halogen. In some embodiments, R^d is OH. In some embodiments, R^d is OR. In some embodiments, R^d is OR^x. In some embodiments, R^d is NR_2. In some embodiments, R^d is NHCOR. In some embodiments, R^d an acyl. In some embodiments, R^d is C_{1-10} aliphatic. In some embodiments, R^d is C_{1-6} heteroaliphatic. In some embodiments, R^d is 6-10-membered aryl. In some embodiments, R^d is arylalkyl. In some embodiments, R^d is 5-10-membered heteroaryl having 1-4 heteroatoms independently selected from nitrogen, oxygen, sulfur. In some embodiments, R^d is 4-7-membered heterocyclyl having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur.

In some embodiments, R^2 is hydrogen. In some embodiments, R^2 is a halogen. In some embodiments, R^2 is OH. In some embodiments, R^2 is OR. In some

embodiments, R^2 is $OC(O)R^4$. In some embodiments, R^2 is $OC(O)OR^4$. In some embodiments, R^2 is $OC(O)NHR^4$. In some embodiments, R^2 is $OC(O)NRR^4$. In some embodiments, R^2 is $OC(O)SR^4$. In some embodiments, R^2 is $NHC(O)R^4$. In some embodiments, R^2 is $NRC(O)R^4$. In some embodiments, R^2 is $NHC(O)OR^4$. In some embodiments, R^2 is $NHC(O)NHR^4$. In some embodiments, R^2 is $NHC(O)NRR^4$. In some embodiments, R^2 is NHR^4. In some embodiments, R^2 is $N(R^4)_2$. In some embodiments, R^2 is NHR^4. In some embodiments, R^2 is NRR^4. In some embodiments, R^2 is N_3. In some embodiments, R^2 is C_{1-10} aliphatic. In some embodiments, R^2 is C_{1-6} heteroaliphatic. In some embodiments, R^2 is 6-10-membered aryl. In some embodiments, R^2 is arylalkyl. In some embodiments, R^2 is 5-10 membered heteroaryl having 1-4 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur. In some embodiments, R^2 is 4-7-membered heterocyclyl having 1–2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur.

In some embodiments, R^3 is hydrogen. In some embodiments, R^3 is a halogen. In some embodiments, R^3 is CH_2OR^1. In some embodiments, R^3 is an acyl. In some embodiments, R^3 is C_{1-10} aliphatic. In some embodiments, R^3 is C_{1-6} heteroaliphatic. In some embodiments, R^3 is 6-10-membered aryl. In some embodiments, R^3 is arylalkyl. In some embodiments, R^3 is 5-10-membered heteroaryl having 1-4 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur. In some embodiments, R^3 is 4-7-membered heterocyclyl having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur.

In some embodiments, R^4 is $-T-R^z$. In some embodiments, R^4 is $-C(O)-T-R^z$. In some embodiments, R^4 is $-NH-T-R^z$. In some embodiments, R^4 is $-O-T-R^z$. In some embodiments, R^4 is $-S-T-R^z$. In some embodiments, R^4 is $-C(O)NH-T-R^z$. In some embodiments, R^4 is $C(O)O-T-R^z$. In some embodiments, R^4 is $C(O)S-T-R^z$. In some embodiments, R^4 is $C(O)NH-T-O-T-R^z$. In some embodiments, R^4 is $-O-T-R^z$. In some embodiments, R^4 is $-T-O-T-R^z$. In some embodiments, R^4 is $-T-S-T-R^z$. In some embodiments, R^4 is



In some embodiments, X is —O—. In some embodiments, X is —NR—. In some embodiments, X is T-R^z.

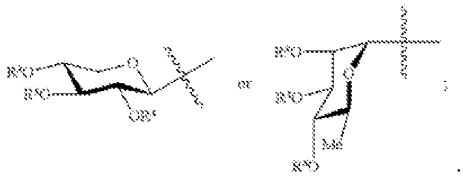
In some embodiments, T is a covalent bond or a bivalent C_{1-26} saturated or unsaturated, straight or branched, aliphatic or heteroaliphatic chain.

In some embodiments, R^z is hydrogen. In some embodiments, R^z is a halogen. In some embodiments, R^z is —OR. In some embodiments, R^z is —OR^x. In some embodiments, R^z is —OR^1. In some embodiments, R^z is —$OR^{1'}$. In some embodiments, R^z is —SR. In some embodiments, R^z is NR_2. In some embodiments, R^z is —C(O)OR. In some embodiments, R^z is —C(O)R. In some embodiments, R^z is -NHC(O)R. In some embodiments, R^z is -NHC(O)OR. In some embodiments, R^z is NC(O)OR. In some embodiments, R^z is an acyl. In some embodiments, R^z is arylalkyl. In some embodiments, R^z is heteroarylalkyl. In some embodiments, R^z is C_{1-6} aliphatic. In some embodiments, R^z is 6-10-membered aryl. In some embodiments, R^z is 5-10-membered heteroaryl having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R^z is 4-7-membered heterocyclyl having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur.

In some embodiments, R^x is hydrogen. In some embodiments, R^x is an oxygen protecting group. In some embodiments, R^x is an alkyl ethers. In some embodiments, R^x is a benzyl ether. In some embodiments, R^x is silyl ether. In some embodiments, R^x is an acetal. In some embodiments, R^x is ketal. In some embodiments, R^x is ester. In some embodiments, R^x is carbamate. In some embodiments, R^x is carbonate.

In some embodiments, R^y is —OH. In some embodiments, R^y is —OR. In some embodiments, R^y is a carboxyl protecting group. In some embodiments, R^y is an ester. In some embodiments, R^y is an amide. In some embodiments, R^y is a hydrazide.

In some embodiments, R^s is



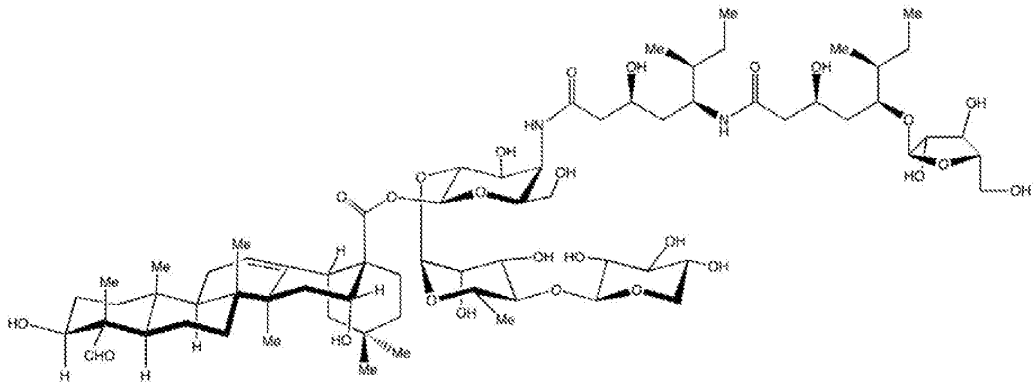
In some embodiments, $R^{x'}$ is optionally substituted 6-10-membered aryl. In some embodiments, $R^{x'}$ is optionally substituted C_{1-6} aliphatic. In some embodiments, $R^{x'}$ is optionally substituted or C_{1-6} heteroaliphatic having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur. In some embodiments, two $R^{x'}$ are taken together to form a 5-7-membered heterocyclic ring having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur.

In some embodiments, R is hydrogen. In some embodiments, R is an acyl. In some embodiments, R is arylalkyl. In some embodiments, R is 6-10-membered aryl. In some embodiments, R is C_{1-6} aliphatic. In some embodiments, R is C_{1-6} heteroaliphatic having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur. In some embodiments, two R on the same nitrogen atom are taken with the nitrogen atom to form a 4-7-membered heterocyclic ring having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur.

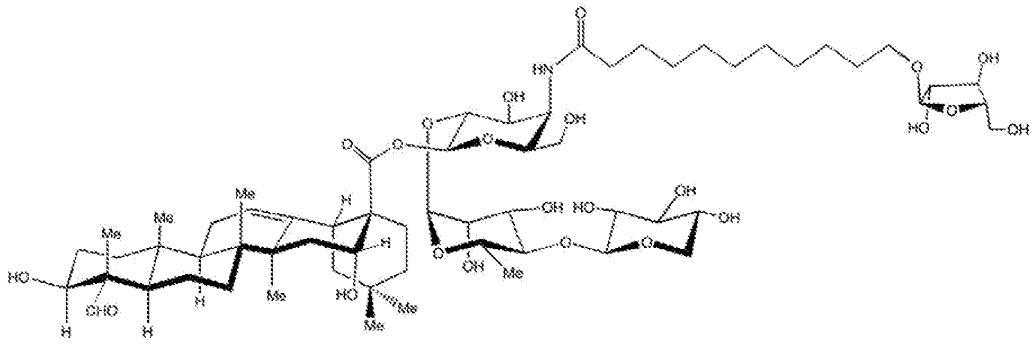
In some embodiments, $R^{1'}$ has the same embodiments as R^1.

Exemplary compounds of Formula I are set forth in Table 1 below:

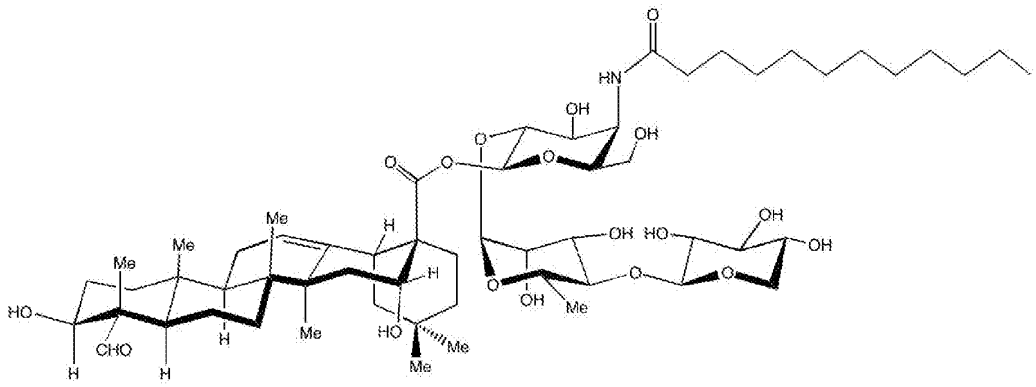
TABLE 1. EXEMPLARY COMPOUNDS OF FORMULA I



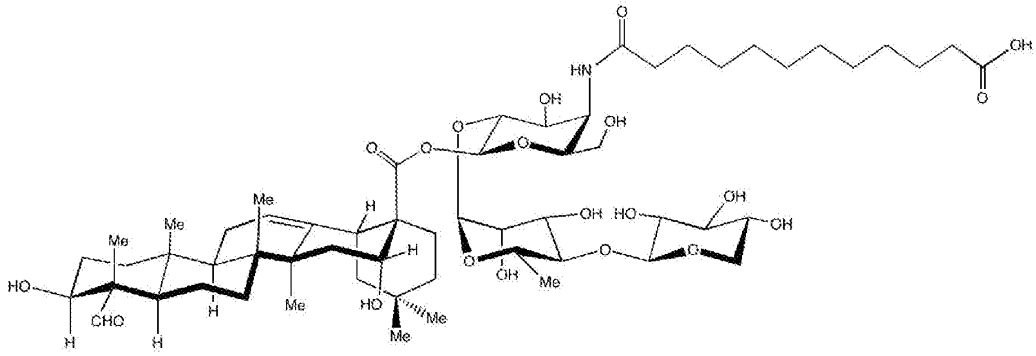
I-1



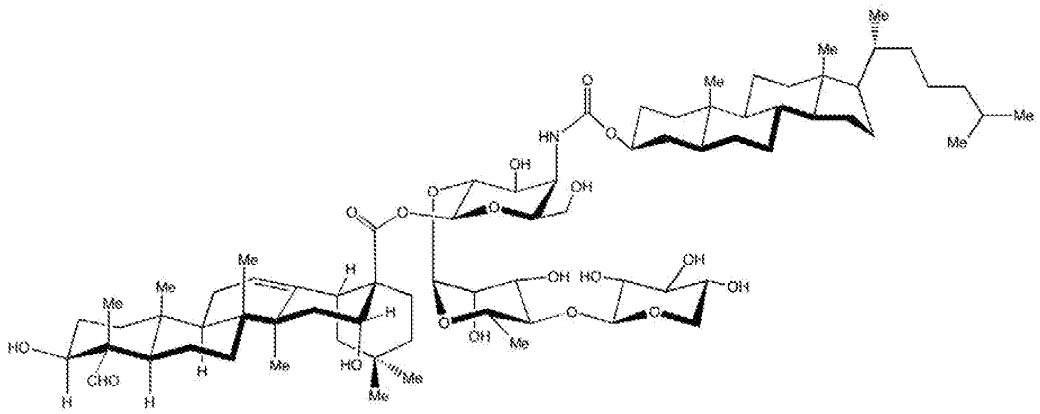
I-2



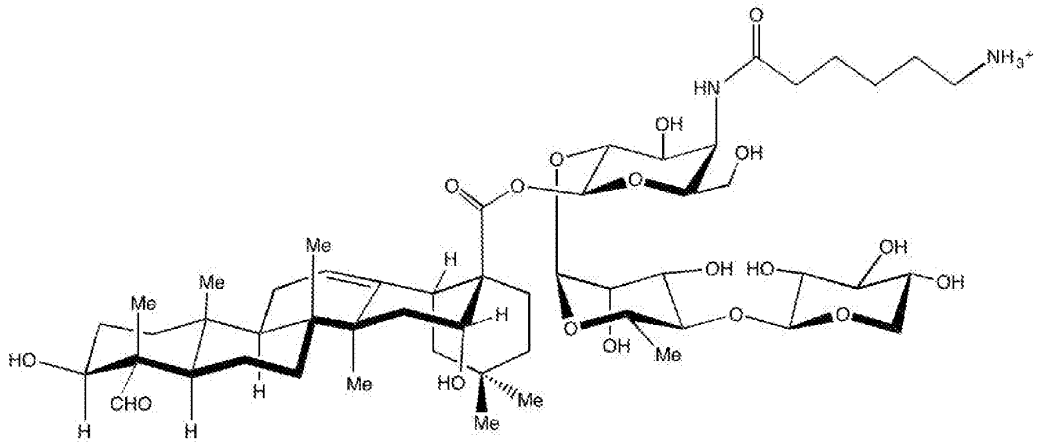
I-3



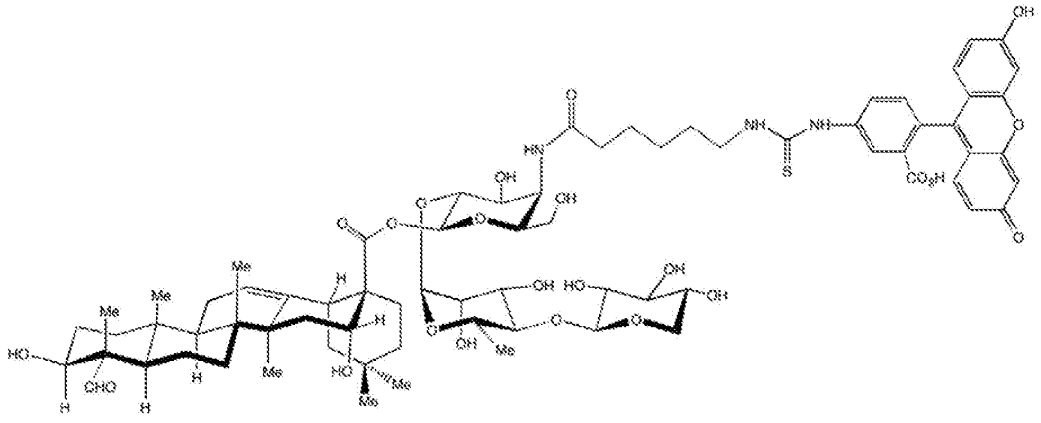
I-4



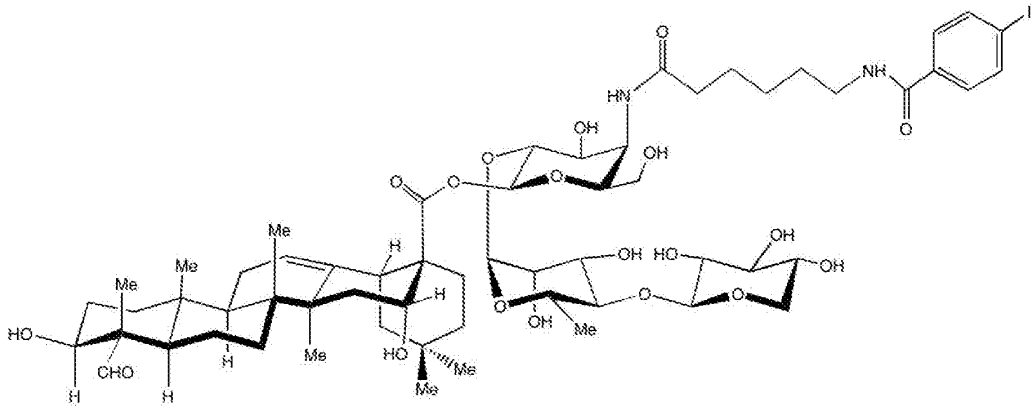
I-5



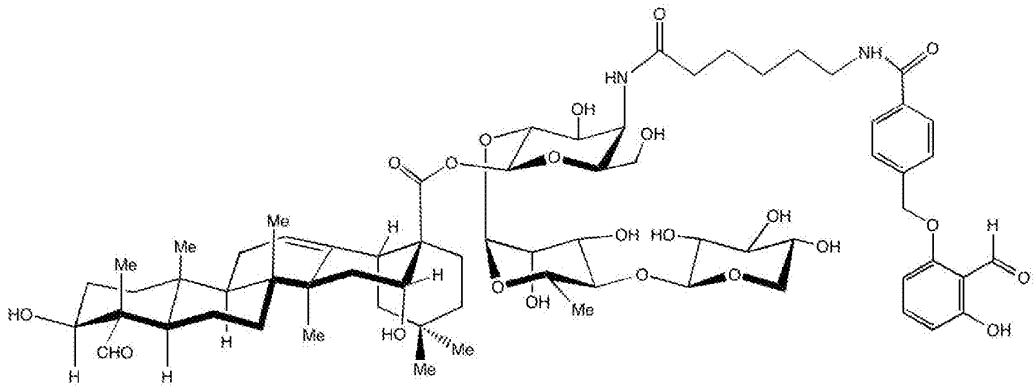
I-6



I-7



I-8



I-9

It will be appreciated that it is not an object of the present subject matter to claim compounds disclosed in the prior art that are the result of isolation or degradation studies on naturally occurring prosapogenins or saponins.

Synthesis of Adjuvant Compounds

Adjuvant compounds of the present application may be synthesized according to the approaches set forth in WO2017079582A1 and/or WO2018191598A1.

Varicella Zoster Antigens

The VZV antigen for use in the present application is an optionally truncated VZV glycoprotein, a fragment thereof, or an immunogenic derivative thereof. The optionally truncated VZV glycoprotein may be VZV glycoprotein E (gE) (VZV gE is also known as gp1), a fragment thereof, or an immunogenic derivative thereof, and may have the sequence of SEQ ID No. 1.

In one aspect the VZV gE is a truncated gE having the sequence of SEQ ID No. 1 (FIG. 1), and as disclosed in Virus research, vol 40, 1996 p 199 ff, herein incorporated fully by reference. In another aspect, the VZV gE is a truncated gE having the sequence of SEQ ID No. 2 (FIG. 2). In another aspect, the VZV gE is a truncated gE having the sequence of SEQ ID No. 9 (FIG. 9). In another aspect, the VZV gE is gE having the sequence of SEQ ID No. 3 (FIG. 3). In another aspect, the VZV gE is gE having the sequence of SEQ ID No. 4 (FIG. 4). In another aspect, the VZV gE is a truncated version of SEQ ID No. 3 or 4.

In another aspect, the VZV antigen may include, by way of example, VZV gB (SEQ ID No. 5, FIG. 5), VZV gH (SEQ ID No. 6, FIG. 6), VZV gC (SEQ ID No. 8, FIG. 8), VZV gI (SEQ ID No. 7, FIG 7), IE63 (e.g. see, Huang et al. J. Virol. 1992, 66: 2664, Sharp et al. J. Inf. Dis. 1992, 165:852, Debrus, J. Virol. 1995 May; 69(5):3240-5 and references therein), IE62 (e.g. see Arvin et al. J. Immunol. 1991 146:257, Sabella J. Virol. 1993 December; 67(12):7673-6 and references therein) ORF4 or ORF 10 (Arvin et al. Viral Immunol. 2002 15: 507.). In another aspect, the VZV antigen may be a truncated version of any of the foregoing.

The present application herein also contemplates that antigen combinations may be used with the live attenuated or killed VZV, and in one aspect a truncated gE as discussed above (SEQ ID Nos. 1, 2, or 9) may be included in any such combination. In one aspect the present application relates to combinations of truncated gE (SEQ ID Nos. 1, 2, or 9) with IE63 and truncated gE (SEQ ID Nos. 1, 2, or 9) with IE62, for example.

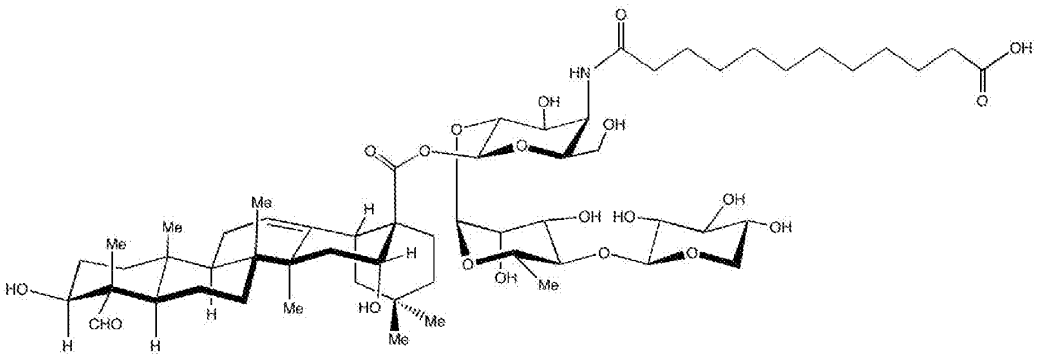
Embodiments of a gE antigen, derivatives thereof, and production thereof is described in EP0405867 and references therein (*see also* Vafai A. Antibody binding sites on truncated forms of varicella-zoster virus gpI(gE) glycoprotein Vaccine 1994 12:1265-9). EP0192902 also discloses embodiments of gE and production thereof.

Vaccine Compositions

Vaccine preparation is generally described in New Trends and Developments in Vaccines, Voller et al. (eds), University Park Press, Baltimore, Md., 1978.

In an embodiment of the present application, a composition which induces an immune response against Varicella zoster virus comprises an optionally truncated VZV glycoprotein or a fragment thereof in combination with a triterpene glycoside saponin-derived adjuvant. The optionally truncated VZV glycoprotein may be VZV glycoprotein E or a fragment thereof or may have the sequence of any of SEQ ID Nos. 1-4, and 9 (FIGs. 1-4, and 9). The saponin-derived adjuvant may be a compound according to Formula I as described previously.

In a preferred embodiment of the present application, a composition which induces an immune response against Varicella zoster virus comprises a VZV glycoprotein E truncated to remove the carboxy terminal anchor region, which gE is not in the form of a fusion protein, in combination with an adjuvant comprising a compound of Formula II (or a pharmaceutically acceptable salt thereof, e.g. a choline salt), a TLR4 agonist (e.g. MPLA, PHAD, MPL), and a liposome-forming compound (e.g. DOPC, DMPC, DMPG, cholesterol, and/or combinations of the foregoing).



(II)

In some of the preferred embodiments, the VZV glycoprotein contains a non-native signal peptide on the N-terminus to improve cleavage. In the context of the present application, such non-native signal peptides together with truncated or untruncated glycoproteins are not considered fusion proteins.

In another preferred embodiment, the present application utilizes an emulsion-based technology instead of or in conjunction with a liposome-forming compound.

Vaccine compositions, VZV antigens, and derivatives of VZV antigens can be tested for suitable immunogenic activity by use in the model systems by clinical trials in humans. One or more of the following indicators of activity are suitable for consideration in assessment of immunogenic activity: (1) Increased CD4 or CD8 T cell responses to VZV or antigen derivatives; (2) Elevation in VZV or antigens derivative specific antibodies; (3) Enhanced production of cytokines such as interferon γ or IL-2 or TNF α, (4) Enhanced expression of CD40L on CD4 and CD8 T cells; and/or (5) Reduction in the incidence of zoster below the incidence found in the general population of similarly at risk individuals, and likewise reduced disease severity and/or associated pain below the incidence found in the general population of similarly at risk individuals.

In another aspect, the present application relates to vaccine compositions comprising VZV antigen in combination with live attenuated or killed VZV. Suitable combinations of antigens include, for example, optionally truncated gE (SEQ ID No. 1, 2, 9), fragments thereof, or immunogenic derivatives thereof. The combined composition, or either or both of the individual components may additionally comprise an adjuvant composition as set forth in the present application.

Where a live attenuated strain is used, in one aspect the live attenuated VZV strain is the OKA strain, a strain well known in the art, for example as disclosed in Arbeter et al. (Journal of Pediatrics, vol 100, No 6, p 886 ff), WO9402596, and references therein, such as U.S. Pat. No. 3,985,615, all incorporated herein by reference. Any other suitable live attenuated strain may also be used in the present application. For example, the VARILRIX and VARIVAX strains are both appropriate and commercially available and could be employed. VZV-Dumas (either attenuated or inactivated) could also be employed. Whole inactivated VZV strains, such as inactivated VZV OKA are also suitable for use in the subject matter of the present application.

The amount of VZV antigen used in vaccine compositions of the present application is selected as an amount which induces an immunoprotective response without significant, adverse side effects. Such amount will vary depending upon which specific immunogen is employed and how it is presented. Generally, it is expected that each dose will comprise 1-1000 µg of protein, such as 2-100 µg, or 5-60 µg. Where gE (SEQ ID No 1, 2, 3, 4, or 9) is used then in one aspect 25-100 µg of gE (SEQ ID No 1, 2, 3, 4, or 9) may be used in humans, such as 40-100 µg of gE (SEQ ID No 1,

2, 3, 4, or 9) for human use, in one aspect about 25 µg, about 50 µg or about 100 µg of gE (SEQ ID No 1, 2, 3, 4, or 9), suitably 25 µg, 50 µg or 100 µg gE (SEQ ID No 1, 2, 3, 4, or 9). For the OKA strain, for example, a suitable dose is 500-50000 pfu/0.5 ml, such as 2000-6000 pfu/0.5 ml, with a suitable dose of the Oka strain for example being 6000-25,000 per dose, for example 10,000 pfu/dose. Higher doses such as 30,000 pfu, 40000 pfu, 50,000 pfu 60,000 pfu, 70000 pfu, 80000 pfu, 90000 pfu or even 100000 pfu may be employed.

An optimal amount for a particular vaccine can be ascertained by standard studies involving observation of appropriate immune responses in subjects. Following an initial vaccination, subjects may receive one or several booster immunizations adequately spaced. The composition(s) of the present application may be formulated for any appropriate manner of administration, including for example, topical, oral, nasal, mucosal, intravenous, intradermal, intraperitoneal, subcutaneous and intramuscular administration. Delivery of the OKA strain is, in one aspect, by subcutaneous delivery.

In another embodiment, a gE antigen (SEQ ID No. 1, 2, 3, 4, or 9), or immunogenic derivative or immunogenic fragment thereof, may be used with an adjuvant composition of the present application to provide an immunogenic composition or vaccine. That is, the gE antigen (SEQ ID No. 1, 2, 3, 4, or 9) or immunogenic derivative or immunogenic fragment thereof may be used in a vaccination schedule in the absence of a live attenuated strain or whole inactivated strain. Thus, the application relates to an immunogenic composition or vaccine comprising gE (SEQ ID No. 1, 2, 3, 4, or 9) or immunogenic derivative or immunogenic fragment thereof in combination with an adjuvant composition according to the present application.

In one aspect of the present application, a gE truncate is used in which gE has a C terminal truncation. In one aspect the truncation removes from 4 to 20 percent of the total amino acid residues at the carboxy terminal end. In one aspect the gE is lacking the carboxy terminal anchor region (suitably approximately amino acids 547-623 of the wild type sequence). In one aspect gE is a truncated gE having the sequence of SEQ ID No. 1 (FIG. 1) and as disclosed in Virus research, (Haumont et al Vol 40, 1996 p 199-204), herein incorporated fully by reference. In one aspect, with respect to SEQ ID No. 1, Thr 40 is substituted for Ile 40 (i.e. p.Ile40Thr). In one aspect,

with respect to SEQ ID No. 1, Leu 536 is substituted for Ile 536 (i.e. p.Ile536Leu). In one aspect, both substitutions are made.

In one aspect gE is a truncated gE having the sequence of SEQ ID No. 2 (FIG. 2). In one aspect gE is a truncated gE having the sequence of SEQ ID No. 9 (FIG. 9). In another aspect of the present application, the composition comprises full length gE (SEQ ID Nos. 3 or 4, FIGs. 3 or 4).

In another aspect, the composition comprises a truncated gE having a portion of SEQ ID Nos. 3 or 4. In one aspect, with respect to SEQ ID No. 3, Ile 40 is substituted for Thr 40 (i.e. p.Thr40Ile). In one aspect, with respect to SEQ ID No. 3, Ile 536 is substituted for Leu 536 (i.e. p.Leu536Ile). In one aspect, both substitutions are made.

In another aspect the gE or derivative or fragment thereof is lyophilized. In another aspect the gE or derivative or fragment thereof is reconstituted in a solution containing an adjuvant composition according to the present application (such as an adjuvant containing Formula II, cholesterol, DOPC and a TLR4 agonist) before delivery.

In one embodiment the composition or vaccine comprises gE and an adjuvant composition according to the present application and does not comprise an IE63 antigen or portion thereof. In one embodiment the composition or vaccine comprises gE (SEQ ID No. 1, 2, 3, 4, or 9) and an adjuvant composition according to the present application and does not comprise any other VZV antigen. In one embodiment the composition or vaccine comprises gE (SEQ ID No. 1, 2, 3, 4, or 9) and an adjuvant according to the present application and does not comprise any other viral antigen.

In one aspect the gE or immunogenic fragment thereof is not in the form of a fusion protein. In the context of the present application, non-native signal peptides together with truncated or untruncated glycoproteins are not considered fusion proteins.

In one aspect the composition or vaccine consists essentially of the compound of Formula II, a truncated VZV gE antigen and liposomes comprising DOPC, DMPC, DMPG, cholesterol, and/or combinations of the foregoing, and a TLR4 agonist.

In one aspect the composition or vaccine consists of the compound of Formula II, a truncated VZV gE antigen and liposomes comprising cholesterol and a TLR4 agonist, and a pharmaceutically acceptable carrier.

The term ‘immunogenic derivative’ encompasses any molecule which retains the ability to induce an immune response to VZV following administration to man.

Immunogenic compounds herein are suitably capable of reacting detectably within an immunoassay (such as an ELISA or T-cell stimulation assay) with antisera and/or T-cells from a patient with VZV. Screening for immunogenic activity can be performed using techniques well known to the skilled artisan. For example, such screens can be performed using methods such as those described in Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988.

Immunogenic fragments as described herein are immunogenic derivatives which retain the ability to induce an immune response to VZV following administration to man.

Suitable methods for the generation of derivatives are well known in the art and include standard molecular biology techniques as disclosed, for example, in Sambrook et al [Molecular Cloning: A Laboratory Manual, third edition, 2000, Cold Spring Harbor Laboratory Press], such as techniques for the addition, deletion, substitution or rearrangement of amino acids or chemical modifications thereof. In one aspect derivatives include, for example, truncations or other fragments.

In one aspect derivatives in the context of this application are amino acid sequences comprising epitopes, i.e., antigenic determinants substantially responsible for the immunogenic properties of a polypeptide and being capable of eliciting an immune response, in one aspect being T cell epitopes.

In one aspect, the level of immunogenic activity of the immunogenic derivative is at least about 50%, in one aspect at least about 70% and in one aspect at least or greater than about 90% of the immunogenicity for the polypeptide from which it is derived, suitably as assessed by immunoassay techniques described above. In some aspects of the present application, immunogenic portions may be identified that have a level of immunogenic activity greater than that of the corresponding full-length polypeptide, e.g., having greater than about 100% or 150% or more immunogenic activity.

Vaccine Regimes

In one aspect the present application relates to a prime boost regime wherein a VZV antigen, in one aspect an adjuvanted antigen, is delivered first, after which the immune system is boosted with delivery of an attenuated VZV.

A prime boost regime in humans comprises, in one aspect, priming with 25-100 µg gE (SEQ ID No. 1, 2, 3, 4, or 9), in one aspect 40-100 µg gE (SEQ ID No. 1, 2, 3, 4, or 9), such as 50 or about 50 µg gE (SEQ ID No. 1, 2, 3, 4, or 9), or an immunogenic derivative thereof, adjuvanted with an adjuvant of Formula II, and boosting with the OKA strain of VZV.

Where prime boost regimes are used, or where multiple vaccination regimes are used, then 2, 3, 4 or more immunizations may be employed. Suitable regimes for prime boost include 1, 2, 3, 4, 5 or 6 months between individual immunizations. A prime boost schedule comprises, in one aspect, delivery of a VZV antigen or immunogenic derivative thereof, suitably an adjuvanted VZV antigen or derivative, at 0 months and boosting with a live attenuated VZV at 2 months.

In an alternative delivery schedule, there is concomitant delivery of both of the two individual components (VZV antigen or derivative and live attenuated VZV) at both 0 and 2 months.

In an alternative delivery schedule, there is delivery of VZV antigen or derivative thereof (no live attenuated or killed VZV) at both 0 and 2 months. The VZV antigen may be, for example, VZV gE (SEQ ID No. 1, 2, 3, 4, or 9).

In an alternative delivery schedule, there is delivery of a VZV antigen or a derivate thereof in a single dose. The VZV antigen may be, for example. VZV gE (SEQ ID No. 1, 2, 3, 4, or 9).

The composition or vaccine is suitably used in the population of people 50 or older than 50. Suitably the population is the population of those older than 55, 60, 65, 70, 75, 80, or older than 80. Suitably the population is 50-70 years.

In one aspect the population of individuals are those who have had varicella or who have had a live varicella vaccine.

Thus, the present application relates to use of a composition as described above in the preparation of a medicament for the prevention or amelioration of herpes zoster reactivation and/or post herpetic neuralgia in a population of people 50 or above.

The present application thus also relates to a method for the prevention or amelioration of herpes zoster reactivation and/or post herpetic neuralgia, the method comprising delivering to an individual in need thereof a composition of the present application.

In one aspect the composition of the first and second aspects of the present application are used in those individuals in whom the varicella zoster virus has not reactivated.

The composition may be used at doses and delivery routes as outlined above for the first aspect of the invention. Specifically, the amount of gE antigen (SEQ ID No. 1, 2, 3, 4, or 9) is selected as an amount which induces an immunoprotective response without significant, adverse side effects in typical vaccines. Such amount will vary depending upon which specific immunogen is employed and how it is presented. Generally, it is expected that each dose will comprise 1-1000 µg of protein, such as 2-100 µg, or 5-60 µg. Where gE (SEQ ID No. 1, 2, 3, 4, or 9) is used then suitably 25-100 µg gE (SEQ ID No. 1, 2, 3, 4, or 9) is used, in one aspect 40-100 µg of gE (SEQ ID No. 1, 2, 3, 4, or 9), such as about 25 µg, 50 µg or about 100 µg of gE (SEQ ID No. 1, 2, 3, 4, or 9), suitably 25 µg, 50 µg or 100 µg gE (SEQ ID No. 1, 2, 3, 4, or 9). An optimal amount for a particular vaccine can be ascertained by standard studies involving observation of appropriate immune responses in subjects. Following an initial vaccination, subjects may receive one or several booster immunization adequately spaced.

In one aspect the gE (SEQ ID No. 1, 2, 3, 4, or 9) and adjuvant composition or vaccine is used in a one dose delivery regime. In one aspect the gE (SEQ ID No. 1, 2, 3, 4, or 9) and adjuvant composition or vaccine is used in a two-dose delivery regime. In one aspect the composition or vaccine of the invention is used in a 2 dose regime with a 2 month spacing between doses.

Vaccine Kits and Medicaments

In another embodiment, the present application relates to a kit comprising a live attenuated VZV or inactivated whole VZV and a VZV antigen.

In another aspect, the present application relates to a kit comprising, as separate components, an adjuvant composition according to the present application and a gE antigen or immunogenic fragment thereof, as described above, suitable for extemporaneous preparation of a vaccine composition. In one aspect both components are liquids. In one aspect one component is lyophilized and is suitable for reconstitution with the other component. In one aspect the kit comprises a gE antigen

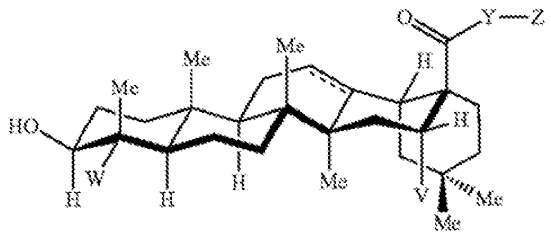
having the sequence SEQ ID No. 1 and an adjuvant comprising the compound of Formula II and liposomes comprising cholesterol and a TLR4 agonist.

In yet another embodiment, the present application relates to use of a VZV antigen, including a composition comprising gE (SEQ ID No. 1, 2, 3, 4, or 9), or an immunogenic derivative or immunogenic fragment thereof in combination with an adjuvant composition according to the present application, in the preparation of a medicament for the prevention or amelioration of herpes zoster reactivation and/or post herpetic neuralgia.

Further embodiments

In a series of further specific or alternate embodiments, the present application also provides:

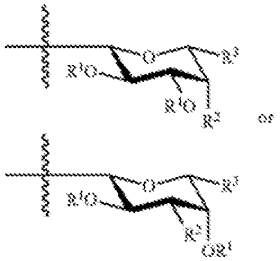
1.1 An immunogenic composition comprising:
a varicella zoster virus antigen, and
a compound of Formula I:



(I)

or a pharmaceutically acceptable salt thereof, wherein
— is a single or double bond;
W is —CHO;
V is hydrogen or OR^x;
Y is CH_2, —O—, —NR-, or —NH—;
Z is hydrogen; a cyclic or acyclic, optionally substituted moiety selected from the group consisting of acyl, aliphatic, heteroaliphatic, aryl,

arylalkyl, heteroacyl, and heteroaryl; or a carbohydrate domain having the structure:



wherein each occurrence of R^1 is R^x or a carbohydrate domain having the structure:



wherein:

each occurrence of a, b, and c is independently 0, 1, or 2;

d is an integer from 1-5, wherein each d bracketed structure may be the same or different; with the proviso that the d bracketed structure represents a furanose or a pyranose moiety, and the sum of b and c is 1 or 2;

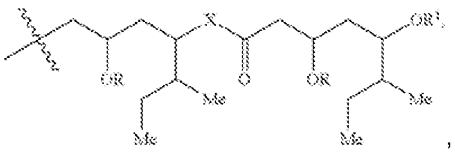
R^0 is hydrogen; an oxygen protecting group selected from the group consisting of alkyl ethers, benzyl ethers, silyl ethers, acetals, ketals, esters, carbamates, and carbonates; or an optionally substituted moiety selected from the group consisting of acyl, C_{1-10} aliphatic, C_{1-6} heteroaliphatic, 6-10-membered aryl, arylalkyl, 5-10 membered heteroaryl having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 4-7 membered heterocyclyl having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur;

each occurrence of R^a, R^b, R^c, and R^d is independently hydrogen, halogen, OH, OR, OR^x, NR_2, NHCOR, or an optionally substituted group selected from acyl, C_{1-10} aliphatic, C_{1-6} heteroaliphatic, 6-10-membered aryl, arylalkyl, 5-10-membered heteroaryl having 1-4 heteroatoms independently selected from nitrogen, oxygen, sulfur; 4-7-membered heterocyclyl having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur;

R^2 is hydrogen, halogen, OH, OR, $OC(O)R^4$, $OC(O)OR^4$, $OC(O)NHR^4$, $OC(O)NRR^4$, $OC(O)SR^4$, $NHC(O)R^4$, $NRC(O)R^4$, $NHC(O)OR^4$, $NHC(O)NHR^4$, $NHC(O)NRR^4$, NHR^4, $N(R^4)_2$, NHR^4, NRR^4, N_3, or an optionally substituted group selected from C_{1-10} aliphatic, C_{1-6} heteroaliphatic, 6-10-membered aryl, arylalkyl, 5-10 membered heteroaryl having 1-4 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur, 4-7-membered heterocyclyl having 1–2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur;

R^3 is hydrogen, halogen, CH_2OR^1, or an optionally substituted group selected from the group consisting of acyl, C_{1-10} aliphatic, C_{1-6} heteroaliphatic, 6-10-membered aryl, arylalkyl, 5-10-membered heteroaryl having 1-4 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur, 4-7-membered heterocyclyl having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur,

R^4 is $-T-R^z$, $-C(O)-T-R^z$, $-NH-T-R^z$, $-O-T-R^z$, $-S-T-R^z$, $-C(O)NH-T-R^z$, $C(O)O-T-R^z$, $C(O)S-T-R^z$, $C(O)NH-T-O-T-R^z$, $-O-T-R^z$, $-T-O-T-R^z$, $-T-S-T-R^z$, or



,

wherein

X is —O—, —NR—, or $T-R^z$;

T is a covalent bond or a bivalent C_{1-26} saturated or unsaturated, straight or branched, aliphatic or heteroaliphatic chain; and

R^z is hydrogen, halogen, —OR, —OR^x, —OR^1, —SR, NR_2, —C(O)OR, —C(O)R, -NHC(O)R, -NHC(O)OR, NC(O)OR, or an optionally substituted group selected from acyl, arylalkyl, heteroarylalkyl, C_{1-6} aliphatic, 6-10-membered aryl, 5-10-membered heteroaryl having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 4-7-membered heterocyclyl having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur;

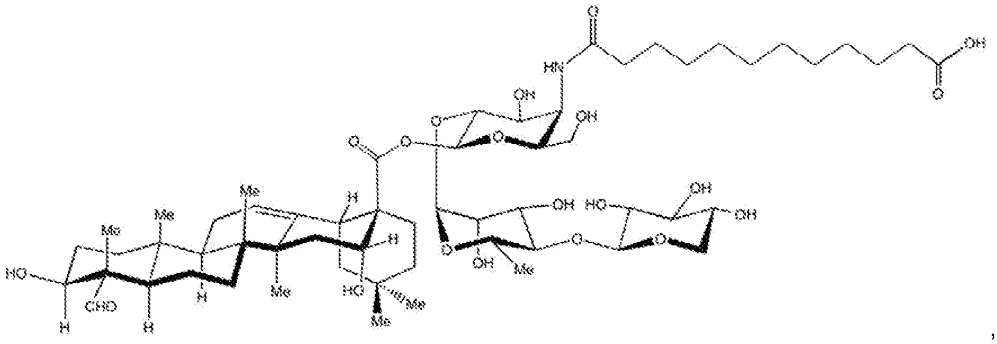
each occurrence of R^x is independently hydrogen or an oxygen protecting group selected from the group consisting of alkyl ethers, benzyl ethers, silyl ethers, acetals, ketals, esters, carbamates, and carbonates;

each occurrence of R is independently hydrogen, an optionally substituted group selected from acyl, arylalkyl, 6-10-membered aryl, C_{1-6} aliphatic, or C_{1-6} heteroaliphatic having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur, or:

two R on the same nitrogen atom are taken with the nitrogen atom to form a 4-7-membered heterocyclic ring having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur.

1.2. The immunogenic composition of 1.1,

wherein the compound of Formula I is:



,

or a pharmaceutically acceptable salt thereof.

1.3. The immunogenic composition of any of 1.1 to 1.2,

wherein the compound of Formula I is in free acid form.

1.4. The immunogenic composition of any of 1.1 to 1.2,
wherein the compound of Formula I is in choline salt form.

1.5. The immunogenic composition of any of 1.1 to 1.4,
wherein the varicella zoster virus antigen is a varicella zoster virus gE antigen truncated to remove the carboxy terminal anchor region.

1.6. The immunogenic composition of any of 1.1 to 1.5,
wherein the varicella zoster virus gE antigen is a truncate.

1.7. The immunogenic composition of any of 1.1 to 1.6,
wherein the varicella zoster virus gE antigen is a C-terminal truncate.

1.8. The immunogenic composition of any of 1.1 to 1.7,
wherein the varicella zoster virus antigen has the sequence of SEQ ID No. 1, SEQ ID No. 2, or SEQ ID No. 9.

1.9. The immunogenic composition of any of 1.1 to 1.7,
wherein the varicella zoster virus antigen has the sequence of SEQ ID No. 1.

1.10. The immunogenic composition of any of 1.1 to 1.7,
wherein the varicella zoster virus antigen has the sequence of SEQ ID No. 9.

1.11. The immunogenic composition of any of 1.1 to 1.10,
further comprising a TLR4 agonist.

1.12. The immunogenic composition of any of 1.1 to 1.11,
further comprising a liposome-forming compound.

1.13. The immunogenic composition of 1.12,
wherein the liposome-forming compound forms liposomes containing the TLR4 agonist.

1.14. The immunogenic composition of any of 1.12 to 1.13,
wherein the liposome-forming compound is selected from the group consisting of DOPC, DMPC, DMPG, cholesterol, and combinations thereof.

1.15. The immunogenic composition according to any of 1.1 to 1.11,
further comprising an emulsion.

1.16. The immunogenic composition according to 1.15,
wherein the emulsion is an oil-in-water emulsion.

1.17. The immunogenic composition according to any of 1.15 to 1.16,
wherein the emulsion contains the compound of Formula I or pharmaceutically acceptable salt thereof.

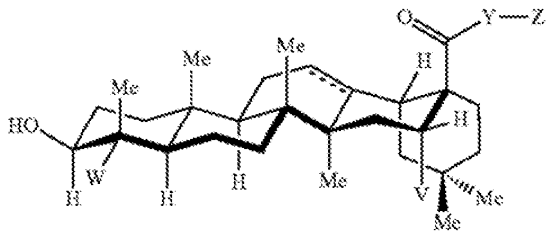
1.18. The immunogenic composition according to any of 1.15 to 1.17,
wherein the emulsion contains the TLR4 agonist.

1.19. The immunogenic composition according to any of 1.1 to 1.13,
further comprising an emulsion.

1.20. The immunogenic composition according to 1.19,
wherein the emulsion is an oil-in-water emulsion.

1.21. The immunogenic composition according to any of 1.19 to 1.20,
wherein the emulsion contains the compound of Formula I or pharmaceutically acceptable salt thereof.

2.1. A method of increasing cell-mediated immunity in a patient, said method comprising administering to said patient an effective amount of an immunogenic composition comprising a varicella zoster virus antigen and a compound of Formula I:



(I)

or a pharmaceutically acceptable salt thereof, wherein

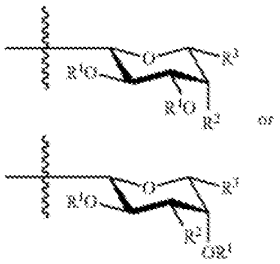
is a single or double bond;

W is —CHO;

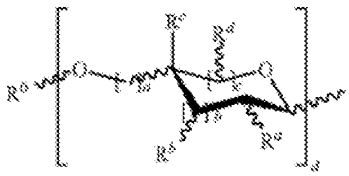
V is hydrogen or OR^x;

Y is CH_2, —O—, —NR-, or —NH—;

Z is hydrogen; a cyclic or acyclic, optionally substituted moiety selected from the group consisting of acyl, aliphatic, heteroaliphatic, aryl, arylalkyl, heteroacyl, and heteroaryl; or a carbohydrate domain having the structure:



wherein each occurrence of R^1 is R^x or a carbohydrate domain having the structure:



wherein:

each occurrence of a, b, and c is independently 0, 1, or 2;

d is an integer from 1-5, wherein each d bracketed structure may be the same or different; with the proviso that the d bracketed structure represents a furanose or a pyranose moiety, and the sum of b and c is 1 or 2;

R^0 is hydrogen; an oxygen protecting group selected from the group consisting of alkyl ethers, benzyl ethers, silyl ethers, acetals, ketals, esters, carbamates, and carbonates; or an optionally substituted moiety selected from the group consisting of acyl, C_{1-10} aliphatic, C_{1-6} heteroaliphatic, 6-10-membered aryl, arylalkyl, 5-10 membered heteroaryl having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 4-7 membered heterocyclyl having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur;

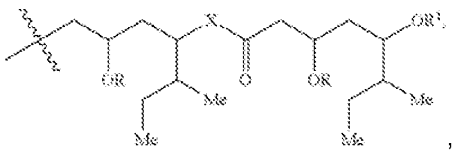
each occurrence of R^a, R^b, R^c, and R^d is independently hydrogen, halogen, OH, OR, OR^x, NR_2, NHCOR, or an optionally substituted group selected from acyl, C_{1-10} aliphatic, C_{1-6} heteroaliphatic, 6-10-membered aryl, arylalkyl, 5-10-membered heteroaryl having 1-4 heteroatoms independently selected from nitrogen, oxygen, sulfur; 4-7-membered heterocyclyl having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur;

R^2 is hydrogen, halogen, OH, OR, $OC(O)R^4$, $OC(O)OR^4$, $OC(O)NHR^4$, $OC(O)NRR^4$, $OC(O)SR^4$, $NHC(O)R^4$, $NRC(O)R^4$, $NHC(O)OR^4$, $NHC(O)NHR^4$, $NHC(O)NRR^4$, NHR^4, $N(R^4)_2$, NHR^4, NRR^4, N_3, or an optionally substituted group selected from C_{1-10} aliphatic, C_{1-6} heteroaliphatic, 6-10-membered aryl, arylalkyl, 5-10 membered heteroaryl having 1-4 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur, 4-7-membered heterocyclyl having 1–2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur;

R^3 is hydrogen, halogen, CH_2OR^1, or an optionally substituted group selected from the group consisting of acyl, C_{1-10} aliphatic, C_{1-6} heteroaliphatic, 6-10-membered aryl, arylalkyl, 5-10-membered heteroaryl having 1-4 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur, 4-7-membered heterocyclyl having 1-2

heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur,

R^4 is -T-R^z, -C(O)-T-R^z, -NH-T-R^z, -O-T-R^z, -S-T-R^z, -C(O)NH-T-R^z, C(O)O-T-R^z, C(O)S-T-R^z, C(O)NH-T-O-T-R^z, -O-T-R^z, -T-O-T-R^z, -T-S-T-R^z, or



wherein

X is —O—, —NR—, or T-R^z;

T is a covalent bond or a bivalent C_{1-26} saturated or unsaturated, straight or branched, aliphatic or heteroaliphatic chain; and

R^z is hydrogen, halogen, —OR, —OR^x, —OR^1, —SR, NR_2, —C(O)OR, —C(O)R, -NHC(O)R, -NHC(O)OR, NC(O)OR, or an optionally substituted group selected from acyl, arylalkyl, heteroarylalkyl, C_{1-6} aliphatic, 6-10-membered aryl, 5-10-membered heteroaryl having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, 4-7-membered heterocyclyl having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur;

each occurrence of R^x is independently hydrogen or an oxygen protecting group selected from the group consisting of alkyl ethers, benzyl ethers, silyl ethers, acetals, ketals, esters, carbamates, and carbonates;

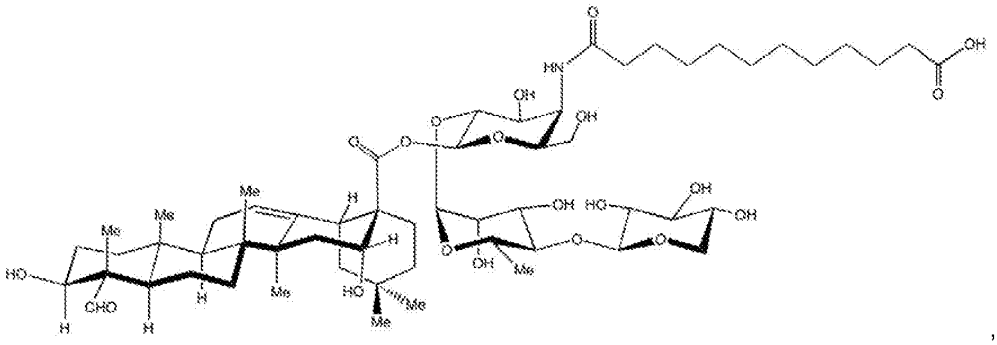
each occurrence of R is independently hydrogen, an optionally substituted group selected from acyl, arylalkyl, 6-10-membered aryl, C_{1-6} aliphatic, or C_{1-6} heteroaliphatic having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur, or:

two R on the same nitrogen atom are taken with the nitrogen atom to form a 4-7-membered heterocyclic ring having 1-2 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur,

wherein the cell mediated immunity prevents herpes zoster reactivation in the patient.

2.2. The method of 2.1,

wherein the compound of Formula I is:



or a pharmaceutically acceptable salt thereof.

2.3. The method of any of 2.1 to 2.2,

wherein the compound of Formula I is in free acid form.

2.4. The method of any of 2.1 to 2.2,

wherein the compound of Formula I is in choline salt form.

2.5. The method of any of 2.1 to 2.4,

wherein the varicella zoster virus antigen is a varicella zoster virus gE antigen truncated to remove the carboxy terminal anchor region.

2.6. The method of any of 2.1 to 2.5,

wherein the varicella zoster virus gE antigen is a truncate.

2.7. The method of any of 2.1 to 2.6,

wherein the varicella zoster virus gE antigen is a C-terminal truncate.

2.8. The method of any of 2.1 to 2.7,

wherein the varicella zoster virus antigen has the sequence of SEQ ID No. 1, SEQ ID No. 2, or SEQ ID No. 9.

2.9. The method of any of 2.1 to 2.7,

wherein the varicella zoster virus antigen has the sequence of SEQ ID No. 1.

2.10. The method of any of 2.1 to 2.7,
wherein the varicella zoster virus antigen has the sequence of SEQ ID No. 9.

2.11. The method of any of 2.1 to 2.10,
further comprising a TLR4 agonist.

2.12. The method of any of 2.1 to 2.11,
further comprising a liposome-forming compound.

2.13. The method of 2.12,
wherein the liposome-forming compound forms liposomes containing the TLR4 agonist.

2.14. The method of any of 2.12 to 2.13,
wherein the liposome-forming compound is selected from the group consisting of DOPC, DMPC, DMPG, cholesterol, and combinations thereof.

2.15. The method according to any of 2.1 to 2.11,
further comprising an emulsion.

2.16. The method according to 2.15,
wherein the emulsion is an oil-in-water emulsion.

2.17. The method according to any of 2.15 to 2.16,
wherein the emulsion contains the compound of Formula I or pharmaceutically acceptable salt thereof.

2.18. The method according to any of 2.15 to 2.17,
wherein the emulsion contains the TLR4 agonist.

2.19. The method according to any of 2.1 to 2.13,
further comprising an emulsion.

2.20. The method according to 2.19,
wherein the emulsion is an oil-in-water emulsion.

2.21. The method according to any of 2.19 to 2.20,
wherein the emulsion contains the compound of Formula I or pharmaceutically acceptable salt thereof.

EXAMPLES

Example 1 – Liposome PHAD + Compound I-4 Free Acid Form

The impact of TQL-1055 free acid (Compound I-4 free acid) on antibody titers induced by gE antigen was tested. Mice were immunized with gE (5 mcg) alone, gE (5mcg) with blank liposomes, gE (5 mcg) with liposomes containing PHAD (7.5 mcg), gE (5 mcg) with liposomes containing PHAD (7.5 mcg) and TQL-1055 free acid (four groups: 5 mcg, 15 mcg, 30 mcg, 50 mcg), and gE (5 mcg) with liposomes containing MPL (7.5 mcg) and QS-21 (5 mcg). Mice were immunized at Day 0 and Day 14. Groups were bled at Day 14 (post Dose 1) and Day 28 (post Dose 2) for serum analysis for anti-gE specific antibody response. The results are shown below.

gE-specific total IgG titers post dose 1

FIG. 10 is a graph depicting gE-specific total IgG titers post Dose 1 for the groups described above (Groups 1 to 7). For each group shown in FIG. 10, Tables 1.1 and 1.2 below contain geometric mean titer (GMT) values, 95% confidence intervals (95% CI) for titer values, and adjusted P-values comparing the GMT for Groups 3-6 vs. Group 7.

Table 1.1 -- gE-specific total IgG titers post dose 1

Group #	Group	GMT	95% CI	Adjusted P-value (compared to Group 7)

1	5 mcg gE alone	N/A: not detected	N/A	--
2	5 mcg gE + blank liposome	N/A: not detected	N/A	--
3	5 mcg gE + 7.5 mcg liposomal PHAD/5 mcg 1055	17,667	[12,027; 25,953]	0.0002
4	5 mcg gE + 7.5 mcg liposomal PHAD/15 mcg 1055	21,077	[14,534; 30566]	<0.0001
5	5 mcg gE + 7.5 mcg liposomal PHAD/30 mcg 1055	17,457	[12,117; 25,150]	0.0002
6	5 mcg gE + 7.5 mcg liposomal PHAD/50 mcg 1055	28,885	[21,778; 38,312]	<0.0001
7	5 mcg gE + 7.5 mcg liposomal MPL + 5 mcg QS21	6,519	[5,176; 8,210]	--

Table 1.2 – Group-wise comparisons between groups in Table 1.1

Comparison (Group vs Group)	**GMT**	**Adjusted P-value**
3 (5 mcg) vs 4 (15 mcg)	17,667 vs 21,077	0.9135
3 (5 mcg) vs 5 (30 mcg)	17,667 vs 17,457	>0.999
3 (5 mcg) vs 6 (50 mcg)	17,667 vs 28,885	0.1436
4 (15 mcg) vs 5 (30 mcg)	21,077 vs 17,457	0.8927
4 (15 mcg) vs 6 (50 mcg)	21,077 vs 28,885	0.5574
5 (30 mcg) vs 6 (50 mcg)	17,457 vs 28,885	0.1276

Log10 transformed GMT were compared using One-way ANOVA. Groupwise comparisons were made and p-values adjusted for multiple comparisons with Tukey's post-hoc test. Groups 1 and 2 were excluded from the analysis. Family-wise $\alpha = 0.05$.

The data demonstrate post dose 1, Liposomal PHAD + TQL-1055 free acid significantly enhanced gE-specific IgG GMT compared to Group 7 at all TQL-1055 free acid doses. There was a trend for increasing GMT with increasing TQL-1055 free acid dose, however, not statistically significant between any TQL-1055 free acid dose groups.

gE-specific total IgG titers post dose 2

FIG. 11 is a graph depicting gE-specific total IgG titers post Dose 2 for the groups described above (Groups 1 to 7). For each group shown in FIG. 11, Tables 1.3 and 1.4 below contain geometric mean titer (GMT) values, 95% confidence intervals (95% CI) for titer values, and adjusted P-values comparing the GMT for Groups 3-6 vs. Group 7 .

Table 1.3 -- gE-specific total IgG titers post dose 2

Group #	**Group**	**GMT**	**95% CI**	**Adjusted P-value (compared to Group 7)**
1	5 µg gE alone	949.8	393.3; 2293	--
2	5 µg gE + blank liposome	510.9	228.9; 1140	--
3	5 µg gE + 7.5 mcg liposomal PHAD/5 µg 1055	173,150	117,302; 255,587	<0.0001
4	5 µg gE +	200,974	156,245; 258,509	<0.0001

	7.5 mcg liposomal PHAD/15 µg 1055			
5	5 µg gE + 7.5 mcg liposomal PHAD/30 µg 1055	336,323	283,817; 398,543	0.2442
6	5 µg gE + 7.5 mcg liposomal PHAD/50 µg 1055	524,413	464,592; 591,936	0.9762
7	5 µg gE + 7.5 µg liposomal MPL + 5 µg QS21	475,612	349,596; 647,051	--

Table 1.4 – Group-wise comparisons between groups in Table 1.3

Comparison (Group vs Group)	**GMT**	**Adjusted P-value**
3 (5 µg) vs 4 (15 µg)	173,150 vs 200,974	0.8966
3 (5 µg) vs 5 (30 µg)	173,150 vs 336,323	0.0021
3 (5 µg) vs 6 (50 µg)	173,150 vs 524,413	<0.0001
4 (15 µg) vs 5 (30 µg)	200,974 vs 336,323	0.0263
4 (15 µg) vs 6 (50 µg)	200,974 vs 524,413	<0.0001
5 (30 µg) vs 6 (50 µg)	336,323 vs 524,413	0.0741

Log10 transformed GMT were compared using One-way ANOVA. Groupwise comparisons were made and p-values adjusted for multiple comparisons with Tukey's post-hoc test. Groups 1 and 2 were excluded from the analysis. Family-wise $\alpha = 0.05$.

The data demonstrate after dose 2, GMT for all PHAD liposome + 1055 groups increased by up to 18-fold compared to post dose 1. Post dose 2, Group 7 resulted in significantly higher GMT compared to PHAD liposomes + TQL-1055 free acid at 5 and

15 mcg doses. GMTs for Group 7 trended higher than PHAD liposomes + TQL-1055 free acid at 30 mcg dose, although not statistically significant. GMT for the 50 mcg TQL-1055 free acid dose trended higher than Group 7, although not statistically significant. There was a trend for increasing GMT with increasing TQL-1055 free acid dose. Increasing the TQL-1055 free acid dose from 5 to 30 mcg and from 15 to 30 mcg significantly increased GMTs.

gE-specific total IgG1 titers post dose 1

FIG. 12 is a graph depicting gE-specific total IgG1 titers post Dose 1 for the groups described above (Groups 1 to 7). For each group shown in FIG. 12, Tables 1.5 and 1.6 below contain geometric mean titer (GMT) values, 95% confidence intervals (95% CI) for titer values, and adjusted P-values comparing the GMT for Groups 3-6 vs. Group 7 .

Table 1.5 -- gE-specific total IgG1 titers post dose 1

Group #	Group	GMT	95% CI	Adjusted P-value (compared to Group 7)
1	5 mcg gE alone	Not Detected	N/A	--
2	5 mcg gE + blank liposome	Not Detected	N/A	--
3	5 mcg gE + 7.5 mcg liposomal PHAD/5 mcg 1055	8,591	4,943; 14,932	0.9854
4	5 mcg gE + 7.5 mcg liposomal PHAD/15 mcg 1055	9,801	6,379; 15,061	0.8641
5	5 mcg gE + 7.5 mcg liposomal PHAD/30 mcg 1055	15,240	11,211; 20,716	0.1038

6	5 mcg gE + 7.5 mcg liposomal PHAD/50 mcg 1055	26,180	14,493; 47,291	0.0008
7	5 mcg gE + 7.5 mcg liposomal MPL + 5 mcg QS21	7,416	4,935; 11,144	--

Table 1.6 – Group-wise comparisons between groups in Table 1.5

Comparison (Group vs Group)	**GMT**	**Adjusted P-value**
3 (5 mcg) vs 4 (15 mcg)	8,591 vs 9,801	0.9903
3 (5 mcg) vs 5 (30 mcg)	8,591 vs 15,240	0.2793
3 (5 mcg) vs 6 (50 mcg)	8,591 vs 26,180	0.0039
4 (15 mcg) vs 5 (30 mcg)	9,801 vs 15,240	0.5389
4 (15 mcg) vs 6 (50 mcg)	9,801 vs 26,180	0.0137
5 (30 mcg) vs 6 (50 mcg)	15,240 vs 26,180	0.3618

Log10 transformed GMT were compared using One-way ANOVA. Groupwise comparisons were made and p-values adjusted for multiple comparisons with Tukey's post-hoc test. Groups 1 and 2 were excluded from the analysis. Family-wise $\alpha = 0.05$.

The data demonstrate that after dose 1, gE-specific IgG1 GMT trended higher in all groups having PHAD Liposomes + TQL-1055 free acid compared to Group 7. GMT for the 50 mcg TQL-1055 free acid dose was significantly higher compared to Group 7. There was a trend for increasing GMT with increasing TQL-1055 free acid dose. Increasing TQL-1055 free acid dose from 5 or 15 mcg to 50 mcg resulted in significantly higher GMTs.

gE-specific total IgG1 titers post dose 2

FIG. 13 is a graph depicting gE-specific total IgG1 titers post Dose 2 for the groups described above (Groups 1 to 7). For each group shown in FIG. 13, Tables 1.7 and 1.8 below contain geometric mean titer (GMT) values, 95% confidence intervals

(95% CI) for titer values, and adjusted P-values comparing the GMT for Groups 3-6 vs. Group 7 .

Table 1.7 -- gE-specific total IgG1 titers post dose 2

Group #	Group	GMT	95% CI	Adjusted P-value (compared to Group 7)
1	5 mcg gE alone	793.1	321; 1,960	--
2	5 mcg gE + blank liposome	539.8	220; 1,319	--
3	5 mcg gE + 7.5 mcg liposomal PHAD/5 mcg 1055	118,756	85,120; 165,684	0.0063
4	5 mcg gE + 7.5 mcg liposomal PHAD/15 mcg 1055	97,959	81,845; 117,245	0.0002
5	5 mcg gE + 7.5 mcg liposomal PHAD/30 mcg 1055	206,466	166,154; 256,558	0.9997
6	5 mcg gE + 7.5 mcg liposomal PHAD/50 mcg 1055	200,551	158,328; 254,034	0.9959
7	5 mcg gE + 7.5 mcg liposomal MPL + 5 mcg QS21	212,822	158,986; 284,890	--

5

Table 1.8 – Group-wise comparisons between groups in Table 1.7

Comparison (Group vs Group)	GMT	Adjusted P-value
3 (5 mcg) vs 4 (15 mcg)	118,756 vs 97,959	0.7534
3 (5 mcg) vs 5 (30 mcg)	118,756 vs 206,466	0.0107

3 (5 mcg) vs 6 (50 mcg)	118,756 vs 200,551	0.0174
4 (15 mcg) vs 5 (30 mcg)	97,959 vs 206,466	0.0003
4 (15 mcg) vs 6 (50 mcg)	97,959 vs 200,551	0.0005
5 (30 mcg) vs 6 (50 mcg)	206,466 vs 200,551	0.9998

Log10 transformed GMT were compared using One-way ANOVA. Groupwise comparisons were made and p-values adjusted for multiple comparisons with Tukey's post-hoc test. Groups 1 and 2 were excluded from the analysis. Family-wise $\alpha = 0.05$.

The data demonstrate after a second dose, IgG1 GMT for all PHAD liposome + TQL-1055 free acid groups increased compared to post dose 1. Post dose 2, Group 7 resulted in significantly higher GMT compared to PHAD liposomes + TQL-1055 free acid at 5 and 15 mcg doses. GMT titers for Group 7 trended higher than PHAD liposomes + 30 mcg and 50 mcg TQL-1055 free acid, although not statistically significant. GMTs for the 30 and 50 mcg TQL-1055 free acid dose were significantly higher than both the 5 and 15 mcg 1055 dose.

gE-specific total IgG2c titers post dose 1

FIG. 14 is a graph depicting gE-specific total IgG2c titers post Dose 1 for the groups described above (Groups 1 to 7). For each group shown in FIG. 14, Tables 1.9 and 1.10 below contain geometric mean titer (GMT) values, 95% confidence intervals (95% CI) for titer values, and adjusted P-values comparing the GMT for Groups 3-6 vs. Group 7 .

Table 1.9 -- gE-specific total IgG2c titers post dose 1

Group #	**Group**	**GMT**	**95% CI**	**Adjusted P-value (compared to Group 7)**
1	5 mcg gE alone	Not Detected	N/A	--
2	5 mcg gE + blank liposome	Not Detected	N/A	--

3	5 mcg gE + 7.5 mcg liposomal PHAD/5 mcg 1055	4,313	2,943; 6,321	0.9892
4	5 mcg gE + 7.5 mcg liposomal PHAD/15 mcg 1055	7,010	4,197; 11,706	0.1058
5	5 mcg gE + 7.5 mcg liposomal PHAD/30 mcg 1055	6,762	4,694; 9,738	0.1448
6	5 mcg gE + 7.5 mcg liposomal PHAD/50 mcg 1055	10,072	7,714; 13,150	0.0019
7	5 mcg gE + 7.5 mcg liposomal MPL + 5 mcg QS21	3,851	2,750; 5,393	--

Table 1.10 – Group-wise comparisons between groups in Table 1.9

Comparison (Group vs Group)	**GMT**	**Adjusted P-value**
3 (5 mcg) vs 4 (15 mcg)	4,313 vs 7,010	0.2652
3 (5 mcg) vs 5 (30 mcg)	4,313 vs 6,762	0.3390
3 (5 mcg) vs 6 (50 mcg)	4,313 vs 10,072	0.0076
4 (15 mcg) vs 5 (30 mcg)	7,010 vs 6,762	0.9999
4 (15 mcg) vs 6 (50 mcg)	7,010 vs 10,072	0.5548
5 (30 mcg) vs 6 (50 mcg)	6,762 vs 10,072	0.4614

Log10 transformed GMT were compared using One-way ANOVA. Groupwise comparisons were made and p-values adjusted for multiple comparisons with Tukey's post-hoc test. Groups 1 and 2 were excluded from the analysis. Family-wise $\alpha = 0.05$.

The data demonstrate post dose 1, gE-specific IgG2c GMT for PHAD Liposomes + TQL-1055 free acid groups trended higher compared to Group 7. GMT for the 50 mcg TQL-1055 free acid dose were significantly higher compared to Group 7. There was a trend for increasing GMT with increasing TQL-1055 free acid dose.

Increasing TQL-1055 free acid dose from 5 to 50 mcg resulted in significantly higher GMTs.

gE-specific total IgG2c titers post dose 2

FIG. 15 is a graph depicting gE-specific total IgG2c titers post Dose 2 for the groups described above (Groups 1 to 7). For each group shown in FIG. 15, Tables 1.11 and 1.12 below contain geometric mean titer (GMT) values, 95% confidence intervals (95% CI) for titer values, and adjusted P-values comparing the GMT for Groups 3-6 vs. Group 7 .

Table 1.11 -- gE-specific total IgG2c titers post dose 2

Group #	Group	GMT	95% CI	Adjusted P-value (compared to Group 7)
1	5 mcg gE alone	Not Detected	N/A	--
2	5 mcg gE + blank liposome	Not Detected	N/A	--
3	5 mcg gE + 7.5 mcg liposomal PHAD/5 mcg 1055	40,409	26,051; 62,679	0.3380
4	5 mcg gE + 7.5 mcg liposomal PHAD/15 mcg 1055	44,104	25,874; 75,181	0.5384
5	5 mcg gE + 7.5 mcg liposomal PHAD/30 mcg 1055	42,295	31,856; 56,154	0.4380
6	5 mcg gE + 7.5 mcg liposomal PHAD/50 mcg 1055	52,170	38,323; 71,020	0.8990
7	5 mcg gE + 7.5 mcg liposomal MPL + 5 mcg QS21	65,517	42,621; 100,715	--

Table 1.12 – Group-wise comparisons between groups in Table 1.11

Comparison (Group vs Group)	GMT	Adjusted P-value
3 (5 mcg) vs 4 (15 mcg)	40,409 vs 44,104	0.9969
3 (5 mcg) vs 5 (30 mcg)	40,409 vs 42,295	0.9998
3 (5 mcg) vs 6 (50 mcg)	40,409 vs 52,170	0.8547
4 (15 mcg) vs 5 (30 mcg)	44,104 vs 42,295	0.9998
4 (15 mcg) vs 6 (50 mcg)	44,104 vs 52,170	0.9645
5 (30 mcg) vs 6 (50 mcg)	42,295 vs 52,170	0.9230

Log10 transformed GMT were compared using One-way ANOVA. Groupwise comparisons were made and p-values adjusted for multiple comparisons with Tukey's post-hoc test. Groups 1 and 2 were excluded from the analysis. Family-wise $\alpha = 0.05$.

The data demonstrate after a second dose, IgG2c GMT for all PHAD liposome + TQL-1055 free acid groups increased compared to post dose 1. Post dose 2, Group 7 IgG2c GMT trended higher compared to PHAD liposomes + TQL-1055 free acid at all doses of TQL-1055 free acid, although not statistically significant. IgG2c GMT for the 50 mcg TQL-1055 free acid dose trended slightly higher compared to lower TQL-1055 free acid doses.

Example 2 – Liposome PHAD + Compound I-4 Choline Salt Form

The impact of TQL-1055 choline salt (Compound I-4 choline salt) on antibody titers induced by gE antigen was tested. Mice were immunized with gE (5 mcg) alone, gE (5 mcg) with liposomes containing PHAD (5 mcg), gE (5 mcg) with liposomes containing PHAD (5 mcg) and TQL-1055 choline salt (five groups: 5 mcg, 10 mcg, 20 mcg, 40 mcg, 80 mcg), and gE (5 mcg) with TQL-1055 choline salt (five groups: 5 mcg, 10 mcg, 20 mcg, 40 mcg, 80 mcg). Mice were immunized at Day 0 and Day 14. Groups were bled at Day 13 (post Dose 1) and Day 28 (post Dose 2) for serum analysis. The results are shown below.

Anti-gE IgG endpoint titers post dose 1

FIG. 16 is a graph depicting gE-specific total IgG titers post Dose 1 for the groups described above. FIGs. 17A-E depict subsets of the data shown in FIG. 16, in which the synergistic effects of PHAD and TQL-1055 choline salt become apparent. For groups shown in FIG. 16, Table 2.1 below contains geometric mean titer (GMT) values and adjusted P-values comparing various groups.

Table 2.1 -- gE-specific total IgG titers post dose 1

	Day 13 Anti-gE GMT titer		**Day 13 Comparison**		**Day 28 Anti-gE GMT titer**		**Day 28 Comparison**	
	without PHAD liposomes	**5 mcg PHAD in liposomes**	**Stat Combo vs PHAD alone**	**Stats Combo vs CS alone**	**without PHAD liposomes**	**5 mcg PHAD in liposomes**	**Stat Combo vs PHAD alone**	**Stats Combo vs CS alone**
5 mcg gE	135	**19,037**	Not statistically significant	Not statistically significant	12,311	**689,865**	Not statistically significant	(40: p=0.025) (80: p<0.0001)
5 mcg gE + 5 mcg 1055 CS	2,456	**253,878**	P=0.0006	P=0.0002	61,703	**2,128,350**	P=0.0005	P<0.0001
5 mcg gE + 10 mcg 1055 CS	9,124	**73,163**	P=0.9423	P=0.8836	425,670	**1,313,264**	P=0.319	P<0.0001
5 mcg gE + 20 mcg 1055 CS	10,000	**193,649**	P=0.0099	P=0.0054	500,000	**1,811,949**	P=0.0066	P=0.04
5 mcg gE + 40 mcg 1055 CS	30,852	**750,022**	P<0.0001	P<0.0001	810,328	**2,128,350**	P=0.0005	P<0.0001
5 mcg gE + 80 mcg 1055 CS	65,561	**572,074**	P<0.0001	P<0.0001	2,128,350	**>2,500,000**	P<0.0001	P=0.0066
+6h 5 mcg gE + 5 mcg 1055 CS	2,091	**85,939**	**vs T=0** Not statistically significant		117,462	**1,313,264**	**vs T=0** Not statistically significant	

+6h 5 mcg gE + 80 mcg 1055 CS	332,839	**572,074**	**vs T=0** **with PHAD:** Not statistically significant Without PHAD: P=0.0016	1,811,949	**2,128,350**	**vs T=0** Not statistically significant

The data demonstrate after the first dose, gE-specific IgG for TQL-1055 choline salt + PHAD in liposome exhibits as synergistic effect as compared to either compound alone. When considered together with data from Example 1, there is a trend to higher titers in groups including TQL-1055 choline salt as compared to groups including TQL-1055 free acid.

Anti-gE IgG endpoint titers post dose 2

FIG. 18 is a graph depicting gE-specific total IgG titers post Dose 2 for the groups described above. FIGs. 19A-E depict subsets of the data shown in FIG. 18, in which the synergistic effects of PHAD and TQL-1055 choline salt once again become apparent. For groups shown in FIG. 16, Table 2.1 above contains geometric mean titer (GMT) values and adjusted P-values comparing various groups.

The data demonstrate after the second dose, gE-specific IgG for TQL-1055 choline salt + PHAD in liposome exhibits as synergistic effect as compared to either compound alone. When considered together with data from Example 1, there is a trend to higher titers in groups including TQL-1055 choline salt as compared to groups including TQL-1055 free acid.

Example 3 – Oil in water emulsions + Compound I-4 Choline Salt Form

The impact of an oil-in-water emulsion containing TQL-1055 free acid (Compound I-4 free acid), TQL-1055 choline salt (Compound I-4 choline salt), PHAD liposomes, and combinations thereof on antibody titers induced by gE antigen was tested. Mice were immunized with PBS alone, gE (5 mcg) alone, gE (5 mcg) in an oil-in-water emulsion (L2), gE (5 mcg) and TQL-1055 choline salt (three groups: 5 mcg, 30 mcg, 100 mcg), gE (5 mcg) in an oil-in-water emulsion (L2) with TQL-1055 free acid (three groups: 5 mcg, 30 mcg, 100 mcg), gE (5 mcg) in an oil-in-water emulsion (L2) with TQL-1055 free acid (three groups: 5 mcg, 30 mcg, 100 mcg) and PHAD liposomes (20 mcg), and gE (5 mcg) with PHAD liposomes (20 mcg). Mice were immunized at

Day 0 and Day 14. Groups were bled at Day 13 (post Dose 1) and Day 28 (post Dose 2) for serum analysis. The results are shown below.

Anti-gE IgG endpoint titers post dose 1

FIG. 20 is a graph depicting gE-specific total IgG titers post Dose 1 for the groups described above. For groups shown in FIG. 20, Table 3.1 below contains geometric mean titer (GMT) values and confidence intervals. Table 3.2 below shows adjusted P-values comparing various groups.

Table 3.1 -- gE-specific total IgG titers post dose 1

	PBS Alone	**5 µg gE Alone**	**5 µg gE + L2**	**5 µg gE + 5 µg 1055 C.S**	**5 µg gE + 30 µg 1055 C.S**	**5 µg gE + 100 µg 1055 C.S**	**5 µg gE + L2 + 5 µg 1055 F.A**
Number of values	10	10	10	10	10	10	10
Geometric mean	100.0	138.0	190.4	388.4	6295	19905	5000
Geometric SD factor	1.000	1.971	2.296	3.240	2.071	3.284	1.000
Lower 95% CI of geo. mean	100.0	84.91	105.0	167.5	3739	8503	5000
Upper 95% CI of geo. mean	100.0	224.2	345.0	900.4	10597	46599	5000

Table 3.1 -- gE-specific total IgG titers post dose 1 (continued)

	5 µg gE + L2 + 30 µg 1055 F.A	**5 µg gE + L2 + 100 µg 1055 F.A**	**5 µg gE + L2 + 5 µg 1055 F.A + 20 µg PHAD**	**5 µg gE + L2 + 30 µg 1055 F.A + 20 µg PHAD**	**5 µg gE + L2 + 100 µg 1055 F.A + 20 µg PHAD**	**5 µg gE + 20 µg PHAD**
Number of values	10	10	10	10	10	10
Geometric mean	5000	25059	50000	50000	1233844	50000

Geometric SD factor	1.000	3.041	1.000	1.000	5.418	1.000
Lower 95% CI of geo. mean	5000	11309	50000	50000	368409	50000
Upper 95% CI of geo. mean	5000	55529	50000	50000	4132283	50000

Table 3.2 -- Group-wise comparisons between groups in Table 3.1

Tukey's multiple comparisons test	**Significant?**	**Summary**	**Adjusted P Value**
PBS Alone vs. 5 µg gE Alone	No	ns	>0.9999
PBS Alone vs. 5 µg gE + L2	No	ns	>0.9999
PBS Alone vs. 5 µg gE + 5 µg 1055 CS	No	ns	>0.9999
PBS Alone vs. 5 µg gE + 30 µg 1055 CS	No	ns	>0.9999
PBS Alone vs. 5 µg gE + 100 µg 1055 CS	No	ns	>0.9999
PBS Alone vs. 5 µg gE + L2 + 5 µg 1055 FA	No	ns	>0.9999
PBS Alone vs. 5 µg gE + L2 + 30 µg 1055 FA	No	ns	>0.9999
PBS Alone vs. 5 µg gE + L2 + 100 µg 1055 FA	No	ns	>0.9999
PBS Alone vs. 5 µg gE + L2 + 5 µg 1055 FA + 20 µg PHAD liposomes	No	ns	>0.9999
PBS Alone vs. 5 µg gE + L2 + 30 µg 1055 FA + 20 µg PHAD liposomes	No	ns	>0.9999
PBS Alone vs. 5 µg gE + L2 + 100 µg 1055 FA + 20 µg PHAD liposomes	Yes	****	<0.0001
PBS Alone vs. 5 µg gE + 20 µg PHAD liposomes	No	ns	>0.9999

Table 3.2 -- Group-wise comparisons between groups in Table 3.1 (continued)

Tukey's multiple comparisons test	**Significant?**	**Summary**	**Adjusted P Value**
5 µg gE Alone vs. 5 µg gE + L2	No	ns	>0.9999

5 µg gE Alone vs. 5 µg gE + 5 µg 1055 CS	No	ns	>0.9999
5 µg gE Alone vs. 5 µg gE + 30 µg 1055 CS	No	ns	>0.9999
5 µg gE Alone vs. 5 µg gE + 100 µg 1055 CS	No	ns	>0.9999
5 µg gE Alone vs. 5 µg gE + L2 + 5 µg 1055 FA	No	ns	>0.9999
5 µg gE Alone vs. 5 µg gE + L2 + 30 µg 1055 FA	No	ns	>0.9999
5 µg gE Alone vs. 5 µg gE + L2 + 100 µg 1055 FA	No	ns	>0.9999
5 µg gE Alone vs. 5 µg gE + L2 + 5 µg 1055 FA + 20 µg PHAD liposomes	No	ns	>0.9999
5 µg gE Alone vs. 5 µg gE + L2 + 30 µg 1055 FA + 20 µg PHAD liposomes	No	ns	>0.9999
5 µg gE Alone vs. 5 µg gE + L2 + 100 µg 1055 FA + 20 µg PHAD liposomes	Yes	****	<0.0001
5 µg gE Alone vs. 5 µg gE + 20 µg PHAD liposomes	No	ns	>0.9999

Table 3.2 -- Group-wise comparisons between groups in Table 3.1 (continued)

Tukey's multiple comparisons test	**Significant?**	**Summary**	**Adjusted P Value**
5 µg gE + L2 vs. 5 µg gE + 5 µg 1055 CS	No	ns	>0.9999
5 µg gE + L2 vs. 5 µg gE + 30 µg 1055 CS	No	ns	>0.9999
5 µg gE + L2 vs. 5 µg gE + 100 µg 1055 CS	No	ns	>0.9999
5 µg gE + L2 vs. 5 µg gE + L2 + 5 µg 1055 FA	No	ns	>0.9999
5 µg gE + L2 vs. 5 µg gE + L2 + 30 µg 1055 FA	No	ns	>0.9999
5 µg gE + L2 vs. 5 µg gE + L2 + 100 µg 1055 FA	No	ns	>0.9999
5 µg gE + L2 vs. 5 µg gE + L2 + 5 µg 1055 FA + 20 µg PHAD liposomes	No	ns	>0.9999
5 µg gE + L2 vs. 5 µg gE + L2 + 30 µg 1055 FA + 20 µg PHAD liposomes	No	ns	>0.9999
5 µg gE + L2 vs. 5 µg gE + L2 + 100 µg 1055 FA + 20 µg PHAD liposomes	Yes	****	<0.0001

5 µg gE + L2 vs. 5 µg gE + 20 µg PHAD liposomes	No	ns	>0.9999

Table 3.2 -- Group-wise comparisons between groups in Table 3.1 (continued)

Tukey's multiple comparisons test	Significant?	Summary	Adjusted P Value
5 µg gE + 5 µg 1055 CS vs. 5 µg gE + 30 µg 1055 CS	No	ns	>0.9999
5 µg gE + 5 µg 1055 CS vs. 5 µg gE + 100 µg 1055 CS	No	ns	>0.9999
5 µg gE + 5 µg 1055 CS vs. 5 µg gE + L2 + 5 µg 1055 FA	No	ns	>0.9999
5 µg gE + 5 µg 1055 CS vs. 5 µg gE + L2 + 30 µg 1055 FA	No	ns	>0.9999
5 µg gE + 5 µg 1055 CS vs. 5 µg gE + L2 + 100 µg 1055 FA	No	ns	>0.9999
5 µg gE + 5 µg 1055 CS vs. 5 µg gE + L2 + 5 µg 1055 FA + 20 µg PHAD liposomes	No	ns	>0.9999
5 µg gE + 5 µg 1055 CS vs. 5 µg gE + L2 + 30 µg 1055 FA + 20 µg PHAD liposomes	No	ns	>0.9999
5 µg gE + 5 µg 1055 CS vs. 5 µg gE + L2 + 100 µg 1055 FA + 20 µg PHAD liposomes	Yes	****	<0.0001
5 µg gE + 5 µg 1055 CS vs. 5 µg gE + 20 µg PHAD liposomes	No	ns	>0.9999

Table 3.2 -- Group-wise comparisons between groups in Table 3.1 (continued)

Tukey's multiple comparisons test	Significant?	Summary	Adjusted P Value
5 µg gE + 30 µg 1055 CS vs. 5 µg gE + 100 µg 1055 CS	No	ns	>0.9999
5 µg gE + 30 µg 1055 CS vs. 5 µg gE + L2 + 5 µg 1055 FA	No	ns	>0.9999
5 µg gE + 30 µg 1055 CS vs. 5 µg gE + L2 + 30 µg 1055 FA	No	ns	>0.9999
5 µg gE + 30 µg 1055 CS vs. 5 µg gE + L2 + 100 µg 1055 FA	No	ns	>0.9999
5 µg gE + 30 µg 1055 CS vs. 5 µg gE + L2 + 5 µg 1055 FA + 20 µg PHAD liposomes	No	ns	>0.9999

5 µg gE + 30 µg 1055 CS vs. 5 µg gE + L2 + 30 µg 1055 FA + 20 µg PHAD liposomes	No	ns	>0.9999
5 µg gE + 30 µg 1055 CS vs. 5 µg gE + L2 + 100 µg 1055 FA + 20 µg PHAD liposomes	Yes	****	<0.0001
5 µg gE + 30 µg 1055 CS vs. 5 µg gE + 20 µg PHAD liposomes	No	ns	>0.9999

Table 3.2 -- Group-wise comparisons between groups in Table 3.1 (continued)

Tukey's multiple comparisons test	**Significant?**	**Summary**	**Adjusted P Value**
5 µg gE + 100 µg 1055 CS vs. 5 µg gE + L2 + 5 µg 1055 FA	No	ns	>0.9999
5 µg gE + 100 µg 1055 CS vs. 5 µg gE + L2 + 30 µg 1055 FA	No	ns	>0.9999
5 µg gE + 100 µg 1055 CS vs. 5 µg gE + L2 + 100 µg 1055 FA	No	ns	>0.9999
5 µg gE + 100 µg 1055 CS vs. 5 µg gE + L2 + 5 µg 1055 FA + 20 µg PHAD liposomes	No	ns	>0.9999
5 µg gE + 100 µg 1055 CS vs. 5 µg gE + L2 + 30 µg 1055 FA + 20 µg PHAD liposomes	No	ns	>0.9999
5 µg gE + 100 µg 1055 CS vs. 5 µg gE + L2 + 100 µg 1055 FA + 20 µg PHAD liposomes	Yes	****	<0.0001
5 µg gE + 100 µg 1055 CS vs. 5 µg gE + 20 µg PHAD liposomes	No	ns	>0.9999

Table 3.2 -- Group-wise comparisons between groups in Table 3.1 (continued)

Tukey's multiple comparisons test	**Significant?**	**Summary**	**Adjusted P Value**
5 µg gE + L2 + 5 µg 1055 FA vs. 5 µg gE + L2 + 30 µg 1055 FA	No	ns	>0.9999
5 µg gE + L2 + 5 µg 1055 FA vs. 5 µg gE + L2 + 100 µg 1055 FA	No	ns	>0.9999
5 µg gE + L2 + 5 µg 1055 FA vs. 5 µg gE + L2 + 5 µg 1055 FA + 20 µg PHAD liposomes	No	ns	>0.9999

5 µg gE + L2 + 5 µg 1055 FA vs. 5 µg gE + L2 + 30 µg 1055 FA + 20 µg PHAD liposomes	No	ns	>0.9999
5 µg gE + L2 + 5 µg 1055 FA vs. 5 µg gE + L2 + 100 µg 1055 FA + 20 µg PHAD liposomes	Yes	****	<0.0001
5 µg gE + L2 + 5 µg 1055 FA vs. 5 µg gE + 20 µg PHAD liposomes	No	ns	>0.9999
5 µg gE + L2 + 30 µg 1055 FA vs. 5 µg gE + L2 + 100 µg 1055 FA	No	ns	>0.9999
5 µg gE + L2 + 30 µg 1055 FA vs. 5 µg gE + L2 + 5 µg 1055 FA + 20 µg PHAD liposomes	No	ns	>0.9999
5 µg gE + L2 + 30 µg 1055 FA vs. 5 µg gE + L2 + 30 µg 1055 FA + 20 µg PHAD liposomes	No	ns	>0.9999
5 µg gE + L2 + 30 µg 1055 FA vs. 5 µg gE + L2 + 100 µg 1055 FA + 20 µg PHAD liposomes	Yes	****	<0.0001
5 µg gE + L2 + 30 µg 1055 FA vs. 5 µg gE + 20 µg PHAD liposomes	No	ns	>0.9999
5 µg gE + L2 + 100 µg 1055 FA vs. 5 µg gE + L2 + 5 µg 1055 FA + 20 µg PHAD liposomes	No	ns	>0.9999
5 µg gE + L2 + 100 µg 1055 FA vs. 5 µg gE + L2 + 30 µg 1055 FA + 20 µg PHAD liposomes	No	ns	>0.9999
5 µg gE + L2 + 100 µg 1055 FA vs. 5 µg gE + L2 + 100 µg 1055 FA + 20 µg PHAD liposomes	Yes	****	<0.0001
5 µg gE + L2 + 100 µg 1055 FA vs. 5 µg gE + 20 µg PHAD liposomes	No	ns	>0.9999

Table 3.2 -- Group-wise comparisons between groups in Table 3.1 (continued)

Tukey's multiple comparisons test	**Significant?**	**Summary**	**Adjusted P Value**
5 µg gE + L2 + 5 µg 1055 FA + 20 µg PHAD liposomes vs. 5 µg gE + L2 + 30 µg 1055 FA + 20 µg PHAD liposomes	No	ns	>0.9999
5 µg gE + L2 + 5 µg 1055 FA + 20 µg PHAD liposomes vs. 5 µg gE + L2 + 100 µg 1055 FA + 20 µg PHAD liposomes	Yes	****	<0.0001

5 µg gE + L2 + 5 µg 1055 FA + 20 µg PHAD liposomes vs. 5 µg gE + 20 µg PHAD liposomes	No	ns	>0.9999
5 µg gE + L2 + 30 µg 1055 FA + 20 µg PHAD liposomes vs. 5 µg gE + L2 + 100 µg 1055 FA + 20 µg PHAD liposomes	Yes	****	<0.0001
5 µg gE + L2 + 30 µg 1055 FA + 20 µg PHAD liposomes vs. 5 µg gE + 20 µg PHAD liposomes	No	ns	>0.9999
5 µg gE + L2 + 100 µg 1055 FA + 20 µg PHAD liposomes vs. 5 µg gE + 20 µg PHAD liposomes	Yes	****	<0.0001

Anti-gE IgG endpoint titers post dose 2

FIG. 21 is a graph depicting gE-specific total IgG titers post Dose 2 for the groups described above. For groups shown in FIG. 21, Table 3.3 below contains geometric mean titer (GMT) values and confidence intervals. Table 3.4 below shows adjusted P-values comparing various groups.

Table 3.3 -- gE-specific total IgG titers post dose 2

	PBS Alone	**5 µg gE Alone**	**5 µg gE + L2**	**5 µg gE + 5 µg 1055 C.S**	**5 µg gE + 30 µg 1055 C.S**	**5 µg gE + 100 µg 1055 C.S**	**5 µg gE + L2 + 5 µg 1055 F.A**
Number of values	10	10	10	10	10	10	10
Geometric mean	125.9	5012	19953	39811	1000001	794329	125893
Geometric SD factor	2.071	8.886	3.041	3.284	1	2.071	2.071
Lower 95% CI of geo. mean	74.78	1050	9004	17006	1000001	471829	74780
Upper 95% CI of geo. mean	211.9	23914	44213	93198	1000001	1337261	211941

Table 3.3 -- gE-specific total IgG titers post dose 2 (continued)

	5 µg gE + L2 + 30 µg 1055 F.A	**5 µg gE + L2 + 100 µg 1055 F.A**	**5 µg gE + L2 + 5 µg 1055 F.A + 20 µg PHAD**	**5 µg gE + L2 + 30 µg 1055 F.A + 20 µg PHAD**	**5 µg gE + L2 + 100 µg 1055 F.A + 20 µg PHAD**	**5 µg gE + 20 µg PHAD**
Number of values	10	10	10	10	10	10
Geometric mean	125893	501188	1000001	1000001	1000001	1000001
Geometric SD factor	2.071	3.041	1	1	1	1
Lower 95% CI of geo. mean	74780	226178	1000001	1000001	1000001	1000001
Upper 95% CI of geo. mean	211941	1110582	1000001	1000001	1000001	1000001

Table 3.4 -- Group-wise comparisons between groups in Table 3.3

Tukey's multiple comparisons test	**Significant?**	**Summary**	**Adjusted P Value**
PBS Alone vs. 5 µg gE Alone	No	ns	>0.9999
PBS Alone vs. 5 µg gE + L2	No	ns	>0.9999
PBS Alone vs. 5 µg gE + 5 µg 1055 CS	No	ns	>0.9999
PBS Alone vs. 5 µg gE + 30 µg 1055 CS	Yes	****	<0.0001
PBS Alone vs. 5 µg gE + 100 µg 1055 CS	Yes	****	<0.0001
PBS Alone vs. 5 µg gE + L2 + 5 µg 1055 FA	No	ns	0.5482
PBS Alone vs. 5 µg gE + L2 + 30 µg 1055 FA	No	ns	0.5482
PBS Alone vs. 5 µg gE + L2 + 100 µg 1055 FA	Yes	****	<0.0001
PBS Alone vs. 5 µg gE + L2 + 5 µg 1055 FA + 20 µg PHAD liposomes	Yes	****	<0.0001
PBS Alone vs. 5 µg gE + L2 + 30 µg 1055 FA + 20 µg PHAD liposomes	Yes	****	<0.0001
PBS Alone vs. 5 µg gE + L2 + 100 µg 1055 FA + 20 µg PHAD liposomes	Yes	****	<0.0001

PBS Alone vs. 5 µg gE + 20 µg PHAD liposomes	Yes	****	<0.0001

Table 3.4 -- Group-wise comparisons between groups in Table 3.3 (continued)

Tukey's multiple comparisons test	**Significant?**	**Summary**	**Adjusted P Value**
5 µg gE Alone vs. 5 µg gE + L2	No	ns	>0.9999
5 µg gE Alone vs. 5 µg gE + 5 µg 1055 CS	No	ns	>0.9999
5 µg gE Alone vs. 5 µg gE + 30 µg 1055 CS	Yes	****	<0.0001
5 µg gE Alone vs. 5 µg gE + 100 µg 1055 CS	Yes	****	<0.0001
5 µg gE Alone vs. 5 µg gE + L2 + 5 µg 1055 FA	No	ns	0.7461
5 µg gE Alone vs. 5 µg gE + L2 + 30 µg 1055 FA	No	ns	0.7461
5 µg gE Alone vs. 5 µg gE + L2 + 100 µg 1055 FA	Yes	****	<0.0001
5 µg gE Alone vs. 5 µg gE + L2 + 5 µg 1055 FA + 20 µg PHAD liposomes	Yes	****	<0.0001
5 µg gE Alone vs. 5 µg gE + L2 + 30 µg 1055 FA + 20 µg PHAD liposomes	Yes	****	<0.0001
5 µg gE Alone vs. 5 µg gE + L2 + 100 µg 1055 FA + 20 µg PHAD liposomes	Yes	****	<0.0001
5 µg gE Alone vs. 5 µg gE + 20 µg PHAD liposomes	Yes	****	<0.0001

Table 3.4 -- Group-wise comparisons between groups in Table 3.3 (continued)

Tukey's multiple comparisons test	**Significant?**	**Summary**	**Adjusted P Value**
5 µg gE + L2 vs. 5 µg gE + 5 µg 1055 CS	No	ns	>0.9999
5 µg gE + L2 vs. 5 µg gE + 30 µg 1055 CS	Yes	****	<0.0001
5 µg gE + L2 vs. 5 µg gE + 100 µg 1055 CS	Yes	****	<0.0001
5 µg gE + L2 vs. 5 µg gE + L2 + 5 µg 1055 FA	No	ns	0.8333
5 µg gE + L2 vs. 5 µg gE + L2 + 30 µg 1055 FA	No	ns	0.8333

5 µg gE + L2 vs. 5 µg gE + L2 + 100 µg 1055 FA	Yes	****	<0.0001
5 µg gE + L2 vs. 5 µg gE + L2 + 5 µg 1055 FA + 20 µg PHAD liposomes	Yes	****	<0.0001
5 µg gE + L2 vs. 5 µg gE + L2 + 30 µg 1055 FA + 20 µg PHAD liposomes	Yes	****	<0.0001
5 µg gE + L2 vs. 5 µg gE + L2 + 100 µg 1055 FA + 20 µg PHAD liposomes	Yes	****	<0.0001
5 µg gE + L2 vs. 5 µg gE + 20 µg PHAD liposomes	Yes	****	<0.0001

Table 3.4 -- Group-wise comparisons between groups in Table 3.3 (continued)

Tukey's multiple comparisons test	**Significant?**	**Summary**	**Adjusted P Value**
5 µg gE + 5 µg 1055 CS vs. 5 µg gE + 30 µg 1055 CS	Yes	****	<0.0001
5 µg gE + 5 µg 1055 CS vs. 5 µg gE + 100 µg 1055 CS	Yes	****	<0.0001
5 µg gE + 5 µg 1055 CS vs. 5 µg gE + L2 + 5 µg 1055 FA	No	ns	0.9527
5 µg gE + 5 µg 1055 CS vs. 5 µg gE + L2 + 30 µg 1055 FA	No	ns	0.9527
5 µg gE + 5 µg 1055 CS vs. 5 µg gE + L2 + 100 µg 1055 FA	Yes	****	<0.0001
5 µg gE + 5 µg 1055 CS vs. 5 µg gE + L2 + 5 µg 1055 FA + 20 µg PHAD liposomes	Yes	****	<0.0001
5 µg gE + 5 µg 1055 CS vs. 5 µg gE + L2 + 30 µg 1055 FA + 20 µg PHAD liposomes	Yes	****	<0.0001
5 µg gE + 5 µg 1055 CS vs. 5 µg gE + L2 + 100 µg 1055 FA + 20 µg PHAD liposomes	Yes	****	<0.0001
5 µg gE + 5 µg 1055 CS vs. 5 µg gE + 20 µg PHAD liposomes	Yes	****	<0.0001

Table 3.4 -- Group-wise comparisons between groups in Table 3.3 (continued)

Tukey's multiple comparisons test	**Significant?**	**Summary**	**Adjusted P Value**
5 µg gE + 30 µg 1055 CS vs. 5 µg gE + 100 µg 1055 CS	No	ns	0.9972

5 µg gE + 30 µg 1055 CS vs. 5 µg gE + L2 + 5 µg 1055 FA	Yes	****	<0.0001
5 µg gE + 30 µg 1055 CS vs. 5 µg gE + L2 + 30 µg 1055 FA	Yes	****	<0.0001
5 µg gE + 30 µg 1055 CS vs. 5 µg gE + L2 + 100 µg 1055 FA	No	ns	0.0792
5 µg gE + 30 µg 1055 CS vs. 5 µg gE + L2 + 5 µg 1055 FA + 20 µg PHAD liposomes	No	ns	>0.9999
5 µg gE + 30 µg 1055 CS vs. 5 µg gE + L2 + 30 µg 1055 FA + 20 µg PHAD liposomes	No	ns	>0.9999
5 µg gE + 30 µg 1055 CS vs. 5 µg gE + L2 + 100 µg 1055 FA + 20 µg PHAD liposomes	No	ns	>0.9999
5 µg gE + 30 µg 1055 CS vs. 5 µg gE + 20 µg PHAD liposomes	No	ns	>0.9999

Table 3.4 -- Group-wise comparisons between groups in Table 3.3 (continued)

Tukey's multiple comparisons test	**Significant?**	**Summary**	**Adjusted P Value**
5 µg gE + 100 µg 1055 CS vs. 5 µg gE + L2 + 5 µg 1055 FA	Yes	****	<0.0001
5 µg gE + 100 µg 1055 CS vs. 5 µg gE + L2 + 30 µg 1055 FA	Yes	****	<0.0001
5 µg gE + 100 µg 1055 CS vs. 5 µg gE + L2 + 100 µg 1055 FA	No	ns	0.6314
5 µg gE + 100 µg 1055 CS vs. 5 µg gE + L2 + 5 µg 1055 FA + 20 µg PHAD liposomes	No	ns	0.9996
5 µg gE + 100 µg 1055 CS vs. 5 µg gE + L2 + 30 µg 1055 FA + 20 µg PHAD liposomes	No	ns	0.9972
5 µg gE + 100 µg 1055 CS vs. 5 µg gE + L2 + 100 µg 1055 FA + 20 µg PHAD liposomes	No	ns	0.9972
5 µg gE + 100 µg 1055 CS vs. 5 µg gE + 20 µg PHAD liposomes	No	ns	0.9972

Table 3.4 -- Group-wise comparisons between groups in Table 3.3 (continued)

Tukey's multiple comparisons test	**Significant?**	**Summary**	**Adjusted P Value**

5 µg gE + L2 + 5 µg 1055 FA vs. 5 µg gE + L2 + 30 µg 1055 FA	No	ns	>0.9999
5 µg gE + L2 + 5 µg 1055 FA vs. 5 µg gE + L2 + 100 µg 1055 FA	Yes	****	<0.0001
5 µg gE + L2 + 5 µg 1055 FA vs. 5 µg gE + L2 + 5 µg 1055 FA + 20 µg PHAD liposomes	Yes	****	<0.0001
5 µg gE + L2 + 5 µg 1055 FA vs. 5 µg gE + L2 + 30 µg 1055 FA + 20 µg PHAD liposomes	Yes	****	<0.0001
5 µg gE + L2 + 5 µg 1055 FA vs. 5 µg gE + L2 + 100 µg 1055 FA + 20 µg PHAD liposomes	Yes	****	<0.0001
5 µg gE + L2 + 5 µg 1055 FA vs. 5 µg gE + 20 µg PHAD liposomes	Yes	****	<0.0001
5 µg gE + L2 + 30 µg 1055 FA vs. 5 µg gE + L2 + 100 µg 1055 FA	Yes	****	<0.0001
5 µg gE + L2 + 30 µg 1055 FA vs. 5 µg gE + L2 + 5 µg 1055 FA + 20 µg PHAD liposomes	Yes	****	<0.0001
5 µg gE + L2 + 30 µg 1055 FA vs. 5 µg gE + L2 + 30 µg 1055 FA + 20 µg PHAD liposomes	Yes	****	<0.0001
5 µg gE + L2 + 30 µg 1055 FA vs. 5 µg gE + L2 + 100 µg 1055 FA + 20 µg PHAD liposomes	Yes	****	<0.0001
5 µg gE + L2 + 30 µg 1055 FA vs. 5 µg gE + 20 µg PHAD liposomes	Yes	****	<0.0001
5 µg gE + L2 + 100 µg 1055 FA vs. 5 µg gE + L2 + 5 µg 1055 FA + 20 µg PHAD liposomes	No	ns	0.3062
5 µg gE + L2 + 100 µg 1055 FA vs. 5 µg gE + L2 + 30 µg 1055 FA + 20 µg PHAD liposomes	No	ns	0.0792
5 µg gE + L2 + 100 µg 1055 FA vs. 5 µg gE + L2 + 100 µg 1055 FA + 20 µg PHAD liposomes	No	ns	0.0792
5 µg gE + L2 + 100 µg 1055 FA vs. 5 µg gE + 20 µg PHAD liposomes	No	ns	0.0792

Table 3.4 -- Group-wise comparisons between groups in Table 3.3 (continued)

Tukey's multiple comparisons test	Significant?	Summary	Adjusted P Value
5 µg gE + L2 + 5 µg 1055 FA + 20 µg PHAD liposomes vs. 5 µg gE + L2 + 30 µg 1055 FA + 20 µg PHAD liposomes	No	ns	>0.9999
5 µg gE + L2 + 5 µg 1055 FA + 20 µg PHAD liposomes vs. 5 µg gE + L2 + 100 µg 1055 FA + 20 µg PHAD liposomes	No	ns	>0.9999
5 µg gE + L2 + 5 µg 1055 FA + 20 µg PHAD liposomes vs. 5 µg gE + 20 µg PHAD liposomes	No	ns	>0.9999
5 µg gE + L2 + 30 µg 1055 FA + 20 µg PHAD liposomes vs. 5 µg gE + L2 + 100 µg 1055 FA + 20 µg PHAD liposomes	No	ns	>0.9999
5 µg gE + L2 + 30 µg 1055 FA + 20 µg PHAD liposomes vs. 5 µg gE + 20 µg PHAD liposomes	No	ns	>0.9999
5 µg gE + L2 + 100 µg 1055 FA + 20 µg PHAD liposomes vs. 5 µg gE + 20 µg PHAD liposomes	No	ns	>0.9999