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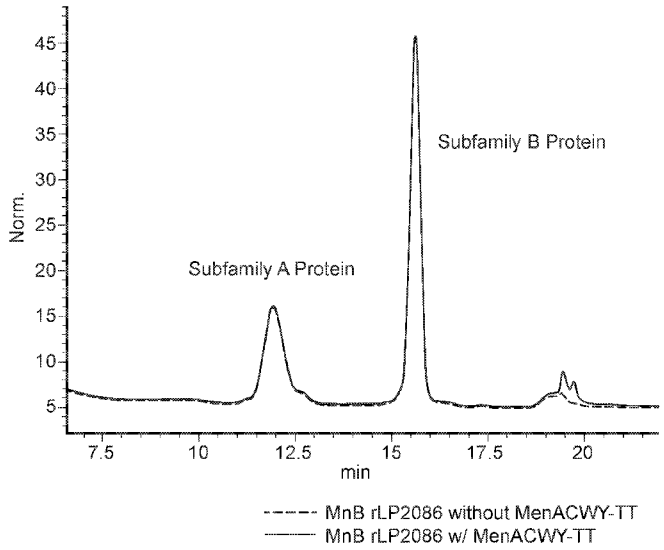
(54) Title: NEISSERIA MENINGITIDIS COMPOSITIONS AND METHODS THEREOF

FIG. 1



(57) Abstract: In one aspect, the invention relates to a composition including a factor H binding protein (fHBP) and a *Neisseria meningitidis* non-serogroup B capsular polysaccharide. The invention further relates to uses of a composition that includes fHBP, such as, for example, uses to elicit an immune response against *N. meningitidis* serogroup B strains and non-serogroup B strains. The compositions and methods described herein are directed to administration in humans, including adults, adolescents, toddlers, and infants.

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[Continued on next page]

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NEISSERIA MENINGITIDIS COMPOSITIONS AND METHODS THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

The present application claims the benefit of U.S. Provisional Patent Application 62/452,963, filed January 31, 2017, U.S. Provisional Patent Application 62/503,295, filed May 8, 2017, U.S. Provisional Patent Application 62/613,945, filed January 5, 2018, and U.S. Provisional Patent Application Number 62/623,233, filed January 29, 2018. All of the foregoing applications are hereby incorporated by reference in their entireties.

FIELD OF THE INVENTION

The present invention relates to *Neisseria meningitidis* compositions and methods thereof.

BACKGROUND OF THE INVENTION

Neisseria meningitidis is a Gram-negative encapsulated bacterium that can cause sepsis, meningitis, and death. *N. meningitidis* can be classified into at least 12 serogroups (including serogroups A, B, C, 29E, H, I, K, L, W-135 (mostly now referred to as W), X, Y and Z) based on chemically and antigenically distinctive polysaccharide capsules. Strains with five of the serogroups (A, B, C, Y, and W135) are responsible for the majority of disease.

Meningococcal meningitis is a devastating disease that can kill children and young adults within hours despite the availability of antibiotics. There is a need for improved immunogenic compositions against meningococcal serogroups A, B, C, Y, and W135 and/or X.

Currently, a cross-protective vaccine or composition effective against a wide range of MnB and meningococcal serogroups A, C, Y, and W and/or X isolates is not yet commercially available. For example, published results-to-date relating to a licensed multi-component composition for protection against serogroup B disease has not demonstrated a direct bactericidal immune response against multiple meningococcal strains that expressnon-serogroup B capsular polysaccharides, at least in adolescents. Accordingly, a cross-protective vaccine or composition effective against diverse MnB and meningococcal serogroups A, C, Y, and W and/or X isolates is needed as is determining real-world vaccine coverage against a panel of diverse or heterologous meningococcal strains (e.g., representing different geographical regions).

It is a further object of the invention to provide improved schedules for administering a meningococcal vaccine, in particular to children. While incidence rates of invasive meningococcal disease (IMD) vary with age, incidence is often highest during

infancy from age 1 month to 1 year, with a second peak in incidence during adolescence. In the United States, during 1998 to 2007, the overall rate of meningococcal disease in infants aged less than 2 years was 3.9 per 100,000. In children aged 2 to 10 years, the incidence was 0.68 per 100,000, with 41% of cases in this age group occurring in children aged 2 to 3 years. National surveillance data from Australia show the peak incidence of disease in children aged 4 years or less, with a secondary peak in adolescents and young adults; approximately 85% of all cases are attributed to serogroup B disease.

SUMMARY OF THE INVENTION

To meet these and other needs, the present invention relates to *Neisseria meningitidis* compositions and methods thereof.

The inventors surprisingly discovered a composition including at least one factor H binding protein (fHBP) and at least one *N. meningitidis* capsular saccharide conjugate. The composition is surprisingly stable and elicited an immune response against strains that express fHBP variants that are homologous to the fHBP variant in the multi-component composition and an immune response against strains that express fHBP variants that are heterologous to the fHBP variant in the multi-component composition.

The composition includes a first lipidated polypeptide including the amino acid sequence set forth in SEQ ID NO: 1; a second lipidated polypeptide including the amino acid sequence set forth in SEQ ID NO: 2; a *Neisseria meningitidis* serogroup A (MenA) capsular saccharide conjugated to tetanus toxoid carrier protein (TT); a *Neisseria meningitidis* serogroup C (MenC) capsular saccharide conjugated to tetanus toxoid carrier protein (TT); a *Neisseria meningitidis* serogroup W135 (MenW) capsular saccharide conjugated to tetanus toxoid carrier protein (TT); and a *Neisseria meningitidis* serogroup Y (MenY) capsular saccharide conjugated to tetanus toxoid carrier protein (TT).

In one embodiment, the composition includes a *Neisseria meningitidis* serogroup A (MenA) capsular saccharide conjugated to an adipic acid dihydrazide (ADH) linker by 1-cyano-4-dimethylamino pyridinium tetrafluoroborate chemistry, wherein the linker is conjugated to tetanus toxoid carrier protein (TT) by carbodiimide chemistry ($MenA_{AH}$-TT conjugate); a *Neisseria meningitidis* serogroup C (MenC) capsular saccharide conjugated to an ADH linker by 1-cyano-4-dimethylamino pyridinium tetrafluoroborate chemistry, wherein the linker is conjugated to tetanus toxoid carrier protein (TT) by carbodiimide chemistry ($MenC_{AH}$-TT conjugate); a *Neisseria meningitidis* serogroup W_{135} (MenW) capsular saccharide directly conjugated to tetanus toxoid carrier protein (TT) by 1-cyano-4-dimethylamino pyridinium tetrafluoroborate chemistry, in the absence of a linker (MenW-TT conjugate); and a *Neisseria meningitidis* serogroup Y (MenY) capsular

saccharide directly conjugated to tetanus toxoid carrier protein (TT) by 1-cyano-4-dimethylamino pyridinium tetrafluoroborate chemistry, in the absence of a linker (MenY-TT conjugate).

In one aspect, the invention relates to a kit including (a) a first composition including a lipidated MenB rLP2086 subfamily A polypeptide and a lipidated MenB rLP2086 subfamily B polypeptide; and (b) a second composition including a *Neisseria meningitidis* serogroup A (MenA) capsular saccharide conjugated to tetanus toxoid carrier protein (TT); a *Neisseria meningitidis* serogroup C (MenC) capsular saccharide conjugated to tetanus toxoid carrier protein (TT); a *Neisseria meningitidis* serogroup W_{135} (MenW) capsular saccharide conjugated to tetanus toxoid carrier protein (TT); and a *Neisseria meningitidis* serogroup Y (MenY) capsular saccharide conjugated to tetanus toxoid carrier protein (TT). In one embodiment, the first composition is a liquid composition and the second composition is a lyophilized composition. In another embodiment, the kit does not further include any one of the following immunogenic compositions: MENACTRA(R), MENVEO(R), ADACEL(R), HAVRIX(R), GARDASIL(R), REPEVAX, or any combination thereof. In one embodiment, the kit includes any one of ibuprofen, paracetamol, and amoxicillin.

In one aspect, the invention relates to an immunogenic composition including a liquid composition including (i) a first lipidated polypeptide including the amino acid sequence set forth in SEQ ID NO: 1; and (ii) a second lipidated polypeptide including the amino acid sequence set forth in SEQ ID NO: 2; and a lyophilized composition including a *Neisseria meningitidis* serogroup A (MenA) capsular saccharide conjugated to an adipic acid dihydrazide (ADH) linker by 1-cyano-4-dimethylamino pyridinium tetrafluoroborate chemistry, wherein the linker is conjugated to tetanus toxoid carrier protein (TT) by carbodiimide chemistry ($MenA_{AH}$-TT conjugate); a *Neisseria meningitidis* serogroup C (MenC) capsular saccharide conjugated to an ADH linker by 1-cyano-4-dimethylamino pyridinium tetrafluoroborate chemistry, wherein the linker is conjugated to tetanus toxoid carrier protein (TT) by carbodiimide chemistry ($MenC_{AH}$-TT conjugate); a *Neisseria meningitidis* serogroup W_{135} (MenW) capsular saccharide directly conjugated to tetanus toxoid carrier protein (TT) by 1-cyano-4-dimethylamino pyridinium tetrafluoroborate chemistry, in the absence of a linker (MenW-TT conjugate); and a *Neisseria meningitidis* serogroup Y (MenY) capsular saccharide directly conjugated to tetanus toxoid carrier protein (TT) by 1-cyano-4-dimethylamino pyridinium tetrafluoroborate chemistry, in the absence of a linker (MenY-TT conjugate). In one embodiment, the lyophilized composition is reconstituted with the liquid composition.

In another aspect, the invention relates to a method of inducing a bactericidal immune response against a *Neisseria meningitidis* serogroup X strain. In some

embodiments, the method includes administering to the human a composition including a fHBP protein. In some embodiments, the method includes administering to the human a composition comprising a polypeptide comprising an amino acid sequence having at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.9% identity to the amino acid sequence set forth in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, and SEQ ID NO: 62. In some embodiments, the method includes administering to the human a composition including a first lipidated polypeptide including the amino acid sequence set forth in SEQ ID NO: 1; a second lipidated polypeptide including the amino acid sequence set forth in SEQ ID NO: 2.

In another aspect, the invention relates to a method of inducing a bactericidal immune response against a *Neisseria meningitidis* serogroup X strain. The method includes administering to the human a composition that includes a first lipidated polypeptide including the amino acid sequence set forth in SEQ ID NO: 1; a second lipidated polypeptide including the amino acid sequence set forth in SEQ ID NO: 2; a *Neisseria meningitidis* serogroup A (MenA) capsular saccharide conjugated to tetanus toxoid carrier protein (TT); a *Neisseria meningitidis* serogroup C (MenC) capsular saccharide conjugated to tetanus toxoid carrier protein (TT); a *Neisseria meningitidis* serogroup W135 (MenW) capsular saccharide conjugated to tetanus toxoid carrier protein (TT); and a *Neisseria meningitidis* serogroup Y (MenY) capsular saccharide conjugated to tetanus toxoid carrier protein (TT).

In one aspect, the invention relates to a method for eliciting an immune response in a patient of any age. The method includes administering to the human a composition including a first lipidated polypeptide including the amino acid sequence set forth in SEQ ID NO: 1; a second lipidated polypeptide including the amino acid sequence set forth in SEQ ID NO: 2. In one embodiment, the composition further includes polysorbate-80. In one embodiment, the composition further includes aluminum. In one embodiment, the

composition further includes histidine. In one embodiment, the composition further includes sodium chloride. In one embodiment, the composition further includes polysorbate-80, aluminum, histidine, and sodium chloride. In yet another embodiment, the composition further includes a *Neisseria meningitidis* serogroup A (MenA) capsular saccharide conjugated to tetanus toxoid carrier protein (TT); a *Neisseria meningitidis* serogroup C (MenC) capsular saccharide conjugated to tetanus toxoid carrier protein (TT); a *Neisseria meningitidis* serogroup W135 (MenW) capsular saccharide conjugated to tetanus toxoid carrier protein (TT); and a *Neisseria meningitidis* serogroup Y (MenY) capsular saccharide conjugated to tetanus toxoid carrier protein (TT).

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 - Overlaid IEX-HPC Chromatograms for the MnB bivalent rLP2086 composition in the Absence and Presence of the MenACWY-TT composition, described in Example 6.

FIG. 2 - Overlay of RP-HPLC Chromatograms showing that the presence of the MenACWY-TT composition does not interfere with evaluation of the MnB bivalent rLP2086 composition purity, as described in Example 7.

FIG. 3 - Overlay of RP-HPLC Chromatograms showing rLP2086 Protein Purity and Peak Ratio in the Combined MenABCWY composition, as described in Example 14.

FIG. 4A – Primary Amino Acid Sequence of MnB rLP2086 Subfamily A A05 Protein and

FIG. 4B - Primary Structure of MnB rLP2086 Subfamily A A05 Protein

FIG. 5A - Primary Amino Acid Sequence of MnB rLP2086 Subfamily B B01 Protein and

FIG. 5B - Primary Structure of MnB rLP2086 Subfamily B B01 Protein

FIG. 6A - Amino acid sequences for a factor H binding protein (fHBP) B16 (SEQ ID NO: 26) from a *N. meningitidis* serogroup A strain (fHBP variant B16 (PMB3257, MenA);

FIG. 6B - a fHBP A10 (SEQ ID NO: 27) from a *N. meningitidis* serogroup C strain (fHBP variant A10 (PMB5208, MenC and PMB5523, MenW);

FIG. 6C - a fHBP A19 (SEQ ID NO: 28) from a *N. meningitidis* serogroup W strain (fHBP variant A19 (PMB5248, MenW);

FIG. 6D - a fHBP A10 (SEQ ID NO: 27) from a *N. meningitidis* serogroup W strain (fHBP variant A10 (PMB5523, MenW);

FIG. 6E - a fHBP B47 (SEQ ID NO: 29) from a *N. meningitidis* serogroup Y strain (fHBP variant B47 (PMB5187, MenY);

FIG. 6F - a fHBP B49 (SEQ ID NO: 30) from a *N. meningitidis* serogroup X strain (fHBP variant B49 (PMB5540, MenX).

FIG. 7 - Serum Bactericidal Activity, the Correlate of Protection for Meningococcal Disease. A titer of ≥1:4 in serum bactericidal assays using human complement (hSBA) is the established correlate of protection for meningococcal disease.

FIG. 8 - MenA, C, W, Y, and X Test Strain Selection

FIG. 9 – Schematic of the Relevant Groups of the Clinical Trial From Which a Subset of Test Sera Were Randomly Selected

FIG. 10 – Distribution of FHbp Surface Expression Levels (MFI) Determined From Flow Cytometric Experiments Using the FHbp Reactive mAb MN 994-11. The FHbp surface expression for each of the strains within a serogroup is noted with a black dot while the FHbp surface expression levels for the selected test strains within each serogroup are noted with a colored star.

FIG. 11 – hSBA Response Rate (Percentage of Subjects With hSBA Titers ≥1:8) for MenA PMB3257 (B16). Response rates and 95% confidence intervals for sera collected at preimmunization (month 0) and 1 month after doses 1, 2, and 3 for MenBFHbp are shown. The geometric mean titers (GMTs) obtained were 2, 3, 4, and 5, respectively. The response rates for subjects in the positive control group were 3% prior to vaccination and 97% one month after receiving MCV4. The GMTs for the positive control group were 2 and 95, respectively.

FIG. 12 – hSBA Response Rate (Percentage of Subjects With hSBA Titers ≥1:8) for MenC PMB5208 (A10). Response rates and 95% confidence intervals for sera collected at preimmunization (month 0) and 1 month after doses 1, 2, and 3 for MenBFHbp are shown. The GMTs obtained were 4, 8, 12, and 29, respectively. The response rates for subjects in the positive control group were 20% prior to vaccination and 90% one month after receiving MCV4. The GMTs for the positive control group were 3 and 119, respectively.

FIG. 13 – hSBA Response Rate (Percentage of Subjects With hSBA Titers ≥1:8) for MenW PMB5248 (A19). Response rates and 95% confidence intervals for sera collected at preimmunization (month 0) and 1 month after doses 1, 2, and 3 for MenBFHbp are shown. The GMTs obtained were 4, 18, 47, and 77, respectively. The response rates for subjects in the positive control group were 40% prior to vaccination and 97% one month after receiving MCV4. The GMTs for the positive control group were 5 and 88, respectively.

FIG. 14 – hSBA Response Rate (Percentage of Subjects With hSBA Titers ≥1:8) for MenW PMB5523 (A10). Response rates and 95% confidence intervals for sera collected at preimmunization (month 0) and 1 month after doses 1, 2, and 3 for MenBFHbp are shown. The GMTs obtained were 7, 15, 21, and 42, respectively. The response rates for subjects in the positive control group were 55% prior to vaccination and 97% one month after receiving MCV4. The GMTs for the positive control group were 8 and 60, respectively.

FIG. 15 – hSBA Response Rate (Percentage of Subjects With hSBA Titers ≥1:8) for MenY PMB5187 (B47). Response rates and 95% confidence intervals for sera collected at preimmunization (month 0) and 1 month after doses 1, 2, and 3 for MenBFHbp are shown. The GMTs obtained were 3, 7, 31, and 58, respectively. The response rates for subjects in the positive control group were 13% prior to vaccination and 97% one month after receiving MCV4. The GMTs for the positive control group were 3 and 79, respectively.

FIG. 16 – hSBA Response Rate (Percentage of Subjects With hSBA Titers ≥1:8) for MenX PMB5540 (B49). Response rates and 95% confidence intervals for sera collected

at preimmunization (month 0) and 1 month after doses 1, 2, and 3 for MenBFHbp are shown. The GMTs obtained were 2, 3, 7, and 20, respectively. The response rates for subjects in the positive control group were 0% prior to vaccination and 0% one month after receiving MCV4 vaccine. The GMTs for the positive control group were 2 and 2, respectively.

SEQUENCE IDENTIFIERS

SEQ ID NO: 1 sets forth the amino acid sequence for a recombinant *N. meningitidis*, serogroup B, 2086 variant A05 polypeptide antigen.

SEQ ID NO: 2 sets forth the amino acid sequence for a recombinant *N. meningitidis*, serogroup B, 2086 variant B01 polypeptide antigen.

SEQ ID NO: 3 sets forth the amino acid residues at positions 1-4 of SEQ ID NO: 1 and SEQ ID NO: 2.

SEQ ID NO: 4 sets forth the amino acid sequence of the N-terminus of a recombinant Neisserial Subfamily A LP2086 polypeptide (rLP2086) (A05) polypeptide.

SEQ ID NO: 5 sets forth the amino acid sequence of the N-terminus of Neisserial Subfamily A LP2086 M98250771 polypeptide (A05) polypeptide.

SEQ ID NO: 6 sets forth the amino acid sequence for *N. meningitidis*, serogroup B, 2086 variant B153.

SEQ ID NO: 7 sets forth the amino acid sequence for *N. meningitidis*, serogroup B, 2086 variant A04.

SEQ ID NO: 8 sets forth the amino acid sequence for *N. meningitidis*, serogroup B, 2086 variant A05

SEQ ID NO: 9 sets forth the amino acid sequence for *N. meningitidis*, serogroup B, 2086 variant A12.

SEQ ID NO: 10 sets forth the amino acid sequence for *N. meningitidis*, serogroup B, 2086 variant A22.

SEQ ID NO: 11 sets forth the amino acid sequence for *N. meningitidis*, serogroup B, 2086 variant B02.

SEQ ID NO: 12 sets forth the amino acid sequence for *N. meningitidis*, serogroup B, 2086 variant B03.

SEQ ID NO: 13 sets forth the amino acid sequence for *N. meningitidis*, serogroup B, 2086 variant B09.

SEQ ID NO: 14 sets forth the amino acid sequence for *N. meningitidis*, serogroup B, 2086 variant B22.

SEQ ID NO: 15 sets forth the amino acid sequence for *N. meningitidis*, serogroup B, 2086 variant B24.

SEQ ID NO: 16 sets forth the amino acid sequence for *N. meningitidis*, serogroup B, 2086 variant B44.

SEQ ID NO: 17 sets forth the amino acid sequence for *N. meningitidis*, serogroup B, 2086 variant B16.

SEQ ID NO: 18 sets forth the amino acid sequence for *N. meningitidis*, serogroup B, 2086 variant A07.

SEQ ID NO: 19 sets forth the amino acid sequence for *N. meningitidis*, serogroup B, 2086 variant A19.

SEQ ID NO: 20 sets forth the amino acid sequence for *N. meningitidis*, serogroup B, 2086 variant A06.

SEQ ID NO: 21 sets forth the amino acid sequence for *N. meningitidis*, serogroup B, 2086 variant A15.

SEQ ID NO: 22 sets forth the amino acid sequence for *N. meningitidis*, serogroup B, 2086 variant A29.

SEQ ID NO: 23 sets forth the amino acid sequence for *N. meningitidis*, serogroup B, 2086 variant B15.

SEQ ID NO: 24 sets forth the amino acid sequence of the N-terminus of a recombinant Neisserial Subfamily B LP2086 polypeptide (rLP2086) (B01) polypeptide.

SEQ ID NO: 25 sets forth the amino acid sequence of the N-terminus of Neisserial Subfamily B LP2086 CDC-1573 polypeptide (B01) polypeptide.

SEQ ID NO: 26 sets forth the amino acid sequence for *N. meningitidis* serogroup A strain expressing factor H binding protein (fHBP) B16.

SEQ ID NO: 27 sets forth the amino acid sequence for a *N. meningitidis* serogroup C strain expressing fHBP A10. SEQ ID NO: 27 also sets forth the amino acid sequence for a *N. meningitidis* serogroup W strain expressing fHBP A10.

SEQ ID NO: 28 sets forth the amino acid sequence for a *N. meningitidis* serogroup W strain expressing fHBP A19.

SEQ ID NO: 29 sets forth the amino acid sequence for a *N. meningitidis* serogroup Y strain expressing fHBP B47.

SEQ ID NO: 30 sets forth the amino acid sequence for a *N. meningitidis* serogroup X strain expressing fHBP B49.

SEQ ID NO: 31 sets forth the amino acid sequence for a non-lipidated *N. meningitidis*, serogroup B, 2086 variant B16.

SEQ ID NO: 32 sets forth the amino acid sequence for a non-lipidated *N. meningitidis*, serogroup B, 2086 variant A07.

SEQ ID NO: 33 sets forth the amino acid sequence for a non-lipidated *N. meningitidis*, serogroup B, 2086 variant A19.

SEQ ID NO: 34 sets forth the amino acid sequence for a non-lipidated *N. meningitidis*, serogroup B, 2086 variant A06.

SEQ ID NO: 35 sets forth the amino acid sequence for a non-lipidated *N. meningitidis*, serogroup B, 2086 variant A15.

SEQ ID NO: 36 sets forth the amino acid sequence for a non-lipidated *N. meningitidis*, serogroup B, 2086 variant A29.

SEQ ID NO: 37 sets forth the amino acid sequence for a non-lipidated *N. meningitidis*, serogroup B, 2086 variant B15.

SEQ ID NO: 38 sets forth the amino acid sequence for a non-lipidated *N. meningitidis* serogroup A strain expressing factor H binding protein (fHBP) B16.

SEQ ID NO: 39 sets forth the amino acid sequence for a non-lipidated *N. meningitidis* serogroup C strain expressing fHBP A10. SEQ ID NO: 39 also sets forth the amino acid sequence for a non-lipidated *N. meningitidis* serogroup W strain expressing fHBP A10.

SEQ ID NO: 40 sets forth the amino acid sequence for a non-lipidated *N. meningitidis* serogroup W strain expressing fHBP A19.

SEQ ID NO: 41 sets forth the amino acid sequence for a non-lipidated *N. meningitidis* serogroup Y strain expressing fHBP B47.

SEQ ID NO: 42 sets forth the amino acid sequence for a non-lipidated *N. meningitidis* serogroup X strain expressing fHBP B49.

SEQ ID NO: 43 sets forth the amino acid sequence for a non-lipidated *N. meningitidis*, serogroup B, 2086 variant B44.

SEQ ID NO: 44 sets forth the amino acid sequence for a non-lipidated *N. meningitidis*, serogroup B, 2086 variant B09.

SEQ ID NO: 45 sets forth the amino acid sequence for a *N. meningitidis*, serogroup B, 2086 variant B09.

SEQ ID NO: 46 sets forth the amino acid sequence for a non-lipidated *N. meningitidis*, serogroup B, 2086 variant A05.

SEQ ID NO: 47 sets forth the amino acid sequence for a non-lipidated *N. meningitidis*, serogroup B, 2086 variant B01.

SEQ ID NO: 48 sets forth the amino acid sequence for a *N. meningitidis*, serogroup B, 2086 variant B01, which includes an N-terminal Cys at amino acid position 1.

SEQ ID NO: 49 sets forth the amino acid sequence for a *N. meningitidis*, serogroup B, 2086 variant B15, which includes an N-terminal Cys at amino acid position 1.

SEQ ID NO: 50 sets forth the amino acid sequence for a *N. meningitidis*, serogroup B, 2086 variant B16, which includes an N-terminal Cys at amino acid position 1.

SEQ ID NO: 51 sets forth the amino acid sequence for a *N. meningitidis*, serogroup B, 2086 variant B22.

SEQ ID NO: 52 sets forth the amino acid sequence for a *N. meningitidis*, serogroup B, 2086 variant A22.

SEQ ID NO: 53 sets forth the amino acid sequence for a non-lipidated *N. meningitidis*, serogroup B, 2086 variant A12.

SEQ ID NO: 54 sets forth the amino acid sequence for a non-lipidated *N. meningitidis*, serogroup B, 2086 variant A22.

SEQ ID NO: 55 sets forth the amino acid sequence for a *N. meningitidis* serogroup B, 2086 variant A62, which includes an N-terminal Cys at amino acid position 1.

SEQ ID NO: 56 sets forth the amino acid sequence for a non-lipidated *N. meningitidis*, serogroup B, 2086 variant A62.

SEQ ID NO: 57 sets forth the amino acid sequence for a *N. meningitidis*, serogroup B, 2086 variant A29, which includes an N-terminal Cys at amino acid position 1.

SEQ ID NO: 58 sets forth the amino acid sequence for a non-lipidated *N. meningitidis*, serogroup B, 2086 variant B22.

SEQ ID NO: 59 sets forth the amino acid sequence for a *N. meningitidis*, serogroup B, 2086 variant A05.

SEQ ID NO: 60 sets forth the amino acid sequence for a non-lipidated *N. meningitidis*, serogroup B, 2086 variant A05.

SEQ ID NO: 61 sets forth the amino acid sequence for a *N. meningitidis*, serogroup B, 2086 variant B24.

SEQ ID NO: 62 sets forth the amino acid sequence for a *N. meningitidis*, serogroup B, 2086 variant B24.

DETAILED DESCRIPTION OF THE INVENTION

The inventors surprisingly discovered a composition including at least one factor H binding protein (fHBP) and at least one *N. meningitidis* capsular saccharide conjugate. The composition is surprisingly stable and elicited an immune response against strains that express fHBP variants that are homologous to the fHBP variant in the multi-component composition and an immune response against strains that express fHBP variants that are heterologous to the fHBP variant in the multi-component composition. The inventors further surprisingly discovered that an fHBP polypeptide effectively elicited an immune response in children, such as, for example, humans aged 12 months and above. Moreover, the inventors surprisingly discovered that an fHBP polypeptide effectively elicited an immune response against a *N. meningitidis* serogroup X strain.

The inventors surprisingly discovered a composition that includes (a) a first lipidated polypeptide including the amino acid sequence set forth in SEQ ID NO: 1; (b) a second lipidated polypeptide including the amino acid sequence set forth in SEQ ID NO: 2; (c) a *Neisseria meningitidis* serogroup A (MenA) capsular saccharide conjugated to an adipic acid dihydrazide (ADH) linker by 1-cyano-4-dimethylamino pyridinium tetrafluoroborate chemistry, wherein the linker is conjugated to tetanus toxoid carrier protein (TT) by carbodiimide chemistry ($MenA_{AH}$-TT conjugate); (d) a *Neisseria meningitidis* serogroup C (MenC) capsular saccharide conjugated to an ADH linker by 1-cyano-4-dimethylamino pyridinium tetrafluoroborate chemistry, wherein the linker is conjugated to tetanus toxoid carrier protein (TT) by carbodiimide chemistry ($MenC_{AH}$-TT conjugate); (e) a *Neisseria meningitidis* serogroup W_{135} (MenW) capsular saccharide directly conjugated to tetanus toxoid carrier protein (TT) by 1-cyano-4-dimethylamino pyridinium tetrafluoroborate chemistry, in the absence of a linker (MenW-TT conjugate); (f) a *Neisseria meningitidis* serogroup Y (MenY) capsular saccharide directly conjugated to tetanus toxoid carrier protein (TT) by 1-cyano-4-dimethylamino pyridinium tetrafluoroborate chemistry, in the absence of a linker (MenY-TT conjugate). The composition includes a lyophilized MenACWY-TT composition that surprisingly is readily reconstituted with a liquid MnB bivalent rLP2086 composition, wherein the composition is in a single vial. The inventors discovered that the lyophilized MenACWY-TT composition and the liquid MnB bivalent rLP2086 composition were compatible and stable, following reconstitution, for at least 24 hours at room temperature.

Moreover, the inventors further discovered that the MnB bivalent rLP2086 composition elicited bactericidal antibodies not only against *N. meningitidis* serogroup B, but also *N. meningitidis* serogroups other than B. For example, the MnB bivalent rLP2086 composition elicited bactericidal antibodies against at least *N. meningitidis* serogroups A, C, W, Y, and X. The surprising discovery that the MnB bivalent rLP2086

composition elicited bactericidal antibodies against *N. meningitidis* serogroup X indicates that the MnB bivalent rLP2086 composition elicits a broadly cross-reactive bactericidal immune response in humans against at least two diverse *Neisseria meningitidis* serogroups.

Futhermore, the inventors surprisingly discovered an immune response as measured by serum bactericidal assay using human complement (hSBA) performed with 4 primary *Neisseria meningitidis* serogroup B (MnB) test strains, 2 expressing an LP2086 subfamily A protein and 2 expressing an LP2086 subfamily B protein, measured 1 month after the third vaccination with bivalent rLP2086, in healthy subjects aged ≥24 months to <4 years at study entry. The inventors also surprisingly discovered an immune response as measured by hSBA performed with 4 primary MnB test strains, 2 expressing an LP2086 subfamily A protein and 2 expressing an LP2086 subfamily B protein, measured 1 month after the third vaccination with bivalent rLP2086, in healthy subjects aged ≥4 years to <10 years at study entry. The inventors further surprisingly discovered an immune response as measured by hSBA performed with 4 primary MnB test strains, 2 expressing an LP2086 subfamily A protein and 2 expressing an LP2086 subfamily B protein, measured 1 month after the third vaccination with bivalent rLP2086, in healthy subjects aged ≥24 months to <10 years at study entry (ie, in the combined age stratum). The inventors also surprisingly discovered an immune response as measured by hSBA performed with 4 primary MnB test strains, 2 expressing an LP2086 subfamily A protein and 2 expressing an LP2086 subfamily B protein, measured 1 month after the second vaccination and 6 months after the third vaccination with bivalent rLP2086, in healthy subjects aged ≥24 months to <4 years at study entry, in healthy subjects aged ≥4 years to <10 years at study entry, and in the combined age stratum. The immune response was further described through additional endpoints, as measured by hSBA performed with 4 primary MnB test strains, 2 expressing an LP2086 subfamily A protein and 2 expressing an LP2086 subfamily B protein, at specified time points, in healthy subjects aged ≥24 months to <4 years at study entry, in healthy subjects aged ≥4 years to <10 years at study entry, and in the combined age stratum.

In addition, the inventors surprisingly discovered an immune response as measured by serum bactericidal assay using human complement (hSBA) performed with 4 primary *Neisseria meningitidis* serogroup B (MnB) strains, 2 expressing an LP2086 subfamily A protein and 2 expressing an LP2086 subfamily B protein, measured 1 month after the third vaccination with bivalent rLP2086, in healthy toddlers aged 12 to <18 months at study entry. The inventors also surprisingly discovered an immune response as measured by hSBA performed with 4 primary MnB strains, 2 expressing an LP2086

subfamily A protein and 2 expressing an LP2086 subfamily B protein, measured 1 month after the third vaccination with bivalent rLP2086, in healthy toddlers aged 18 to <24 months at study entry. The inventors further surprisingly discovered an immune response as measured by hSBA performed with 4 primary MnB test strains, 2 expressing an LP2086 subfamily A protein and 2 expressing an LP2086 subfamily B protein, measured 1 month after the third vaccination with bivalent rLP2086, in healthy toddlers aged 12 to <24 months at study entry (ie, both age strata combined). The inventors also surprisingly discovered an immune response as measured by hSBA performed with 4 primary MnB test strains, 2 expressing an LP2086 subfamily A protein and 2 expressing an LP2086 subfamily B protein, measured 1 month after the second vaccination and at least 6 months after the third vaccination in healthy toddlers aged 12 to <18 months and 18 to <24 months at study entry, and in both age strata combined. For example, the hSBA may be measured at any time, including 12, 24, 36, and 48 months after the third vaccination in healthy toddlers aged 12 to <18 months and 18 to <24 months at study entry, and in both age strata combined. The immune response was further described through additional endpoints, as measured by hSBA performed with 4 primary MnB test strains, 2 expressing an LP2086 subfamily A protein and 2 expressing an LP2086 subfamily B protein, measured 1 month after the second vaccination and at least1 month after the third vaccination with bivalent rLP2086 in healthy toddlers aged 12 to <18 months and 18 to <24 months at study entry, and in both age strata combined. For example, the hSBA may be measured at any time, including 6, 12, 24, 36, and 48 months after the third vaccination with bivalent rLP2086 in healthy toddlers aged 12 to <18 months and 18 to <24 months at study entry, and in both age strata combined. The immune response was also described through additional endpoints, as measured by hSBA to secondary MnB test strains expressing LP2086 subfamily A and B proteins, at 1 month after the second vaccination and at least 1 month after the third vaccination in healthy toddlers aged 12 to <18 months and 18 to <24 months at study entry, and in both age strata combined. For example, the hSBA may be measured at any time, including 6, 12, 24, 36, and 48 months after the third vaccination in healthy toddlers aged 12 to <18 months and 18 to <24 months at study entry, and in both age strata combined.

Accordingly, in one aspect, the invention relates to a method for eliciting an immune response in a patient of any age. In some embodiments, the human is aged at least 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, or 12 weeks old. For example, in a preferred embodiment, the human is aged at least 6 weeks. As is known in the art, a Meningococcal Group A, C, W-135, and Y Conjugate Vaccine, such as NIMENRIX ®, is suitable for infants as early as six weeks of age, and can be administered to any human aged six weeks and above. In some embodiments,

the human is aged at least 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 12 months. For example, in a preferred embodiment, the human is aged at least 12 months. In one embodiment, the human is aged between 12 and 18 months. In another aspect, the invention relates to a method for eliciting an immune response in a patient aged at least 18 months. In one embodiment, the human is aged between 18 and 24 months. In yet another aspect, the invention relates to a method for eliciting an immune response in a patient aged at least 24 months. In one embodiment, the human is aged between 24 months and 10 years. In another aspect, the invention relates to a method for eliciting an immune response in a patient aged 10 years and above. In a further aspect, the invention relates to a method for eliciting an immune response in a patient aged between 10 years and 25 years. The method includes administering to the human a composition including a first lipidated polypeptide including the amino acid sequence set forth in SEQ ID NO: 1; a second lipidated polypeptide including the amino acid sequence set forth in SEQ ID NO: 2. In one embodiment, the composition further includes polysorbate-80. In one embodiment, the composition further includes aluminum. In one embodiment, the composition further includes histidine. In one embodiment, the composition further includes sodium chloride. In one embodiment, the composition further includes polysorbate-80, aluminum, histidine, and sodium chloride. In yet another embodiment, the composition further includes a *Neisseria meningitidis* serogroup A (MenA) capsular saccharide conjugated to tetanus toxoid carrier protein (TT); a *Neisseria meningitidis* serogroup C (MenC) capsular saccharide conjugated to tetanus toxoid carrier protein (TT); a *Neisseria meningitidis* serogroup W135 (MenW) capsular saccharide conjugated to tetanus toxoid carrier protein (TT); and a *Neisseria meningitidis* serogroup Y (MenY) capsular saccharide conjugated to tetanus toxoid carrier protein (TT). As is known in the art, a Meningococcal Group A, C, W-135, and Y Conjugate Vaccine, such as NIMENRIX ®, is suitable for infants as early as six weeks of age, and can be administered to any human aged six weeks and above.

Further descriptions of exemplary compositions are described below.

COMPOSITION AND VACCINE

The inventors further discovered that a composition including fHBP elicits an effective immune response in humans aged at least 12 months. The composition also elicits an immune response against a *N. meningitidis* serogroup X strain. In addition, the inventors surprisingly discovered a composition including at least one factor H binding polypeptide (fHBP) and at least one *N. meningitidis* capsular saccharide conjugate. The composition is surprisingly stable and elicited an immune response against strains that express fHBP variants that are homologous to the fHBP variant in the multi-component composition and an immune response against strains that express fHBP variants that

are heterologous to the fHBP variant in the multi-component composition. In one embodiment, the composition includes any fHBP, such as, for example, any one of the following polypeptides: B24, B16, B44, A22, B03, B09, A12, A19, A05, A07, A06, A15, A29, B01, A62, B15, and any combination thereof. Preferably, the composition includes a combination of A05 and B01 polypeptides. In another preferred embodiment, the composition includes a combination of B24 and A05 polypeptides. In another embodiment, the composition includes a combination of A05, A12, B09, and B44 polypeptides. In one embodiment, the composition includes a lipidated fHBP. In one embodiment, the composition does not include a non-lipidated fHBP.

In another embodiment, the composition includes a non-lipidated fHBP, such as any one of the non-lipidated fHBP described in International Patent Publication No. WO2012/032489, US Patent Publication No. US20120093852, International Patent Publication No. WO2013/132452, and US Patent Publication No. US20160030543, which are each incorporated herein by reference in their entirety. In one embodiment, the composition includes at least one non-lipidated fHBP and at least one lipidated fHBP.

In some embodiments, the composition includes a polypeptide having at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.9% identity to the amino acid sequence set forth in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, and SEQ ID NO: 62.

The inventors further surprisingly discovered that a liquid MnB bivalent rLP2086 composition can readily reconstitute a lyophilized MenACWY-TT composition and that the combined composition is compatible and stable.

In one aspect, the invention relates to a composition against *Neisseria meningitidis*. The composition includes (a) a first lipidated polypeptide including the amino acid sequence set forth in SEQ ID NO: 1; (b) a second lipidated polypeptide including the amino acid sequence set forth in SEQ ID NO: 2; (c) a *Neisseria*

meningitidis serogroup A (MenA) capsular saccharide conjugated to an adipic acid dihydrazide (ADH) linker by 1-cyano-4-dimethylamino pyridinium tetrafluoroborate chemistry, wherein the linker is conjugated to tetanus toxoid carrier protein (TT) by carbodiimide chemistry ($MenA_{AH}$-TT conjugate); (d) a *Neisseria meningitidis* serogroup C (MenC) capsular saccharide conjugated to an ADH linker by 1-cyano-4-dimethylamino pyridinium tetrafluoroborate chemistry, wherein the linker is conjugated to tetanus toxoid carrier protein (TT) by carbodiimide chemistry ($MenC_{AH}$-TT conjugate); (e) a *Neisseria meningitidis* serogroup W_{135} (MenW) capsular saccharide directly conjugated to tetanus toxoid carrier protein (TT) by 1-cyano-4-dimethylamino pyridinium tetrafluoroborate chemistry, in the absence of a linker (MenW-TT conjugate); (f) a *Neisseria meningitidis* serogroup Y (MenY) capsular saccharide directly conjugated to tetanus toxoid carrier protein (TT) by 1-cyano-4-dimethylamino pyridinium tetrafluoroborate chemistry, in the absence of a linker (MenY-TT conjugate).

In another aspect, the invention relates to a composition that includes a combination of a MnB bivalent rLP2086 composition and a MenACWY-TT composition. The **MnB bivalent rLP2086 composition** refers to a composition that includes a single *N. meningitidis* polypeptide component that induces an effective broadly protective immune response against multiple strains of *N. meningitidis* serogroup B. Specifically, in one embodiment, the MnB bivalent rLP2086 composition includes a MnB rLP2086 subfamily A protein (SEQ ID NO: 1) and MnB rLP2086 subfamily B protein (SEQ ID NO: 2). In one embodiment, the composition does not include a fusion protein. In one embodiment, the composition does not include a chimeric protein. In one embodiment, the composition does not include a hybrid protein. In one embodiment, the composition does not further include a peptide fragment. In another embodiment, the composition does not further include a Neisserial polypeptide that is not fHBP. For example, in one embodiment, the composition does not include a PorA protein. In another embodiment, the composition does not include a NadA protein. In another embodiment, the composition does not further include a Neisserial heparin binding antigen (NHBA). In another embodiment, the composition does not further include a Neisserial outer membrane vesicle (OMV). In a preferred embodiment, the composition does not further include antigens, other than the first polypeptide and the second polypeptide. In a preferred embodiment, the MnB bivalent rLP2086 composition further includes polysorbate-80. In one embodiment, the MnB bivalent rLP2086 composition further includes histidine buffer. In one embodiment, the MnB bivalent rLP2086 composition further includes sodium chloride. In one embodiment, the MnB bivalent rLP2086 composition further includes aluminum phosphate. In one embodiment, the MnB bivalent rLP2086 composition further includes polysorbate-80, histidine buffer, sodium

chloride, and aluminum phosphate. Preferably, the MnB bivalent rLP2086 composition is a liquid formulation, wherein the polypeptides are formulated as120 mcg/mL/subfamily in 10 mM histidine buffer, pH 6.0, 150 mM sodium chloride (NaCl) with 0.5 mg/mL aluminum phosphate ($AlPO_4$), and further includes 0.018 mg polysorbate-80 in a 0.5 mL dose.

The MenACWY-TT composition refers to a composition that includes purified capsular polysaccharides of *Neisseria meningitidis* Serogroup A, C, W-135 and Y, each independently conjugated to TT at ratios (TT to polysaccharide) of ~3, ~3, ~1.5 and ~1.3, respectively. Specifically, the composition includes (c) a *Neisseria meningitidis* serogroup A (MenA) capsular saccharide conjugated to an adipic acid dihydrazide (ADH) linker by 1-cyano-4-dimethylamino pyridinium tetrafluoroborate chemistry, wherein the linker is conjugated to tetanus toxoid carrier protein (TT) by carbodiimide chemistry ($MenA_{AH}$-TT conjugate); (d) a *Neisseria meningitidis* serogroup C (MenC) capsular saccharide conjugated to an ADH linker by 1-cyano-4-dimethylamino pyridinium tetrafluoroborate chemistry, wherein the linker is conjugated to tetanus toxoid carrier protein (TT) by carbodiimide chemistry ($MenC_{AH}$-TT conjugate); (e) a *Neisseria meningitidis* serogroup W_{135} (MenW) capsular saccharide directly conjugated to tetanus toxoid carrier protein (TT) by 1-cyano-4-dimethylamino pyridinium tetrafluoroborate chemistry, in the absence of a linker (MenW-TT conjugate); (f) a *Neisseria meningitidis* serogroup Y (MenY) capsular saccharide directly conjugated to tetanus toxoid carrier protein (TT) by 1-cyano-4-dimethylamino pyridinium tetrafluoroborate chemistry, in the absence of a linker (MenY-TT conjugate). Preferably, the MenACWY-TT composition is presented as a lyophilized powder.

$MenA_{AH}$-TT, $MenC_{AH}$-TT, MenW-TT, and MenY-TT conjugates are prepared through the following steps: manufacture of the polysaccharide drug substance intermediate, manufacture of the TT drug substance intermediate, microfluidization of the polysaccharide, derivatization of the polysaccharide (for the MenAAH-TT and MenCAH-TT processes only), additional purification of the TT, and conjugation of the individual polysaccharides to TT.

Regarding the $MenA_{AH}$-TT conjugate, the MenA polysaccharide is first microfluidized to reduce molecular size and viscosity, then activated via cyanylation with 1-cyano-4-dimethylamino-pyridinium tetrafluoroborate (CDAP). Activated MenA is derivatized with adipic acid dihydrazide (ADH) to form the $MenA_{AH}$. $MenA_{AH}$ and Tetanus Toxoid (TT) are coupled through carbodiimide-mediated condensation (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC) coupling technology) to form $MenA_{AH}$-Tetanus Toxoid Conjugate ($MenA_{AH}$-TT).

Regarding the $MenC_{AH}$-TT conjugate, the MenC polysaccharide is first microfluidized to reduce molecular size and viscosity, then activated via cyanylation with CDAP. Activated MenC is derivatized with adipic acid dihydrazide (ADH) to form the $MenC_{AH}$. $MenC_{AH}$ and TT are coupled through carbodiimide-mediated condensation EDAC coupling technology) to form $MenC_{AH}$-Tetanus Toxoid ($MenC_{AH}$-TT).

Regarding the MenW-TT conjugate, MenW polysaccharide is first microfluidized to reduce molecular size and viscosity, then activated via cyanylation with CDAP. Activated MenW is directly coupled to TT to form MenW-Tetanus Toxoid (MenW-TT).

Regarding the MenY-TT conjugate, MenY polysaccharide is first microfluidized to reduce molecular size and viscosity, then activated via cyanylation with CDAP. Activated MenY is directly coupled to TT to form MenY-Tetanus Toxoid (MenY-TT).

In another aspect, the inventors surprisingly discovered that polypeptide antigens derived from at most two *N. meningitidis* serogroup B strains induces an effective broadly protective immune response against multiple strains of *N. meningitidis* serogroup B. Accordingly, in one embodiment, the composition does not further include a polypeptide that is not derived from *N. meningitidis* serogroup B fHBP subfamily A M98250771 strain and/or *N. meningitidis* serogroup B fHBP subfamily B CDC1573 strain.

In one embodiment, the composition does not further include a polypeptide having less than 100% sequence identity to SEQ ID NO: 1. In another embodiment, the composition does not further include a polypeptide having less than 100% sequence identity to SEQ ID NO: 2. For example, the composition does not further include a polypeptide having less than 100% sequence identity to the full length of SEQ ID NO: 1 and/or SEQ ID NO: 2.

In one embodiment, the composition further includes polysorbate-80, aluminum, histidine, and sodium chloride. In one embodiment, the composition includes about 60 µg of a first lipidated polypeptide including the amino acid sequence set forth in SEQ ID NO: 1, about 60 µg of a second lipidated polypeptide including the amino acid sequence set forth in SEQ ID NO: 2, 2.8 molar ratio of polysorbate-80 to each polypeptide, 0.5 mg aluminum/ml as aluminum phosphate, 10 mM histidine, and 150 mM sodium chloride, wherein the composition preferably has a total volume of about 0.5 ml.

In another aspect, the composition includes about 120 µg/ml of a first lipidated polypeptide including the amino acid sequence set forth in SEQ ID NO: 1, about 120 µg/ml of a second lipidated polypeptide including the amino acid sequence set forth in SEQ ID NO: 2, 2.8 molar ratio of polysorbate-80 to each polypeptide, 0.5 mg aluminum/ml as aluminum phosphate, 10 mM histidine, and 150 mM sodium chloride.

In a further aspect, the composition includes a) 60 µg of a first lipidated polypeptide including the amino acid sequence set forth in SEQ ID NO: 1; b) 60 µg of a second lipidated polypeptide including the amino acid sequence set forth in SEQ ID NO: 2; c) 18 µg polysorbate-80 ; d) 250 µg aluminum,; e) 780 µg histidine, and; f) 4380 µg sodium chloride.

In an exemplary embodiment, the composition includes about 60 µg of a first lipidated polypeptide consisting of the amino acid sequence set forth in SEQ ID NO: 1, about 60 µg of a second lipidated polypeptide consisting of the amino acid sequence set forth in SEQ ID NO: 2, 2.8 molar ratio of polysorbate-80 to first lipidated polypeptide and to second lipidated polypeptide, 0.5 mg/ml aluminum phosphate, 10 mM histidine, and 150 mM sodium chloride, wherein the composition has a total volume of about 0.5 ml. In the exemplary embodiment, the composition is a sterile isotonic buffered liquid suspension. In the exemplary embodiment, the composition has a pH 6.0. In the exemplary embodiment, the first polypeptide and the second polypeptide are adsorbed to aluminum.

In one embodiment, the composition includes a $MenA_{AH}$-TT conjugate having a mean TT/polysaccharide ratio 3; a $MenC_{AH}$-TT conjugate having a mean TT/polysaccharide ratio 3; a MenW-TT conjugate having a mean TT/polysaccharide ratio 1.5; and a MenY-TT conjugate having a mean TT/polysaccharide ratio 1.3. In a preferred embodiment, the composition includes a $MenA_{AH}$-TT conjugate having 5 mcg MenA polysaccharide and ~ 15 mcg TT; a $MenC_{AH}$-TT conjugate having 5 mcg MenC polysaccharide and ~ 15 mcg TT; a MenW-TT conjugate having 5 mcg MenW polysaccharide and ~ 7.5 mcg TT; and a MenY-TT conjugate having 5 mcg MenY polysaccharide and ~ 6.5 mcg TT. The composition may further include Tris-HCl, sucrose, and sodium chloride.

In another embodiment, the composition includes a $MenA_{AH}$-TT conjugate; $MenC_{AH}$-TT conjugate; MenW-TT conjugate; and MenY-TT conjugate, which includes MenA polysaccharide; MenC polysaccharide; MenW polysaccharide; and MenY polysaccharide and TT carrier protein. The composition may further include sucrose and Trometanol. For example, in one embodiment, the composition includes 10 µg/mL MenA polysaccharide; 10 µg/mL MenC polysaccharide; 10 µg/mL MenW polysaccharide; and 10 µg/mL MenY polysaccharide; 88 µg/mL TT carrier protein; 164 mM sucrose; and 1.6 mM Trometanol.

In one embodiment, the composition has a total volume of about 0.5 ml. In one embodiment, a first dose of the composition has a total volume of about 0.5 ml. A “first dose” refers to the dose of the composition that is administered on Day 0. A “second dose” or “third dose” refers to the dose of the composition that is administered

subsequently to the first dose, which may or may not be the same amount as the first dose.

In one aspect, the invention relates to a liquid immunogenic composition resulting from the lyophilized MenACWY-TT composition having been reconstituted with the liquid MnB bivalent rLP2086 composition. Reconstitution refers to restoring a dry lyophilized composition to a liquid form by the addition of a liquid diluent. In one preferred embodiment, the liquid MnB bivalent rLP2086 composition is not concomitantly administered, is not coadministered with, and is not simultaneously administered with the lyophilized MenACWY-TT composition, wherein the lyophilized MenACWY-TT composition has been reconstituted with a liquid composition that is not the liquid MnB bivalent rLP2086 composition. For example, in one preferred embombodiment, the lyophilized MenACWY-TT composition is not reconstituted with an aqueous diluent consisting of sodium chloride and water and is not subsequently concomitantly administered, is not coadministered with, and is not simultaneously administered with with the liquid MnB bivalent rLP2086 composition.

Rather, in a preferred embodiment, the lyophilized MenACWY-TT composition is administered with the MnB bivalent rLP2086 composition in one, i.e., a single, administration to the human. The resulting single administration (e.g., the MenABCWY composition) may result from the MnB bivalent rLP2086 composition, from a first container, being mixed with the lyophilized MenACWY-TT composition, from a second container. Alternatively, single administration of the MenABCWY composition may result from one (single) container that includes the MnB bivalent rLP2086 composition and the lyophilized MenACWY-TT composition. Delivery devices for vaccine or immunogenic compositions are known in the art. In one embodiment, the MenABCWY composition is administered concomitantly with any one of ibuprofen, paracetamol, and amoxicillin.

The composition is immunogenic after administration of a first dose to a human. In one embodiment, the first dose is about 0.5 ml in total volume.

The composition induces a bactericidal titer of serum immunoglobulin that is at least greater than 1-fold higher, preferably at least 2-fold higher, in the human after receiving the first dose than a bactericidal titer of serum immunoglobulin in the human prior to receiving the first dose, when measured under identical conditions in a serum bactericidal assay using human complement (hSBA).

The bactericidal titer or bactericidal immune response is against *N. meningitidis* serogroup B. In a preferred embodiment, the bactericidal titer or bactericidal immune response is against a *N. meningitidis* serogroup B fHBP subfamily A strain and against a *N. meningitidis* serogroup B fHBP subfamily B strain. Most preferably, the bactericidal

titer or bactericidal immune response is at least against *N. meningitidis* serogroup B, fHBP subfamily B, B01 strain.

In one embodiment, the composition induces a bactericidal titer of serum immunoglobulin that is at least greater than 1-fold, such as, for example, at least 1.01-fold, 1.1-fold, 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 11-fold, 12-fold, 13-fold, 14-fold, 15-fold, or 16-fold higher in the human after receiving a dose of the composition than a bactericidal titer of serum immunoglobulin in the human prior to receiving said dose, when measured under identical conditions in a serum bactericidal assay using human complement.

In one embodiment, the composition is an immunogenic composition. In one embodiment, the composition is an immunogenic composition for a human. In another embodiment, the composition is a vaccine. A "vaccine" refers to a composition that includes an antigen, which contains at least one epitope that induces an immune response that is specific for that antigen. The vaccine may be administered directly into the subject by subcutaneous, oral, oronasal, or intranasal routes of administration. Preferably, the vaccine is administered intramuscularly. In one embodiment, the composition is a human vaccine. In one embodiment, the composition is an immunogenic composition against *N. meningitidis*.

In one embodiment, the composition is a liquid composition. In a preferred embodiment, the composition is a liquid suspension composition. In another preferred embodiment, the composition is not lyophilized.

FIRST POLYPEPTIDE; MNB RLP2086 SUBFAMILY A (A05) PROTEIN

In one embodiment, the composition includes a first polypeptide having the amino acid sequence set forth in SEQ ID NO: 1. The polypeptide is a modified factor H binding protein (fHBP) from *N. meningitidis* strain M98250771. A description of fHBP is disclosed in WO2012032489 and US patent publication US 2012/0093852, which are each incorporated by reference in their entirety. The polypeptide is N-terminally lipidated with three predominant fatty acids C16:0, C16:1, and C18:1 covalently linked at three positions of the polypeptide. The first polypeptide includes a total of 258 amino acids.

The representative primary structure of the MnB rLP2086 A05 protein is presented in FIG. 4. The primary structure of the protein is illustrated in FIG. 4 using a single letter notation for all amino acids except for the N-terminal cysteine and glyceryl moieties (illustrated using full chemical formula). This structure includes the primary structure of the protein sequence in which the N-terminal cysteine residue is lipidated. The amino group of the N-terminal cysteine residue at the protein N-terminus is attached to a fatty acid (R1) forming an amide linkage and the cysteinyl sulfhydryl group is

attached to a glycerol moiety containing two ester-bound fatty acids (R2). The structure of R1 is deduced to be hexadecanoic acid (C16:0) and the structures of R2 vary depending on the MnB rLP2086 isoforms.

The first polypeptide includes two modifications introduced in the N-terminal region of the polypeptide, as compared to the corresponding wild-type sequence from *N. meningitidis* strain M98250771. A glycine in the second position is added as a consequence of introducing a cloning site. A second modification includes the deletion of four amino acids. Accordingly, in one embodiment, the first polypeptide includes a C-G-S-S sequence (SEQ ID NO: 3) at the N-terminus. See SEQ ID NO: 1, first four amino acid residues.

The N-terminal differences between the first polypeptide sequence and the wild-type Neisserial sequence is shown below. Accordingly, in one embodiment, the first polypeptide includes at least the first 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or more amino acid residues of the amino acid sequence set forth in SEQ ID NO: 1. Preferably, the first polypeptide includes at least the first 4, more preferably at least the first 6, and most preferably, at least the first 8 amino acid residues of SEQ ID NO: 1.

Comparison of Predicted N-Terminal Sequences of Recombinant and Neisserial Subfamily A LP2086 Polypeptide

rLP2086 M98250771	CGSS-----GGGGVAAD	(SEQ ID NO: 4)
Neisserial LP2086 M98250771	C-SSGS-GSGGGGVAAD	(SEQ ID NO: 5)

>A05 (SEQ ID NO: 1)
CGSSGGGGVAADIGTGLADALTAPLDHKDKGLKSLTLEDSISQNGTLTLSAQGAEKTFK
VGDKDNSLNTGKLKNDKISRFDFVQKIEVDGQTITLASGEFQIYKQDHSAVVALQIEKINN
PDKIDSLINQRSFLVSGLGGEHTAFNQLPSGKAEYHGKAFSSDDAGGKLTYTIDFAAKQ
GHGKIEHLKTPEQNVELASAELKADEKSHAVILGDTRYGSEEKGTYHLALFGDRAQEIA
GSATVKIREKVHEIGIAGKQ

In one embodiment, the first polypeptide includes the amino acid sequence set forth in SEQ ID NO: 1. In one embodiment, the first polypeptide has a total of 258 amino acids. In one embodiment, the first polypeptide does not include an amino acid sequence having less than 100% sequence identity to SEQ ID NO: 1. In another embodiment, the first polypeptide consists of the amino acid sequence set forth in SEQ ID NO: 1. In another embodiment, the first polypeptide includes the amino acid sequence KDN. See for example, amino acid residues 73-75 of SEQ ID NO: 1. In another embodiment, the first polypeptide includes the amino acid sequence set forth in SEQ ID NO: 3 at the N-terminus of the polypeptide. In another embodiment, the first

polypeptide includes the amino acid sequence set forth in SEQ ID NO: 4 at the N-terminus of the polypeptide.

In a preferred embodiment, the first polypeptide is readily expressed in a recombinant host cell using standard techniques known in the art. In another preferred embodiment, the first polypeptide includes a bactericidal epitope on the N- and/or C-domain of SEQ ID NO: 1. In one embodiment, the first polypeptide includes at least the first 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 amino acid residues of the amino acid sequence set forth in SEQ ID NO: 1. Preferably, the first polypeptide includes at least the first 2, more preferably at least the first 4, and most preferably, at least the first 8 amino acid residues of SEQ ID NO: 1.

In another embodiment, the first polypeptide includes at least the last 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 amino acid residues of the amino acid sequence set forth in SEQ ID NO: 1.

In one embodiment, the composition includes about 30 µg/ml of a first polypeptide including the amino acid sequence set forth in SEQ ID NO: 1. In one preferred embodiment, the composition includes about 60 µg of a first polypeptide including the amino acid sequence set forth in SEQ ID NO: 1. In one preferred embodiment, the composition includes about 60 µg of a first polypeptide including the amino acid sequence set forth in SEQ ID NO: 1, wherein the composition preferably has a total volume of 0.5 ml. In another embodiment, the composition includes about 120 µg/ml of a first polypeptide including the amino acid sequence set forth in SEQ ID NO: 1.

SECOND POLYPEPTIDE; MNB RLP2086 SUBFAMILY B (B01) PROTEIN

In one embodiment, the composition includes a second polypeptide having the amino acid sequence set forth in SEQ ID NO: 2. The polypeptide is a factor H binding protein (fHBP) from *N. meningitidis* strain CDC1573. A description of fHBP is disclosed in WO2012032489 and US patent publication US 2012/0093852, which are each incorporated by reference in their entirety. The polypeptide is N-terminally lipidated with three predominant fatty acids C16:0, C16:1, and C18:1 covalently linked at three positions of the polypeptide. The second polypeptide includes a total of 261 amino acids.

The representative primary structure of the MnB rLP2086 B01 protein is presented in FIG. 5. The primary structure of the protein is illustrated in FIG. 5 using a single letter notation for all amino acids except for the N-terminal cysteine and glyceryl moieties (illustrated using full chemical formula). This structure includes the primary structure of the protein sequence in which the N-terminal cysteine residue is lipidated. The amino group of the N-terminal cysteine residue at the protein N-terminus is attached to a fatty acid (R1) forming an amide linkage and the cysteinyl sulfhydryl group is attached to a glycerol moiety containing two-ester bound fatty acids (R2). The structure of R1 is deduced to be hexadecanoic acid (C16:0) and the structures of R2 vary depending on the rLP2086 isoforms.

The second polypeptide includes one modification introduced in the N-terminal region for the rLP2086 subfamily B protein, as compared to the corresponding wild-type sequence from *N. meningitidis* strain CDC-1573. A glycine in the second position is a consequence of introducing a cloning site.

The N-terminal differences from the original Neisserial sequences are shown below.

Comparison of Predicted N-Terminal Sequences of Recombinant and Neisserial Subfamily B LP2086 Protein

rLP2086 CDC-1573 CGSSGGGGSGGGGVTAD (SEQ ID NO: 24)

Neisserial LP2086 CDC-1573 C-SSGGGGSGGGGVTAD (SEQ ID NO: 25)

In one embodiment, the second polypeptide includes a C-G-S-S sequence (SEQ ID NO: 3) at the N-terminus. See the first four amino acid residues of SEQ ID NO: 2.

>B01 (SEQ ID NO: 2)

CGSSGGGGSGGGGVTADIGTGLADALTAPLDHKDKGLKSLTLEDSISQNGTLTLSAQG
AEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQLITLESGEFQVYKQSHSALTALQT
EQEQDPEHSEKMVAKRRFRIGDIAGEHTSFDKLPKDVMATYRGTAFGSDDAGGKLTYTI
DFAAKQGHGKIEHLKSPELNVDLAVAYIKPDEKHHAVISGSVLYNQDEKGSYSLGIFGEK
AQEVAGSAEVETANGIHHIGLAAKQ

In one embodiment, the second polypeptide includes the amino acid sequence set forth in SEQ ID NO: 2. In one embodiment, the second polypeptide has a total of 261 amino acids. In one embodiment, the second polypeptide consists of the amino acid sequence set forth in SEQ ID NO: 2. In another embodiment, the second polypeptide does not further include a polypeptide having less than 100% sequence identity to SEQ ID NO: 2. In a preferred embodiment, the first polypeptide and the second polypeptide includes a C-G-S-S (SEQ ID NO: 3) sequence at the N-terminus of the respective polypeptide.

In a preferred embodiment, the second polypeptide is readily expressed in a recombinant host cell using standard techniques known in the art. In another preferred embodiment, the second polypeptide includes a bactericidal epitope on the N- and/or C-domain of SEQ ID NO: 2. In one embodiment, the second polypeptide includes at least the first 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 amino acid residues of the amino acid sequence set forth in SEQ ID NO: 2. Preferably, the second polypeptide includes at least the first 2, more preferably at least the first 4, and most preferably, at least the first 8 amino acid residues of SEQ ID NO: 2.

In another embodiment, the second polypeptide includes at least the last 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76,

77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 amino acid residues of the amino acid sequence set forth in SEQ ID NO: 2.

In one embodiment, the composition includes about 30 µg/ml of a first polypeptide including the amino acid sequence set forth in SEQ ID NO: 2. In one preferred embodiment, the composition includes about 60 µg of a first polypeptide including the amino acid sequence set forth in SEQ ID NO: 2. In one preferred embodiment, the composition includes about 60 µg of a second polypeptide including the amino acid sequence set forth in SEQ ID NO: 2, wherein the composition preferably has a total volume of 0.5 ml. In another embodiment, the composition includes 120 µg/ml of a second polypeptide including the amino acid sequence set forth in SEQ ID NO: 2.

SACCHARIDES

The term "saccharide" throughout this specification may indicate polysaccharide or oligosaccharide and includes both. Polysaccharides are isolated from bacteria or isolated from bacteria and sized to some degree by known methods and optionally by microfluidisation. Polysaccharides can be sized in order to reduce viscosity in polysaccharide samples and/or to improve filterability for conjugated products. Oligosaccharides have a low number of repeat units (typically 5-30 repeat units) and are typically hydrolysed polysaccharides.

Each *N. meningitidis* capsular saccharide may be conjugated to a carrier protein independently selected from the group consisting of TT, DT, CRM197, fragment C of TT and protein D. Although one or more *N. meningitidis* capsular saccharide may be conjugated to different carrier proteins from the others, in one embodiment they are all conjugated to the same carrier protein. For instance they may all be conjugated to the same carrier protein selected from the group consisting of TT, DT, CRM197, fragment C of TT and protein D. In this context CRM197 and DT may be considered to be the same carrier protein as they differ by only one amino acid. In a preferred embodiment all the *N. meningitidis* capsular saccharides present are conjugated to TT.

If the protein carrier is the same for 2 or more saccharides in the composition, the saccharide could be conjugated to the same molecule of the protein carrier (carrier molecules having 2 more different saccharides conjugated to it) [see for instance WO 04/083251; for example, a single carrier protein might be conjugated to MenA and MenC; MenA and MenW; MenA and MenY; MenC and MenW; MenC and MenY; Men W and MenY; MenA, MenC and MenW; MenA, MenC and MenY; MenA, MenW and MenY; MenC, MenW and MenY; MenA, MenC, MenW and MenY. Alternatively the saccharides may each be separately conjugated to different molecules of the protein carrier (each molecule of protein carrier only having one type of saccharide conjugated to it).

In one embodiment, at least 2 different saccharide conjugates are conjugated separately to the same type of carrier protein, wherein one or more saccharide(s) is/are conjugated to the carrier protein via a first type of chemical group on the protein carrier, and one or more saccharide(s) is/are conjugated to the carrier protein via a second (different) type of chemical group on the protein carrier.

In one embodiment the 2 conjugates involve the same saccharide linked to the same carrier, but by different conjugation chemistries. In an alternative embodiment 2 different saccharides are conjugated to different groups on the protein carrier.

By “conjugated separately to the same type of carrier protein” it is meant that the saccharides are conjugated to the same carrier individually (for example, MenA is conjugated to tetanus toxoid through an amine group on the tetanus toxoid and MenC is conjugated to tetanus toxoid through a carboxylic acid group on a different molecule of tetanus toxoid.)

The capsular saccharide(s) may be conjugated to the same carrier protein independently selected from the group consisting of TT, DT, CRM197, fragment C of TT and protein D. A more complete list of protein carriers that may be used in the conjugates of the invention is presented below. In this context CRM197 and DT may be considered to be the same carrier protein as they differ by only one amino acid. In an embodiment all the capsular saccharides present are conjugated to TT.

The saccharides may include any one of: *N. meningitidis* serogroup A capsular saccharide (MenA), *N. meningitidis* serogroup C capsular saccharide (MenC), *N. meningitidis* serogroup Y capsular saccharide (MenY), and *N. meningitidis* serogroup W capsular saccharide (MenW), or any combination thereof.

The first and second chemical groups present on the protein carrier are different from each other and are ideally natural chemical groups that may be readily used for conjugation purposes. They may be selected independently from the group consisting of: carboxyl groups, amino groups, sulphydryl groups, Hydroxyl groups, Imidazolyl groups, Guanidyl groups, and Indolyl groups. In one embodiment the first chemical group is carboxyl and the second is amino, or vice versa. These groups are explained in greater detail below.

In a specific embodiment the immunogenic composition comprises at least 2 different *N. meningitidis* capsular saccharides, wherein one or more is/are selected from a first group consisting of MenA and MenC which is/are conjugated to the carrier protein via the first type of chemical group on the protein carrier (for instance carboxyl), and one or more different saccharides is/are selected from a second group consisting of MenC, MenY and MenW which is/are conjugated to the carrier protein via the second type of chemical group on the protein carrier (for instance amino).

In a further embodiment the immunogenic composition of the invention comprises MenA conjugated via the first type of chemical group (for instance carboxyl), and MenC conjugated via the second type of chemical group (for instance amino).

In another embodiment the immunogenic composition comprises MenC conjugated via the first type of chemical group (for instance carboxyl), and MenY conjugated via the second type of chemical group (for instance amino).

In another embodiment the immunogenic composition comprises MenA conjugated via the first type of chemical group (for instance carboxyl), and MenC, MenY and MenW conjugated via the second type of chemical group (for instance amino).

In another embodiment the immunogenic composition comprises MenA and MenC conjugated via the first type of chemical group (for instance carboxyl), and MenY and MenW conjugated via the second type of chemical group (for instance amino).

The saccharides of the invention included in pharmaceutical (immunogenic) compositions of the invention are conjugated to a carrier protein such as tetanus toxoid (TT), tetanus toxoid fragment C, non-toxic mutants of tetanus toxin [note all such variants of TT are considered to be the same type of carrier protein for the purposes of this invention], diphtheria toxoid (DT), CRM197, other non-toxic mutants of diphtheria toxin [such as CRM176, CRM 197, CRM228, CRM 45 (Uchida et al J. Biol. Chem. 218; 3838-3844, 1973); CRM 9, CRM 45, CRM102, CRM 103 and CRM107 and other mutations described by Nicholls and Youle in Genetically Engineered Toxins, Ed: Frankel, Maecel Dekker Inc, 1992; deletion or mutation of Glu-148 to Asp, Gln or Ser and/or Ala 158 to Gly and other mutations disclosed in US 4709017 or US 4950740; mutation of at least one or more residues Lys 516, Lys 526, Phe 530 and/or Lys 534 and other mutations disclosed in US 5917017 or US 6455673; or fragment disclosed in US 5843711] (note all such variants of DT are considered to be the same type of carrier protein for the purposes of this invention), pneumococcal pneumolysin (Kuo et al (1995) Infect Immun 63; 2706-13), OMPC (meningococcal outer membrane protein – usually extracted from *N. meningitidis* serogroup B – EP0372501), synthetic peptides (EP0378881, EP0427347), heat shock proteins (WO 93/17712, WO 94/03208), pertussis proteins (WO 98/58668, EP0471177), cytokines, lymphokines, growth factors or hormones (WO 91/01146), artificial proteins comprising multiple human CD4+ T cell epitopes from various pathogen derived antigens (Falugi et al (2001) Eur J Immunol 31; 3816-3824) such as N19 protein (Baraldoi et al (2004) Infect Immun 72; 4884-7) pneumococcal surface protein PspA (WO 02/091998), iron uptake proteins (WO 01/72337), toxin A or B of *C. difficile* (WO 00/61761) or Protein D (EP594610 and WO 00/56360).

In an embodiment, the immunogenic composition of the invention uses the same type of carrier protein (independently) in at least two, three, four or each of the saccharides contained therein.

In an embodiment, the immunogenic composition of the invention comprises a *N. meningitidis* saccharide conjugated to a carrier protein selected from the group consisting of TT, DT, CRM197, fragment C of TT and protein D.

The immunogenic composition of the invention optionally comprises at least one meningococcal saccharide (for example MenA; MenC; MenW; MenY; MenA and MenC;

MenA and MenW; MenA and MenY; MenC and Men W; Men C and MenY; Men W and MenY; MenA, MenC and MenW; MenA, MenC and MenY; MenA, MenW and MenY; MenC, MenW and MenY or MenA, MenC, MenW and MenY) conjugate having a ratio of Men saccharide to carrier protein of between 1:5 and 5:1, between 1:2 and 5:1, between 1:0.5 and 1:2.5 or between 1:1.25 and 1:2.5(w/w). In one preferred embodiment, the composition includes MenA, MenC, MenW and MenY each conjugated to tetanus toxoid at ratios (toxoid to polysaccharide) of about 3, about 3, about 1.5 and about 1.3, respectively.

The ratio of saccharide to carrier protein (w/w) in a conjugate may be determined using the sterilized conjugate. The amount of protein is determined using a Lowry assay (for example Lowry et al (1951) J. Biol. Chem. 193, 265-275 or Peterson et al Analytical Biochemistry 100, 201-220 (1979)) and the amount of saccharide is determined using ICP-OES (inductively coupled plasma-optical emission spectroscopy) for MenA, DMAP assay for MenC and Resorcinol assay for MenW and MenY (Monsigny et al (1988) Anal. Biochem. 175, 525-530).

In an embodiment, the immunogenic composition of the invention comprises *N. meningitidis* saccharide conjugate(s) wherein the *N. meningitidis* saccharide(s) is conjugated to the carrier protein via a linker, for instance a bifunctional linker. The linker is optionally heterobifunctional or homobifunctional, having for example a reactive amino group and a reactive carboxylic acid group, 2 reactive amino groups or two reactive carboxylic acid groups. The linker has for example between 4 and 20, 4 and 12, 5 and 10 carbon atoms. A possible linker is ADH. Other linkers include B-propionamido (WO 00/10599), nitrophenyl-ethylamine (Gever et al (1979) Med. Microbiol. Immunol. 165; 171-288), haloalkyl halides (US4057685), glycosidic linkages (US4673574, US4808700), hexane diamine and 6-aminocaproic acid (US4459286).

The saccharide conjugates present in the immunogenic compositions of the invention may be prepared by any known coupling technique. The conjugation method may rely on activation of the saccharide with 1-cyano-4-dimethylamino pyridinium tetrafluoroborate (CDAP) to form a cyanate ester. The activated saccharide may thus be coupled directly or via a spacer (linker) group to an amino group on the carrier protein. For example, the spacer could be cystamine or cysteamine to give a thiolated polysaccharide which could be coupled to the carrier via a thioether linkage obtained after reaction with a maleimide-activated carrier protein (for example using GMBS) or a holoacetylated carrier protein (for example using iodoacetimide or N-succinimidyl bromoacetatebromoacetate). Optionally, the cyanate ester (optionally made by CDAP chemistry) is coupled with hexane diamine or ADH and the amino-derivatised saccharide is conjugated to the carrier protein using using carbodiimide (e.g. EDAC or EDC)

chemistry via a carboxyl group on the protein carrier. Such conjugates are described in PCT published application WO 93/15760 Uniformed Services University and WO 95/08348 and WO 96/29094.

Other suitable techniques use carbiinides, hydrazides, active esters, norborane, p-nitrobenzoic acid, N-hydroxysuccinimide, S-NHS, EDC, TSTU. Many are described in WO 98/42721. Conjugation may involve a carbonyl linker which may be formed by reaction of a free hydroxyl group of the saccharide with CDI (Bethell et al J. Biol. Chem. 1979, 254; 2572-4, Hearn et al J. Chromatogr. 1981. 218; 509-18) followed by reaction of with a protein to form a carbamate linkage. This may involve reduction of the anomeric terminus to a primary hydroxyl group, optional protection/deprotection of the primary hydroxyl group' reaction of the primary hydroxyl group with CDI to form a CDI carbamate intermediate and coupling the CDI carbamate intermediate with an amino group on a protein.

The conjugates can also be prepared by direct reductive amination methods as described in US 4365170 (Jennings) and US 4673574 (Anderson). Other methods are described in EP-0-161-188, EP-208375 and EP-0-477508.

A further method involves the coupling of a cyanogen bromide (or CDAP) activated saccharide derivatised with adipic acid hydrazide (ADH) to the protein carrier by Carbodiimide condensation (Chu C. et al Infect. Immunity, 1983 245 256), for example using EDAC.

In an embodiment, a hydroxyl group (optionally an activated hydroxyl group for example a hydroxyl group activated by a cyanate ester) on a saccharide is linked to an amino or carboxylic group on a protein either directly or indirectly (through a linker). Where a linker is present, a hydroxyl group on a saccharide is optionally linked to an amino group on a linker, for example by using CDAP conjugation. A further amino group in the linker for example ADH) may be conjugated to a carboxylic acid group on a protein, for example by using carbodiimide chemistry, for example by using EDAC. In an embodiment, *N. meningitidis* capsular saccharide(s) (or saccharide in general) is conjugated to the linker first before the linker is conjugated to the carrier protein. Alternatively the linker may be conjugated to the carrier before conjugation to the saccharide.

In general the following types of chemical groups on a protein carrier can be used for coupling / conjugation:

A) Carboxyl (for instance via aspartic acid or glutamic acid). In one embodiment this group is linked to amino groups on saccharides directly or to an amino group on a linker with carbodiimide chemistry e.g. with EDAC.

B) Amino group (for instance via lysine). In one embodiment this group is linked to carboxyl groups on saccharides directly or to a carboxyl group on a linker with carbodiimide chemistry e.g. with EDAC. In another embodiment this group is linked to hydroxyl groups activated with CDAP or CNBr on saccharides directly or to such groups on a linker; to saccharides or linkers having an aldehyde group; to saccharides or linkers having a succinimide ester group.

C) Sulphydryl (for instance via cysteine). In one embodiment this group is linked to a bromo or chloro acetylated saccharide or linker with maleimide chemistry. In one embodiment this group is activated/modified with bis diazobenzidine.

D) Hydroxyl group (for instance via tyrosine). In one embodiment this group is activated/modified with bis diazobenzidine.

E) Imidazolyl group (for instance via histidine). In one embodiment this group is activated/modified with bis diazobenzidine.

F) Guanidyl group (for instance via arginine).

G) Indolyl group (for instance via tryptophan).

On a saccharide, in general the following groups can be used for a coupling: OH, COOH or NH2. Aldehyde groups can be generated after different treatments known in the art such as: periodate, acid hydrolysis, hydrogen peroxide, etc.

Direct coupling approaches:

Saccharide-OH + CNBr or CDAP -----> cyanate ester + NH2-Prot ----> conjugate

Saccharide-aldehyde + NH2-Prot ----> Schiff base + NaCNBH3 ----> conjugate

Saccharide-COOH + NH2-Prot + EDAC ----> conjugate

Saccharide-NH2 + COOH-Prot + EDAC ----> conjugate

Indirect coupling via spacer (linker) approaches:

Saccharide-OH + CNBr or CDAP ---> cyanate ester + NH2----NH2 ----> saccharide----NH2 + COOH-Prot + EDAC -----> conjugate

Saccharide-OH + CNBr or CDAP ----> cyanate ester + NH2-----SH -----> saccharide----SH + SH-Prot (native Protein with an exposed cysteine or obtained after modification of amino groups of the protein by SPDP for instance) -----> saccharide-S-S-Prot

Saccharide-OH + CNBr or CDAP ---> cyanate ester + NH2----SH -------> saccharide----SH + maleimide-Prot (modification of amino groups) ----> conjugate

Saccharide-COOH + EDAC + NH2-----NH2 ---> saccharide------NH2 + EDAC + COOH-Prot ----> conjugate

Saccharide-COOH + EDAC+ NH2----SH -----> saccharide----SH + SH-Prot (native Protein with an exposed cysteine or obtained after modification of amino groups of the protein by SPDP for instance) -----> saccharide-S-S-Prot

Saccharide-COOH + EDAC+ NH2----SH -----> saccharide----SH + maleimide-Prot (modification of amino groups) ----> conjugate

Saccharide-Aldehyde + NH2-----NH2 ----> saccharide---NH2 + EDAC + COOH-Prot ----> conjugate

Note: instead of EDAC above, any suitable carbodiimide may be used.

In summary, the types of protein carrier chemical group that may be generally used for coupling with a saccharide are amino groups (for instance on lysine residues), COOH groups (for instance on aspartic and glutamic acid residues) and SH groups (if accessible) (for instance on cysteine residues).

In an embodiment, at least one of the *N. meningitidis* capsular saccharides (or saccharide in general) is directly conjugated to a carrier protein; optionally Men W and/or MenY and/or MenC saccharide(s) is directly conjugated to a carrier protein. For example MenW; MenY; MenC; MenW and MenY; MenW and MenC; MenY and MenC; or MenW, MenY and MenC are directly linked to the carrier protein. Optionally, at least one of the *N. meningitidis* capsular saccharides is directly conjugated by CDAP. For example MenW; MenY; MenC; MenW and MenY; MenW and MenC; MenY and MenC; or MenW, MenY and MenC are directly linked to the carrier protein by CDAP (see WO 95/08348 and WO 96/29094). In an embodiment, all *N. meningitidis* capsular saccharides are conjugated to tetanus toxoid.

In an embodiment, the ratio of Men W and/or Y saccharide to carrier protein is between 1:0.5 and 1:2 (w/w) and/or the ratio of MenC saccharide to carrier protein is between 1:0.5 and 1:4 or 1:0.5 and 1:1.5 (w/w), especially where these saccharides are directly linked to the protein, optionally using CDAP.

In an embodiment, at least one of the *N. meningitidis* capsular saccharide(s) (or saccharide in general) is conjugated to the carrier protein via a linker, for instance a bifunctional linker. The linker is optionally heterobifunctional or homobifunctional, having for example a reactive amine group and a reative carboxylic acid group, 2 reactive amine groups or 2 reactive carboxylic acid groups. The linker has for example between 4 and 20, 4 and 12, 5 and 10 carbon atoms. A possible linker is ADH.

In an embodiment, MenA; MenC; or MenA and MenC is conjugated to a carrier protein (for example tetanus toxoid) via a linker.

In an embodiment, at least one *N. meningitidis* saccharide is conjugated to a carrier protein via a linker using CDAP and EDAC. For example, MenA; MenC; or MenA

and MenC are conjugated to a protein via a linker (for example those with two hydrazino groups at its ends such as ADH) using CDAP and EDAC as described above. For example, CDAP is used to conjugate the saccharide to a linker and EDAC is used to conjugate the linker to a protein. Optionally the conjugation via a linker results in a ratio of saccharide to carrier protein of between 1:0.5 and 1:6; 1:1 and 1:5 or 1:2 and 1:4, for MenA; MenC; or MenA and MenC.

In an embodiment, the MenA capsular saccharide, where present is at least partially O-acetylated such that at least 50%, 60%, 70%, 80%, 90%, 95% or 98% of the repeat units are O-acetylated at at least one position. O-acetylation is for example present at least at the O-3 position of at least 50%, 60%, 70%, 80%, 90%, 95% or 98% of the repeat units.

In an embodiment, the MenC capsular saccharide, where present is at least partially O-acetylated such that at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or 98% of (α2 →9)-linked NeuNAc repeat units are O-acetylated at at least one or two positions. O-acetylation is for example present at the O-7 and/or O-8 position of at least 30%. 40%, 50%, 60%, 70%, 80%, 90%, 95% or 98% of the repeat units.

In an embodiment, the MenW capsular saccharide, where present is at least partially O-acetylated such that at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or 98% of the repeat units are O-acetylated at at least one or two positions. O-acetylation is for example present at the O-7 and/or O-9 position of at least 30%. 40%, 50%, 60%, 70%, 80%, 90%, 95% or 98% of the repeat units.

In an embodiment, the MenY capsular saccharide, where present is at least partially O-acetylated such that at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or 98% of the repeat units are O-acetylated at at least one or two positions. O-acetylation is present at the 7 and/or 9 position of at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or 98% of the repeat units.

The percentage of O-acetylation refers to the percentage of the repeat units containing O-acetylation. This may be measured in the saccharide prior to conjugate and/or after conjugation.

In one embodiment of the invention the immunogenic composition, saccharide present, or each *N. meningitidis* capsular saccharide present, is conjugated to TT. In a further embodiment each *N. meningitidis* capsular saccharide is separately conjugated to a separate carrier protein. In a further embodiment each *N. meningitidis* capsular saccharide conjugate has a saccharide:carrier ratio of 1:5-5:1 or 1:1-1:4(w/w). In a further embodiment at least one, two or three *N. meningitidis* capsular saccharide conjugate(s) is directly conjugated to a carrier protein. In a further embodiment Men W and/or MenY, MenW and/or MenC, MenY and/or MenC, or MenW and MenC and MenY

are directly conjugated to a carrier protein. In a further embodiment at least one, two or three *N. meningitidis* saccharide conjugate(s) is directly conjugated by CDAP chemistry. In a further embodiment the ratio of Men W and/or Y saccharide to carrier protein is between 1:0.5 and 1:2 (w/w). In a further embodiment the ratio of MenC saccharide to carrier protein is between 1:0.5 and 1:2 (w/w). In a further embodiment at least one, two or three *N. meningitidis* capsular saccharide(s) are conjugated to the carrier protein via a linker (which may be bifunctional such as having two reactive amino groups (such as ADH) or two reactive carboxyl groups, or a reactive amino group at one end and a reactive carboxyl group at the other). The linker can have between 4 and 12 carbon atoms. In a further embodiment the or each *N. meningitidis* capsular saccharide(s) conjugated via a linker are conjugated to the linker with CDAP chemistry. In a further embodiment the carrier protein is conjugated to the linker using carbodiimide chemistry, for example using EDAC. In a further embodiment the or each *N. meningitidis* capsular saccharide is conjugated to the linker before the carrier protein is conjugated to the linker. In a further embodiment MenA is conjugated to a carrier protein via a linker (the ratio of MenA saccharide to carrier protein may be between 1:2 and 1:5 (w/w)). In a further embodiment MenC is conjugated to a carrier protein via a linker (the ratio of MenC saccharide to carrier protein may be between 1:2 and 1:5 (w/w)).

By using native or slightly sized polysaccharide conjugates, one or more of the following advantages may be realised: 1) a conjugate having high immungenicity which is filterable through a 0.2 micron filter; 2) immune memory may be enhanced (as in example three); 3) the alteration of the ratio of polysaccharide to protein in the conjugate such that the ratio of polysaccharide to protein (w/w) in the conjugate may be increased (this can result in a reduction of the carrier suppression effect); 4) immunogenic conjugates prone to hydrolysis (such as MenA conjugates) may be stabilised by the use of larger polysaccharides for conjugation.The use of larger polysaccharides can result in more cross-linking with the conjugate carrier and may lessen the liberation of free saccharide from the conjugate. The conjugate vaccines described in the prior art tend to depolymerise the polysaccharides prior to conjugation in order to improve conjugation. Meningococcal (or saccharide) conjugate vaccines retaining a larger size of saccharide can provide a good immune response against meningococcal disease.

The immunogenic composition of the invention may thus comprise one or more saccharide conjugates wherein the average size of each saccharide before conjugation is above 50kDa, 75kDa, 100kDa, 110kDa, 120kDa or 130kDa. In one embodiment the conjugate post conjugation should be readily filterable through a 0.2 micron filter such that a yield of more than 50, 60, 70, 80, 90 or 95% is obtained post filtration compared with the pre filtration sample.

In particular, the immunogenic composition of the invention comprises *N. meningitidis* capsular saccharides from at least one, two, three or four of serogroups A, C, W and Y conjugated to a carrier protein, wherein the average size (weight-average molecular weight; Mw) of at least one, two, three or four or each *N. meningitidis* saccharide is above 50kDa, 60kDa, 75kDa, 100kDa, 110kDa, 120kDa or 130kDa.

In a preferred embodiment, the average Mw of the $MenA_{AH}$-TT conjugate is at least 250 kDa, 260 kDa, 270 kDa, 280 kDa, or 290 kDa, most preferably about 300 kDa, and at most 350 kDa or 330 kDa. In a preferred embodiment, the average Mw of the $MenC_{AH}$-TT conjugate is at least 150 kDa, 160 kDa, 170 kDa, 180 kDa, or 190 kDa, most preferably about 200 kDa, and at most 250 kDa or 230 kDa. In a preferred embodiment, the average Mw of the MenW-TT conjugate is at least 240, 250 kDa, 260 kDa, or 270 kDa, most preferably about 280 kDa, and at most 330 kDa or 310 kDa. In a preferred embodiment, the average Mw of the MenY-TT conjugate is at least 220 kDa, 230 kDa, 240 kDa, or 250 kDa, most preferably about 270 kDa, and at most 320 kDa or 300 kDa.

The immunogenic composition may comprise *N. meningitidis* capsular saccharides from at least one, two, three or four of serogroups A, C, W and Y conjugated to a carrier protein, wherein at least one, two, three or four or each *N. meningitidis* saccharide is either a native saccharide or is sized by a factor up to x2, x3, x4, x5, x6, x7, x8, x9 or x10 relative to the weight average molecular weight of the native polysaccharide.

For the purposes of the invention, “native polysaccharide” refers to a saccharide that has not been subjected to a process, the purpose of which is to reduce the size of the saccharide. A polysaccharide can become slightly reduced in size during normal purification procedures. Such a saccharide is still native. Only if the polysaccharide has been subjected to sizing techniques would the polysaccharide not be considered native.

For the purposes of the invention, “sized by a factor up to x2” means that the saccharide is subject to a process intended to reduce the size of the saccharide but to retain a size more than half the size of the native polysaccharide. X3, x4 etc. are to be interpreted in the same way i.e. the saccharide is subject to a process intended to reduce the size of the polysaccharide but to retain a size more than a third, a quarter etc. the size of the native polysaccharide.

In an aspect of the invention, the immunogenic composition comprises *N. meningitidis* capsular saccharides from at least one, two, three or four of serogroups A, C, W and Y conjugated to a carrier protein, wherein at least one, two, three or four or each *N. meningitidis* saccharide is native polysaccharide.

In an aspect of the invention, the immunogenic composition comprises *N. meningitidis* capsular saccharides from at least one, two, three or four of serogroups A,

C, W and Y conjugated to a carrier protein, wherein at least one, two, three or four or each *N. meningitidis* saccharide is sized by a factor up to x1.5, x2, x3, x4, x5, x6, x7, x8, x9 or x10.

The immunogenic compositions of the invention optionally comprise conjugates of : *N. meningitidis* serogroup C capsular saccharide (MenC), serogroup A capsular saccharide (MenA), serogroup W135 capsular saccharide (MenW), serogroup Y capsular saccharide (MenY), serogroup C and Y capsular saccharides (MenCY), serogroup C and A capsular saccharides (MenAC), serogroup C and W capsular saccharides (MenCW), serogroup A and Y capsular saccharide (MenAY), serogroup A and W capsular saccharides (MenAW), serogroup W and Y capsular saccharides (Men WY), serogroup A, C and W capsular saccharide (MenACW), serogroup A, C and Y capsular saccharides (MenACY); serogroup A, W135 and Y capsular saccharides (MenAWY), serogroup C, W135 and Y capsular saccharides (MenCWY); or serogroup A, C, W135 and Y capsular saccharides (MenACWY). This is the definition of “one , two, three or four”, or “at least one of” of serogroups A, C, W and Y, or of each *N. meningitidis* saccharide where mentioned herein.

In an embodiment, the average size of at least one, two, three, four or each *N. meningitidis* saccharide is between 50KDa and 1500kDa, 50kDa and 500kDa, 50 kDa and 300 KDa, 101kDa and 1500kDa, 101kDa and 500kDa, 101kDa and 300kDa as determined by MALLS.

In an embodiment, the MenA saccharide, where present, has a molecular weight of 50-500kDa, 50-100kDa, 100-500kDa, 55-90KDa, 60-70kDa or 70-80kDa or 60-80kDa.

In an embdiment, the MenC saccharide, where present, has a molecular weight of 100-200kDa, 50-100kDa, 100-150kDa, 101-130kDa, 150-210kDa or 180-210kDa.

In an ebodiment the MenY saccharide, where present, has a molecular weight of 60-190kDa, 70-180kDa, 80-170kDa, 90-160kDa, 100-150kDa or 110-140kDa, 50-100kDa, 100-140kDa, 140-170kDa or 150-160kDa.

In an mbodiment the MenW saccharide, where present, has a molecular weight of 60-190kDa, 70-180kDa, 80-170kDa, 90-160kDa, 100-150kDa, 110-140kDa, 50-100kDa or 120-140kDa.

The molecular weight or average molecular weight of a saccharide herein refers to the weight-average molecular weight (Mw) of the saccharide measured prior to conjugation and is measured by MALLS.

The MALLS technique is well known in the art and is typically carried out as described in example 2. For MALLS analysis of meningococcal saccharides, two columns (TSKG6000 and 5000PWxl) may be used in combination and the saccharides are eluted in water. Saccharides are detected using a light scattering detector (for

instance Wyatt Dawn DSP equipped with a 10mW argon laser at 488nm) and an inferometric refractometer (for instance Wyatt Otilab DSP equipped with a P100 cell and a red filter at 498nm).

In an embodiment the *N. meningitidis* saccharides are native polysaccharides or native polysaccharides which have reduced in size during a normal extraction process.

In an embodiment, the *N. meningitidis* saccharides are sized by mechanical cleavage, for instance by microfluidisation or sonication. Microfluidisation and sonication have the advantage of decreasing the size of the larger native polysaccharides sufficiently to provide a filterable conjugate (fro example through a 0.2 micron filter). Sizing is by a factor of no more than x20, x10, x8, x6, x5, x4, x3, x2 or x1.5.

In an embodiment, the immunogenic composition comprises *N. meningitidis* conjugates that are made from a mixture of native polysaccharides and saccharides that are sized by a factor of no more than x20. For example, saccharides from MenC and/or MenA are native. For example, saccharides from MenY and/or MenW are sized by a factor of no more than x20, x10, x8, x6, x5, x4, x3 or x2. For example, an immunogenic composition contains a conjugate made from MenY and/or MenW and/or MenC and/or MenA which is sized by a factor of no more then x10 and/or is microfluidised. For example, an immunogenic composition contains a conjugate made from native MenA and/or MenC and/or MenW and/or MenY. For example, an immunogenic composition comprises a conjugate made from native MenC. For example, an immunogenic composition comprises a conjugate made from native MenC and MenA which is sized by a factor of no more then x10 and/or is microfluidised. For example, an immunogenic composition comprises a conjugate made from native MenC and MenY which is sized by a factor of no more then x10 and/or is microfluidised.

In an embodiment, the polydispersity of the saccharide is 1-1.5, 1-1.3, 1-1.2, 1-1.1 or 1-1.05 and after conjugation to a carrier protein, the polydispersity of the conjugate is 1.0-2.5, 1.0-2.0. 1.0-1.5, 1.0-1.2, 1.5-2.5, 1.7-2.2 or 1.5-2.0. All polydispersity measurements are by MALLS.

Saccharides are optionally sized up to 1.5, 2, 4, 6, 8, 10, 12, 14, 16, 18 or 20 times from the size of the polysaccharide isolated from bacteria.

In one embodiment each *N. meningitidis* saccharide is either a native polysaccharide or is sized by a factor of no more than x10. In a further embodiment each *N. meningitidis* capsular saccharide is a native polysaccharide. In a further embodiment at least one, two, three or four *N. meningitidis* capsular saccharide(s) is sized by microfluidization. In a further embodiment each *N. meningitidis* capsular saccharide is sized by a factor of no more than x10. In a further embodiment the *N. meningitidis* conjugates are made from a mixture of native polysaccharides and saccharides that are

sized by a factor of no more than x10. In a further embodiment the capsular saccharide from serogroup Y is sized by a factor of no more than x10. In a further embodiment capsular saccharides from serogroups A and C are native polysaccharides and saccharides from serogroups W135 and Y are sized by a factor of no more than x10. In a further embodiment the average size of each *N. meningitidis* capular saccharide is between 50 kDa and 300 KDa or 50kDa and 200kDa. In a further embodiment the immunogenic composition comprises a MenA capsular saccharide having an average size of above 50kDa, 75kDa, 100kDa or an average size of between 50-100kDa or 55-90KDa or 60-80kDa. In a further embodiment the immunogenic composition comprises a MenC capsular saccharide having an average size of above 50kDa, 75kDa, 100kDa or between 100-200kDa, 100-150kDa, 80-120kDa , 90-110kDa, 150-200kDa, 120-240kDa, 140-220kDa, 160-200kDa or 190-200kDa. In a further embodiment the immunogenic composition comprises a MenY capsular saccharide, having an average size of above 50kDa, 75kDa, 100kDa or between 60-190kDa or 70-180kDa or 80-170kDa or 90-160kDa or 100-150kDa , 110-145kDa or 120-140kDa. In a further embodiment the immunogenic composition comprises a MenW capsular saccharide having an average size of above 50kDa, 75kDa, 100kDa or between 60-190kDa or 70-180kDa or 80-170kDa or 90-160kDa or 100-150kDa, 140-180kDa, 150-170kDa or 110-140kDa.

In an embodiment of the invention, the saccharide dose of each of the at least two, three, four or each of the *N. meningitidis* saccharide conjugates is optionally the same, or approximately the same.

In an embodiment, the immunogenic composition of the invention is adjusted to or buffered at, or adjusted to between pH 7.0 and 8.0, pH 7.2 and 7.6 or around or exactly pH 7.4.

The immunogenic composition or vaccines of the invention are optionally lyophilised in the presence of a stabilising agent for example a polyol such as sucrose or trehalose.

For the *N. meningitidis* saccharide combinations discussed above, it may be advantageous not to use any aluminium salt adjuvant or any adjuvant at all.

The active agent can be present in varying concentrations in the pharmaceutical composition or vaccine of the invention. Typically, the minimum concentration of the substance is an amount necessary to achieve its intended use, while the maximum concentration is the maximum amount that will remain in solution or homogeneously suspended within the initial mixture. For instance, the minimum amount of a therapeutic agent is optionally one which will provide a single therapeutically effective dosage. For bioactive substances, the minimum concentration is an amount necessary for bioactivity

upon reconstitution and the maximum concentration is at the point at which a homogeneous suspension cannot be maintained.

In another embodiment, the composition includes a conjugate of a *Neisseria meningitidis* serogroup X capsular polysaccharide and a carrier molecule. The structure of the group X capsular polysaccharide consists of N-acetylglucosamine-4-phosphate residues held together by al-4 phosphodiester bonds without O-acetyl groups. The carrier molecule may be a diphtheria or tetanus toxoid, CRM 197 or protein D. In a preferred embodiment, as exemplified in the Examples, the composition does not include a conjugate of a *N. meningitidis* serogroup X capsular polysaccharide.

STABILITY

The terms "stable" and "stability" refer the ability of an antigen to remain immunogenic over a period of time. Stability may be measured in potency over time. The terms "stable" and "stability" further refer to the physical, chemical, and conformational stability of the immunogenic composition. Instability of a protein composition may be caused by chemical degradation or aggregation of the protein molecules to form higher order polymers, by dissociation of the heterodimers into monomers, deglycosylation, modification of glycosylation, or any other structural modification that reduces at least one biological activity of the protein composition included in the present invention. Stability may be assessed by methods well- known in the art, including measurement of a sample's light scattering, apparent attenuation of light (absorbance, or optical density), size (e.g. by size exclusion chromatography), in vitro or in vivo biological activity and/or properties by differential scanning calorimetry (DSC). Other methods for assessing stability are known in the art and can also be used according to the present invention.

In some embodiments, an antigen in a stable formulation of the invention may maintain at least 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% potency, as compared to a reference standard, for at least 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 9 months, 12 months, 18 months, 24 months, 30 months, 36 months, 42 months, 48 months, 54 months, or 60 months. In some embodiments, an antigen in a stable formulation of the invention may maintain at least 50% potency, as compared to a reference standard, for at least 1 year, 2 years, 3 years, 4 years or 5 years. The terms "stable" and "stability" also refer to the ability of an antigen to maintain epitopes or immunoreactivity over a period of time. For example, an antigen in a stable formulation of the invention may maintain at least 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% of its epitopes or immunoreactivity, as compared to a reference standard, for at least 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 9 months, 12 months, 18 months, 24 months, 30 months, 36 months, 42 months, 48 months, 54 months, or 60 months. In some embodiments, stability is

measured with respect to an environmental condition. Non-limiting examples of environmental conditions include light, temperature, freeze/thaw cycles, agitation, and pH. One of skill in the art would be able to determine the presence of antigenic epitopes or immunoreactivity using the methods disclosed herein or other methods known in the art. In some embodiments, the stability of an antigen is measured from the date of its formulation. In some embodiments, the stability of an antigen is measured from the date of a change in its storage conditions. Non-limiting examples of changes in storage conditions include changing from frozen to refrigerated, changing from frozen to room temperature, changing from refrigerated to room temperature, changing from refrigerated to frozen, changing from room temperature to frozen, changing from room temperature to refrigerated, changing from light to dark, or introduction of agitation.

In one embodiment, the terms "stable" and "stability" includes the ability of an antigen to be bound to aluminum. For example, a stable formulation of the invention includes at least 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% of a protein that is bound to aluminum (e.g., aluminum phosphate) in the formulation, as compared to a reference standard, for at least 1 hour, 6 hours, 12 hours, 18 hours, 24 hours, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 9 months, 12 months, 18 months, 24 months, 30 months, 36 months, 42 months, 48 months, 54 months, or 60 months. See, for example Example 13. In a preferred embodiment, at least 90%, more preferably at least 95%, and most preferably at least 99% of the total Subfamily A rLP2086 polypeptide (e.g., a polypeptide that includes the amino acid sequence set forth in SEQ ID NO: 1) is bound to aluminum in the composition. In a preferred embodiment, at least 90%, more preferably at least 95%, and most preferably at least 99% of the total Subfamily B rLP2086 polypeptide (e.g., a polypeptide that includes the amino acid sequence set forth in SEQ ID NO: 2) is bound to aluminum in the composition.

Determination of Aluminum Binding. A composition comprising aluminum and at least one protein antigen was centrifuged such that the aluminum was pelleted. Centrifugation of aluminum absorbed proteins is known in the art. See e.g., Egan et al., Vaccine, Vol. 27(24): 3175-3180 (2009). Aluminum-bound protein was also pelleted, while non- aluminum-bound protein remained in the supernatant. Total protein in the supernatant and pellet were determined by Lowry Assay. The percentage bound protein was calculated by dividing the total protein in the supernatant by the total protein added to the composition and multiplying by 100%. Similarly, the percentage unbound protein was calculated by dividing the total protein in the supernatant by the total protein added to the composition and multiplying by 100%. For compositions comprising both Subfamily A and Subfamily B antigens, the individual Subfamily A and B protein

concentrations in the supernatant were determined by ion-exchange chromatography. The separation and elution of Subfamily A and B proteins was carried out using a strong anion column and a high salt concentration eluent. Both Subfamily A and B proteins were detected and quantified using a fluorescence detector set at Excitation = 280 run and Emission = 310 run. Subfamily A and Subfamily B proteins elute at distinct retention times and were quantified using a standard curve generated against a rLP2086 protein reference material. The percentage unbound protein was calculated by dividing the total protein in the supernatant by the total protein added to the composition and multiplying by 100%. The percentage bound protein was calculated by subtracting the percentage unbound protein from 100%.

POLYSORBATE-80

Polysorbate 80 (PS-80) is a non-ionic surfactant. Accelerated stability studies using an in vitro monoclonal antibody based potency assay demonstrated instability of the subfamily B protein at higher molar ratios of PS-80 to MnB rLP2086 protein in the final formulation. Further experiments with varying ratios of PS-80 have demonstrated that the optimal molar ratio of PS-80 to MnB rLP2086 protein is approximately 2.8 ± 1.4 to retain potency.

The concentration of PS-80 in the composition is dependent on a molar ratio of PS-80 to the polypeptide. In one embodiment, the composition includes a 2.8±1.4 molar ratio of PS-80 to the first polypeptide and to the second polypeptide. In one embodiment, the composition includes a 2.8±1.1 molar ratio of PS-80 to the first polypeptide and to the second polypeptide. In one embodiment, the composition includes at least 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, or 3.3 molar ratio of PS-80 to polypeptide. In one embodiment, the composition includes at most 4.0, 3.9, 3.8, 3.7, 3.6, 3.5, 3.4, 3.3, 3.2, 3.1, 3.0, or 2.9 molar ratio of PS-80 to polypeptide. Any minimum value may be combined with any maximum value described herein to define a range. Preferably, the composition includes a 2.8 molar ratio of PS-80 to polypeptide.

The PS-80 to polypeptide molar ratio is determined by calculation from the measured concentration of PS-80 and the measured total polypeptide concentration, in which both values are expressed in moles. For example, PS-80 to Protein molar ratio is determined by calculation of the measured concentration of PS-80 (e.g., by reverse phase high pressure liquid chromatography (RP-HPLC)) to the measured total protein concentration (e.g., by ion exchange-high pressure liquid chromatography (IEX-HPLC)) in the final drug substance, where both values are expressed in moles.

A RP-HPLC is used to quantitate the concentration of Polysorbate 80 in vaccine formulations. The concentration of detergent is determined by saponification of the fatty acid moiety; Polysorbate 80 is converted to free oleic acid by alkaline hydrolysis at 40°C. The sample is separated by RP-HPLC using a C18 column and quantitated using a UV detector at a wavelength of 200 nm.

The first and the second polypeptides are resolved by anion-exchange HPLC. rLP2086(fHBP) Subfamily A and B proteins elute at distinct retention times and are quantitated using a standard curve generated against the respective rLP2086 protein reference material.

The term “molar ratio” and a description of an immunogenic composition including a fHBP and PS-80 is further disclosed in WO2012025873 and US patent publication US 2013/0171194, which are each incorporated by reference in their entirety.

The term "molar ratio" as used herein refers to the ratio of the number of moles of two different elements in a composition. In some embodiments, the molar ratio is the ratio of moles of detergent to moles of polypeptide. In some embodiments, the molar ratio is the ratio of moles of PS-80 to moles of protein. In one embodiment, based on the protein and Polysorbate 80 concentrations, the Molar Ratio may be calculated using the following equation:

$$\text{Molar Ratio} = \frac{\%\ \text{PS-80}}{\text{mg/ml protein}} \times 216$$

In one embodiment, the composition includes a molar ratio of PS-80 to MnB rLP2086 protein between 1.4 to 4.2 to retain potency. In one embodiment, the composition includes at least 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, or 2.8. In one embodiment, the composition includes at most 4.2, 4.1, 4.0, 3.9, 3.8, 3.7, 3.7, 3.6, 3.5, 3.4, 3.3, 3.2, 3.1, 3.0, 2.9, or 2.8. Any minimum value may be combined with any maximum value described herein to define a range.

In one embodiment, the composition includes about 0.0015, 0.0017, 0.0019, 0.0021, 0.0023, 0.0025, 0.0027, 0.0029, 0.0031, 0.0033, 0.0035, 0.0037, 0.0039, 0.0041, 0.0043, 0.0045, 0.0047, 0.0049, 0.0051 mg/mL PS-80. Preferably, the composition includes about 0.0035 mg/mL PS-80.

In another embodiment, the composition includes at least 10 µg, 11 µg, 12 µg, 13 µg, 14 µg, 15 µg, 16 µg, 17 µg, 18 µg, 19 µg, 20 µg, 21 µg , 22 µg , 23 µg, 24 µg, or 25

µg PS-80. In another embodiment, the composition includes at most 30 µg, 29 µg, 28 µg, 27 µg, 26 µg, 25 µg, 24 µg, 23 µg, 22 µg, 21 µg, 20 µg, 19 µg, or 18 µg PS-80. Any minimum value may be combined with any maximum value described herein to define a range. In a preferred embodiment, the composition includes at least 10 µg and at most 20 µg PS-80. In a most preferred embodiment, the composition includes about 18 µg PS-80.

In another embodiment, the composition includes a PS-80 concentration ranging from 0.0005% to 1%. For example, the PS-80 concentration in the composition may be at least 0.0005%, 0.005%, 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.10%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, or 1.1% PS-80. In one embodiment, the PS-80 concentration in the composition may be at most 2.0%, 1.9%, 1.8%, 1.7%, 1.6%, 1.5%, 1.4%, 1.3%, 1.2%, 1.1%, 1.0%, 0.9%, 0.8%, or 0.7% PS-80. In a preferred embodiment, the composition includes about 0.07% PS-80. Any minimum value may be combined with any maximum value described herein to define a range.

The inventors surprisingly discovered that while a composition that includes a combination of the first composition and the second composition may have a different molar ratio of polysorbate-80 in relation to the MnB rLP2086 polypeptides, as compared to the molar ratio of polysorbate-80 in relation to the MnB rLP2086 polypeptides in the first composition, additional surfactant for the combined composition was surprisingly not necessary to maintain solubility and stability of the MnB rLP2086 polypeptides in the combined composition. Accordingly, in one embodiment, the kit does not comprise greater than 0.02 mg polysorbate-80.

ALUMINUM

The composition includes aluminum as aluminum phosphate. $AlPO_4$ is added as a stabilizer to provide enhanced manufacturability and stability. The process for producing an aluminum phosphate is described in US patent publication US 2009/0016946, which is incorporated by reference in its entirety. In one embodiment, the composition does not further include a multivalent cation, other than aluminum. In one embodiment, the composition does not further include $Al(OH)_3$ or $Al(SO_4)_3$.

In one embodiment, the composition includes at least 50 µg, 60 µg, 70 µg, 80 µg, 90 µg,100 µg,110 µg, 120 µg, 130 µg, 140 µg, 150 µg, 160 µg, 170 µg, 180 µg, 190 µg, 200 µg, 210 µg, 220 µg, 230 µg, 240 µg, or 250 µg aluminum. In one embodiment, the composition includes at most 500 µg, 490 µg, 480 µg, 470 µg, 460 µg, 450 µg, 440 µg, 430 µg, 420 µg, 410 µg, 400 µg, 390 µg, 380 µg, 370 µg, 360 µg, 350 µg, 340 µg, 330 µg, 320 µg, 310 µg, 300 µg, 290 µg, 280 µg, 270 µg, 260 µg, or 250 µg aluminum. Any minimum value may be combined with any maximum value described herein to define a range. In a most preferred embodiment, the composition includes 250 µg aluminum.

In one embodiment, the composition includes at least 0.005 mg/ml, 0.01 mg/ml, 0.02 mg/ml, 0.03 mg/ml, 0.04 mg/ml, 0.05 mg/ml, 0.06 mg/ml, 0.07 mg/ml, 0.08 mg/ml, 0.09 mg/ml, 0.10 mg/ml, 0.2 mg/ml, 0.3 mg/ml, 0.4 mg/ml, or 0.5 mg/ml aluminum phosphate. In one embodiment, the composition includes at most 2.0 mg/ml, 1.9 mg/ml, 1.8 mg/ml, 1.7 mg/ml, 1.6 mg/ml, 1.5 mg/ml, 1.4 mg/ml, 1.3 mg/ml, 1.2 mg/ml, 1.1 mg/ml, 1.0 mg/ml, 0.9 mg/ml, 0.8 mg/ml, or 0.7 mg/ml PS-80. In a preferred embodiment, the composition includes about 0.07 mg/ml PS-80. Any minimum value may be combined with any maximum value described herein to define a range. In a preferred embodiment, the composition includes 0.5 mg/ml aluminum phosphate. In a most preferred embodiment, the composition includes 0.5 mg aluminum/ml as aluminum phosphate ($AlPO_4$). This concentration maintains binding (at least 90% binding or better) of the subfamily A and B proteins to aluminum.

The inventors surprisingly discovered that while a composition that a combination of the first composition and the second composition could change the percentage of MnB rLP2086 polypeptides bound to the aluminum, when compared to the percentage of MnB rLP2086 polypeptides bound to the aluminum in the first composition, the combination of the first and second compositions surprisingly maintained binding of at least 90% of the total MnB rLP2086 polypeptides to the aluminum. Accordingly, in one embodiment, the percentage of total MnB rLP2086 polypeptides to the aluminum in the combined

composition is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%. Preferably, the percentage of total MnB rLP2086 polypeptides to the aluminum in the combined composition is at least 90%, more preferably at least 95%, and most preferably at least 100%.

In another embodiment, the concentration of polypeptides bound to the aluminum in the immunogenic composition is not decreased after 24 hours, as compared to the concentration of polypeptides bound to the aluminum in the liquid composition prior to reconstituting the lyophilized composition. In another embodiment, the concentration of $MenA_{AH}$-TT conjugate in the immunogenic composition is not decreased after 24 hours, as compared to the concentration of the $MenA_{AH}$-TT conjugate in the lyophilized composition. In one embodiment, the concentration is decreased by at most 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, or 20% after 24 hours, as compared to the respective concentration in the liquid composition prior to reconsititution.

In another embodiment, the concentration of $MenC_{AH}$-TT conjugate in the immunogenic composition is not decreased after 24 hours, as compared to the concentration of the $MenC_{AH}$-TT conjugate in the lyophilized composition. In another embodiment, the concentration of MenW-TT conjugate in the immunogenic composition is not decreased after 24 hours, as compared to the concentration of the MenW-TT conjugate in the lyophilized composition. In another embodiment, the concentration of MenY-TT conjugate in the immunogenic composition is not decreased after 24 hours, as compared to the concentration of the MenY-TT conjugate in the lyophilized composition. In one embodiment, the concentration is decreased by at most 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, or 20% after 24 hours, as compared to the respective concentration in the lyophilized composition prior to reconsititution.

EXCIPIENTS

In one embodiment, the composition includes histidine. In one embodiment, the composition includes at least 650 µg, 660 µg, 670 µg, 680 µg, 690 µg, 700 µg, 710 µg, 720 µg, 730 µg, 740 µg, 750 µg, 760 µg, 770 µg, 780 µg, 790 µg, 800 µg, 810 µg, 820 µg, 830 µg, 840 µg, or 850 µg of histidine. In one embodiment, the composition includes at most 1560 µg, 1500 µg, 1400 µg, 1300 µg, 1200 µg, 1100 µg, 1000 µg, 950 µg, 900 µg, 890 µg, 880 µg, 870 µg, 860 µg, 850 µg, 840 µg, 830 µg, 820 µg, 810 µg, 800 µg, 790 µg, or 780 µg of histidine. Any minimum value may be combined with any maximum value described herein to define a range. Preferably, the composition includes 780 µg histidine.

In one embodiment, the composition includes a tris, phosphate, or succinate buffer. In a preferred embodiment, the composition does not include tris buffer. In a preferred, the composition does not include phosphate buffer. In one preferred embodiment, the composition does not include succinate buffer. In a preferred embodiment, the composition includes histidine buffer.

In one embodiment, the composition includes sodium chloride. Sodium chloride concentration in MenABCWY composition may vary between 160.5-161.1 mM.

In one embodiment, the pH of the composition is between 5.5 and 7.5. In a preferred embodiment, the pH of the composition is between 5.8 and 7.0, most preferably pH 5.8 to pH 6.0. In one embodiment, the pH of the composition is at most 6.1. In one embodiment, the pH of the composition is 5.8.

KITS

A further aspect of the invention is a kit for administering a dose of a composition for eliciting bactericidal antibodies against *Neisseria meningitidis* in a mammal.

In one aspect, the kit includes a first composition including a first polypeptide as described above and a second polypeptide as described above. In a preferred embodiment, the first polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 1. In another preferred embodiment, the second polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 2. The kit further includes a second composition including a $MenA_{AH}$-TT conjugate, a $MenC_{AH}$-TT conjugate, a MenW-TT conjugate, and a MenY-TT conjugate. In one embodiment, the kit includes at least two containers, wherein a first container includes the first composition, a second container includes the second composition.

In one embodiment, the kit includes a liquid first composition and a lyophilized second composition. Preferably, the kit includes a liquid MnB bivalent rLP2086 composition and a lyophilized MenACWY-TT composition.

The inventors surprisingly discovered that while a composition that includes a combination of the first composition and the second composition changes the molar ratio of polysorbate-80 in relation to the MnB rLP2086 polypeptides in the first composition, additional surfactant for the combined composition was surprisingly not necessary to maintain solubility and stability of the MnB rLP2086 polypeptides in the combined composition. Accordingly, in one embodiment, the kit does not comprise greater than 0.02 mg polysorbate-80.

In one embodiment of the invention, the kit does not further comprise any one of the following commercial immunogenic compositions: MENACTRA(R), MENVEO(R), ADACEL(R), HAVRIX(R), GARDASIL(R), REPEVAX, or any combination thereof. For example, the kit preferably does not further include a meningococcal A, C, Y and W-135 polysaccharide conjugate (MCV4) composition, wherein the carrier protein is diphtheria toxoid. In one embodiment, the kit does not further include a meningococcal A, C, Y and W-135 polysaccharide conjugate (MCV4) composition, wherein the carrier protein is CRM_{197}. In one embodiment, the kit does not further comprise NIMENRIX vaccine, wherein NIMENRIX comprises a diluent consisting of sodium chloride and water.

BACTERICIDAL ACTIVITY

Disease incidence of MnB is approximately 1 in 100,000, meaning that extremely large numbers of subjects (400,000 to over 6 million) would be required to support a statistically significant assessment of efficacy. Thus, a serum bactericidal assay using human complement (hSBA), which is a surrogate of protection and vaccine efficacy, is used to assess immunogenicity in clinical trials.

Pfizer has built an extensive MnB strain collection (N=at least1263) comprising IMD-causing isolates from Years 2000 to 2006. The MnB isolates were systematically collected from the US Centers for Disease Control and Prevention (CDC) and health and reference laboratories from European countries.

In one embodiment, immune response induced by administering the composition to a human is determined using a serum bactericidal assay using human complement (hSBA) against four *N. meningitidis* serogroup B (MnB) strains. The MnB strains used in the hSBA were selected from the strain pool. The strain pool represented a collection of systematically collected clinically relevant *N. meningitidis* strains.

The high proportion of hSBA response to all test strains, especially strains expressing lipoprotein 2086 variants with sequences heterologous to both the first polypeptide and the second polypeptide suggests that the composition is a broadly protective vaccine are sufficient to confer high seroprotection against *N. meningitidis* strains expressing rLP2086 (FHBP) from at least serogroup B, including additional serogroups, such as serogroup X.

Subfamily A strains

In one embodiment, the hSBA strain is an *N. meningitidis* strain that expresses LP2086 (fHBP) subfamily A protein. In one embodiment, the hSBA strain is an LP2086 (fHBP) subfamily A strain that expresses a lipoprotein 2086 variant that is heterologous to a *N. meningitidis* strain expressing A05. For example, in one embodiment, the hSBA strain is an LP2086 (fHBP) subfamily A strain that expresses a lipoprotein 2086 variant that is heterologous to strain M98250771.

In one embodiment, the hSBA strain is a *N. meningitidis* strain expressing fHBP A10. In one embodiment, the hSBA strain is a *N. meningitidis* strain expressing LP2086 (fHBP) A22. In one embodiment, the hSBA strain is a *N. meningitidis* strain expressing LP2086 (fHBP) A56. In a further embodiment, the hSBA strains are LP2086 (fHBP) A22

and LP2086 (fHBP) A56 strains. In another embodiment, the hSBA strain is a *N. meningitidis* strain expressing LP2086 A04. In one embodiment, the hSBA strain is a *N. meningitidis* strain expressing LP2086 A05. In one embodiment, the hSBA strain is a *N. meningitidis* strain expressing LP2086 A12. In one embodiment, the hSBA strain is a *N. meningitidis* strain expressing LP2086 A22. In one embodiment, the hSBA strain is a *N. meningitidis* strain expressing LP2086 A12. In one embodiment, the hSBA strain is a *N. meningitidis* strain expressing LP2086 A04. In one embodiment, the hSBA strain is a *N. meningitidis* strain expressing LP2086 A19. In one embodiment, the hSBA strain is a *N. meningitidis* strain expressing LP2086 A07. In a further embodiment, the hSBA strain includes any one of an A22-, A12-, A19-, A05-, and A07-expressing strain. In one embodiment, the hSBA strains include any one of an A06-, A15-, and A29- expressing strain.

In one embodiment, the immune response is bactericidal against a *N. meningitidis* serogroup B fHPB subfamily A strain that is heterologous to a *N. meningitidis* strain expressing A05. In one embodiment, the immune response is against *N. meningitidis* serogroup B A22 strain. In one embodiment, the immune response is against *N. meningitidis* serogroup B A56 strain. In one embodiment, the immune response is against *N. meningitidis* serogroup B A06 strain. In one embodiment, the immune response is against *N. meningitidis* serogroup B A15 strain. In one embodiment, the immune response is against *N. meningitidis* serogroup B A29 strain. In one embodiment, the immune response is against *N. meningitidis* serogroup B A62 strain. In one embodiment, the immune response is bactericidal against a *N. meningitidis* serogroup B subfamily A strain that is heterologous to *N. meningitidis* strain M98250771.

In one embodiment, the immune response is bactericidal against a *N. meningitidis* serogroup B subfamily A strain that expresses a factor H binding protein including an amino acid sequence that has at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the first polypeptide. In another embodiment, the immune response is bactericidal against a *N. meningitidis* serogroup B subfamily A strain that expresses a factor H binding protein including an amino acid sequence that has at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to a factor H binding protein expressed by *N. meningitidis* strain M98250771. In a preferred embodiment, the immune response is bactericidal against a *N. meningitidis* serogroup B subfamily A strain that expresses a

factor H binding protein including an amino acid sequence that has at least 80%, more preferably at least 84%, identity to a factor H binding protein expressed by *N. meningitidis* strain M98250771.

In another embodiment, the immune response is bactericidal against a *N. meningitidis* serogroup B subfamily A strain that expresses a factor H binding protein including an amino acid sequence that has at most 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the first polypeptide. In another embodiment, the immune response is bactericidal against a *N. meningitidis* serogroup B subfamily A strain that expresses a factor H binding protein including an amino acid sequence that has at most 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to a factor H binding protein expressed by *N. meningitidis* strain M98250771. In a preferred embodiment, the immune response is bactericidal against a *N. meningitidis* serogroup B subfamily A strain that expresses a factor H binding protein including an amino acid sequence that has at most 85%, more preferably at most 99%, identity to a factor H binding protein expressed by *N. meningitidis* strain M98250771. Any minimum value may be combined with any maximum value described herein to define a range.

In one embodiment, the immune response elicited by the composition is bactericidal not only against a *N. meningitidis* serogroup B fHPB subfamily A strain but also a *N. meningitidis* strain expressing an fHBP subfamily A polypeptide, wherein the serogroup is not serogroup B. For example, in one preferred embodiment, the immune response elicited by the composition is bactericidal against a *N. meningitidis* serogroup B subfamily A strain and against a *N. meningitidis* serogroup C strain that expresses an fHBP subfamily A polypeptide heterologous to fHBP A05. For example, in one embodiment, the immune response is against a *N. meningitidis* serogroup C strain expressing fHBP A10. In another embodiment, the immune response is against a *N. meningitidis* serogroup W strain expressing fHBP A19. In one embodiment, the immune response is bactericidal against a *N. meningitidis* strain that expresses an fHBP subfamily A polypeptide, wherein the strain is heterologous to *N. meningitidis* strain M98250771.

Subfamily B strains

In one embodiment, the hSBA strain is an LP2086 (fHBP) subfamily B strain. In one embodiment, the hSBA strain is an LP2086 (fHBP) subfamily B strain that expresses

a lipoprotein 2086 variant that is heterologous to a *N. meningitidis* strain expressing B01. For example, in one embodiment, the hSBA strain is an LP2086 (fHBP) subfamily B strain that expresses a lipoprotein 2086 variant that is heterologous to strain CDC1127. In a preferred embodiment, the hSBA strain is an LP2086 (fHBP) subfamily B strain that expresses a lipoprotein 2086 variant that is heterologous to strain CDC1573.

In one embodiment, the immune response is bactericidal against a *N. meningitidis* serogroup B fHPB subfamily B strain that is heterologous to a *N. meningitidis* strain expressing B01. In one embodiment, the immune response is against *N. meningitidis* serogroup B B24 strain. In one embodiment, the immune response is against *N. meningitidis* serogroup B B44 strain. In one embodiment, the immune response is against *N. meningitidis* serogroup B B16 strain. In one embodiment, the immune response is against *N. meningitidis* serogroup B B03 strain. In one embodiment, the immune response is against *N. meningitidis* serogroup B B09 strain. In one embodiment, the immune response is against *N. meningitidis* serogroup B B15 strain. In one embodiment, the immune response is against *N. meningitidis* serogroup B B153 strain. In one embodiment, the immune response is bactericidal against a *N. meningitidis* serogroup B subfamily B strain that is heterologous to *N. meningitidis* strain CDC1573.

In one embodiment, the immune response is bactericidal against a *N. meningitidis* serogroup B subfamily B strain that expresses a factor H binding protein including an amino acid sequence that has at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the second polypeptide. In another embodiment, the immune response is bactericidal against a *N. meningitidis* serogroup B subfamily B strain that expresses a factor H binding protein including an amino acid sequence that has at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to a factor H binding protein expressed by *N. meningitidis* strain CDC1573. In a preferred embodiment, the immune response is bactericidal against a *N. meningitidis* serogroup B subfamily B strain that expresses a factor H binding protein including an amino acid sequence that has at least 80% identity, more preferably at least 87% identity, to a factor H binding protein expressed by *N. meningitidis* strain CDC1573. In another preferred embodiment, the immune response is bactericidal against a *N. meningitidis* serogroup B subfamily B strain that expresses a factor H binding protein including an amino acid sequence that has 100% identity to a factor H binding protein expressed by *N. meningitidis* strain CDC1573.

In another embodiment, the immune response is bactericidal against a *N. meningitidis* serogroup B subfamily B strain that expresses a factor H binding protein including an amino acid sequence that has at most 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the second polypeptide. In another embodiment, the immune response is bactericidal against a *N. meningitidis* serogroup B subfamily B strain that expresses a factor H binding protein including an amino acid sequence that has at most 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to a factor H binding protein expressed by *N. meningitidis* strain CDC1573. In a preferred embodiment, the immune response is bactericidal against a *N. meningitidis* serogroup B subfamily B strain that expresses a factor H binding protein including an amino acid sequence that has at most 88% identity, more preferably at least 99% identity, to a factor H binding protein expressed by *N. meningitidis* strain CDC1573. Any minimum value may be combined with any maximum value described herein to define a range.

In one embodiment, the hSBA strain is an LP2086 (fHBP) B24 strain. In another embodiment, the hSBA strains is an LP2086 (fHBP) B44 strain. In a further embodiment, the hSBA strains includes LP2086 (fHBP) B24 and LP2086 (fHBP) B44 strains. In one embodiment, the hSBA strains includes LP2086 (fHBP) A22, LP2086 (fHBP) A56, LP2086 (fHBP) B24, and LP2086 (fHBP) B44 strains. In one embodiment, the hSBA strain includes B15. In one embodiment, the hSBA strain includes B153. In another embodiment, the hSBA strain is an LP2086 B16 strain. In one embodiment, the hSBA strain is an LP2086 B03 strain. In one embodiment, the hSBA strain is an LP2086 B09 strain. In a further embodiment, the hSBA strains include B24, B16, B44, B03, and B09, or any combination thereof. In another embodiment, the hSBA strains include B24, B16, B44, A22, B03, B09, A12, A19, A05, and A07, or any combination thereof. In another embodiment, the hSBA strains include A06, A07, A12, A15, A19, A29, B03, B09, B15, and B16, or any combination thereof.

In one embodiment, the method induces an immune response against a *N. meningitidis* serogroup B fHPB subfamily A strain and against a *N. meningitidis* serogroup B fHPB subfamily B strain. Preferably, the immune response is bactericidal against a *N. meningitidis* serogroup B fHPB subfamily A strain and against a *N. meningitidis* serogroup B fHPB subfamily B strain.

In one embodiment, the immune response elicited by the composition is bactericidal not only against a *N. meningitidis* serogroup B fHPB subfamily B strain but

also a *N. meningitidis* strain expressing an fHBP subfamily B polypeptide, wherein the serogroup is not serogroup B. For example, in one preferred embodiment, the immune response elicited by the composition is bactericidal against a *N. meningitidis* serogroup B subfamily B strain and against a *N. meningitidis* serogroup Y strain that expresses an fHBP subfamily B polypeptide heterologous to fHBP B01. For example, in one embodiment, the immune response is against a *N. meningitidis* serogroup A strain expressing fHBP B16. In another embodiment, the immune response is against a *N. meningitidis* serogroup Y strain expressing fHBP B47. In another embodiment, the immune response is against a *N. meningitidis* serogroup X strain expressing fHBP B49. In one embodiment, the immune response is bactericidal against a *N. meningitidis* strain that expresses an fHBP subfamily B polypeptide, wherein the strain is heterologous to *N. meningitidis* serogroup B strain CDC1573.

TITERS

In one embodiment, the composition induces an increase in bactericidal titer in the human, as compared to the bactericidal titer in the human prior to administration of a dose of the composition, when measured under identical conditions in an hSBA. In one embodiment, the increase in bactericidal titer is compared to the bactericidal titer in the human before administration of the first dose of the composition, as compared to the bactericidal titer in the human prior to administration of the first dose of the composition, when measured under identical conditions in an hSBA. In one embodiment, the increase in titer is observed after a second dose of the composition, as compared to the bactericidal titer in the human prior to administration of the second dose of the composition, when measured under identical conditions in an hSBA. In another embodiment, the increase in bactericidal titer is observed after a third dose of the composition, as compared to the bactericidal titer in the human prior to administration of the third dose of the composition, when measured under identical conditions in an hSBA.

In one embodiment, the composition induces a bactericidal titer in the human after administration of a dose, wherein the bactericidal titer is at least greater than 1-fold higher than the bactericidal titer in the human prior to administration of the dose, when measured under identical conditions in an hSBA. For example, the bactericidal titer may be at least 1.01-fold, 1.1-fold, 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 11-fold, 12-fold, 13-fold, 14-fold, 15-fold, or 16-fold higher in the human after receiving a dose of the composition, as compared to the bactericidal titer in the human prior to administration of the dose, when measured under identical conditions in an hSBA.

In one embodiment, a “responder” refers to a human, wherein the composition induces a bactericidal titer in the human after administration of a dose, wherein the bactericidal titer is at least greater than 1-fold higher than the bactericidal titer in the human prior to administration of the dose. In a preferred embodiment, the responder achieves at least a ≥ 4-fold rise in hSBA titer, as compared to a bactericidal titer in the human prior to administration of the dose. Such a responder may be referred to as having a protective titer. In some embodiments, a protective titer is one that is greater than 1:4.

In one embodiment, the hSBA titer is the reciprocal of the highest dilution of a serum sample that produces a measurable effect. For example, in one embodiment, the hSBA titer is the reciprocal of the highest 2-fold dilution of a test serum that results in at

least a 50% reduction of MnB bacteria (50% bacterial survival) compared to the T30 CFU value (i.e., the number of bacteria surviving after incubation in assay wells containing all assay components except test serum; 100% bacterial survival).

In one embodiment, the composition induces a bactericidal titer in the human after receiving the first dose that is at least 2-fold higher than the bactericidal titer in the human prior to receiving the first dose (e.g., higher than the bactericidal titer in the human in the absence of the first dose), when measured under identical conditions in the hSBA. In one embodiment, the composition induces a bactericidal titer in the human that is at least 4-fold higher than the bactericidal titer in the human prior to receiving the first dose, when measured under identical conditions in a human serum bactericidal assay that utilizes human complement (hSBA). In one embodiment, the composition induces a bactericidal titer in the human that is at least 8-fold higher than the bactericidal titer in the human prior to receiving the first dose, when measured under identical conditions in a human serum bactericidal assay that utilizes human complement (hSBA).

In a preferred embodiment, the human serum complement is derived from a human having low intrinsic bactericidal activity for a given hSBA test strain. Low intrinsic bactericidal activity refers to, for example, a bactericidal titer that is at least less than a 1:4 dilution against the given hSBA test strain. In one embodiment, the human complement is derived from a human having an hSBA titer that is at least less than 1:4, such as a 1:2 dilution, against the given hSBA test strain, wherein the composition was not administered to the human.

A human may exhibit an hSBA titer of less than 1:4 prior to administration of a composition, such as the bivalent rLP2086 composition, or a human may exhibit an hSBA titer of ≥1:4 prior to administration of the composition. Accordingly, in preferred embodiments and examples, administration of at least one dose of the composition to the human results in an hSBA titer that is at least 4-fold greater than the titer in the human prior to the administration. In some embodiments, administration of at least one dose of the composition to the human results in an hSBA titer that is at least greater than 1:4, such as, for example, an hSBA titer of ≥1:8, an hSBA titer of ≥1:16, and an hSBA titer of ≥1:32. The respective Examples described herein include assessments of the proportion of human subjects having an hSBA titer ≥1:8 and/or ≥1:16, wherein the bivalent rLP2086 composition was administered to the human. In some embodiments, a 4-fold rise in titer in the human after administration of the composition as compared to before administration of the composition show that protection is associated with the composition. In some embodiments, such preferred assessments of hSBA titers greater

than 1:4 show that the protection, i.e., the bactericidal immune response induced in the human, is associated with the composition.

In one embodiment, the human has an hSBA titer equal to or greater than the hSBA's lower limit of quantitation (LLOQ) after administration of the first dose of the composition. In another embodiment, the human has an hSBA titer equal to or greater than the hSBA's LLOQ after administration of the second dose of the composition. In another embodiment, the human has an hSBA titer equal to or greater than the hSBA's LLOQ after administration of the third dose of the composition.

METHODS AND ADMINISTRATION

In one aspect, the invention relates to a method of inducing an immune response against *N. meningitidis* in a human. In another aspect, the invention relates to a method of vaccinating a human. In one embodiment, the method includes administering to the human at least one dose of the composition described above. In a preferred embodiment, the method includes administering to the human at most one dose of the composition described above. In another embodiment, the method includes administering to the human at least a first dose and a second dose of the composition described above.

In one embodiment, the second dose is administered at least 20, 30, 50, 60, 100, 120, 160, 170, or 180 days after the first dose, and at most 250, 210, 200, or 190 days after the first dose. Any minimum value may be combined with any maximum value described herein to define a range.

In another embodiment, the second dose is administered about 30 days after the first dose. In another embodiment, the second dose is administered about 60 days after the first dose, such as, for example, in a 0, 2 month immunization schedule. In another embodiment, the second dose is administered about 180 days after the first dose, such as, for example, in a 0, 6 month immunization schedule. In yet another embodiment, the second dose is administered about 120 days after the first dose, such as, for example, in a 2, 6 month immunization schedule.

In one embodiment, the method includes administering to the human two doses of the composition and at most two doses. In one embodiment, the two doses are administered within a period of about 6 months after the first dose. In one embodiment, the method does not include further administration of a booster to the human. A "booster" as used herein refers to an additional administration of the composition to the

human. Administering to the human at most two doses of the composition may be advantageous. Such advantages include, for example, facilitating a human to comply with a complete administration schedule and facilitating cost-effectiveness of the schedule.

In one embodiment, the first dose and the second dose are administered to the human over a period of about 25, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200 days, and most 400, 390, 380, 370, 365, 350, 340, 330, 320, 310, 300, 290, 280, 270, 260, 250, 240, 230, 220, 210, or 200 days after the first dose. Any minimum value may be combined with any maximum value described herein to define a range. Preferably, the first and second doses will be administered at least 4 weeks apart e.g. ≥8 weeks apart, ≥2 months apart, ≥3 months apart, ≥6 months apart, etc.

In one embodiment, the first dose and the second dose are administered to the human over a period of about 30 days. In another embodiment, the first dose and the second dose are administered to the human over a period of about 60 days. In another embodiment, the first dose and the second dose are administered to the human over a period of about 180 days.

Conveniently, the first dose can be administered at substantially the same time as (e.g. during the same medical consultation or visit to a healthcare professional or within 24 hours of the first dose of the meningococcal vaccine) another vaccine e.g. at substantially the same time as a hepatitis B virus vaccine, a diphtheria vaccine, a tetanus vaccine, a pertussis vaccine (either cellular or, preferably, acellular), a *Haemophilus influenzae* type b vaccine, a *Streptococcus pneumoniae* vaccine, and/or a polio vaccine (preferably in inactivated poliovirus vaccine). Each of these optionally co-administered vaccines may be a monovalent vaccine or may be part of a combination vaccine (e.g. as part of a DTP vaccine).

Conveniently, the second dose can be administered at substantially the same time as (e.g. during the same medical consultation or visit to a healthcare professional or within 24 hours of the second dose of the meningococcal vaccine) another vaccine e.g. at substantially the same time as a hepatitis B virus vaccine, a diphtheria vaccine, a tetanus vaccine, a pertussis vaccine (either cellular or acellular), a *Haemophilus influenzae* type b vaccine, a *Streptococcus pneumoniae* vaccine, a polio vaccine (preferably in inactivated poliovirus vaccine), an influenza vaccine, a chickenpox vaccine, a measles vaccine, a mumps vaccine, and/or a rubella vaccine. Each of these optionally

co-administered vaccines may be a monovalent vaccine or may be part of a combination vaccine (e.g. as part of an MMR vaccine).

Conveniently, the third dose can be administered at substantially the same time as (e.g. during the same medical consultation or visit to a healthcare professional or within 24 hours of the third dose of the meningococcal vaccine) another vaccine e.g. at substantially the same time as a hepatitis B virus vaccine, a diphtheria vaccine, a tetanus vaccine, a pertussis vaccine (either cellular or acellular), a *Haemophilus influenzae* type b vaccine, a *Streptococcus pneumoniae* vaccine, a polio vaccine (preferably in inactivated poliovirus vaccine), an influenza vaccine, a chickenpox vaccine, a measles vaccine, a mumps vaccine, and/or a rubella vaccine. Each of these optionally co-administered vaccines may be a monovalent vaccine or may be part of a combination vaccine (e.g. as part of an MMR vaccine).

THREE DOSES

In one embodiment, a three-dose schedule of the composition induces a bactericidal titer against multiple strains expressing LP2086 (fHBP) heterologous to the first and/or second polypeptide in a greater percentage of humans than a two-dose schedule.

In one embodiment, the method includes administering to the human three doses of the composition. In another embodiment, the method includes administering at most three doses of the composition. In one embodiment, the three doses are administered within a period of about 6 months after the first dose. In one embodiment, the method includes an administration of a booster dose to the human after the third dose. In another embodiment, the method does not include administration of a booster dose to the human after the third dose. In another embodiment, the method does not further include administering a fourth or booster dose of the composition to the human. In a further embodiment, at most three doses within a period of about 6 months are administered to the human.

In an exemplary embodiment, the second dose is administered about 30 days after the first dose, and the third dose is administered about 150 days after the second dose, such as, for example, in a 0, 1, 6 month immunization schedule. In another exemplary embodiment, the second dose is administered about 60 days after the first dose, and the third dose is administered about 120 days after the second dose, such as, for example, in a 0, 2, 6 month immunization schedule.

In one embodiment, the first dose, second dose, and third dose are administered to the human over a period of about 150, 160, 170, or 180 days, and at most 240, 210 200, or 190 days. Any minimum value may be combined with any maximum value described herein to define a range. Preferably, the first dose, second dose, and third dose is administered to the human over a period of about 180 days or 6 months. For example, the second dose may be administered to the human about 60 days after the first dose, and the third dose may be administered to the human about 120 days after the second dose. Accordingly, an exemplary schedule of administration includes administering a dose to the human at about months 0, 2, and 6.

As described above, multiple doses of the immunogenic composition may be administered to the human, and the number of days between each dose may vary. An advantage of the method includes, for example, flexibility for a human to comply with the administration schedules.

In one embodiment, the method includes administering to the human at most three doses of the identical immunogenic composition. For example, in a preferred embodiment, the method does not include administering to the human a first dose of a first composition, administering to the human a second dose of a second composition, and administering to the human a third dose of a third composition, wherein the first, second, and third compositions are not identical. In another embodiment, the method includes administering to the human at most four doses of the identical immunogenic composition.

EXAMPLES

The following Examples illustrate embodiments of the invention. Unless noted otherwise herein, reference is made in the following Examples to a MnB bivalent rLP2086 composition, at the 120-μg bivalent rLP2086 dose level, which is a preferred exemplary embodiment of a composition including: 60 μg of a first lipidated polypeptide including the amino acid sequence set forth in SEQ ID NO: 1 per 0.5 mL dose, 60 μg of a second lipidated polypeptide including the amino acid sequence set forth in SEQ ID NO: 2 per 0.5 mL dose, 2.8 molar ratio polysorbate-80 to the first polypeptide, 2.8 molar ratio polysorbate-80 to the second polypeptide, 0.5 mg Al^{3+}/ml of the composition, 10 mM histidine, and 150 mM sodium chloride.

More specifically, the investigational bivalent recombinant rLP2086 vaccine at the 120-μg bivalent rLP2086 dose level includes (a) 60 μg of a first lipidated polypeptide including the amino acid sequence set forth in SEQ ID NO: 1; (b) 60 μg of a second lipidated polypeptide including the amino acid sequence set forth in SEQ ID NO: 2; (c) 18 μg polysorbate-80; (d) 250 μg aluminum; (e) 780 μg histidine, and (f) 4380 μg sodium chloride. Each dose is 0.5 mL.

Unless noted otherwise herein, reference is made in the following Examples to a MenACWY-TT composition, which is a preferred exemplary embodiment of a tetravalent meningococcal polysaccharide conjugated composition that includes *Neisseria meningitidis* capsular polysaccharides A, C, W-135 and Y each coupled to tetanus toxoid as a carrier protein. The *Neisseria meningitidis* serogroups A and C polysaccharides are conjugated with an adipic dihydrazide (AH) spacer and indirectly conjugated to the tetanus toxoid whereas the W-135 and Y polysaccharides are conjugated directly to tetanus toxoid. The composition does not contain any preservatives or adjuvants.

More specifically, the lyophilized MenACWY-TT composition described in the examples below includes 5 micrograms of *Neisseria meningitidis* serogroup A polysaccharide conjugated to tetanus toxoid carrier protein; 5 micrograms of *Neisseria meningitidis* serogroup C polysaccharide conjugated to tetanus toxoid carrier protein; 5 micrograms of *Neisseria meningitidis* serogroup W-135 polysaccharide conjugated to tetanus toxoid carrier protein; 5 micrograms of *Neisseria meningitidis* serogroup Y polysaccharide conjugated to tetanus toxoid carrier protein; 28 mg sucrose; 97 μg trometamol, per dose (0.5 mL).

EXAMPLE 1: THE MenABCWY COMPOSITION

The final MenABCWY composition is prepared by reconstituting the lyophilized MenACWY-TT Drug Product (described in Example 2 below) vial with 0.67 mL of MnB Bivalent rLP2086 Drug Product (described in Example 3 below) in order to withdraw 0.5 mL dose of MenABCWY vaccine for intramuscular injection. All components used in the preparation of the MenABCWY vaccine and their functions are provided in Table 1 below.

Table 1. Composition of MenABCWY vaccine

Ingredients	Amount / dose
MnB rLP2086 subfamily A (SEQ ID NO: 1)	60 mcg
MnB rLP2086 subfamily B (SEQ ID NO: 2)	60 mcg
$MenA_{AH}$-TT conjugate (mean TT/polysaccharide ratio: ~3)	5 mcg MenA ~7.5 mcg TT
$MenC_{AH}$-TT conjugate (mean TT/polysaccharide ratio: ~3)	5 mcg MenC ~7.5 mcg TT
MenW-TT conjugate (mean TT/polysaccharide ratio: ~1.5)	5 mcg MenW ~3.75 mcg TT
MenY-TT conjugate (mean TT/polysaccharide ratio: ~1.3)	5 mcg MenY ~3.25 mcg TT
Tris-HCl	97 mcg
Sodium Chloride[a]	4.69- 4.71 mg
Sucrose	28 mg
L-Histidine	0.78 mg
Polysorbate 80 (PS80)	0.02 mg
Aluminum phosphate	0.25 mg aluminum
Water for injection	qs to 0.5 mL

[a]Sodium chloride concentration in MenABCWY Vaccine may vary between 160.5-161.1 mM based on the composition of the clinical and commercial NIMENRIX Drug Product (DP) lots.

EXAMPLE 2: DESCRIPTION AND COMPOSITION OF THE MnB BIVALENT rLP2086 DRUG PRODUCT

MnB bivalent rLP2086 drug product is a sterile liquid formulation composed of rLP2086 subfamily A and B proteins formulated at 120 µg/mL/subfamily in 10 mM histidine buffer, 150 mM sodium chloride (NaCl) at pH 6.0 with 0.5 mg/mL aluminum as aluminum phosphate (AlPO4). Polysorbate 80 (PS-80) is added to drug substance to obtain the target PS-80 to protein molar ratio. Therefore, PS-80 is not added during the drug product formulation but is present in the final drug product at the same ratio. The drug product is filled into 1 mL syringes. A single dose of vaccine is 0.5 mL with no preservatives.

Table 2. Composition of MnB Bivalent rLP2086 Drug Product

Ingredients	Quantity / dose
MnB rLP2086 subfamily A (SEQ ID NO: 1)	120 µg/mL
MnB rLP2086 subfamily B (SEQ ID NO: 2)	120 µg/mL
Sodium chloride	150 mM
L-Histidine	10 mM
Aluminum phosphate	0.50 mg Aluminum phosphate/mL
Water for injection	qs to 1 mL

[a] Polysorbate 80 (PS-80) is part of drug substance. PS-80 functions as a surfactant in the drug product.

[b] Equivalent to 0.25 mg aluminum per dose

The Effect of Polysorbate 80 Concentration

Polysorbate 80 (PS-80) is a non-ionic surfactant. It is used to stabilize and solubilize MnB rLP2086 subfamily A and B proteins in the formulation by preventing aggregation and adsorption that may be caused by temperature, filter, tubing, container/closure contact and process mixing. Stability studies using an *in vitro* monoclonal antibody based potency assay demonstrated instability of the subfamily B protein at higher molar ratios of PS-80 to MnB rLP2086 protein in the final formulation. Experiments with varying molar ratios of PS-80 to protein have demonstrated that the optimal molar ratio of PS-80 to MnB rLP2086 protein is approximately between 1.4 to 4.2 to retain potency.

EXAMPLE 3: DESCRIPTION AND COMPOSITION OF THE MenACWY-TT COMPOSITION

MenACWY-TT drug product is composed of the purified polysaccharides of *Neisseria meningitidis* serogroups A, C, W and Y, each conjugated to Tetanus Toxoid (TT) at ratios to polysaccharide of ~3, ~3, ~1.5 and ~1.3, respectively.

The MenACWY-TT drug product is presented as a lyophilized powder, supplied in a 3 mL glass vial with bromobutyl rubber closures suitable for lyophilization and aluminum flip-off caps. All components used in the manufacture of the MenACWY-TT Drug product and their functions are provided in Table 3.

Table 3. Composition of MenACWY-TT Drug product

Ingredients	Quantity / dose
$MenA_{AH}$-TT conjugate (mean TT/polysaccharide ratio: ~3)	5 mcg MenA ~15 mcg TT
$MenC_{AH}$-TT conjugate (mean TT/polysaccharide ratio: ~3)	5 mcg MenC ~15 mcg TT
MenW-TT conjugate (mean TT/polysaccharide ratio: ~1.5)	5 mcg MenW ~7.5 mcg TT
MenY-TT conjugate (mean TT/polysaccharide ratio: ~1.3)	5 mcg MenY ~6.5 mcg TT
Tris-HCl	97 mcg
Sucrose	28 mg
Sodium Chloride[a]	306.0-325.0 mg

[a]Lyophilized cake also contains sodium chloride resulting from the salt present in each of the bulk purified TT conjugates. Sodium chloride concentration varies between 10.5-11.1 mM based on the composition of the clinical and commercial lots.

EXAMPLE 4: PREPARATION OF THE MenABCWY COMPOSITION

The final MenABCWY composition is prepared in the clinic by reconstituting the lyophilized MenACWY-TT drug product vial with 0.67 mL of MnB Bivalent rLP2086. The resulting MenABCWY composition (a vaccine liquid drug product) contains rLP2086 subfamily A and B proteins at 120 mcg/mL/subfamily, purified polysaccharides of *Neisseria meningitis* types A, C, W and Y at concentration of 10 mcg/mL/type conjugated to Tetanus Toxoid at ratios of ~ 3, ~ 3, ~ 1.5, and ~ 3 respectively in 10 mM histidine and 1.6 mM tris buffer containing 160.5-161.1 mM sodium chloride, 0.5 mg/mL aluminum as aluminum phosphate (AlPO4), 0.035 mg/mL polysorbate 80 and 56 mg/mL sucrose at pH of 6.05 for intramuscular injection.

The MenABCWY vaccine is prepared by mixing of two drug products, MenACWY-TT and MnB Bivalent rLP2086. Buffering components and excipients were chosen based on the individual development of each component and are shown to provide the necessary stability profile for extended shelf life.

Dosage verification studies were performed to demonstrate that MenACWY-TT drug product and MnB Bivalent rLP2086 drug product are compatible when mixed together for administration of MenABCWY vaccine and that all drug product and dosing solutions are compatible with the administration components and that dosing solutions are stable in the administration components for a period of time adequate to perform the dose preparation and administration operations. The stability of MenABCWY vaccine prepared by reconstitution of MenACWY-TT drug product with 0.67 mL of MnB Bivalent rLP2086 drug product over the hold time at ambient temperature and light conditions was confirmed in reconstituted vials and in dosing syringes.

Samples representing the dosing solutions of MenABCWY vaccine were tested using stability indicating methods such as RP-HPLC for antigen binding and purity, bioplex activity assay, ELISA, and ICP-MS with predefined acceptance criteria. The results of this study show acceptable stability of MenABCWY vaccine for 24 hours at room temperature and light conditions. These data are shown and described in Examples 5-15 below.

EXAMPLE 5: EVALUATION OF THE MenABCWY VACCINE

A study was carried out to assess whether there is acceptable physical compatibility and short-term stability when a lyophilized MenACWY-TT composition is reconstituted with the MnB bivalent rLP2086 composition. The lyophilized MenACWY-TT composition and the liquid MnB bivalent rLP2086 composition were combined and stored for up to 24 hours in an uncontrolled room temperature environment to approximate real life conditions. It was demonstrated that lyophilized MenACWY-TT composition could be reconstituted with the liquid MnB bivalent rLP2086 composition with gentle hand mixing and the combined pH and osmolality were within typical range for an injectable. All key attributes for the conjugates and proteins were similar to those of a control for up to 24 hours in the uncontrolled room temperature environment.

The physical compatibility was evaluated through assessing pH, appearance, ease of reconstitution, and osmolality of the combined drug product. The stability of the antigens was evaluated through assessing concentration, purity and the in-vitro relative antigenicity (IVRA) of the rLP2086 subfamily A and subfamily B proteins as well as the concentrations of the conjugated Meningococcal A, C, Y, and W-135 polysaccharides by ELISA.

Example 5 through Example 15 demonstrate that the combination of the lyophilized MenACWY-TT composition and the liquid MnB bivalent rLP2086 composition, i.e., the MenABCWY composition, was found to be compatible and stable for at least 24 hours at room temperature.

ELISAs for Determining Mening A, C, Y, and W-135 Polysaccharide Concentrations in the MenABCWY composition- Development of the Mening A, C, Y and W-135 ELISA & Screening of the pAb for Detection

Six antibodies were selected for screening for use in the ELISA assays. Each of four groups of ten rabbits was immunized with either Men A, C, Y or W-135 polysaccharide TT conjugates with rabbits subsequently exsanguinated after antibody development. Each rabbit was individually screened using Men A, C, Y or W-135 polysaccharides conjugated to a carrier protein CRM_{197} for binding and specificity. Rabbit sera was screened for a positive binding signal, which is equivalent to an absorbance reading greater than three-fold above the background absorbance. Additionally, rabbit sera was screened for low non-specific binding, which was any absorbance readings for sera combinations without antigen, secondary or detection

above the absorbance reading for the blank, as well as low cross-reactivity, which was any absorbance readings for heterologous serotypes that was above the background absorbance. Rabbits that met the screening criteria were pooled. The standard curve range was established using CRM conjugates and confirmed with reconstituted the lyophilized MenACWY-TT composition. The standard curve range was established using CRM conjugates and confirmed with reconstituted lyophilized MenACWY-TT composition.

The feasibility of quantitating the A, C, Y and W conjugates in the combined drug product (MenABCWY composition) was established. It was determined that the MnB Bivalent rLP2086 composition alone was not detected in the assay. Additionally, when aluminum phosphate in the MenABCWY composition samples was solubilized, full recovery of conjugates was obtained. Therefore, it was determined that the MnB bivalent rLP2086 composition does not interfere with the quantitation of the MenACWY-TT conjugates by ELISA.

EXAMPLE 6: Evaluation of Suitability of Methods to Assess the MnB bivalent rLP2086 composition in the Presence of the MenACWY-TT composition

IEX-HPLC was evaluated for its suitability to determine the strength of the MnB bivalent rLP2086 composition subfamily A and B proteins in the presence of the MenACWY-TT composition. The total protein and bound protein results for the MnB bivalent rLP2086 composition in the presence of the MenACWY-TT composition and in the absence of the MenACWY-TT composition were accessed. The overlaid chromatograms are shown in **FIG. 1**.

Table 4

Sample	Subfamily	Bound Protein, %
the MnB bivalent rLP2086 composition no the MenACWY-TT composition	Protein A (SEQ ID NO: 1)	107
	Protein B (SEQ ID NO: 2)	104
the MnB bivalent rLP2086 composition with the MenACWY-TT composition	Protein A (SEQ ID NO: 1)	108
	Protein B (SEQ ID NO: 2)	103

EXAMPLE 7: Evaluation of the MnB bivalent rLP2086 composition Purity and Peak Ratio in the Presence of the MenACWY-TT composition

RP-HPLC was evaluated for its suitability to determine the purity of the MnB bivalent rLP2086 composition in the presence of the MenACWY-TT composition. The purity results for the MnB bivalent rLP2086 composition in the presence of the MenACWY-TT composition and in the absence of the MenACWY-TT composition were compared. The overlaid chromatograms are shown in **FIG. 2**. An example of the integration of the impurity peak is shown as an insert in FIG. 2. The evaluation results show that the presence of the MenACWY-TT composition does not interfere with evaluation of the MnB bivalent rLP2086 composition purity using the RP-HPLC method.

EXAMPLE 8: Evaluation of the MnB bivalent rLP2086 composition IVRA in the Presence of the MenACWY-TT composition

The IVRA method was evaluated for its suitability for determination of in-vitro relative antigenicity of the MnB bivalent rLP2086 composition Subfamily A (SEQ ID NO: 1) and Subfamily B (SEQ ID NO: 2) proteins in the presence of the MenACWY-TT composition.

The IVRA results for the MnB bivalent rLP2086 composition Subfamily A and Subfamily B proteins in the presence and in the absence of the MenACWY-TT composition were compared. The feasibility evaluation results show that, within the assay variability the results are comparable and that the presence of the MenACWY-TT composition does not interfere with determination of in-vitro relative antigenicity.

EXAMPLE 9: Reconstitution of the MenACWY-TT composition Vials With the MnB bivalent rLP2086 composition

The MenACWY-TT composition and the MnB bivalent rLP2086 composition drug products were performed using the MenACWY-TT composition vials reconstituted with the MnB bivalent rLP2086 composition drug product. The MenACWY-TT composition vials reconstituted with either saline or the MenACWY-TT composition matrix placebo were used as controls depending on the method.

Table 5

Product	Component	Composition
The MenACWY-TT composition	*Neisseria meningitidis* Group A polysaccharide Group C polysaccharide Group W-135 polysaccharide Group Y polysaccharide Tetanus toxoid carrier protein Sucrose Trometanol	 10 μg/mL 10 μg/mL 10 μg/mL 10 μg/mL 88 μg/mL 164 mM 1.6 mM
the MnB bivalent rLP2086 composition	Sub-family A rLP2086 protein (SEQ ID NO: 1) Sub-family B rLP2086 protein (SEQ ID NO: 2) AlPO4 Histidine NaCl	120 μg/mL 120 μg/mL 0.5 mg/mL 10 mM 150 mM pH 6.0
the MnB bivalent rLP2086 composition Placebo	AlPO4 Histidine NaCl	0.5 mg/mL 10 mM 150 mM pH 6.01
Bulk MnB bivalent rLP2086 composition DP	Sub-family A rLP2086 protein (SEQ ID NO: 1) Sub-family B rLP2086 protein (SEQ ID NO: 2) AlPO4 Histidine NaCl	120 μg/mL 120 μg/mL 0.5 mg/mL 10 mM 150 mM pH 6.0

Determination of Saline Reconstitution Volume for the MenACWY-TT composition

The NIMENRIX® commercial product package contains both a vial containing the lyophilized MenACWY-TT composition and a syringe containing 0.9% saline used for reconstitution. In order to reproduce the final NIMENRIX® concentration in the commercial vaccine upon reconstitution with the MnB bivalent rLP2086 composition, the amount of saline dispensed using the syringe from the commercial product had to be determined. This same volume of the MnB bivalent rLP2086 composition would then be used for all reconstitution studies.

Reconstitution of the MenACWY-TT Composition Vials with the MnB bivalent rLP2086 Composition

The MnB bivalent rLP2086 composition was pooled in a 10 mL glass vial. Approximately 800 µL of the solution was withdrawn into a 1 mL syringe. The adjusted contents of the syringe were injected into a vial containing the MenACWY-TT composition. The vial was swirled to dissolve the contents.

The pH and appearance were determined on duplicate samples on the MenABCWY composition. Osmolality was measured in triplicate on the MenACWY-TT composition reconstituted with saline and on the MenACWY-TT composition reconstituted with the MnB bivalent rLP2086 composition.

EXAMPLE 10: SEC-MALLS to Evaluate Mening A, C, Y, and W-135 Polysaccharide Stability in DP Matrix.

Mening A, C, Y, and W-135 Polysaccharides were used as surrogates to assess if any instability of the conjugated Meningococcal A, C, Y, and W-135 polysaccharides in the combined drug product (the MenABCWY composition) could be expected.

Treatment of Mening A, C, Y, and W-135 Polysaccharides

Reagent Preparation ("Full MenABCWY Composition Buffer Matrix")

2.24 g of sucrose and 7.8 mg of Tris (Tromethamine) was added to 20 ml of 2X MnB bivalent rLP2086 composition buffer matrix with MnB rLP2086 proteins (Histidine 20mM pH 6.0, NaCl 300mM, PS 80 0.07mg/ml, AlPO4 1mg/ml (8mM), rLP2086 subfamily A (SEQ ID NO: 1) and subfamily B (SEQ ID NO: 2) proteins 240 µg/mL each).

Sample Preparation

Each Mening Polysaccharide was diluted 1:1 with Full MenABCWY composition Buffer Matrix and incubated for 0, 6 and 24 hours at 5 °C, 25 °C and 37 °C. After incubation the sample suspension was spun for 1 minute at 14,000 r.p.m. The supernatant was analyzed by SEC-MALLS.

EXAMPLE 11: Stability of the MenABCWY Composition - Evaluation of pH, Appearance, and Osmolality of the Combined MnB bivalent rLP2086 and MenACWY-TT Compositions

The pH and appearance of the combined MnB bivalent rLP2086 composition and the MenACWY-TT composition, i.e., the MenABCWY composition, were evaluated immediately after reconstitution and again after 24 hours.

All results were as expected (Table 6).

Table 6. Appearance and pH of the MenABCWY composition

Sample #	Sample	Time Point, hours	Appearance	pH
1	MenABCWY composition, Rep1	0	Homogeneous white suspension	5.8
2	MenABCWY composition, Rep2	0	Homogeneous white suspension	5.8
3	MenABCWY composition, Rep 1	24	Homogeneous white suspension	5.8
4	MenABCWY composition, Rep 2	24	Homogeneous white suspension	5.8
5	MenACWY-TT composition w/Saline	0	Clear, Colorless	6.3
6	MenACWY-TT composition w/Saline	24	Clear, Colorless	6.4

The average osmolality of the MenACWY-TT composition reconstituted with the MnB bivalent rLP2086 composition was within 3% of the average osmolality of the MenACWY-TT composition reconstituted with saline.

Table 7

Vial	Reconstituting Agent	Reading 1 mOsm	Reading 2 mOsm	Reading 3 mOsm	Average mOsm
MenACWY-TT composition	Saline	471	473	478	474
MenACWY-TT composition	MnB bivalent rLP2086 composition	487	487	489	488

EXAMPLE 12: Mening A, C, Y, and W-135 Polysaccharide Conjugate Concentrations in the Combined Drug Product

The concentration of the Mening A, C, Y and W-135-TT conjugates in the MenABCWY composition was assessed initially and again after 24 hours. The concentrations of the four conjugates were stable over the twenty four hour time period (Table 8).

Table 8. Short Term Stability Results of the MnA, C, Y and W Conjugates in the MenABCWY composition by ELISA

Serotype	MenACWY-TT composition + MnB bivalent rLP2086 composition			The MenACWY-TT composition + Saline		
	Initial, μg/mL	After 24 hrs, μg/mL	Stability Ratio	Initial, μg/mL	After 24 hrs, μg/mL	Stability Ratio
A	6.7	6.8	1.0	6.4	6.7	1.1
C	6.9	6.6	1.0	6.5	6.7	1.0
Y	8.1	8.7	1.1	9.6	9.8	1.0
W	8.5	8.8	1.0	8.8	9.0	1.0

EXAMPLE 13: Evaluation of the Stability of the MnB bivalent rLP2086 Proteins in the MenABCWY composition

Total and Bound rLP2086 Subfamily A (SEQ ID NO: 1) and Subfamily B (SEQ ID NO: 2) Protein Concentrations in the Combined Drug Product

The MenABCWY composition samples were analyzed by IEX-HPLC to determine the protein concentrations. As shown in Table 9, the total protein, bound protein (to aluminum), and % bound of both MnB bivalent rLP2086 Subfamily A (SEQ ID NO: 1) and Subfamily B (SEQ ID NO: 2) proteins (bound to aluminum) did not change within 24 hours indicating that the rLP2086 Subfamily A and Subfamily B proteins were stable over the twenty four hour time period.

Table 9. Total and Bound Protein Stability

Time Point, hours	Subfamily	Total Protein, µg/mL	Bound Protein, µg/mL	Bound Protein, %
0	A	83	85	102
	B	88	87	99
24	A	87	88	101
	B	92	91	99

EXAMPLE 14: rLP2086 Protein Purity and Peak Ratio in the Combined MenABCWY composition

The MenABCWY composition samples were analyzed by RP-HPLC to determine purity and peak ratios for the rLP2086 proteins. See **FIG. 3.** The peak at 11.9 min is excluded from the purity calculation.

rLP2086 Subfamily A and Subfamily B Protein IVRA in the Combined Drug Product

The IVRA of the MenABCWY composition samples was assessed for up to 24 hours after mixing. It was determined that the relative antigenicity of the rLP2086 Subfamily A (SEQ ID NO: 1) and Subfamily B (SEQ ID NO: 2) proteins in the MenABCWY composition was stable over the twenty four hour time period.

EXAMPLE 15: Mening A, C, Y, and W-135 Polysaccharide Stability in Full MenABCWY Composition Buffer Matrix by SEC-MALS

Stability of Mening A PS in Full MenABCWY Composition Buffer Matrix by SEC-MALLS after 6 and 24 Hours Incubation at Various Temperatures

Mening A, C, W, and Y polysaccharides were mixed with the Full MenABCWY Composition Buffer Matrix and evaluated for stability by SEC-MALS after incubation at 5°C, 25°C and 37°C for up to 24 hours. All four polysaccharides appear to be stable for up to 24 hours at 5°C and 25°C. Some degradation was observed at 37 °C for Mening A and Y. The degree of degradation could not be determined for Mening Y Polysaccharides due to formation of high Mw aggregates under all tested conditions except initial.

Table 10

Sample	Incubation Time, hours	Incubation Temperature	Mw (kDa)	Δ Mw (%)
Mening A PS	0	NA	169	N/A
	24	5°C	171	1
		25°C	157	-7
		37°C	126	-26
Mening C PS	0	NA	213	N/A
	24	5°C	216	1
		25°C	215	1
		37°C	220	3
Mening Y PS*	0	NA	294	N/A
	24	5°C	734	149
		25°C	756	157
		37°C	696	136
Mening W-135 PS	0	NA	205	N/A
	24	5°C	230	12
		25°C	211	3
		37°C	219	7

EXAMPLE 16: EVALUATION OF THE MNB BIVALENT RLP2086 COMPOSITION-INDUCED HSBA ACTIVITY CROSS PROTECTION AGAINST MENACWXY

The TRUMENBA MnB bivalent rLP2086 composition contains two lipidated factor H binding proteins (fHBP), one from each subfamily and provides protection against *Neisseria meningitides* serogroup B. fHBP has been found to be expressed at variable levels in serogroups A, C, W, Y, and X suggesting that the MnB bivalent rLP2086 composition might offer protection to these serogroups. We used sera from study B1971015 to investigate the proof of concept that the TRUMENBA MnB bivalent rLP2086 composition may offer protection against serogroups A, C, W, Y, and X. Strains were collected globally (including MnW strains from a recent UK outbreaks and MnX which is newly emerging in Africa). The process of selecting a candidate strain to develop an hSBA and the immune response from a subset of subjects immunized with the MnB bivalent rLP2086 composition and a subset immunized with a four valent meningococcal capsular polysaccharide conjugate vaccine (MCV4) as a comparison is described.

Sera used for immunogenicity determinations

Subsets of sera from the study B1971015, a phase 2, randomized, active-controlled, observer-blinded study in which two group of subjects received either MENACTRA (meningococcal A, C, Y and W-135 polysaccharide conjugate vaccine [MCV4]) and ADACEL (tetanus toxoid, reduced diphtheria toxoid, acellular pertussis vaccine [Tdap]) or bivalent rLP2086 (TRUMENBA [meningococcal serogroup B vaccine], approved in the United States) were used to access the immunogenicity in hSBA using the MnACWYX strains selected as described below.

Baseline seropositivity rates

Baseline seropositivity rates in the microcolony-based hSBA were estimated using non-immune or prevaccination sera obtained from adolescents from study B1971015. For this purpose, seropositivity is defined as a hSBA titer ≥ 1:8, which was the anticipated assay LLOQ. To be considered for assay development, the strains must demonstrate low baseline seropositivity (i.e., a rate of hSBA titers ≥ 1:8 using baseline sera < ~40%).

Sera used for immunogenicity determinations

Subsets of sera from the study B1971015, a phase 2, randomized, active-controlled, observer-blinded study in which two group of subjects received either MENACTRA (meningococcal A, C, Y and W-135 polysaccharide conjugate vaccine [MCV4]) and

ADACEL (tetanus toxoid, reduced diphtheria toxoid, acellular pertussis vaccine [Tdap]) or bivalent rLP2086 (TRUMENBA [meningococcal serogroup B vaccine], approved in the United States) were used to access the immunogenicity in hSBA using the MnACWYX strains selected as described below.

RESULTS AND DISCUSSION

Serogroup A

There were 17 strains from South Africa, 4 strains from US and one strain from Netherlands in the Serogroup A SBA strain pool. Seventy-three percent of the serogroup A strains in this collection express B16 and 23% B22. The prevalent clonal complexes in this collection of serogroup A strains are ST-1 and ST-5. Two strains expressing B16 and one strain expressing B22 with fHBP expression levels above 1100 MFI were selected for further testing. 36 lots of C' were used for C' T30/T0 ratio screening. PMB1546 was excluded since appropriate human serum complement sources that did not non-specifically kill the strain could not be identified according the C' passing rate (Table 11). PMB3143 was excluded due to high baseline seropositivity rates, 52%, with adolescent preimmune sera. PMB3257 met the selection criteria: appropriate complement sources were identified, low baseline seropositivity rates with adolescent preimmune sera e.g. 0%, and hSBAs was technically feasible.

Table 11. Serogroup A Test Strain Candidates

Strain	fHBP Variant	fHBP Expression[a]	Epidemio-logical Marker	Country of Isolation	C' Pass Rate	hSBA Compatible	Baseline Seropositivity Rate
PMB3257	**B16**	**1725**	**ST-1 complex/ subgroup I/II**	**South Africa**	**33%**	**Yes[b]**	**0%[c]**
PMB3143	B16	1657	**ST-1 complex/ subgroup I/II**	U. S.	14%	**Yes**	52%[d]
PMB1546	B22	2657	ST-5 complex/ subgroup III	South Africa	0%	ND[e]	ND

a.mean fluorescent intensity as determined in the MEASURE assay
b.strain formed defined colonies, and was killed by indicator sera in hSBA
c.adolescents, prevaccinated sera
d.pre-immunity rate high
e. not done
The selected Serogroup A strain is indicated in **bold** font.

Serogroup C

There were 49 strains from U. S. and 10 strains from Netherlands in the Serogroup C SBA strain pool. The fHBP sequence is more heterogeneous in serogroup C. The strain pool contains at least17%, namely 19%, of MenC strains were fHBP A10 expressing strains and 10% A15 expressing strains. The prevalent clonal complexes in this collection of serogroup C strains are ST11/ET-37 complex and ST-103. One strain expresing A10 and three strains epxresing A15 with fHBP expression levels above 1100 MFI were selected for further testing. 36 lots of C' were used for C' T30/T0 ratio screening. PMB5180, and PMB5196 expressing A15 for fHBP were excluded since appropriate human serum complement sources that did not non-specifically kill the strain could not be identified according the C' passing rate (Table 12). For PMB5043, the C' passed initial screening test failed in SBA assay due to T30/T0 ratio. PMB5208 met the selection criteria: appropriate complement sources were identified, low baseline seropositivity rates with adolescent preimmune sera were confirmed 17%, and hSBAs was technically feasible.

Table 12. Serogroup C Test Strain Candidates

Strain	fHBP Variant	fHBP Expression[a]	Epidemio-logical marker	Country of Isolation	C' Pass Rate	hSBA Compatible	Baseline Seropositivity Rate
PMB5208	**A10**	**1563**	**ST11/ET-37 complex**	**United States**	**56%**	**Yes[b]**	**17%[d]**
PMB5180	A15	3237	ST-103	United States	0%	ND[e]	ND
PMB5196	A15	3353	ST-103	United States	3%	ND	ND
PMB5043	A15	2803	ST-103	United States	8%	No[c]	ND

a.mean fluorescent intensity as determined in the MEASURE assay
b.strain formed defined colonies, and was killed by indicator sera, in hSBA
c. no appropriate complement sources available, failed T30/T0 ratio in SBA
d.adolescents, prevaccinated sera
e.not done
The selected Serogroup C strain is indicated in **bold** font.

Serogroup W

There were 14 strains from U. S., 9 strains from Netherlands and 6 recent outbreak strains from U. K. in the Serogroup W SBA strain pool. The fHBP sequence is more homogeneous in this collection of serogroup W strains. Forty-five percent of the strains in this pool expressed fHBP A19 variant and 45% fHBP A10. The prevalent clonal complex for fHBP A19 variant strains in the serogroup W pool is ST-22 and the prevalent clonal complex for fHBP A10 variant strains is ST-11/ET-37 complex. From the US, one strain expressing A19 and one strain expressing A10 were selected. Two UK outbreak strains expressing A10 with fHBP expression levels above 1100 MFI were selected for further testing. 36 lots of C' were used for C' T30/T0 ratio screening. Four strains all passed this initial C' screening test. Based on the preimmunity rate, PMB5524 and PMB5163 were excluded since the seropositivity rates with adolescent preimmune sera were 74% and 48%, respectively. PMB5248 and PMB5523 met the selection criteria: appropriate complement sources were identified, low baseline seropositivity rates with adolescent preimmune sera e.g. 26%, and 28%, respectively and hSBAs were technically feasible (Table 13).

Table 13. Serogroup W Test Strain Candidates

Strain	fHBP Variant	fHBP Expression[a]	Epidemio-logical Marker	Country of Isolation	C' Pass Rate	hSBA Compatible	Baseline Seropositivity Rate
PMB5248	**A19**	**2684**	**ST-22 complex**	United States	**14%**	**Yes[b]**	**26%[c]**
PMB5524	A10	1197	ST-11/ET-37 complex	U. K.	34%	Yes	74%[d]
PMB5163	A10	1565	ST-11 complex/ET-37 complex	United States	6%	Yes	48%[d]
PMB5523	**A10**	**1796**	**ST-11 complex/ET-37 complex**	**U. K.**	**32%**	**Yes**	**28%**

a.mean fluorescent intensity as determined in the MEASURE assay
b.strain formed defined colonies, and was killed by indicator sera, in hSBA
c.adolescents, prevaccinated sera
d.pre-immunity rate high
The selected Serogroup W strain is indicated in **bold** font.

Serogroup X

There were 8 strains from Africa and one strain from Netherlands in the Serogroup X SBA strain pool. Five strains from Africa express fHBP B49 variant, three strains had a new fHBP type and the one strain from Netherland expressed fHBP B09. The fHBP expression for all nine strains was over 1100 MFI. Three Africa outbreak strains and one from the Netherlands were selected for further testing. 36 lots of C' were used for C' T30/T0 ratio screening. Four strains all passed C' initial screening test. Based on the preimmunity rate, PMB5467 was excluded since the seropositivity rates with adolescent preimmune sera was 68%. PMB5537, PMB5540 and PMB5539 met the selection criteria: appropriate complement sources were identified, low baseline seropositivity rates with adolescent preimmune sera e.g. 0, and hSBAs were technically feasible (Table 14). PMB5540 was selected for evaluation.

Table 14. Serogroup X Test Strain Candidates

Strain	fHBP Variant	fHBP Expression[a]	Epidemio-logical Marker	Country of Isolation	C' Pass Rate	hSBA Compatible	Baseline Seropositivity Rate
PMB5467	B09	1795	ST-1157 complex	Netherland	6%	Yes[b]	68%[d]
PMB5537	B239	4896	ST-181 complex	Burkina Faso	8%	Yes[b]	4%[c]
PMB5540	**B49**	**8612**	**ST-181 complex**	**Burkina Faso**	**53%**	Yes[b]	**0%**
PMB5539	B49	13706	To be assigned	Uganda	83%	Yes[b]	0%

a.mean fluorescent intensity as determined in the MEASURE assay
b.strain formed defined colonies, and was killed by indicator sera in hSBA
c. no appropriate complement sources available, failed T30/T0 ratio in SBA
c.adolescents, prevaccinated sera
d.pre-immunity rate high
The selected Serogroup X strain is indicated in **bold** font.

Serogroup Y

There were 87 strains from U. S. and 30 strains from Netherlands in the Serogroup Y SBA strain pool. The fHBP sequence is more homogeneous in this collection of serogroup Y strains. Sixty-six percent of the serogroup Y strains in the pool express fHBP A15. The common clonal complex for fHBP A15 variant expressing strains in this serogroup Y collection is ST-23/Cluster A3. Only 15% of the serogroup Y strains in this collection have fHBP expression levels above 1100 MFI. Three strains from the A15 variant group with fHBP expression levels above 1100 MFI were selected for further testing. 36 lots of C' were used for C' T30/T0 ratio screening. PMB5122, PMB5053 and PMB5050 were excluded since appropriate human serum complement sources that did not non-specifically kill the strain could not be identified according the C' passing rate (Table 15). PMB5187 with fHBP B47 was selected for C' T30/T0 screening and C' sources that did not kill the strain non-specfically could be identified (Table 15). Even though PMB5187 did not express the prevalent fHBP variant for serogroup Y, this strain met the other selection criteria: appropriate complement sources were identified, low baseline seropositivity rates with adolescent preimmune sera e.g. 4%, and hSBAs was technically feasible.

Table 15. Serogroup Y Test Strain Candidates:

Strain	fHBP Variant	fHBP Expression[a]	Epidemio-logical Marker	Country of Isolation	C' Pass Rate	hSBA Compatible	Baseline Seropositivity Rate
PMB5122	A15	1011	ST-23/Cluster A3	United States	0%	ND[d]	ND
PMB5053	A15	1608	ST-23/Cluster A3	United States	0%	ND	ND
PMB5050	A15	1811	ST-23/Cluster A3	United States	0%	ND	ND
PMB5187	**B47**	**5063**	**ST-23/Cluster A3**	United States	**64%**	**Yes[b]**	**4%[c]**

a.mean fluorescent intensity as determined in the MEASURE assay
b.strain formed defined colonies, and was killed by indicator sera, in hSBA
c.adolescents, prevaccinated sera
d.not done
The selected Serogroup Y strain is indicated in **bold** font.

MnACYWX strains were selected for hSBA development based on the prevalent variants of fHBP for the respective serogroup, fHBP expression above 1100 MFI and assay feasibility (e.g. identification of human complement and baseline seropositivity). Characteristics of the six selected MnACYWX strains, as well as the median fHBP expression for each variant group are summarized in Table 16.

Table 16. MnACYWX Test Strain Characteristics

Strain	Serogroup	fHBP Variant	fHBP Expression	Clonal Complex	Country of Isolation
PMB3257	A	B16	1725	ST-1 complex	South Africa
PMB5208	C	A10	1563	CC11/ET-37 complex	United States
PMB5248	W	A19	2684	CC-22	United States
PMB5523	W	A10	1796	CC-11/ET-37 complex	U. K.
PMB5187	Y	B47	5063	CC-23/Cluster A3	United States
PMB5540	X	B49	8612	ST-181 complex	Burkina Faso

Immunogenicity Analysis

The MnACYWX test strains were used in hSBAs to assess the immune response elicited in subsets of healthy adolescents aged 10 to <13 years enrolled in the Phase 2 concomitant study B1971015. TRUMENBA-elicited hSBA responses were compared to MENACTRA- elicited (meningococcal A, C, Y and W-135 polysaccharide conjugate vaccine [MCV4]) hSBA responses for the six strains selected (Table 17).

Substantial bactericidal antibody responses were observed in a high proportion of vaccinated individuals based on an hSBA titer ≥1:8, a more stringent criterion than the accepted correlate of protection (ie, an hSBA titer ≥1:4) for MenCWYX strains except MenA with 28% from those subjects immunized with TRUMENBA compared to 97% from those immunized with MCV4. The TRUMENBA response reached a peak at one month after dose 2 for two serogroup W strains (PMB5248 100%, PMB5523, 97%) and one month after dose 3 for serogroup C, Y, X strains with response rates of 83%-100%. MCV4 responses reached peak one month after dose 1, with no response for MenX. TRUMENBA elicited antibodies demonstrated the potential to protect against MenX, which is not covered by MCV4. The TRUMENBA response rate (i.e. % ≥ 1:8) after 3 doses against MnC, W or Y strains is comparable to MCV4 response rate after 1 dose. The immunogenicity data obtained from the MnACYWX hSBA test strains provide proof of concept for protection against serogroups other than B.

Table 17: The percentage of SBA titers ≥ 1:8 for MnACWYX strains

			Vaccine Group					
			Group 2: MCV4 0-month			Group 3: Trumenba 0, 2, 6-month		
Strain	Serogroup	Sampling Time	N	n	%(GMT)	N	n	%(GMT)
PMB3257	A	Month 0	30	1	3% (2)	30	1	3% (2)
		Month 1	30	29	97% (95)	30	4	13% (3)
		Month 3	30	22	73% (31)	30	6	20% (4)
		Month 7	30	14	47% (11)	29	8	28% (5)
PMB5208	C	Month 0	30	6	20% (3)	30	7	23% (4)
		Month 1	30	27	90% (119)	30	16	53% (8)
		Month 3	30	28	93% (88)	30	21	70% (12)
		Month 7	30	27	90% (38)	30	28	93% (29)
PMB5248	W	Month 0	30	12	40% (5)	30	8	27% (4)
		Month 1	30	29	97% (88)	30	22	73% (18)
		Month 3	29	28	97% (58)	30	30	100% (47)
		Month 7	29	26	90% (41)	30	30	100% (77)
PMB5523	W	Month 0	29	16	55% (8)	30	13	43% (7)
		Month 1	30	29	97% (60)	30	20	67% (15)
		Month 3	30	29	97% (58)	30	29	97% (21)
		Month 7	30	29	97% (44)	30	30	100% (42)
PMB5187	Y	Month 0	30	4	13% (3)	30	3	10% (3)
		Month 1	30	29	97% (79)	30	14	47% (7)
		Month 3	30	28	93% (58)	30	28	93% (31)
		Month 7	30	27	90% (36)	30	30	100% (58)
PMB5540	X	Month 0	30	0	0% (2)	30	2	7% (2)
		Month 1	30	0	0% (2)	30	5	17% (3)
		Month 3	30	0	0% (2)	30	16	53% (7)
		Month 7	30	0	0% (2)	30	25	83% (20)

CONCLUSION MnACYWX strains were selected for hSBA development based on the prevalent variants of fHBP for the respective serogroup, fHBP expression above the fHBP-expression threshold for MnB and assay feasibility (e.g. identification of human complement and baseline seropositivity). Using sera from subjects immunized with Trumenba in hSBA using MnACYWX strains provided proof for the concept that Trumenba elicited antibodies can provide protection against serogroups other than B. Bivalent rLP2086-elicited responses (e.g. percentage of subjects with titers ≥1:8) after 3 doses are comparable to MCV4-elicited responses after 1 dose for strains from serogroups C, W and Y, but lower for a serogroup A strain. Moreover, bivalent rLP2086-elicited hSBA titers ≥1:8 in a high proportion of subjects, indicative of protection against MenX, which is not provided by MCV4.

EXAMPLE 17: Trumenba Elicits Bactericidal Antibodies Against Non–Serogroup B Meningococci

Introduction. *Neisseria meningitidis* (Men) is the leading cause of bacterial meningitis and septicemia in infants, adolescents, and young adults. There are 5 major disease-causing meningococcal serogroups, A, B, C, Y, and W, with a sixth serogroup, X, emerging in Africa. Quadrivalent meningococcal conjugate vaccines (MCV4) are used to protect from disease caused by meningococcal serogroups A, C, Y, and W in various regions of the world. The recently approved vaccine TRUMENBA® (MenB-FHbp, bivalent rLP2086, Pfizer Inc, Collegeville, PA), intended to provide protection against serogroup B disease, consists of 2 recombinant lipoproteins, 1 from each of the 2 factor H binding protein (FHbp) phylogenetic subfamilies.2 The gene coding for FHbp is found in nearly all invasive Men strains, independent of serogroup classification. Preclinical studies have demonstrated the potential for MenB-FHbp to protect against other disease-causing serogroups. In preliminary studies, the Men B vaccine BEXSERO® (MenB-4C; Novartis Inc, Cambridge, MA), which includes a single FHbp variant antigen, was able to elicit a bactericidal immune response against MenX and MenW strains. The aim of this study was to extend these observations using exploratory assays to investigate whether antibodies elicited in adolescents by MenB-FHbp are bactericidal against MenA, C, W, Y, and X strains (**FIG. 7**), thereby providing the potential for protection against meningococcal disease across serogroups.

Experimental Overview. From the candidate strains (**FIG. 8**), select strains that

• Were not susceptible to human complement killing alone

• Were killed in hSBA using sera shown to have bactericidal antibodies directed against FHbp expressed by MenB strains

• Have low baseline titers with prevaccination sera (25 prevaccination sera; see **FIG. 9**)

For the selected strains (**Table 18**, **FIG. 10**): Develop exploratory hSBA assays. See **FIG. 9** for the sera tested in the hSBA. Response rate=percentage of subjects with hSBA titers ≥1:8, greater than the established correlate of protection (≥1:4) (**FIG. 11-16**).

Table 18. Characteristics of Selected MenA, MenC, MenY, MenW, and MenX Test Strains

Strain	Serogroup	fHBP Variant	fHBP Expression (MFI)	Median of fHBP Expression (MFI) for Sergroup	Clonal Complex	Country of Isolation
PMB3257	A	B16	1725	1192	ST-1 complex/subgroup I/II	South Africa
PMB5208	C	A10	1563	1563	ST-11 complex/ET-37 complex	United States
PMB5248	W	A19	2684	460	ST-22 complex	United States
PMB5523	W	A10	1796	460	ST-11 complex/ET-37 complex	England/Wales
PMB5187	Y	B47	5063	290	ST-23 complex/cluster A3	United States
PMB5540	X	B49	8612	7555	ST-181 complex	Burkina Faso

With reference to **FIG. 9**, 25 sera from Group A month 0 were used to study pre-immunity. Sera from 30 subjects from Group A drawn at month 0 and 1 and Group B drawn at month 0, 1, 3 and 7 were used for hSBA testing. Endpoint of the response rate is the percentage of sera with titers > 1:8.

FIG. 10 – Distribution of FHbp Surface Expression Levels (MFI) Determined From Flow Cytometric Experiments Using the FHbp Reactive mAb MN 994-11. The FHbp surface expression for each of the strains within a serogroup is noted with a black dot while the FHbp surface expression levels for the selected test strains within each serogroup are noted with a colored star.

FIG. 11 – hSBA Response Rate (Percentage of Subjects With hSBA Titers ≥1:8) for MenA PMB3257 (B16). Response rates and 95% confidence intervals for sera collected at preimmunization (month 0) and 1 month after doses 1, 2, and 3 for MenBFHbp are shown. The geometric mean titers (GMTs) obtained were 2, 3, 4, and 5, respectively. The response rates for subjects in the positive control group were 3% prior to vaccination

and 97% one month after receiving MCV4. The GMTs for the positive control group were 2 and 95, respectively.

FIG. 12 – hSBA Response Rate (Percentage of Subjects With hSBA Titers ≥1:8) for MenC PMB5208 (A10). Response rates and 95% confidence intervals for sera collected at preimmunization (month 0) and 1 month after doses 1, 2, and 3 for MenBFHbp are shown. The GMTs obtained were 4, 8, 12, and 29, respectively. The response rates for subjects in the positive control group were 20% prior to vaccination and 90% one month after receiving MCV4. The GMTs for the positive control group were 3 and 119, respectively.

FIG. 13 – hSBA Response Rate (Percentage of Subjects With hSBA Titers ≥1:8) for MenW PMB5248 (A19). Response rates and 95% confidence intervals for sera collected at preimmunization (month 0) and 1 month after doses 1, 2, and 3 for MenBFHbp are shown. The GMTs obtained were 4, 18, 47, and 77, respectively. The response rates for subjects in the positive control group were 40% prior to vaccination and 97% one month after receiving MCV4. The GMTs for the positive control group were 5 and 88, respectively.

FIG. 14 – hSBA Response Rate (Percentage of Subjects With hSBA Titers ≥1:8) for MenW PMB5523 (A10). Response rates and 95% confidence intervals for sera collected at preimmunization (month 0) and 1 month after doses 1, 2, and 3 for MenBFHbp are shown. The GMTs obtained were 7, 15, 21, and 42, respectively. The response rates for subjects in the positive control group were 55% prior to vaccination and 97% one month after receiving MCV4. The GMTs for the positive control group were 8 and 60, respectively.

FIG. 15 – hSBA Response Rate (Percentage of Subjects With hSBA Titers ≥1:8) for MenY PMB5187 (B47). Response rates and 95% confidence intervals for sera collected at preimmunization (month 0) and 1 month after doses 1, 2, and 3 for MenBFHbp are shown. The GMTs obtained were 3, 7, 31, and 58, respectively. The response rates for subjects in the positive control group were 13% prior to vaccination and 97% one month after receiving MCV4. The GMTs for the positive control group were 3 and 79, respectively.

FIG. 16 – hSBA Response Rate (Percentage of Subjects With hSBA Titers ≥1:8) for MenX PMB5540 (B49). Response rates and 95% confidence intervals for sera collected at preimmunization (month 0) and 1 month after doses 1, 2, and 3 for MenBFHbp are

shown. The GMTs obtained were 2, 3, 7, and 20, respectively. The response rates for subjects in the positive control group were 0% prior to vaccination and 0% one month after receiving MCV4 vaccine. The GMTs for the positive control group were 2 and 2, respectively.

Summary. Response rates elicited by MenB-FHbp peaked at 1 month after dose 2 (Month 3) for the MenW strain, PMB5248 (100%), and 1 month after dose 3 (Month 7) for the MenC, MenY, and MenX strains, as well as for the second MenW strain (response rates ranging from 83%–100%).

The response rates measured by hSBA for the MenA test strain were substantially lower, peaking at 28% after 3 doses of MenB-FHbp.

The recognized correlate of protection against meningococcal disease is an hSBA titer ≥1:4. The ability of MenB-FHbp to elicit hSBA titers of at least 1:8 provides proof of concept that MenB-FHbp may protect against disease caused by meningococcal serogroups other than B, including MenX, which is not covered by MCV4.

EXAMPLE 18: A Phase 2, Randomized, Controlled, Observer-Blinded Study to Describe the Immunogenicity, Safety, and Tolerability of *Neisseria meningitidis* Serogroup B Bivalent Recombinant Lipoprotein 2086 Vaccine (Bivalent rLP2086, i.e., now TRUMENBA® vaccine) in Healthy Subjects Aged ≥24 Months to <10 Years (B1971017-syn)

Study Design: This was a Phase 2, randomized, controlled, observer-blinded, multicenter study designed to assess the immunogenicity, safety, and tolerability of bivalent rLP2086 at the 120-μg dose level administered to healthy subjects aged ≥24 months to <10 years as part of a Month 0, 2, and 6 schedule (Table 19). Approximately 400 subjects were planned to be randomly assigned to 1 of 2 groups in a 3:1 ratio. Group 1 received bivalent rLP2086 at Month 0 (Visit 1) followed by subsequent vaccinations at Months 2 and 6. Group 2 received a licensed pediatric hepatitis A virus (HAV) vaccine at Month 0 (Visit 1) and Month 6 and an injection with saline at Month 2 to maintain the study blind. Follow-up visits were conducted 1 month after each vaccination and 6 months after the third vaccination to collect safety data and/or obtain a blood sample. Subjects participated in the study for up to 13 months.

Table 19. Study Design

	Vaccination 1	**Post-Vaccination 1 Follow-up**	**Vaccination 2**	**Post-Vaccination 2 Blood Draw**	**Vaccination 3**	**Post-Vaccination 3 Blood Draw**	**Month 12 Follow-up and Blood Draw**
Visit	1	2	3	4	5	6	7
Approximate month	0	1	2	3	6	7	12
Group 1 (300 subjects)	Bivalent rLP2086		Bivalent rLP2086		Bivalent rLP2086		
Group 2 (100 subjects)	HAV vaccine		Saline		HAV vaccine		
Blood draw (all subjects)	5-10 mL			5-10 mL		5-10 mL	5-10 mL

HAV = hepatitis A virus

Vaccines Administered: Subjects in Group 1 were administered bivalent rLP2086 by intramuscular injection into the upper deltoid muscle of the arm at Months 0, 2, and 6.

Subjects in Group 2 were administered HAV vaccine/saline/HAV vaccine into the upper deltoid muscle of the arm at Months 0, 2, and 6, respectively.

Immunogenicity Evaluations: To facilitate immunogenicity analysis, subjects had approximately 5 to 10 mL (dependent upon age) of blood collected immediately before Vaccination 1, 1 month after Vaccination 2, and 1 and 6 months after Vaccination 3.

For assessment of the immune response to bivalent rLP2086, functional antibodies were analyzed in validated hSBAs with 4 primary MnB test strains selected using an unbiased algorithm, and adjusted for epidemiological prevalence based on regulatory input, from Pfizer's MnB serum bactericidal assay (SBA) strain pool. The hSBA measures antibodies in human sera that initiate complement-dependent destruction of the target meningococcal strain. Four (4) primary MnB test strains, PMB80 (A22), PMB2001 (A56), PMB2948 (B24), and PMB2707 (B44), each expressing an factor H binding protein (fHBP) variant heterologous to the vaccine component antigens, were used in the hSBAs for determination of the immunogenicity endpoints in this study. Sera obtained from all subjects prior to the first study vaccination, 1 month after the second study vaccination, and 1 and 6 months after the third study vaccination were used in these assays.

For the primary analyses, 2 of the primary test strains (PMB80 [A22] and PMB2948 [B24]) were tested at each blood sampling time point for half of the subjects (in both groups), and the other 2 primary test strains (PMB2001 [A56] and PMB2707 [B44]) were tested at each blood sampling time point for the remaining half of the subjects.

The primary immunogenicity endpoints were: Proportion of subjects aged ≥24 months to <4 years (at study entry) with hSBA titer ≥ lower limit of quantitation (LLOQ) for each of the 4 primary MnB test strains 1 month after the third vaccination with bivalent rLP2086; and Proportion of subjects aged ≥4 years to <10 years (at study entry) with hSBA titer ≥ LLOQ for each of the 4 primary MnB test strains 1 month after the third vaccination with bivalent rLP2086.

The secondary immunogenicity endpoints were:

- In healthy subjects aged ≥24 months to <10 years at study entry:
  - Proportion of subjects with hSBA titer ≥ LLOQ for each of the 4 primary MnB test strains 1 month after the third vaccination with bivalent rLP2086.
- In healthy subjects aged ≥24 months to <4 years at study entry, in healthy subjects aged ≥4 years to <10 years at study entry, and in the combined age stratum:
  - Proportion of subjects with hSBA titer ≥ LLOQ for each of the 4 primary MnB test strains 1 month after the second vaccination and 1 and 6 months after the third vaccination with bivalent rLP2086.

- Proportions of subjects achieving hSBA titers of ≥1:4, ≥1:8, ≥1:16, ≥1:32, ≥1:64, and ≥1:128 for each of the 4 primary test strains at baseline, 1 month after the second vaccination, and 1 and 6 months after the third vaccination with bivalent rLP2086.
- hSBA GMTs for each of the 4 primary test strains at baseline, 1 month after the second vaccination, and 1 and 6 months after the third vaccination with bivalent rLP2086.

The secondary immunogenicity endpoints were summarized for both the evaluable immunogenicity population and the mITT population.

The following exploratory endpoints were used to describe responses in healthy subjects aged ≥24 months to <4 years at study entry, in healthy subjects aged ≥4 years to <10 years at study entry, and in the combined age stratum:

- Proportions of subjects with hSBA titers ≥1:4, ≥1:8, ≥1:16, ≥1:32, ≥1:64, and ≥1:128 at each applicable blood sampling time point.
- hSBA GMTs for each of the 4 primary strains at each applicable blood sampling time point.
- Proportion of subjects achieving at least a 4-fold increase in hSBA titer from baseline to 1 month after the third vaccination with bivalent rLP2086 for each of the 4 primary test strains:
  - For subjects with a baseline hSBA titer below the limit of detection (LOD) or an hSBA titer of <1:4, a 4-fold response was defined as an hSBA titer of ≥1:16 or the LLOQ (whichever titer was higher).
  - For subjects with a baseline hSBA titer of ≥ LOD (ie, hSBA titer of ≥1:4) and < LLOQ, a 4-fold response was defined as an hSBA titer of ≥4 times the LLOQ.
  - For subjects with a baseline hSBA titer of ≥ LLOQ, a 4-fold response was defined as an hSBA titer of ≥4 times the baseline titer.

RESULTS

Subjects: A total of 400 subjects aged ≥24 months to <10 years were randomized in this study. Of the subjects randomized, 294 subjects were in Group 1 (bivalent rLP2086) and 106 subjects were in Group 2 (HAV/saline). There were 200 subjects randomized in each of the ≥24-month to <4-year and ≥4-year to <10-year age strata.

Of the 400 randomized subjects, 390 (97.5%) subjects completed the vaccination phase (through 1 month after last study vaccination) of the study. A total of 387 (96.8%) subjects completed the 6-month follow-up telephone contact. Only subjects who completed the vaccination phase and the 6-month follow-up telephone contact were

considered to have completed the study. Overall, a total of 375 (93.8%) subjects completed all study procedures and completion was similar in each age strata.

A total of 371 (92.8%) subjects were included in the evaluable immunogenicity population, and 29 (7.3%) subjects were excluded from the evaluable immunogenicity population. All 400 randomized subjects were included in the mITT population.

Immunogenicity Results: The primary objectives of this study were to describe subject immune response to bivalent rLP2086 as measured by hSBA against 4 primary MnB test strains, 2 expressing an LP2086 subfamily A protein and 2 expressing an LP2086 subfamily B protein, measured 1 month after the third vaccination in healthy subjects aged ≥24 months to <4 years and ≥4 years to <10 years. The description of immune responses for the combined age stratum (≥24 months to <10 years) was a secondary objective. The endpoints for the primary objectives were the proportions of subjects in each age stratum achieving hSBA titers ≥ LLOQ for each of the 4 primary MnB strains 1 month after the third vaccination.

A robust immune response was observed for children aged ≥24 months to <10 years 1 month after the third dose of bivalent rLP2086, as confirmed by the proportion of subjects achieving an hSBA titer ≥ LLOQ (1:8 for A56, B24 and B44; 1:16 for A22) for each of the 4 primary MnB test strains ranging from 80.0% to 100.0% for subjects aged ≥24 months to <4 years and from 78.3% to 100.0% for subjects ≥4 years to <10 years after 3 doses. The proportion of subjects in the combined age stratum with an hSBA titer ≥ LLOQ for each of the 4 primary MnB test strains 1 month after the third vaccination ranged from 79.1% to 100.0%. These findings are further supported by substantial GMTs (range 19.1 to 191) and in the proportion of subjects achieving an hSBA titer ≥1:4 (81.5% to 100%) or ≥1:16 (75.4% to 100%) against each of the 4 primary MnB test strains after 3 doses of bivalent rLP2086 compared to baseline across both age strata. Additionally, the proportion of subjects in the combined age stratum achieving an hSBA fold rise ≥4 from baseline to 1 month after the third vaccination for each of the 4 primary MnB test strains ranged from 76.9% to 93.5%.

The secondary objective of the study was to describe immune responses 1 month after the second dose of bivalent rLP2086, as assessed by ≥ LLOQ responses, defined hSBA titers and hSBA GMTs for the 2 age strata and the combined age stratum. For the combined age stratum, the proportion of subjects achieving an hSBA titer ≥ LLOQ ranged from 48.5% to 100.0% with no meaningful differences observed between the younger and older age strata. These findings are further supported by the combined age stratum with increases in GMTs (range 11.1 to 96.6) and in the proportion of subjects achieving an hSBA titer ≥1:4 (57.7% to 100%) or ≥1:16 (43.1% to 100%) after 2 doses of

bivalent rLP2086 compared to baseline against each of the 4 primary MnB test strains. GMTs were similar between the 2 age strata. Additionally, the proportion of subjects in the combined age stratum achieving an hSBA fold rise ≥4 from baseline to 1 month after the second vaccination for each of the 4 primary MnB test strains ranged from 42.3% to 91.0%.

Immunopersistence was also assessed at 6 months after the third dose of bivalent rLP2086 with the proportion of subjects with an hSBA titer ≥ LLOQ declining from 79.1% to 100% 1 month after Vaccination 3 to 10.4% to 82.4% at 6 months after the third vaccination for the combined age stratum. No differences between the 2 age strata were observed except for A22, for which older children had a higher proportion of subjects achieving a titer ≥ LLOQ than the younger children (46%, 95% CI 33.4, 59.1 vs 19%, 95% CI 10.2, 30.9). However, baseline prevaccination rates of titers ≥ LLOQ were greater for A22 in the older age stratum (13.6% vs 4.4%). A similar trend was also observed for the combined age stratum for the proportion of subjects with a protective hSBA titer ≥1:4, ranging from 13.3% to 84.0% and GMTs, ranging from 5.1 to 31.3 at 6 months after the third vaccination.

In summary, bivalent rLP2086 given as 3 doses on a 0-, 2-, and 6-month schedule elicits a robust immune response among toddlers and children aged ≥24 months to <10 years with protective antibody titers achieved as measured by hSBA in a high proportion of subjects after the third dose. No clinically meaningful differences were observed between toddlers aged ≥24 months to <4 years and children aged ≥4 years to <10 years. Antibody responses decline 6 months after the third dose, but remain higher than prevaccination baseline rates.

Conclusion(s): In conclusion, bivalent rLP2086 administered to toddlers and children aged ≥24 months to <10 years in a 3-dose series on a 0-, 2-, and 6-month schedule elicits a robust immune response by the majority of subjects after the second and third doses, with protective antibody titers achieved after the third dose as measured by hSBAs. hSBA titers decreased 6 months after a 3-dose series. The vaccine, as administered in this study, was safe and well tolerated with an acceptable safety profile for toddlers and children aged ≥24 months to <10 years.

EXAMPLE 19: A Phase 2, Randomized, Controlled, Observer-Blinded Study to Describe the Immunogenicity, Safety, and Tolerability of *Neisseria meningitidis* Serogroup B Bivalent Recombinant Lipoprotein 2086 Vaccine (Bivalent rLP2086, i.e., now TRUMENBA® vaccine) in Healthy Subjects Aged ≥24 Months to <10 Years (B1971017-CSR)

The initial formulation of bivalent rLP2086 (which did not include polysorbate-80) has been assessed in Phase 1 studies in adults, adolescents, children, and toddlers with satisfactory safety, tolerability, and immunogenicity profiles demonstrated in these populations. The initial formulation has been shown to have an acceptable safety profile up to a dose of 200 μg, and to be immunogenic as measured by hSBA. In toddlers aged 18 to 36 months, the initial formulation has been studied at dose levels of 20 μg, 60 μg, and 200 μg (Study 6108A1-502-AU). In the 6108A1-502-AU study, frequencies of local reactions in each dose group were generally higher than those in the hepatitis A virus (HAV) vaccine/placebo group, but in most cases the reactions were of mild or moderate severity. The incidence of fever tended to increase with increasing dose level. The proportions of subjects reporting any fever after any dose were 36.4% in the 20-μg group, 39.1% in the 60-μg group, and 54.5% in the 200-μg group. The frequencies of other systemic events in each vaccine group were generally comparable to those for HAV/placebo. The majority of subjects had an hSBA response for the MnB strains following the third vaccination.

Compared to the initial formulation, the drug substance manufacturing process and the drug product formulation have undergone enhancements (including the addition of polysorbate-80) designed to increase scalability for manufacture and to ensure long-term stability of the final formulation of the vaccine. Safety data from adults and adolescents participating in studies with the final formulation of bivalent rLP2086 are consistent with the safety of the initial formulation. Local reactions and systemic events were generally mild or moderate in severity in all age groups. Severe events were relatively infrequent. Furthermore, follow-up adverse event (AE) data from immunopersistence studies 6108A1-1002-AU, 6108A1-2001, and B1971033 ranging from 6 months to 48 months after the third dose of bivalent rLP2086 raised no safety concerns. Serious adverse events (SAEs) were infrequent, and mostly considered not related to the study vaccine. There were few withdrawals from the studies due to AEs.

Two (2) Phase 2 studies (B1971017 and B1971035) were conducted to explore the immunogenicity and safety of the vaccine in children (12 months to<10 years) with the final formulation of the vaccine. Study B1971035 is ongoing and designed to assess the safety, tolerability, and immunogenicity of 2 different dose levels (60-μg and 120-μg)

among healthy toddlers aged 12 months to <24 months. This study (B1971017) assessed the immunogenicity, safety, and tolerability of bivalent rLP2086 at the 120-μg dose level (final formulation) administered to healthy subjects aged ≥24 months to <10 years as part of a Month 0, 2, and 6 schedule. Approximately 400 subjects were planned to be randomized to 1 of 2 groups in a 3:1 ratio. Group 1 received bivalent rLP2086 at Month 0 (Visit 1) followed by subsequent vaccinations at Months 2 and 6. Group 2 received HAV vaccine at Month 0 (Visit 1) and Month 6 and an injection with saline at Month 2. Randomization was stratified to ensure that equal numbers of subjects were included in the ≥24-month to <4-year and ≥4-year to <10-year age strata.

This (B1971017) was a Phase 2, randomized, controlled, observer-blinded, multicenter study in which approximately 400 subjects were planned to be randomly assigned to 1 of 2 groups in a 3:1 ratio. Group 1 received bivalent rLP2086 at Month 0 (Visit 1) followed by subsequent vaccinations at Months 2 and 6. Group 2 received a licensed pediatric HAV vaccine at Month 0 (Visit 1) and Month 6 and an injection with saline at Month 2 to maintain the study blind. Randomization was stratified according to age to ensure that equal numbers of subjects were included in the ≥24-month to <4-year age stratum and the ≥4-year to <10-year age stratum. The study was planned to enroll approximately 200 subjects aged ≥24 months to <4 years and approximately 200 subjects aged ≥4 years to <10 years.

This study assessed the immunogenicity, safety, and tolerability of bivalent rLP2086 at the 120-μg dose level administered to healthy subjects aged ≥24 months to <10 years as part of a Month 0, 2, and 6 schedule. Follow-up visits were conducted 1 month after each vaccination and 6 months after the third vaccination to collect safety data and/or obtain a blood sample. Subjects participated in the study for up to 13 months.

Bivalent rLP2086 (containing 60 μg each of a purified subfamily A and subfamily B rLP2086 protein, adsorbed to aluminum in a sterile buffered isotonic suspension) was provided in a 0.5-mL dose for injection.

A licensed pediatric HAV vaccine was provided in a 0.5-mL dose for injection.

For Group 1, 59.31% of subjects aged ≥24 months to <4 years and 57.05% of subjects aged ≥4 years to <10 years received concomitant treatment. For Group 2, 58.18% of subjects aged ≥24 months to <4 years and 52.94% of subjects aged ≥4 years to <10 years received concomitant treatment. The most common concomitant treatments received during the study were ibuprofen, paracetamol, and amoxicillin.

Bivalent rLP2086 Serum Bactericidal Assay – Primary Test Strains

For assessment of the immune response to bivalent rLP2086, functional antibodies were analyzed in validated hSBAs with 4 primary MnB test strains selected using an unbiased

algorithm, and adjusted for epidemiological prevalence based on regulatory input, from Pfizer's MnB serum bactericidal assay (SBA) strain pool. The hSBA measures antibodies in human sera that initiate complement-dependent destruction of the target meningococcal strain. Four (4) primary MnB test strains, PMB80 (A22), PMB2001 (A56), PMB2948 (B24), and PMB2707 (B44), each expressing an fHBP variant heterologous to the vaccine component antigens, were used in the hSBAs for determination of the immunogenicity endpoints in this study. Sera obtained from all subjects prior to the first study vaccination, 1 month after the second study vaccination, and 1 and 6 months after the third study vaccination were used in these assays.

For the primary analyses, 2 of the primary test strains (PMB80 [A22] and PMB2948 [B24]) were tested at each blood sampling time point for half of the subjects (in both groups), and the other 2 test primary strains (PMB2001 [A56] and PMB2707 [B44]) were tested at each blood sampling time point for the remaining half of the subjects.

Immunogenicity Analysis

There was no hypotheses testing for immunogenicity analysis. An estimation approach was used to assess the primary, secondary, and exploratory objectives.

The proportions of subjects in each group achieving hSBA titer ≥ lower limit of quantitation (LLOQ) 1 month after the second and third vaccination and 6 months after the third vaccination were computed for each test strain, along with 2-sided 95% exact confidence intervals (CIs), for each of the age strata and the combined age stratum.

Primary Immunogenicity Endpoints

The primary immunogenicity endpoints were:

- Proportion of subjects aged ≥24 months to <4 years (at study entry) with hSBA titer ≥ LLOQ for each of the 4 primary MnB test strains 1 month after the third vaccination with bivalent rLP2086.
- Proportion of subjects aged ≥4 years to <10 years (at study entry) with hSBA titer ≥ LLOQ for each of the 4 primary MnB test strains 1 month after the third vaccination with bivalent rLP2086.

Secondary Immunogenicity Endpoints

The secondary immunogenicity endpoints were:

- In healthy subjects aged ≥24 months to <10 years at study entry:
  - Proportion of subjects with hSBA titer ≥ LLOQ for each of the 4 primary MnB test strains 1 month after the third vaccination with bivalent rLP2086.
- In healthy subjects aged ≥24 months to <4 years at study entry, in healthy subjects aged ≥4 years to <10 years at study entry, and in the combined age stratum:

- Proportion of subjects with hSBA titer ≥ LLOQ for each of the 4 primary MnB test strains 1 month after the second vaccination and 1 and 6 months after the third vaccination with bivalent rLP2086.
- Proportions of subjects achieving hSBA titers of ≥1:4, ≥1:8, ≥1:16, ≥1:32, ≥1:64, and ≥1:128 for each of the 4 primary test strains at baseline, 1 month after the second vaccination, and 1 and 6 months after the third vaccination with bivalent rLP2086.
- hSBA GMTs for each of the 4 primary test strains at baseline, 1 month after the second vaccination, and 1 and 6 months after the third vaccination with bivalent rLP2086.

Exploratory Immunogenicity Endpoints

For exploratory endpoints, testing was not performed on all 4 primary MnB test strains. Instead, 50% of subjects were tested using strains PMB2001 (A56) and PMB2707 (B44), but not PMB80 (A22) or PMB2948 (B24). The remaining 50% of subjects were tested using strains PMB80 (A22) or PMB2948 (B24), but not PMB2001 (A56) or PMB2707 (B44). All of the exploratory endpoints specified below may have been applied to hSBA results from all subjects who received bivalent rLP2086 and may have been tested for the appropriate strain at the indicated time point(s).

The following exploratory endpoints were used to describe responses in healthy subjects aged ≥24 months to <4 years at study entry, in healthy subjects aged ≥4 years to <10 years at study entry, and in the combined age stratum:

- Proportions of subjects with hSBA titers ≥1:4, ≥1:8, ≥1:16, ≥1:32, ≥1:64, and ≥1:128 at each applicable blood sampling time point.
- hSBA GMTs for each of the 4 primary strains at each applicable blood sampling time point.
- Proportion of subjects achieving at least a 4-fold increase in hSBA titer from baseline to 1 month after the third vaccination with bivalent rLP2086 for each of the 4 primary test strains:
  - For subjects with a baseline hSBA titer below the limit of detection (LOD) or an hSBA titer of <1:4, a 4-fold response was defined as an hSBA titer of ≥1:16 or the LLOQ (whichever titer was higher).
  - For subjects with a baseline hSBA titer of ≥ LOD (ie, hSBA titer of ≥1:4) and < LLOQ, a 4-fold response was defined as an hSBA titer of ≥4 times the LLOQ.
  - For subjects with a baseline hSBA titer of ≥ LLOQ, a 4-fold response was defined as an hSBA titer of ≥4 times the baseline titer.

Methods of Analysis

The primary analysis for immunogenicity included an estimate for the proportion of subjects in each group achieving an hSBA titer ≥ LLOQ 1 month after the third vaccination for each test strain, along with 2-sided 95% exact confidence CIs, for each of the age strata and in the combined age stratum.

All of the binary endpoints (including primary endpoints) were summarized with 2-sided 95% CIs using the exact method. GMTs on hSBA results were also summarized with 95% CIs.

The LLOQ was 1:16 for PMB80 (A22), 1:8 for PMB2001 (A56), 1:8 for PMB2707 (B44), and 1:8 for PMB2948 (B24).

For the calculation of GMTs, hSBA results below the LLOQ were set as 0.5 × LLOQ for the primary analysis.

Analysis of Primary Endpoints

The primary analysis for the primary objectives was based on the evaluable immunogenicity population. The proportion of subjects in each group achieving hSBA titer ≥ LLOQ 1 month after the third vaccination was computed for each test strain with 2-sided 95% exact CIs. To address the 2 primary objectives, these data are presented for the 2 age strata: ≥24 months to <4 years and ≥4 years to <10 years.

All of the binary endpoints (including primary endpoints) were summarized with 2-sided 95% CIs using the exact method. GMTs on hSBA results were also summarized with 95% CIs.

To support the interpretation of the primary analyses, an identical analysis based on the mITT population was conducted.

Analysis of Secondary and Exploratory Endpoints

The following analyses addressed the secondary and exploratory immunogenicity objectives:

- The proportions of subjects achieving hSBA titers ≥ LLOQ for each of the 4 primary strains at 1 month after the second vaccination and 6 months after the third vaccination were analyzed in the 2 age strata separately and combined, in the evaluable immunogenicity and the mITT populations.
- The proportions of subjects achieving hSBA titers of ≥1:4, ≥1:8, ≥1:16, ≥1:32, ≥1:64, and ≥1:128 for each of the 4 primary test strains at baseline, 1 month after the second vaccination, and 1 and 6 months after the third vaccination were analyzed in the 2 age strata separately and combined, in the evaluable immunogenicity and the mITT populations.

- The hSBA GMTs for each of the 4 primary test strains at baseline, 1 month after the second vaccination, and 1 and 6 months after the third vaccination were analyzed in the 2 age strata separately and combined, in the evaluable immunogenicity and the mITT populations.

The exploratory endpoints were summarized, in the 2 age strata separately and combined, for each applicable time point for both the evaluable immunogenicity population and the mITT population.

Reverse Cumulative Distribution Curves

The empirical reverse cumulative distribution curves (RCDCs) were assessed graphically for each of the 4 primary strains and at each sampling time point, for the evaluable immunogenicity population.

IMMUNOGENICITY EVALUATION

Populations Analyzed

The evaluable immunogenicity population was the primary analysis population for the immunogenicity analyses. The mITT population was used as a supportive immunogenicity population for the immunogenicity analyses.

A total of 371 (92.8%) subjects were included in the evaluable immunogenicity population, and 29 (7.3%) subjects were excluded from the evaluable immunogenicity population. Subjects could have been excluded from the immunogenicity populations for more than 1 reason. A total of 21 (5.3%) subjects were excluded from the evaluable immunogenicity population because they did not have baseline blood drawn prior to the first dose of vaccine or after Vaccination 3, 15 (3.8%) subjects did not have a valid and determinate assay result at any visit, 11 (2.8%) subjects were not eligible or became ineligible for the study before or at the 1-month post-Vaccination 3 visit, 11 (2.8%) subjects did not receive vaccine as randomized at all vaccination visits, and 4 (1.0%) subjects had an important protocol deviation as identified by the medical monitor. Overall, the 2 study groups and 2 age strata were comparable with respect to the percentages of subjects who were excluded from the evaluable immunogenicity population.

All 400 randomized subjects were included in the mITT population.

Immunogenicity Results

The results of the analyses for the primary immunogenicity endpoints, secondary immunogenicity endpoints, and exploratory immunogenicity endpoints are provided in the following sections.

Primary and Secondary Endpoints

Proportion of Subjects Achieving an hSBA Titer ≥ LLOQ

The primary immunogenicity endpoints were the proportion of subjects aged ≥24 months to <4 years (at study entry), and aged ≥4 years to <10 years (at study entry), with an hSBA titer ≥ LLOQ for each of the 4 primary MnB test strains 1 month after the third vaccination with bivalent rLP2086. The proportion of all subjects in the combined age stratum (at study entry) with an hSBA titer ≥ LLOQ for each of the 4 primary MnB test strains 1 month after the third vaccination with bivalent rLP2086, along with the proportion of subjects in the individual and combined age strata with an hSBA titer ≥ LLOQ for each of the 4 primary MnB test strains 1 month after the second vaccination with bivalent rLP2086, were secondary endpoints.

The proportion of subjects in each age stratum with an hSBA titer ≥ LLOQ for each of the 4 primary MnB test strains is presented in Table 20 for the evaluable immunogenicity population.

The proportion of subjects aged ≥24 months to <4 years and ≥4 years to <10 years in Group 1 with an hSBA titer ≥ LLOQ at baseline was 4.4% and 13.6%, respectively, for PMB80 (A22); 1.5% and 15.4%, respectively, for PMB2001 (A56); 3.0% and 7.5%, respectively, for PMB2948 (B24); and 0.0%, for both age strata for PMB2707 (B44). Overall, the proportion of subjects in the combined age stratum with an hSBA titer ≥ LLOQ at baseline was 9.0% for PMB80 (A22), 8.3% for PMB2001 (A56), 5.2% for PMB2948 (B24), and 0.0% for PMB2707 (B44) in Group 1.

The proportion of subjects aged ≥24 months to <4 years and ≥4 years to <10 years in Group 1 with an hSBA titer ≥ LLOQ at 1 month after the second vaccination was 59.4% and 78.8%, respectively, for PMB80 (A22); 100.0% for both age strata for PMB2001 (A56); 49.2% and 65.1%, respectively, for PMB2948 (B24); and 57.1% and 40.3%, respectively, for PMB2707 (B44).

Overall, the proportion of subjects in the combined age stratum with an hSBA titer ≥ LLOQ at 1 month after the second vaccination was 69.2% for PMB80 (A22), 100.0% for PMB2001 (A56), 57.0% for PMB2948 (B24), and 48.5% for PMB2707 (B44) in Group 1.

The proportion of subjects aged ≥24 months to <4 years and ≥4 years to <10 years in Group 1 with an hSBA titer ≥ LLOQ at 1 month after the third vaccination was 83.8% and 91.0%, respectively, for PMB80 (A22); 100.0% for both age strata for PMB2001 (A56); 85.7% and 92.1%, respectively, for PMB2948 (B24); and 80.0% and 78.3%, respectively, for PMB2707 (B44).

Overall, the proportion of subjects in the combined age stratum with an hSBA titer ≥ LLOQ at 1 month after the third vaccination was 87.4% for PMB80 (A22), 100.0% for PMB2001 (A56), 88.9% for PMB2948 (B24), and 79.1% for PMB2707 (B44) in Group 1.

In general, the proportion of Group 2 subjects with an hSBA titer ≥ LLOQ did not change over time compared to baseline. The proportion of subjects in the combined age stratum with an hSBA titer ≥ LLOQ at any time point ranged from 4.4% to 8.5% for PMB80 (A22); 14.9% to 20.9% for PMB2001 (A56); 0.0% to 8.9% for PMB2948 (B24); and 0.0% at each time point for PMB2707 (B44).

Results for the mITT population were similar to those of the evaluable immunogenicity population.

Subgroup analyses of the proportion of subjects with an hSBA titer ≥ LLOQ for each of the 4 primary MnB test strains are presented for the evaluable immunogenicity population by sex, race, and country. There were no clinically important differences observed in the subgroup analyses performed.

Immunopersistence: Proportion of Subjects Achieving hSBA Titer ≥ LLOQ 6 Months After Third Vaccination

The proportion of subjects aged ≥24 months to <4 years, ≥4 years to <10 years, and in the combined age stratum (≥24 months to <10 years), with an hSBA titer ≥ LLOQ for each of the 4 primary MnB test strains 6 months after the third vaccination with bivalent rLP2086 was a secondary immunogenicity endpoint. The proportion of subjects with an hSBA titer ≥ LLOQ for each of the 4 primary MnB test strains in each age stratum is presented in Table 20 for the evaluable immunogenicity population.

In general, there was a decline in the proportion of subjects with an hSBA titer ≥ LLOQ for each of the 4 primary MnB test strains observed among Group 1 subjects in both age strata at 6 months after the third vaccination.

For subjects aged ≥24 months to <4 years, from 1 month after the third vaccination to 6 months after the third vaccination, the proportion of subjects with an hSBA titer ≥ LLOQ decreased from 83.8% to 19.0%, respectively, for PMB80 (A22); 100.0% to 80.3%, respectively, for PMB2001 (A56); 85.7% to 9.2%, respectively for PMB2948 (B24); and 80.0% to 12.1%, respectively, for PMB2707 (B44).

For subjects aged ≥4 years to <10 years, from 1 month after the third vaccination to 6 months after the third vaccination, the proportion of subjects with an hSBA titer ≥ LLOQ decreased from 91.0% to 46.0%, respectively, for PMB80 (A22); 100.0% to 84.3%, respectively, for PMB2001 (A56); 92.1% to 21.9%, respectively for PMB2948 (B24); and 78.3% to 8.7%, respectively, for PMB2707 (B44).

Overall for the combined age stratum, from 1 month after the third vaccination to 6 months after the third vaccination, the proportion of subjects with an hSBA titer ≥ LLOQ decreased from 87.4% to 32.5% for PMB80 (A22); 100.0% to 82.4% for PMB2001 (A56); 88.9% to 15.5% for PMB2948 (B24); and 79.1% to 10.4% for PMB2707 (B44).

In general, the proportion of Group 2 subjects with an hSBA titer ≥ LLOQ did not change over time compared to baseline.

Table 20. Subjects With hSBA Titer ≥ LLOQ for Primary Strains – Evaluable Immunogenicity Population

Strain (Variant) Sampling Time Point Age Strata	Vaccine Group (as Randomized) Group 1 rLP2086 N[a]	n[b]	(%)	(95% CI)[c]	Group 2 HAV/Saline N[a]	n[b]	(%)	(95% CI)[c]
PMB80 (A22)								
Before Vaccination 1								
≥24 Months to <10 years	134	12	(9.0)	(4.7, 15.1)	47	3	(6.4)	(1.3, 17.5)
≥24 Months to <4 years	68	3	(4.4)	(0.9, 12.4)	26	1	(3.8)	(0.1, 19.6)
≥4 Years to <10 years	66	9	(13.6)	(6.4, 24.3)	21	2	(9.5)	(1.2, 30.4)
1 Month after Vaccination 2								
≥24 Months to <10 years	130	90	(69.2)	(60.5, 77.0)	45	2	(4.4)	(0.5, 15.1)
≥24 Months to <4 years	64	38	(59.4)	(46.4, 71.5)	24	0	(0.0)	(0.0, 14.2)
≥4 Years to <10 years	66	52	(78.8)	(67.0, 87.9)	21	2	(9.5)	(1.2, 30.4)
1 Month after Vaccination 3								
≥24 Months to <10 years	135	118	(87.4)	(80.6, 92.5)	45	3	(6.7)	(1.4, 18.3)
≥24 Months to <4 years	68	57	(83.8)	(72.9, 91.6)	25	1	(4.0)	(0.1, 20.4)
≥4 Years to <10 years	67	61	(91.0)	(81.5, 96.6)	20	2	(10.0)	(1.2, 31.7)
6 Months after Vaccination 3								
≥24 Months to <10 years	126	41	(32.5)	(24.5, 41.5)	47	4	(8.5)	(2.4, 20.4)
≥24 Months to <4 years	63	12	(19.0)	(10.2, 30.9)	26	2	(7.7)	(0.9, 25.1)
≥4 Years to <10 years	63	29	(46.0)	(33.4, 59.1)	21	2	(9.5)	(1.2, 30.4)
PMB2001 (A56)								
Before Vaccination 1								
≥24 Months to <10 years	132	11	(8.3)	(4.2, 14.4)	47	7	(14.9)	(6.2, 28.3)
≥24 Months to <4 years	67	1	(1.5)	(0.0, 8.0)	24	2	(8.3)	(1.0, 27.0)
≥4 Years to <10 years	65	10	(15.4)	(7.6, 26.5)	23	5	(21.7)	(7.5, 43.7)
1 Month after Vaccination 2								
≥24 Months to <10 years	133	133	(100.0)	(97.3, 100.0)	43	7	(16.3)	(6.8, 30.7)
≥24 Months to <4 years	66	66	(100.0)	(94.6, 100.0)	21	2	(9.5)	(1.2, 30.4)
≥4 Years to <10 years	67	67	(100.0)	(94.6, 100.0)	22	5	(22.7)	(7.8, 45.4)
1 Month after Vaccination 3								
≥24 Months to <10 years	139	139	(100.0)	(97.4, 100.0)	43	9	(20.9)	(10.0, 36.0)
≥24 Months to <4 years	68	68	(100.0)	(94.7, 100.0)	24	1	(4.2)	(0.1, 21.1)
≥4 Years to <10 years	71	71	(100.0)	(94.9, 100.0)	19	8	(42.1)	(20.3, 66.5)
6 Months after Vaccination 3								
≥24 Months to <10 years	131	108	(82.4)	(74.8, 88.5)	46	9	(19.6)	(9.4, 33.9)
≥24 Months to <4 years	61	49	(80.3)	(68.2, 89.4)	24	4	(16.7)	(4.7, 37.4)
≥4 Years to <10 years	70	59	(84.3)	(73.6, 91.9)	22	5	(22.7)	(7.8, 45.4)
PMB2948 (B24)								
Before Vaccination 1								
≥24 Months to <10 years	134	7	(5.2)	(2.1, 10.5)	47	2	(4.3)	(0.5, 14.5)
≥24 Months to <4 years	67	2	(3.0)	(0.4, 10.4)	26	1	(3.8)	(0.1, 19.6)
≥4 Years to <10 years	67	5	(7.5)	(2.5, 16.6)	21	1	(4.8)	(0.1, 23.8)
1 Month after Vaccination 2								
≥24 Months to <10 years	128	73	(57.0)	(48.0, 65.7)	45	4	(8.9)	(2.5, 21.2)
≥24 Months to <4 years	65	32	(49.2)	(36.6, 61.9)	24	2	(8.3)	(1.0, 27.0)
≥4 Years to <10 years	63	41	(65.1)	(52.0, 76.7)	21	2	(9.5)	(1.2, 30.4)
1 Month after Vaccination 3								
≥24 Months to <10 years	126	112	(88.9)	(82.1, 93.8)	46	2	(4.3)	(0.5, 14.8)
≥24 Months to <4 years	63	54	(85.7)	(74.6, 93.3)	26	2	(7.7)	(0.9, 25.1)
≥4 Years to <10 years	63	58	(92.1)	(82.4, 97.4)	20	0	(0.0)	(0.0, 16.8)
6 Months after Vaccination 3								

Table 20. Subjects With hSBA Titer ≥ LLOQ for Primary Strains – Evaluable Immunogenicity Population

Strain (Variant) Sampling Time Point Age Strata	Vaccine Group (as Randomized) Group 1 rLP2086 N[a]	n[b]	(%)	(95% CI)[c]	Group 2 HAV/Saline N[a]	n[b]	(%)	(95% CI)[c]
≥24 Months to <10 years	129	20	(15.5)	(9.7, 22.9)	47	0	(0.0)	(0.0, 7.5)
≥24 Months to <4 years	65	6	(9.2)	(3.5, 19.0)	26	0	(0.0)	(0.0, 13.2)
≥4 Years to <10 years	64	14	(21.9)	(12.5, 34.0)	21	0	(0.0)	(0.0, 16.1)
PMB2707 (B44)								
Before Vaccination 1								
≥24 Months to <10 years	138	0	(0.0)	(0.0, 2.6)	50	0	(0.0)	(0.0, 7.1)
≥24 Months to <4 years	67	0	(0.0)	(0.0, 5.4)	26	0	(0.0)	(0.0, 13.2)
≥4 Years to <10 years	71	0	(0.0)	(0.0, 5.1)	24	0	(0.0)	(0.0, 14.2)
1 Month after Vaccination 2								
≥24 Months to <10 years	130	63	(48.5)	(39.6, 57.4)	50	0	(0.0)	(0.0, 7.1)
≥24 Months to <4 years	63	36	(57.1)	(44.0, 69.5)	26	0	(0.0)	(0.0, 13.2)
≥4 Years to <10 years	67	27	(40.3)	(28.5, 53.0)	24	0	(0.0)	(0.0, 14.2)
1 Month after Vaccination 3								
≥24 Months to <10 years	134	106	(79.1)	(71.2, 85.6)	50	0	(0.0)	(0.0, 7.1)
≥24 Months to <4 years	65	52	(80.0)	(68.2, 88.9)	26	0	(0.0)	(0.0, 13.2)
≥4 Years to <10 years	69	54	(78.3)	(66.7, 87.3)	24	0	(0.0)	(0.0, 14.2)
6 Months after Vaccination 3								
≥24 Months to <10 years	135	14	(10.4)	(5.8, 16.8)	49	0	(0.0)	(0.0, 7.3)
≥24 Months to <4 years	66	8	(12.1)	(5.4, 22.5)	26	0	(0.0)	(0.0, 13.2)
≥4 Years to <10 years	69	6	(8.7)	(3.3, 18.0)	23	0	(0.0)	(0.0, 14.8)

Abbreviation: hSBA = serum bactericidal assay using human complement; LLOQ = lower limit of quantitation.

Note: LLOQ = 1:16 for A22; 1:8 for A56, B24, and B44.

a. N = number of subjects with valid and determinate hSBA titers for the given strain.

b. n = Number of subjects with observed hSBA titer ≥ LLOQ for the given strain at the given time point.

c. Exact 2-sided CI based upon observed proportion of subjects, using the Clopper and Pearson method.

Program ID: Study B1971017/CP IMM_LLOQ.SAS. Date of Reporting Dataset Creation: 01JUN2017. Runtime ID: 16JUN2017 11:22. File ID: T_2_2_IMM_LLOQ_EVL.HTM.

hSBA GMTs. The hSBA GMTs for each of the 4 primary test strains at baseline, 1 month after the second vaccination, and 1 month after the third vaccination with bivalent rLP2086 was a secondary endpoint. Table 21 provides hSBA GMTs for the 4 primary MnB strains for the evaluable immunogenicity population.

For Group 1 subjects aged ≥24 months to <4 years and aged ≥4 years to <10 years, hSBA GMTs at baseline were 8.3 and 9.1, respectively, for PMB80 (A22); 4.1 and 5.8, respectively, for PMB2001 (A56); 4.3 and 4.6, respectively, for PMB2948 (B24); and 4.0 in both age strata for PMB2707 (B44).

For Group 1 subjects aged ≥24 months to <10 years, hSBA GMTs at baseline were 8.7 for PMB80 (A22), 4.9 for PMB2001 (A56), 4.5 for PMB2948 (B24), and 4.0 for PMB2707 (B44).

For Group 1 subjects aged ≥24 months to <4 years and aged ≥4 years to <10 years, hSBA GMTs at 1 month after Vaccination 2 were 17.4 and 23.1, respectively, for PMB80 (A22); 103.8 and 90.0, respectively, for PMB2001 (A56); 9.1 and 13.7, respectively, for PMB2948 (B24); and 17.1 and 8.2, respectively, for PMB2707 (B44).

For Group 1 subjects aged ≥24 months to <10 years, hSBA GMTs at 1 month after Vaccination 2 were 20.1 for PMB80 (A22), 96.6 for PMB2001 (A56); 11.1 for PMB2948 (B24), and 11.7 for PMB2707 (B44).

For Group 1 subjects aged ≥24 months to <4 years and aged ≥4 years to <10 years, hSBA GMTs at 1 month after Vaccination 3 were 33.7 and 38.2, respectively, for PMB80 (A22); 175.6 and 191.0, respectively, for PMB2001 (A56); 19.1 and 26.8, respectively, for PMB2948 (B24); and 43.6 and 36.5, respectively, for PMB2707 (B44).

For Group 1 subjects aged ≥24 months to <10 years, hSBA GMTs at 1 month after Vaccination 3 were 35.8 for PMB80 (A22), 183.3 for PMB2001 (A56); 22.6 for PMB2948 (B24), and 39.8 for PMB2707 (B44).

In general, the hSBA GMTs for subjects in Group 2 did not change over time compared to baseline. For the combined age stratum, hSBA GMTs at any time point ranged from 8.6 to 8.9 for PMB80 (A22); 5.6 to 6.0 for PMB2001 (A56); 4.0 to 4.8 for PMB2948 (B24); and 4.0 at each time point for PMB2707 (B44).

Table 21. hSBA GMTs for Primary Strains – Evaluable Immunogenicity Population

Strain (Variant) Sampling Time Point Age Strata	Vaccine Group (as Randomized) Group 1 rLP2086 N[a]	GMT[b]	(95% CI)[c]	Group 2 HAV/Saline N[a]	GMT[b]	(95% CI)[c]
PMB80 (A22)						
Before Vaccination 1						
≥24 Months to <10 years	134	8.7	(8.3, 9.1)	47	8.9	(7.8, 10.1)
≥24 Months to <4 years	68	8.3	(7.9, 8.8)	26	8.2	(7.8, 8.7)
≥4 Years to <10 years	66	9.1	(8.3, 9.9)	21	9.8	(7.2, 13.2)
1 Month after Vaccination 2						
≥24 Months to <10 years	130	20.1	(17.4, 23.2)	45	8.6	(7.7, 9.7)
≥24 Months to <4 years	64	17.4	(14.2, 21.4)	24	8.0	(NE, NE)
≥4 Years to <10 years	66	23.1	(18.9, 28.3)	21	9.4	(7.4, 12.0)
1 Month after Vaccination 3						
≥24 Months to <10 years	135	35.8	(30.5, 42.2)	45	8.8	(7.8, 9.8)
≥24 Months to <4 years	68	33.7	(26.4, 42.9)	25	8.7	(7.3, 10.3)
≥4 Years to <10 years	67	38.2	(30.6, 47.6)	20	8.9	(7.6, 10.4)
6 Months after Vaccination 3						
≥24 Months to <10 years	126	12.4	(10.9, 14.2)	47	8.7	(7.9, 9.7)
≥24 Months to <4 years	63	10.9	(9.0, 13.1)	26	8.4	(7.8, 9.1)
≥4 Years to <10 years	63	14.2	(11.8, 17.0)	21	9.1	(7.4, 11.3)
PMB2001 (A56)						
Before Vaccination 1						
≥24 Months to <10 years	132	4.9	(4.3, 5.5)	47	5.6	(4.4, 7.2)
≥24 Months to <4 years	67	4.1	(3.9, 4.3)	24	4.9	(3.7, 6.6)
≥4 Years to <10 years	65	5.8	(4.6, 7.3)	23	6.5	(4.3, 9.8)
1 Month after Vaccination 2						
≥24 Months to <10 years	133	96.6	(83.0, 112.5)	43	5.8	(4.4, 7.6)
≥24 Months to <4 years	66	103.8	(84.2, 127.9)	21	5.0	(3.6, 7.1)
≥4 Years to <10 years	67	90.0	(71.9, 112.7)	22	6.6	(4.2, 10.5)
1 Month after Vaccination 3						
≥24 Months to <10 years	139	183.3	(156.7, 214.4)	43	6.0	(4.6, 7.7)
≥24 Months to <4 years	68	175.6	(139.1, 221.6)	24	4.5	(3.5, 5.7)
≥4 Years to <10 years	71	191.0	(153.9, 237.1)	19	8.6	(5.4, 13.8)
6 Months after Vaccination 3						
≥24 Months to <10 years	131	31.3	(25.3, 38.7)	46	6.0	(4.6, 7.8)
≥24 Months to <4 years	61	27.0	(19.7, 36.9)	24	6.0	(4.0, 8.9)
≥4 Years to <10 years	70	35.7	(26.6, 47.8)	22	6.0	(4.2, 8.7)
PMB2948 (B24)						
Before Vaccination 1						
≥24 Months to <10 years	134	4.5	(4.1, 4.9)	47	4.4	(3.9, 4.9)
≥24 Months to <4 years	67	4.3	(3.8, 4.9)	26	4.3	(3.7, 5.1)
≥4 Years to <10 years	67	4.6	(4.0, 5.2)	21	4.4	(3.6, 5.4)
1 Month after Vaccination 2						
≥24 Months to <10 years	128	11.1	(9.2, 13.5)	45	4.8	(4.0, 5.8)
≥24 Months to <4 years	65	9.1	(7.0, 11.9)	24	4.8	(3.7, 6.2)
≥4 Years to <10 years	63	13.7	(10.3, 18.2)	21	4.9	(3.6, 6.6)
1 Month after Vaccination 3						
≥24 Months to <10 years	126	22.6	(19.1, 26.8)	46	4.3	(3.9, 4.8)
≥24 Months to <4 years	63	19.1	(14.9, 24.5)	26	4.6	(3.8, 5.6)
≥4 Years to <10 years	63	26.8	(21.3, 33.9)	20	4.0	(NE, NE)
6 Months after Vaccination 3						
≥24 Months to <10 years	129	5.6	(4.8, 6.5)	47	4.0	(NE, NE)

Table 21. hSBA GMTs for Primary Strains – Evaluable Immunogenicity Population

Strain (Variant) Sampling Time Point Age Strata	Vaccine Group (as Randomized) Group 1 rLP2086 N[a]	GMT[b]	(95% CI)[c]	Group 2 HAV/Saline N[a]	GMT[b]	(95% CI)[c]
≥24 Months to <4 years	65	5.1	(4.1, 6.3)	26	4.0	(NE, NE)
≥4 Years to <10 years	64	6.2	(4.9, 7.7)	21	4.0	(NE, NE)
PMB2707 (B44)						
Before Vaccination 1						
≥24 Months to <10 years	138	4.0	(NE, NE)	50	4.0	(NE, NE)
≥24 Months to <4 years	67	4.0	(NE, NE)	26	4.0	(NE, NE)
≥4 Years to <10 years	71	4.0	(NE, NE)	24	4.0	(NE, NE)
1 Month after Vaccination 2						
≥24 Months to <10 years	130	11.7	(9.3, 14.7)	50	4.0	(NE, NE)
≥24 Months to <4 years	63	17.1	(11.8, 24.8)	26	4.0	(NE, NE)
≥4 Years to <10 years	67	8.2	(6.3, 10.6)	24	4.0	(NE, NE)
1 Month after Vaccination 3						
≥24 Months to <10 years	134	39.8	(30.6, 51.6)	50	4.0	(NE, NE)
≥24 Months to <4 years	65	43.6	(29.9, 63.6)	26	4.0	(NE, NE)
≥4 Years to <10 years	69	36.5	(25.2, 52.7)	24	4.0	(NE, NE)
6 Months after Vaccination 3						
≥24 Months to <10 years	135	5.1	(4.4, 5.9)	49	4.0	(NE, NE)
≥24 Months to <4 years	66	5.2	(4.2, 6.4)	26	4.0	(NE, NE)
≥4 Years to <10 years	69	5.0	(4.1, 6.2)	23	4.0	(NE, NE)

Abbreviations: GMT = geometric mean titer; hSBA = serum bactericidal assay using human complement; LLOQ = lower limit of quantitation; NE = not estimable.

Note: LLOQ = 1:16 for A22; 1:8 for A56, B24, and B44. Titers below the LLOQ were set to 0.5 × LLOQ for analysis.

a. N = number of subjects with valid and determinate hSBA titers for the given strain.

b. GMTs were calculated using all subjects with valid and determinate hSBA titers at the given time point.

c. CIs are back transformations of confidence levels based on the Student t distribution for the mean logarithm of the hSBA titers.

Subgroup analyses of hSBA GMTs for each of the 4 primary MnB test strains are presented for the evaluable immunogenicity population by sex, race, and country, and for the mITT population. There were no clinically important differences observed in the subgroup analyses performed.

Immunopersistence: hSBA GMTs

The hSBA GMTs for each of the 4 primary test strains at 6 months after the third vaccination with bivalent rLP2086 was a secondary endpoint. Table 21 provides hSBA GMTs for the 4 primary MnB strains for the evaluable immunogenicity population. Overall, there was a decrease observed from 1 month after the third vaccination to 6 months after the third vaccination in hSBA GMTs for each of the 4 primary test strains for Group 1 subjects in both age strata.

For Group 1 subjects aged ≥24 months to <4 years, from 1 month after the third vaccination to 6 months after the third vaccination, hSBA GMTs decreased from 33.7 to 10.9 for PMB80 (A22), 175.6 to 27.0 for PMB2001(A56), 19.1 to 5.1 for PMB2948 (B24), and 43.6 to 5.2 for PMB2707 (B44).

For Group 1 subjects aged ≥4 years to <10 years, from 1 month after the third vaccination to 6 months after the third vaccination, hSBA GMTs decreased from 38.2 to 14.2 for PMB80 (A22), 191.0 to 35.7 for PMB2001 (A56), 26.8 to 6.2 for PMB2948 (B24), and 36.5 to 5.0 for PMB2707 (B44).

For Group 1 subjects aged ≥24 months to <10 years, from 1 month after the third vaccination to 6 months after the third vaccination, hSBA GMTs decreased from 35.8 to 12.4 for PMB80 (A22), 183.3 to 31.3 for PMB2001(A56), 22.6 to 5.6 for PMB2948 (B24), and 39.8 to 5.1 for PMB2707 (B44).

In general, the hSBA GMTs for subjects in Group 2 did not change over time compared to baseline.

Defined hSBA Titers

The proportions of subjects aged ≥24 months to <4 years, ≥4 years to <10 years, and in the combined age stratum, achieving hSBA titers of ≥1:4, ≥1:8, ≥1:16, ≥1:32, ≥1:64, and ≥1:128 for each of the 4 primary test strains at baseline, 1 month after the second vaccination, and 1 month after the third vaccination with bivalent rLP2086 was a secondary immunogenicity endpoint.

The proportion of subjects achieving defined hSBA titers for the 4 primary MnB strains was assessed for the evaluable immunogenicity population.

Subjects who achieved an hSBA titer ≥1:4 and ≥1:16 are described below. An hSBA titer of ≥1:4 is widely recognized as the correlate of protection against IMD; however, a

more conservative hSBA titer of ≥1:16 has been considered a level indicative of a 4-fold vaccine effect for subjects seronegative before vaccination.

The proportion of subjects aged ≥24 months to <4 years, and ≥4 years to <10 years, in Group 1 with an hSBA titer ≥1:4 at baseline was 5.9% and 19.7%, respectively, for PMB80 (A22); 3.0% and 18.5%, respectively, for PMB2001 (A56); 4.5% and 9.0%, respectively, for PMB2948 (B24); and 0.0% and 1.4%, respectively for PMB2707 (B44). Subjects aged ≥24 months to <4 years, and≥4 years to <10 years, in Group 1 with an hSBA titer ≥1:16 at baseline was 4.4% and 13.6%, respectively, for PMB80 (A22); 1.5% and 15.4%, respectively, for PMB2001 (A56); 3.0% and 6.0%, respectively, for PMB2948 (B24); and 0.0% for both age strata for PMB2707 (B44).

The proportion of Group 1 subjects in the combined age stratum with an hSBA titer □1:4 and □1:16 at baseline was 12.7% and 9.0%, respectively, for PMB80 (A22); 10.6% and 8.3%, respectively, for PMB2001 (A56); 6.7% and 4.5%, respectively, for PMB2948 (B24); and 0.7% and 0.0%, respectively, for PMB2707 (B44).

The proportion of subjects aged ≥24 months to <4 years, and ≥4 years to <10 years, in Group 1 with an hSBA titer ≥1:4 at 1 month after the second vaccination was 65.6% and 83.3%, respectively, for PMB80 (A22); 100.0% for both age strata for PMB2001 (A56); 53.8% and 68.3%, respectively, for PMB2948 (B24); and 49.3% and 66.7%, respectively, for PMB2707 (B44). Subjects aged ≥24 months to <4 years, and ≥4 years to <10 years, in Group 1 with an hSBA titer ≥1:16 at 1 month after the second vaccination was 59.4% and 78.8%, respectively, for PMB80 (A22); 98.5% and 100.0%, respectively, for PMB2001 (A56); 43.1% and 58.7%, respectively, for PMB2948 (B24); and 31.3% and 55.6%, respectively, for PMB2707 (B44).

The proportion of Group 1 subjects in the combined age stratum with an hSBA titer ≥1:4 and ≥1:16 at 1 month after the second vaccination was 74.6% and 69.2%, respectively, for PMB80 (A22); 100.0% and 99.2%, respectively, for PMB2001 (A56); 60.9% and 50.8%, respectively, for PMB2948 (B24); and 57.7% and 43.1%, respectively, for PMB2707 (B44).

The proportion of subjects aged ≥24 months to <4 years, and ≥4 years to <10 years, in Group 1 with an hSBA titer ≥1:4 at 1 month after the third vaccination was 86.8% and 98.5%, respectively, for PMB80 (A22); 100.0% for each age strata for PMB2001 (A56); 90.5% and 95.2% for PMB2948 (B24); and 81.5% and 82.6%, respectively for PMB2707 (B44). Subjects aged ≥24 months to <4 years, and ≥4 years to <10 years, in Group 1 with an hSBA titer ≥1:16 at 1 month after the third vaccination was 83.8% and 91.0%, respectively, for PMB80 (A22); 100.0% for each age stratum for PMB2001 (A56); 81.0%

and 88.9%, respectively, for PMB2948 (B24); and 81.5% to 82.6% and 80.0% and 75.4%, respectively, for PMB2707 (B44).

The proportion of Group 1 subjects in the combined age stratum with an hSBA titer ≥1:4 and ≥1:16 at 1 month after the third vaccination was 92.6% and 87.4%, respectively, for PMB80 (A22); 100.0% and 100.0%, respectively, for PMB2001 (A56); 92.9% and 84.9%, respectively, for PMB2948 (B24); and 82.1% and 77.6%, respectively, for PMB2707 (B44).

In general, the proportion of Group 2 subjects achieving defined hSBA titers did not change over time compared to baseline.

Results for the mITT population were similar to those of the evaluable immunogenicity population.

Immunopersistence: Defined hSBA Titers

The proportions of subjects aged ≥24 months to <4 years, ≥4 years to <10 years, and in the combined age stratum, achieving hSBA titers of ≥1:4, ≥1:8, ≥1:16, ≥1:32, ≥1:64, and ≥1:128 for each of the 4 primary test strains at 6 months after the third vaccination with bivalent rLP2086 was a secondary immunogenicity endpoint. The proportion of subjects achieving defined hSBA titers for the 4 primary MnB strains was assessed for the evaluable immunogenicity population.

Overall, there was a decrease observed in the proportion of Group 1 subjects in both age strata who achieved defined hSBA titers from 1 month after the third vaccination to 6 months after the third vaccination.

For Group 1 subjects aged ≥24 months to <4 years and aged ≥4 years to <10 years, from 1 month after the third vaccination to 6 months after the third vaccination, the proportion of subjects with an hSBA titer ≥1:4 decreased from 86.8% to 25.4% and 98.5% to 55.6%, respectively, for PMB80 (A22); 100.0% to 82.0% and 100.0% to 85.7%, respectively for PMB2001 (A56); 90.5% to 13.8% and 95.2% to 26.6%, respectively, for PMB2948 (B24); and 81.5% to 13.6% and 82.6% to 13.0%, respectively, for PMB2707 (B44).

For Group 1 subjects in the combined age stratum, from 1 month after the third vaccination to 6 months after the third vaccination, the proportion of subjects with an hSBA titer ≥1:4 decreased from 92.6% to 40.5% for PMB80 (A22); 100.0% to 84.0% for PMB2001 (A56); 92.9% to 20.2% for PMB2948 (B24); and 82.1% to 13.3% for PMB2707 (B44).

For Group 1 subjects aged ≥24 months to <4 years and aged ≥4 years to <10 years, from 1 month after the third vaccination to 6 months after the third vaccination, the proportion of subjects with an hSBA titer ≥1:16 decreased from 83.8% to 19.0% and

91.0% to 46.0%, respectively, for PMB80 (A22); 100.0% to 77.0% and 100.0% to 82.9%, respectively, for PMB2001 (A56); 81.0% to 9.2% and 88.9% to 20.3%, respectively, for PMB2948 (B24); and 80.0% to 9.1% and 75.4% 7.2%, respectively, for PMB2707 (B44).

For Group 1 subjects in the combined age stratum, from 1 month after the third vaccination to 6 months after the third vaccination, the proportion of subjects with an hSBA titer ≥1:16 decreased from 87.4% to 32.5% for PMB80 (A22); 100.0% to 80.2% for PMB2001 (A56); 84.9% to 14.7% for PMB2948 (B24); and 77.6% to 8.1% for PMB2707 (B44).

In general, the proportion of Group 2 subjects achieving defined hSBA titers did not change over time compared to baseline.

Exploratory Immunogenicity Endpoints

The analysis of some exploratory immunogenicity endpoints was based on hSBA results for strains PMB2001 (A56) and PMB2707 (B44) for half of the subjects, and strains PMB80 (A22) and PMB2948 (B24) for the remaining half. The exploratory endpoint analyzed was hSBA titer with a ≥4-fold increase from baseline.

hSBA Titer 4-Fold Increase From Baseline

Table 22 presents the proportion of subjects with hSBA titers with a ≥4-fold rise from baseline for the 4 primary test strains.

The proportion of Group 1 subjects aged ≥24 months to <4 years and aged ≥4 years to <10 years achieving a ≥4-fold rise in hSBA titer from baseline to 1 month after Vaccination 2 was 56.3% and 63.6%, respectively for PMB80 (A22); 100.0% and 82.1%, respectively for PMB2001 (A56); 43.1% and 54.0%, respectively, for PMB2948 (B24); and 54.0% and 31.3%, respectively, for PMB2707 (B44).

The proportion of Group 1 subjects in the combined age stratum achieving a ≥4-fold rise in hSBA titer from baseline to 1 month after Vaccination 2 was 60.0% for PMB80 (A22), 91.0% for PMB2001 (A56), 48.4% for PMB2948 (B24), and 42.3% for PMB2707 (B44).

The proportion of Group 1 subjects aged ≥24 months to <4 years and aged ≥4 years to <10 years, achieving a ≥4-fold rise in hSBA titer from baseline to 1 month after Vaccination 3 was 79.4% and 77.6%, respectively for PMB80 (A22); 98.5% and 88.7%, respectively for PMB2001 (A56); 77.8% and 82.5%, respectively, for PMB2948 (B24); and 78.5% and 75.4%, respectively, for PMB2707 (B44).

The proportion of Group 1 subjects in the combined age stratum achieving a ≥4-fold rise in hSBA titer from baseline to 1 month after Vaccination 3 was 78.5% for PMB80 (A22), 93.5% for PMB2001 (A56), 80.2% for PMB2948 (B24), and 76.9% for PMB2707 (B44).

The proportion of Group 1 subjects aged ≥24 months to <4 years and aged ≥4 years to <10 years achieving a ≥4-fold rise in hSBA titer from baseline to 6 months after

Vaccination 3 was 19.0% and 36.5%, respectively for PMB80 (A22); 75.4% and 64.3%, respectively for PMB2001 (A56); 6.2% and 17.2%, respectively, for PMB2948 (B24); and 7.6% and 7.2%, respectively, for PMB2707 (B44).

The proportion of Group 1 subjects in the combined age stratum achieving a ≥4-fold rise in hSBA titer from baseline to 6 months after Vaccination 3 was 27.8% for PMB80 (A22), 69.5% for PMB2001 (A56), 11.6% for PMB2948 (B24), and 7.4% for PMB2707 (B44). Similar results were observed for the mITT population.

Table 22. Subjects With hSBA Titer ≥4-Fold Rise for Primary Strains – Evaluable Immunogenicity Population

	Vaccine Group (as Randomized)							
	Group 1 rLP2086				Group 2 HAV/Saline			
Strain (Variant) Sampling Time Point Age Strata	N[a]	n[b]	(%)	(95% CI)[c]	N[a]	n[b]	(%)	(95% CI)[c]
hSBA titer fold rise ≥4 from baseline[d]								
PMB80 (A22)								
1 Month after Vaccination 2								
≥24 Months to <10 years	130	78	(60.0)	(51.0, 68.5)	45	0	(0.0)	(0.0, 7.9)
≥24 Months to <4 years	64	36	(56.3)	(43.3, 68.6)	24	0	(0.0)	(0.0, 14.2)
≥4 Years to <10 years	66	42	(63.6)	(50.9, 75.1)	21	0	(0.0)	(0.0, 16.1)
1 Month after Vaccination 3								
≥24 Months to <10 years	135	106	(78.5)	(70.6, 85.1)	45	1	(2.2)	(0.1, 11.8)
≥24 Months to <4 years	68	54	(79.4)	(67.9, 88.3)	25	1	(4.0)	(0.1, 20.4)
≥4 Years to <10 years	67	52	(77.6)	(65.8, 86.9)	20	0	(0.0)	(0.0, 16.8)
6 Months after Vaccination 3								
≥24 Months to <10 years	126	35	(27.8)	(20.2, 36.5)	47	2	(4.3)	(0.5, 14.5)
≥24 Months to <4 years	63	12	(19.0)	(10.2, 30.9)	26	2	(7.7)	(0.9, 25.1)
≥4 Years to <10 years	63	23	(36.5)	(24.7, 49.6)	21	0	(0.0)	(0.0, 16.1)
PMB2001 (A56)								
1 Month after Vaccination 2								
≥24 Months to <10 years	133	121	(91.0)	(84.8, 95.3)	43	4	(9.3)	(2.6, 22.1)
≥24 Months to <4 years	66	66	(100.0)	(94.6, 100.0)	21	1	(4.8)	(0.1, 23.8)
≥4 Years to <10 years	67	55	(82.1)	(70.8, 90.4)	22	3	(13.6)	(2.9, 34.9)
1 Month after Vaccination 3								
≥24 Months to <10 years	139	130	(93.5)	(88.1, 97.0)	43	8	(18.6)	(8.4, 33.4)
≥24 Months to <4 years	68	67	(98.5)	(92.1, 100.0)	24	1	(4.2)	(0.1, 21.1)
≥4 Years to <10 years	71	63	(88.7)	(79.0, 95.0)	19	7	(36.8)	(16.3, 61.6)
6 Months after Vaccination 3								
≥24 Months to <10 years	131	91	(69.5)	(60.8, 77.2)	46	6	(13.0)	(4.9, 26.3)
≥24 Months to <4 years	61	46	(75.4)	(62.7, 85.5)	24	3	(12.5)	(2.7, 32.4)
≥4 Years to <10 years	70	45	(64.3)	(51.9, 75.4)	22	3	(13.6)	(2.9, 34.9)
PMB2948 (B24)								
1 Month after Vaccination 2								
≥24 Months to <10 years	128	62	(48.4)	(39.5, 57.4)	45	3	(6.7)	(1.4, 18.3)
≥24 Months to <4 years	65	28	(43.1)	(30.8, 56.0)	24	1	(4.2)	(0.1, 21.1)
≥4 Years to <10 years	63	34	(54.0)	(40.9, 66.6)	21	2	(9.5)	(1.2, 30.4)
1 Month after Vaccination 3								
≥24 Months to <10 years	126	101	(80.2)	(72.1, 86.7)	46	2	(4.3)	(0.5, 14.8)
≥24 Months to <4 years	63	49	(77.8)	(65.5, 87.3)	26	2	(7.7)	(0.9, 25.1)
≥4 Years to <10 years	63	52	(82.5)	(70.9, 90.9)	20	0	(0.0)	(0.0, 16.8)
6 Months after Vaccination 3								
≥24 Months to <10 years	129	15	(11.6)	(6.7, 18.5)	47	0	(0.0)	(0.0, 7.5)
≥24 Months to <4 years	65	4	(6.2)	(1.7, 15.0)	26	0	(0.0)	(0.0, 13.2)
≥4 Years to <10 years	64	11	(17.2)	(8.9, 28.7)	21	0	(0.0)	(0.0, 16.1)
PMB2707 (B44)								
1 Month after Vaccination 2								
≥24 Months to <10 years	130	55	(42.3)	(33.7, 51.3)	50	0	(0.0)	(0.0, 7.1)
≥24 Months to <4 years	63	34	(54.0)	(40.9, 66.6)	26	0	(0.0)	(0.0, 13.2)
≥4 Years to <10 years	67	21	(31.3)	(20.6, 43.8)	24	0	(0.0)	(0.0, 14.2)
1 Month after Vaccination 3								

Table 22. Subjects With hSBA Titer ≥4-Fold Rise for Primary Strains – Evaluable Immunogenicity Population

	Vaccine Group (as Randomized)							
	Group 1 rLP2086				Group 2 HAV/Saline			
Strain (Variant) Sampling Time Point Age Strata	N[a]	n[b]	(%)	(95% CI)[c]	N[a]	n[b]	(%)	(95% CI)[c]
≥24 Months to <10 years	134	103	(76.9)	(68.8, 83.7)	50	0	(0.0)	(0.0, 7.1)
≥24 Months to <4 years	65	51	(78.5)	(66.5, 87.7)	26	0	(0.0)	(0.0, 13.2)
≥4 Years to <10 years	69	52	(75.4)	(63.5, 84.9)	24	0	(0.0)	(0.0, 14.2)
6 Months after Vaccination 3								
≥24 Months to <10 years	135	10	(7.4)	(3.6, 13.2)	49	0	(0.0)	(0.0, 7.3)
≥24 Months to <4 years	66	5	(7.6)	(2.5, 16.8)	26	0	(0.0)	(0.0, 13.2)
≥4 Years to <10 years	69	5	(7.2)	(2.4, 16.1)	23	0	(0.0)	(0.0, 14.8)

Abbreviations: hSBA = serum bactericidal assay using human complement; LLOQ = lower limit of quantitation; LOD = limit of detection.
Note: LLOQ = 1:16 for A22; 1:8 for A56, B24, and B44.
Note: The 4-fold increase is defined as follows: (1) For subjects with a baseline hSBA titer below the LOD (hSBA titer <1:4), a response is defined as an hSBA titer ≥1:16 or the LLOQ (whichever titer is higher). (2) For subjects with a baseline hSBA titer ≥ LOD and < LLOQ, a response is defined as an hSBA titer ≥4 times the LLOQ. (3) For subjects with a baseline hSBA titer ≥LLOQ, a response is defined as an hSBA titer ≥4 times the baseline titer.
a. For hSBA titer fold rise ≥4 from baseline, N = number of subjects with valid and determinate hSBA titers for the given strain at both the specified time point and baseline.
b. For hSBA titer fold rise ≥4 from baseline, n = number of subjects who achieved hSBA titer fold rise ≥4 from baseline for the given strain.
c. Exact 2-sided CI based upon observed proportion of subjects, using the Clopper and Pearson method.
d. Baseline is defined as the blood draw prior to Vaccination 1.

Additional Immunogenicity Analyses

Assessment of Missing hSBA Data for Primary MnB Test Strains

Only valid and determinate hSBA results were included in all immunogenicity analyses. The hSBA results were excluded from the immunogenicity analysis (or considered to be missing) for the following reasons:

- The subject withdrew from the study.
- The subject did not have blood samples for testing but was not withdrawn from the study.
- The quantity of blood was insufficient to perform the assay. This was entered as "quantity not sufficient" for the assay results.
- The sample was tested but a numerical titer could not be reliably determined. This was entered as "indeterminate" for the assay results.

Reverse Cumulative Distribution Curves

The RCDCs of the proportions of subjects exhibiting an hSBA response (≥ LLOQ) for each of the 4 primary strains and at each sampling time point, for the combined age stratum were assessed. RCDCs for each of the 4 primary strains and at each sampling

time point, for subjects aged ≥24 months to <4 years were also assessed. RCDCs for each of the 4 primary strains and at each sampling time point, for subjects aged ≥4 years to <10 years were assessed.

The RCDCs showed the majority of subjects in both age strata exhibited a measurable hSBA response to each of the primary MnB test strains at 1 month after the second and third dose of bivalent rLP2086.

Immunogenicity Conclusions

The primary objectives of this study were to describe subject immune response to bivalent rLP2086 as measured by hSBA against 4 primary MnB test strains, 2 expressing an LP2086 subfamily A protein and 2 expressing an LP2086 subfamily B protein, measured 1 month after the third vaccination in healthy subjects aged ≥24 months to <4 years and ≥4 years to <10 years. The description of immune responses for the combined age stratum (≥24 months to <10 years) was a secondary objective. The endpoints for the primary objectives were the proportions of subjects in each age stratum achieving hSBA titers ≥ LLOQ for each of the 4 primary MnB strains 1 month after the third vaccination.

A robust immune response was observed for children aged ≥24 months to <10 years 1 month after the third dose of bivalent rLP2086, as confirmed by the proportion of subjects achieving an hSBA titer ≥ LLOQ (1:8 for A56, B24 and B44; 1:16 for A22) for each of the 4 primary MnB test strains ranging from 80.0% to 100.0% for subjects aged ≥24 months to <4 years and from 78.3% to 100.0% for subjects ≥4 years to <10 years after 3 doses. The proportion of subjects in the combined age stratum with an hSBA titer ≥ LLOQ for each of the 4 primary MnB test strains 1 month after the third vaccination ranged from 79.1% to 100.0%. These findings are further supported by substantial GMTs (range 19.1 to 191) and in the proportion of subjects achieving an hSBA titer ≥1:4 (81.5% to 100%) or ≥1:16 (75.4% to 100%) against each of the 4 primary MnB test strains after 3 doses of bivalent rLP2086 compared to baseline across both age strata. Additionally, the proportion of subjects in the combined age stratum achieving an hSBA fold rise ≥4 from baseline to 1 month after the third vaccination for each of the 4 primary MnB test strains ranged from 76.9% to 93.5%.

The secondary objective of the study was to describe immune responses 1 month after the second dose of bivalent rLP2086, as assessed by ≥ LLOQ responses, defined hSBA titers and hSBA GMTs for the 2 age strata and the combined age stratum. For the combined age stratum, the proportion of subjects achieving an hSBA titer ≥ LLOQ ranged from 48.5% to 100.0% with no meaningful differences observed between the younger and older age strata. These findings are further supported by the combined age

stratum with increases in GMTs (range 11.1 to 96.6) and in the proportion of subjects achieving an hSBA titer ≥1:4 (57.7% to 100%) or ≥1:16 (43.1% to 100%) after 2 doses of bivalent rLP2086 compared to baseline against each of the 4 primary MnB test strains. GMTs were similar between the 2 age strata. Additionally, the proportion of subjects in the combined age stratum achieving an hSBA fold rise ≥4 from baseline to 1 month after the second vaccination for each of the 4 primary MnB test strains ranged from 42.3% to 91.0%.

Immunopersistence was also assessed at 6 months after the third dose of bivalent rLP2086 with the proportion of subjects with an hSBA titer ≥ LLOQ declining from 79.1% to 100% 1 month after Vaccination 3 to 10.4% to 82.4% at 6 months after the third vaccination for the combined age stratum. No differences between the 2 age strata were observed except for A22, for which older children had a higher proportion of subjects achieving a titer ≥ LLOQ than the younger children (46%, 95% CI 33.4, 59.1 vs 19%, 95% CI 10.2, 30.9). However, baseline prevaccination rates of titers ≥ LLOQ were greater for A22 in the older age stratum (13.6% vs 4.4%). A similar trend was also observed for the combined age stratum for the proportion of subjects with a protective hSBA titer ≥1:4, ranging from 13.3% to 84.0% and GMTs, ranging from 5.1 to 31.3 at 6 months after the third vaccination.

In summary, bivalent rLP2086 given as 3 doses on a 0-, 2-, and 6-month schedule elicits a robust immune response among toddlers and children aged ≥24 months to <10 years with protective antibody titers achieved as measured by hSBA in a high proportion of subjects after the third dose. No clinically meaningful differences were observed between toddlers aged ≥24 months to <4 years and children aged ≥4 years to <10 years. Antibody responses decline 6 months after the third dose, but remain higher than prevaccination baseline rates.

DISCUSSION AND OVERALL CONCLUSIONS

Immunogenicity Discussion

Immunogenicity results from this Phase 2 study of a 3-dose regimen (0-, 2-, and 6-month schedule) of bivalent rLP2086 given to toddlers and children aged ≥24 months to <10 years are consistent with previous studies in adolescents and young adults. Immunogenicity responses to bivalent rLP2086 vaccination were measured in validated hSBAs using 4 primary MnB test strains, each expressing fHBP variants heterologous to the vaccine component antigens, using criteria more stringent than the accepted correlate of protection (hSBA titer ≥1:4). Based on an hSBA titer ≥ LLOQ for the 4 primary MnB test strains 1 month after Vaccination 3, the toddlers and children participating in this study had similar immune responses compared to adolescents

(10 years to <19 years) participating in Study B1971009, with proportions of subjects achieving an hSBA titer ≥ LLOQ after the third vaccination (0-, 2-, 6-month schedule) ranging from 79.1% to 100% in this study and 87.1% to 99.5% in Study B1971009. Meaningful differences in the proportion of subjects with an hSBA titer ≥ LLOQ for the 4 primary test strains 1 month after Vaccination 3 between these 2 studies are not apparent, despite the fact that Study B1971009 had a much higher proportion of adolescent subjects with a prevaccination hSBA titer ≥ LLOQ compared to the toddlers and children in this study, particularly for the A22 (33.2% vs 9%, respectively) and A56 (27.5% vs 8.3%, respectively) test strains. Bivalent rLP2086 appears to be highly immunogenic in the ≥24 months to <10 years age population and is likely to offer protection against MnB infection similarly to that expected for adolescents based on the hSBA correlate of protection.

With regard to the secondary objectives, immune responses in this study for the combined age stratum (≥24 months to <10 years) 1 month after the second dose of bivalent rLP2086 were relatively robust for the 4 primary MnB test strains, with the proportion of subjects achieving an hSBA titer ≥ LLOQ ranging from 48.5% to 100%. With exception of strain A56, these responses were lower than immune responses observed among adolescents (10 years to <19 years) participating in Study B1971009 receiving 2 doses of bivalent rLP2086 given 2 months apart, ranging from 64% to 99.1%. Immunopersistence was assessed in this study for toddlers and children by measuring hSBA titers 6 months after Vaccination 3. The proportion of subjects achieving an hSBA titer ≥ LLOQ declined from a range of 79.1% to 100% 1 month after Vaccination 3 to a range of 10.4% to 82.4% 6 months after Vaccination 3. GMTs and the proportion of subjects with defined hSBA titers also declined 6 months after Vaccination 3. Although the proportions of subjects achieving an hSBA titer ≥ LLOQ 6 months after Vaccination 3 in this study (10.4% to 82.4%) were lower than for adolescents (11 years to <19 years) participating in Study 6108A1-2001 (36.7% to 89.4%), they are still higher than the proportion of subjects aged ≥24 months to <10 years with an hSBA titer ≥ LLOQ at baseline (0% to 9%). It is well established that meningococcal colonization rates increase with age through early adulthood. Differences between subjects aged ≥24 months to <10 years and older age groups may be partially attributable to the proportion of subjects with a baseline titer ≥ LLOQ, which were as high as 27.5% (A56) and 33.2% (A22) in Study B1971009, and 7.4% to 13.4% for subfamily A strains in Study 6108A1-2001. Additionally, a recent study showed that carriage of disease-associated serogroup B strains was higher in subjects with protective hSBA titers (before vaccination), and that vaccination did not impact subsequent carriage of

those disease-associated strains. This suggests that carriage may impact baseline hSBA titers and that persistence or recolonization after vaccination may be contributing to a greater proportion of subjects with an hSBA titer ≥ LLOQ observed among adolescents 6 months after vaccination compared to the toddlers and children in this study, who we speculate are less likely to be colonized and have lower percentages of hSBA titers ≥ LLOQ at baseline. Immunopersistence studies with monovalent conjugate meningococcal serogroup C vaccine in infants and children similarly show that protective immunity rapidly wanes. However, even among a cohort of children with percentages of hSBA titers >1:8 for serogroup C at only 46.9% 4 years after completion of the primary dose, a subsequent booster dose provided titers >1:8 in 100% of subjects 1 month and 1 year after the booster. This indicates that even those considered seronegative prior to the booster dose have a strong anamnestic response which persists for up to 1 year after booster vaccination. Postbooster response and persistence studies are therefore warranted among individuals who received their primary series of bivalent rLP2086 as toddlers and children to provide further insights into the utility of a booster dose in providing protection against IMD through adolescence and early adulthood.

In summary, bivalent rLP2086 administered on a 0-, 2-,and 6-month schedule is highly immunogenic among toddlers and children aged ≥24 months to <10 years with protective immune responses achieved as measured by hSBA in a high proportion of subjects after the third dose. Immune responses, as measured in this study, appear to be similar to that observed in prior studies among adolescents 1 month after the second and third doses. The 3-dose regimen appears to provide high rates of protective immunity in toddlers and children aged ≥24 months to <10 years.

Overall Conclusions

In conclusion, bivalent rLP2086 administered to toddlers and children aged ≥24 months to <10 years in a 3-dose series on a 0-, 2-, and 6-month schedule elicits a robust immune response by the majority of subjects after the second and third doses, with protective antibody titers achieved after the third dose as measured by hSBAs. hSBA titers decreased 6 months after a 3-dose series. The vaccine, as administered in this study, was safe and well tolerated with an acceptable safety profile for toddlers and children aged ≥24 months to <10 years.

EXAMPLE 20: A Phase 2, Randomized, Controlled, Observer-Blinded Study Conducted to Describe the Immunogenicity, Safety, and Tolerability of a *Neisseria meningitidis* Serogroup B Bivalent Recombinant Lipoprotein 2086 Vaccine (Bivalent rLP2086) When Administered to Healthy Toddlers Aged 12 to <18 Months or 18 to <24 Months (B1971035-syn)

OBJECTIVES

Primary Immunogenicity Objectives:

- To describe the immune response as measured by serum bactericidal assay using human complement (hSBA) performed with 4 primary *Neisseria meningitidis* serogroup B (MnB) strains, 2 expressing an LP2086 subfamily A protein and 2 expressing an LP2086 subfamily B protein, measured 1 month after the third vaccination with bivalent rLP2086, in healthy toddlers aged 12 to <18 months at study entry.
- To describe the immune response as measured by hSBA performed with 4 primary MnB strains, 2 expressing an LP2086 subfamily A protein and 2 expressing an LP2086 subfamily B protein, measured 1 month after the third vaccination with bivalent rLP2086, in healthy toddlers aged 18 to <24 months at study entry.

Primary Safety Objective:

- To evaluate the safety profile of bivalent rLP2086 compared to a control (hepatitis A virus [HAV] vaccine), as measured by local reactions, systemic events, adverse events (AEs), serious adverse events (SAEs), newly diagnosed chronic medical conditions (NDCMCs), medically attended events (MAEs), and immediate AEs in healthy toddlers 12 to <18 months and 18 to <24 months of age at study entry, and in both age strata combined.

Secondary Immunogenicity Objectives:

- To describe the immune response as measured by hSBA performed with 4 primary MnB test strains, 2 expressing an LP2086 subfamily A protein and 2 expressing an LP2086 subfamily B protein, measured 1 month after the third vaccination with bivalent rLP2086, in healthy toddlers aged 12 to <24 months at study entry (ie, both age strata combined).
- To describe the immune response as measured by hSBA performed with 4 primary MnB test strains, 2 expressing an LP2086 subfamily A protein and 2 expressing an LP2086 subfamily B protein, measured 1 month after the second vaccination and 6, 12, 24, 36, and 48 months after the third vaccination in healthy toddlers aged 12 to <18 months and 18 to <24 months at study entry, and in both age strata combined.

Exploratory Immunogenicity Objectives:

- To further describe the immune response as measured by hSBA performed with 4 primary MnB test strains, 2 expressing an LP2086 subfamily A protein and 2 expressing an LP2086 subfamily B protein, measured 1 month after the second vaccination and 1, 6, 12, 24, 36, and 48 months after the third vaccination with bivalent rLP2086 in healthy toddlers aged 12 to <18 months and 18 to <24 months at study entry, and in both age strata combined.
- To further describe the immune response as measured by hSBA to secondary MnB test strains expressing LP2086 subfamily A and B proteins, at 1 month after the second vaccination and 1, 6, 12, 24, 36, and 48 months after the third vaccination in healthy toddlers aged 12 to <18 months and 18 to <24 months at study entry, and in both age strata combined.

METHODS

Study Design:

The study was a Phase 2, randomized, active-controlled, observer-blinded, sponsor-unblinded, multicenter study in which approximately 396 healthy toddlers stratified by age, 12 to <18 months or 18 to <24 months old, were randomly assigned in a 2:1 ratio to receive bivalent rLP2086 (either of 2 dose levels [60 μg or 120 μg]) or a licensed pediatric HAV vaccine (0.5 mL)/sterile saline solution for injection (0.5-mL of 0.85% sodium chloride).

The study was conducted in 2 stages. Stage 1 assessed vaccine immunogenicity, safety, and tolerability across 2 phases: a sentinel-enrollment phase and an expanded-enrollment phase. Stage 2 assessed the duration of the immune response to bivalent rLP2086.

For immunogenicity, all data through 1 month after Vaccination 3 (Visit 7) are presented with the exception of secondary MnB test strain data supporting an exploratory objective. The final report includes all immunogenicity data through the completion of Stage 2 and safety data for the period after Visit 8 until the end of the study (Visit 13, 48 months after Vaccination 3).

The Stage 1 sentinel-enrollment phase was planned to include a total of 4 sentinel cohorts: 2 age strata for each dose level (60 μg or 120 μg) of bivalent rLP2086. The younger-aged sentinel cohorts were composed of subjects aged 12 to <15 months and the older-aged sentinel cohorts were composed of subjects aged 18 to <24 months. Each of the 4 sentinel cohorts was planned to enroll approximately 33 subjects. The sentinel-enrollment phase was staggered with reviews by an IRC at pre-specified points and stopping rules applied. The 120-μg dose level sentinel cohorts did not proceed until the 60-μg dose level was evaluated by the IRC as safe and tolerable in the sentinel

cohort of the same age. The younger-aged 120-μg dose level sentinel cohort did not proceed until this dose level was evaluated by the IRC as safe and tolerable in the older-aged 120-μg dose level sentinel cohort.

Prior to the Stage 1 expanded-enrollment phase, the IRC reviewed all post–Vaccination 1, 7-day e-diary and SAE data obtained from sentinel subjects. Based on the review, the IRC selected the 120-μg bivalent rLP2086 dose level to be studied in the Stage 1 expanded-enrollment phase for both age strata. The younger-aged expanded-enrollment cohort (enrolling an additional 132 subjects) was extended to subjects aged 12 to <18 months and stratified by age into 2 subsets: aged 12 to <15 months and aged 15 to <18 months. The older-aged expanded enrollment cohort (enrolling an additional 132 subjects) enrolled subjects aged 18 to <24 months during the expanded-enrollment phase. The total study duration for subjects completing only Stage 1 will be approximately 18 months. The visit schedule for Stage 1 is presented in Table 23.

Stage 2 includes only those subjects randomly assigned to bivalent rLP2086 (irrespective of dose level). The total study duration for subjects who complete Stage 2 will be approximately 4.5 years (54 months). The visit schedule for Stage 2 is presented in Table 24.

Bivalent rLP2086 was administered at Months 0, 2, and 6 (Visits 1, 4, and 6). Pediatric HAV vaccine was administered at Months 0 and 6 (Visits 1 and 6), and saline was administered at Month 2 (Visit 4) to maintain the study blind.

Table 23. Stage 1 Visit Schedule

Visit Identifier	1	2	3	4	5	6	7	8	9[a]	10[a]
Time Period	**Month 0**	**Week 1**	**Month 1**	**Month 2**	**Month 3**	**Month 6**	**Month 7**	**Month 12**	**Month 18**	**End of Stage 1**
Visit Description	Vaccination 1	Post–Vaccination 1 Follow-up Visit	Telephone Contact	Vaccination 2	Post–Vaccination 2 Blood Draw	Vaccination 3	Post–Vaccination 3 Blood Draw	6-Month Follow-up Visit[b]	Antibody Persistence Blood Draw[b]	Telephone Contact
	Vaccination Phase							Follow-up Phase		
Vaccination & 30-minute observation[c]	X			X		X				
Obtain 5-mL blood sample	X[d]				X		X	X[e]	X	

Abbreviations: CRF = case report form; e-diary = electronic diary.

a. Visits 9 and 10 are not included in this primary analysis clinical study report.

b. Relative to Vaccination 3.

c. Injection performed by unblinded administrator; acute reactions assessed by blinded observer. Location of vaccination was noted in the source, the CRF, and the e-diary.

d. Blood was collected before vaccination and only after eligibility was confirmed.

e. Immunogenicity results from blood draw at Visit 8 are not included in this primary analysis clinical study report.

Source: Protocol schedule of activities for Stage 2.

Table 24. Stage 2 Visit Schedule

Visit Identifier	11	12	13
Time Period	**Month 30**	**Month 42**	**Month 54**
Visit Description (Time After Vaccination 3)	Immunogenicity 1 (24 Months)	Immunogenicity 2 (36 Months)	Immunogenicity 3 (48 Months)
Obtain 5-mL blood sample	X	X	X

Source: Protocol schedule of activities for Stage 2.

Vaccines Administered: Bivalent rLP2086 (60 μg or 120 μg) was administered 3 times over the course of the study: the first vaccination at Visit 1 (Month 0), second vaccination at Visit 4 (Month 2), and third vaccination at Visit 6 (Month 6). Bivalent rLP2086 was administered as an intramuscular injection into either the deltoid muscle or anterolateral thigh muscle. HAV vaccine was administered twice over the course of the study: the first vaccination at Visit 1 (Month 0) and third vaccination at Visit 6 (Month 6). Saline was administered at the second vaccination (Month 2) time point. HAV vaccine/saline was administered as an intramuscular injection into either the deltoid muscle or anterolateral thigh muscle. Only a third-party unblinded medically qualified member of the study staff administered the investigational product. If muscle mass in the deltoid was not adequate for intramuscular injection, then the thigh was the preferred injection site.

Immunogenicity Evaluations: To facilitate immunogenicity analysis, subjects had approximately 5 mL of blood collected at the following time points during Stage 1: before Vaccination 1 (Visit 1), 1 month after Vaccination 2 (Visit 5), 1 month after Vaccination 3 (Visit 7), 6 months after Vaccination 3 (Visit 8), 12 months after Vaccination 3 (Visit 9). In total, 25 mL was collected over the 18-month period. Local/topical anesthetic could be used prior to blood draws.

To determine duration of immune response, Stage 2 subjects will have approximately 5 mL of blood collected at the following time points: 2 years after Vaccination 3, 3 years after Vaccination 3, and 4 years after Vaccination 3. In total, 15 mL will be collected over approximately 2.5 years.

For assessment of the immune response to bivalent rLP2086, functional antibodies were analyzed in hSBAs with meningococcal serogroup B strains. The hSBA measures antibodies in human sera that result in complement-dependent killing of the target meningococcal strain. Four (4) primary MnB test strains, PMB80 (A22), PMB2001 (A56), PMB2948 (B24), and PMB2707 (B44), each expressing a factor H binding protein (fHBP)

variant heterologous to the vaccine component antigens, were used in the hSBAs for determination of the immunogenicity endpoints in this study.

Due to serum volume limitations, 2 of the primary strains (PMB80 [A22] and PMB2948 [B24]) were tested at each blood sampling time point for half of the subjects (in both age strata), and the other 2 primary strains (PMB2001 [A56] and PMB2707 [B44]) were tested at each blood sampling time point for the remaining half of the subjects.

Once all subjects completed enrollment (Visit 1), the independent statistical center (ISC), a statistical team not involved in the conduct of the study, provided 2 subject listings (randomly selected, 50% of subjects to be tested for PMB80 [A22]/PMB2948 [B24] and the remaining 50% of subjects to be tested for PMB2001 [A56]/PMB2707 [B44]) to the sponsor's sample management team. Both listings followed the same randomization ratio (2:1) and age-strata distribution as in the study design. The same strain pair (PMB80 [A22]/PMB2948 [B24] or PMB2001 [A56]/PMB2707 [B44]) was tested across all visits for the same subjects.

Once testing for the primary analyses was completed, and if sufficient volume of sera was available, additional testing to assess the immune response to bivalent rLP2086 could be considered as follows: PMB80 (A22) and PMB2948 (B24) could be tested in serum samples from the 50% of subjects who received bivalent rLP2086 and whose serum samples were originally tested for PMB2001 (A56) and PMB2707 (B44). Conversely, PMB2001 (A56) and PMB2707 (B44) could be tested in serum samples from the 50% of subjects who received bivalent rLP2086 and were originally tested for PMB80 (A22) and PMB2948 (B24). Testing for secondary strains could be performed.

Statistical Methods:

The primary immunogenicity endpoints were:

- Proportions of subjects achieving an hSBA titer ≥ lower limit of quantitation(LLOQ) 1 month after the third vaccination, for each of the 4 primary MnB test strains in healthy toddlers 12 to <18 months of age at study entry.
- Proportions of subjects achieving an hSBA titer ≥ LLOQ 1 month after the third vaccination, for each of the 4 primary MnB test strains in healthy toddlers 18 to <24 months of age at study entry.

All secondary immunogenicity endpoints for the entire study are described here but this Example presents results for the immunogenicity endpoints applicable to Visits 1 to 7 only. Subsequent endpoints will be analyzed.

The following endpoint applied to results in healthy subjects 12 months to <24 months of age (i.e., both age strata combined) at study entry:

- Proportion of subjects with hSBA titers ≥ LLOQ for each of the 4 primary MnB test strains 1 month after the third vaccination with bivalent rLP2086 and 6, 12, 24, 36, and 48 months after the third vaccination with bivalent rLP2016.

The following endpoints applied to results in healthy subjects 12 to <18 months of age and 18 to <24 months of age at study entry, and in both age strata combined:

- Proportions of subjects with hSBA titers ≥ LLOQ for each of the 4 primary MnB test strains at 1 month after the second vaccination with bivalent rLP2086.
- Proportions of subjects with hSBA titers ≥ LLOQ, ≥1:4, ≥1:8, ≥1:16, ≥1:32, ≥1:64, and ≥1:128 for each of the 4 primary MnB strains at each applicable blood sampling visit.
- hSBA geometric mean titers (GMTs) for each of the 4 primary MnB test strains at each applicable blood sampling visit.

All of the exploratory endpoints specified below applied to hSBA results from all healthy subjects 12 to <18 months of age or 18 to <24 months of age at study entry, and in both age strata combined, who received bivalent rLP2086 and were tested for the appropriate strain at the appropriate time point:

- Proportions of subjects with hSBA titers ≥ LLOQ, ≥1:4, ≥1:8, ≥1:16, ≥1:32, ≥1:64, and ≥1:128 at each applicable blood sampling time point.
- hSBA GMTs for each of the 4 primary MnB strains at each applicable blood sampling visit.
- Proportions of subjects achieving at least a 4-fold increase in hSBA titer from baseline to 1 month after the third vaccination with bivalent rLP2086 for each of the 4 primary test strains:
    - For subjects with a baseline hSBA titer below the limit of detection (LOD) or an hSBA titer of <1:4, a 4-fold response was defined as an hSBA titer of ≥1:16.
    - For subjects with a baseline hSBA titer of ≥ LOD (ie, hSBA titer of ≥1:4) and < LLOQ, a 4-fold response was defined as an hSBA titer of ≥4 times the LLOQ.
    - For subjects with a baseline hSBA titer of ≥ LLOQ, a 4-fold response was defined as an hSBA titer of ≥4 times the baseline titer.

The following endpoints were planned if there had been sufficient sera available to test each subject for all 4 primary strains and/or to test subjects for the secondary strains.

- Proportions of subjects achieving an hSBA titer ≥ LLOQ for all 4 primary test strains (PMB80 [A22], PMB2948 [B24], PMB2001 [A56], and PMB2707 [B44]) combined, 1 month after the third vaccination with bivalent rLP2086. This applied only to those subjects who had all 4 primary strains tested.
- Additional exploratory assays to test hSBA on the secondary MnB strains as follows:
  - hSBA GMTs to each secondary MnB strain tested, at 1 month after the second and third vaccinations and/or at each blood sampling time point thereafter.
  - Proportions of subjects with an hSBA titer ≥ LLOQ, to each secondary MnB strain at 1 month after the second and third vaccinations and/or at each blood sampling time point thereafter.

Analysis of Primary Endpoints

The primary analysis for the primary objectives was the proportion of subjects with an hSBA titer ≥ LLOQ 1 month after the third vaccination, for each of the 4 primary MnB test strains in healthy toddlers aged 12 to <18 months, and 18 to <24 months, at study entry respectively. The evaluable immunogenicity population was used for this summary and both percentages and confidence intervals (CIs) are displayed.

Analysis of Secondary Endpoints

All of the analyses performed on the mITT population were considered as secondary analyses.

Secondary analyses also included percent of subjects with hSBA titers ≥ LLOQ for each of the 4 primary MnB test strains 1 month following the third vaccination for both the evaluable immunogenicity population and for the mITT population.

For this Example, the percentage of subjects with hSBA titers ≥ LLOQ for each of the 4 primary MnB test strains 1 month following the second vaccination and 1 month after the third vaccination were analyzed using both the evaluable immunogenicity population and the mITT population. For the final analysis, the percentage of subjects with hSBA titers ≥ LLOQ for each of the 4 primary MnB test strains 6, 12, 24, 36, and 48 months after the third vaccination will be analyzed using both the evaluable immunogenicity population and the mITT population.

The percentage of subjects with hSBA titers ≥ LLOQ, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128 for each of the 4 primary MnB test strains at each applicable blood sampling visit were analyzed using both the evaluable immunogenicity population and the mITT population.

The GMTs for each of the primary MnB test strains at each applicable blood sampling visit were summarized for the evaluable immunogenicity population and the mITT population.

Analysis of Exploratory Endpoints

The proportion of subjects achieving at least a 4-fold increase in hSBA titer from baseline to 1 month after the second and third vaccinations with bivalent rLP2086 was summarized for the evaluable immunogenicity population and the mITT population.

Subgroup Analysis

Some immunogenicity and safety endpoints were descriptively summarized by sex, by country, and within age strata.

RESULTS

Subject Disposition and Demography:

A total of 396 subjects 12 to <24 months of age were randomized in this study. Of the subjects randomized, a total of 44 subjects received 60 μg of bivalent rLP2086, 220 subjects received 120 μg of bivalent rLP2086, and 132 subjects received HAV vaccine/saline. There were 198 subjects randomized in each of the age strata (12 to <18 months and 18 to <24 months).

Of the 396 randomized subjects, 385 (97.2%) subjects completed the vaccination phase (from the first study vaccination [Visit 1] through 1 month after Vaccination 3 [Visit 7]) of the study. A total of 386 (97.5%) subjects completed the follow-up phase (from Visit 7 to 6 months after Vaccination 3 [Visit 8]). Overall, a total of 381 (96.2%) subjects completed all study visits up to the 6-month follow-up visit (Visit 8) and completion status was similar for each age stratum (189 [95.5%] subjects and 192 [97.0%] subjects in the 12 to <18 months and 18 to <24 months age strata, respectively).

Overall, 52.8% of subjects were female, and the majority of the subjects were white (94.4%) and non-Hispanic/non-Latino (99.5%). The mean age (SD) at first vaccination was 17.3 (3.61) months (range of 12 to 23 months). Demographic characteristics were generally similar among the vaccine groups.

The characteristics of ITT and mITT populations were similar to the characteristics of the safety population.

Immunogenicity Results:

The primary objectives of this study were to describe the immune response to bivalent rLP2086 as measured by hSBA against 4 primary MnB test strains, 2 expressing an LP2086 subfamily A protein and 2 expressing an LP2086 subfamily B protein, measured 1 month after the third vaccination in healthy subjects 12 to <18 months of age and 18 to <24 months

of age. The description of immune responses for the combined age stratum (12 to <24 months) was a secondary objective. The primary endpoints for the primary objectives were the proportions of subjects in each age stratum achieving hSBA titers ≥ LLOQ for each of the 4 primary MnB strains 1 month after the third vaccination.

A robust immune response was observed at both dose levels for toddlers 12 to <18 months of age and for toddlers 18 to <24 months of age, as well as for the combined age stratum (12 to <24 months) 1 month after the third dose of bivalent rLP2086, as confirmed by the proportion of subjects achieving an hSBA titer ≥ LLOQ (1:8 for A56, B24 and B44; 1:16 for A22) for each of the 4 primary MnB test strains. For the 60-μg group, the proportion of subjects achieving an hSBA titer ≥ LLOQ ranged from 88.9% to 100.0% for the younger toddlers (12 to <18 months) and from 81.8% to 100.0% for the older toddlers (18 to <24 months) after 3 doses. For the 120-μg group, the proportion of subjects achieving an hSBA titer ≥ LLOQ ranged from 71.1% to 100.0% for toddlers 12 to <18 months of age and from 72.0% to 100.0% for toddlers 18 to <24 months of age after 3 doses. For the combined age stratum the proportion of subjects achieving an hSBA titer ≥ LLOQ for each of the 4 primary MnB test strains 1 month after the third vaccination ranged from 85.0% to 100.0% for the 60-μg group and from 71.6% to 100.0% for the 120-μg group . These findings are further supported by increases in GMTs (range from 4.0 to 8.5 at baseline to 15.1 to 171.4 at 1 month after Vaccination 3) and in the proportion of subjects achieving an hSBA titer ≥1:4 (71.1% to 100.0%) or ≥1:16 (63.6% to 100.0%) against each of the 4 primary MnB test strains after 3 doses of bivalent rLP2086 compared to baseline across both age strata and dose levels. Additionally, the proportion of subjects for the combined age stratum achieving an hSBA fold rise ≥4 from baseline to 1 month after the third vaccination for each of the 4 primary MnB test strains ranged from 67.4% to 100.0% for both dose levels. In conclusion, 3 doses of either 60 μg or 120 μg of bivalent rLP2086 administered on a 0-, 2-, 6-month schedule, induced robust immune responses in toddlers 12 to <24 months of age (both individual and combined age strata).

The secondary objective of the study was to describe immune responses 1 month after the second dose of bivalent rLP2086, as assessed by ≥ LLOQ responses, defined hSBA titers, and hSBA GMTs for the 2 age strata and the combined age stratum. For the combined age stratum, the proportion of subjects achieving an hSBA titer ≥ LLOQ after the second dose of bivalent rLP2086 (administered 2 months after the first dose) ranged from 57.9% to 94.7% for subjects in the 60-μg group and from 33.7% to 100.0% for subjects in the 120-μg group.

Similar results were obtained for the 2 individual age strata with no clinically meaningful differences between the younger and older age strata. These findings are supported by increases in GMTs (range 7.2 to 110.6) over baseline and in the proportion of subjects achieving an hSBA titer ≥1:4 (36.0% to 100.0%) or ≥1:16 (32.6% to 100%) against each of the 4 primary MnB test strains after 2 doses of bivalent rLP2086 compared to baseline across both dose levels for the combined age stratum. Similar results were obtained for the 2 individual age strata. Additionally, the proportion of subjects for the combined age stratum achieving an hSBA fold rise ≥4 from baseline to 1 month after the second vaccination for each of the 4 primary MnB test strains ranged from 30.2% to 98.9%. In conclusion, 2 doses of either 60 μg or 120 μg of bivalent rLP2086 administered 2 months apart induced immune responses in toddlers 12 to <24 months of age (both individual and combined age strata). In summary, at both the 60-μg and 120-μg dose levels, bivalent rLP2086 given as 3 doses on a 0-, 2-, and 6-month schedule elicits a robust immune response among toddlers 12 to <24 months of age with protective antibody titers achieved as measured by hSBA in a high proportion of subjects after the third dose.

Conclusions:

In conclusion, the 60-μg and 120-μg dose levels of bivalent rLP2086 when administered to toddlers 12 to <24 months of age on a 0-, 2-, and 6-month schedule elicit protective antibody titers after the third dose as measured by hSBAs. The vaccine, as administered in this study, was safe and well tolerated with an acceptable safety profile for toddlers 12 to <24 months of age.

EXAMPLE 21: A Phase 2, Randomized, Controlled, Observer-Blinded Study Conducted to Describe the Immunogenicity, Safety, and Tolerability of a *Neisseria meningitidis* Serogroup B Bivalent Recombinant Lipoprotein 2086 Vaccine (Bivalent rLP2086) When Administered to Healthy Toddlers Aged 12 to <18 Months or 18 to <24 Months (B1971035-CSR)

This Phase 2 study was conducted in 2 stages. Stage 1 was designed and conducted to assess the safety, tolerability, and immunogenicity of bivalent rLP2086 in healthy toddlers aged 12 to <24 months through 12 months after the last study vaccination vaccination (Visit 10). Stage 1 was composed of a sentinel-enrollment phase with 4 sentinel cohorts and an expanded-enrollment phase. In the sentinel cohorts, bivalent rLP2086 was administered at 2 dose levels (60 μg and 120 μg) in 2 age strata (12 to <15 months and 18 to <24 months). Selection of dose level for the expanded-enrollment phase was based on an internal review committee (IRC) review of the safety profile of the 2 dose levels. Stage 2 was planned to assess the duration of the immune response up through 4 years after the final study vaccination.

Primary Immunogenicity Objectives

- To describe the immune response as measured by hSBA performed with 4 primary MnB strains, 2 expressing an LP2086 subfamily A protein and 2 expressing an LP2086 subfamily B protein, measured 1 month after the third vaccination with bivalent rLP2086, in healthy toddlers aged 12 to <18 months at study entry.
- To describe the immune response as measured by hSBA performed with 4 primary MnB strains, 2 expressing an LP2086 subfamily A protein and 2 expressing an LP2086 subfamily B protein, measured 1 month after the third vaccination with bivalent rLP2086, in healthy toddlers aged 18 to <24 months at study entry.

Secondary Immunogenicity Objectives

- To describe the immune response as measured by hSBA performed with 4 primary MnB test strains, 2 expressing an LP2086 subfamily A protein and 2 expressing an LP2086 subfamily B protein, measured 1 month after the third vaccination with bivalent rLP2086, in healthy toddlers aged 12 to <24 months at study entry (ie, both age strata combined).
- To describe the immune response as measured by hSBA performed with 4 primary MnB test strains, 2 expressing an LP2086 subfamily A protein and 2 expressing an LP2086 subfamily B protein, measured 1 month after the second vaccination and 6, 12, 24, 36, and 48 months after the third vaccination in healthy toddlers aged 12 to <18 months and 18 to <24 months at study entry, and in both age strata combined.

Exploratory Immunogenicity Objectives

- To further describe the immune response as measured by hSBA performed with 4 primary MnB test strains, 2 expressing an LP2086 subfamily A protein and 2 expressing an LP2086 subfamily B protein, measured 1 month after the second vaccination and 1, 6, 12, 24, 36, and 48 months after the third vaccination with bivalent rLP2086 in healthy toddlers aged 12 to <18 months and 18 to <24 months at study entry, and in both age strata combined.
- To further describe the immune response as measured by hSBA to secondary MnB test strains expressing LP2086 subfamily A and B proteins, at 1 month after the second vaccination and 1, 6, 12, 24, 36, and 48 months after the third vaccination in healthy toddlers aged 12 to <18 months and 18 to <24 months at study entry, and in both age strata combined.

INVESTIGATIONAL PLAN

Overall Study Design and Plan

The study is a Phase 2, randomized, active-controlled, observer-blinded, sponsor-unblinded, multicenter study in which approximately 396 healthy toddlers stratified by age, 12 to <18 months or 18 to <24 months old, were randomly assigned in a 2:1 ratio to receive bivalent rLP2086 (either of 2 dose levels [60 μg or 120 μg]) or a licensed pediatric HAV vaccine (0.5 mL)/sterile saline solution for injection (0.5-mL of 0.85% sodium chloride).

The study was conducted in 2 stages. Stage 1 assessed vaccine immunogenicity, safety, and tolerability across 2 phases: a sentinel-enrollment phase and an expanded-enrollment phase. Stage 2 assessed the duration of the immune response to bivalent rLP2086.

The Stage 1 sentinel-enrollment phase was planned to include a total of 4 sentinel cohorts: 2 age strata for each dose level (60 μg or 120 μg) of bivalent rLP2086. The younger-aged sentinel cohorts were composed of subjects aged 12 to <15 months and the older-aged sentinel cohorts were composed of subjects aged 18 to <24 months. Each of the 4 sentinel cohorts was planned to enroll approximately 33 subjects. The sentinel-enrollment phase was staggered with reviews by an IRC at pre-specified points and stopping rules applied. The 120-μg dose level sentinel cohorts did not proceed until the 60-μg dose level was evaluated by the IRC as safe and tolerable in the sentinel cohort of the same age. The younger-aged 120-μg dose level sentinel cohort did not proceed until this dose level was evaluated by the IRC as safe and tolerable in the older-aged 120-μg dose level sentinel cohort.

Prior to the Stage 1 expanded-enrollment phase, the IRC reviewed all post–Vaccination 1, 7-day e-diary and SAE data obtained from sentinel subjects. Based on the review, the IRC selected the 120-μg bivalent rLP2086 dose level to be studied in the Stage 1 expanded-enrollment phase for both age strata. The younger-aged expanded-enrollment cohort (enrolling an additional 132 subjects) was extended to subjects aged 12 to <18 months and stratified by age into 2 subsets: aged 12 to <15 months and aged 15 to <18 months. The older-aged expanded enrollment cohort (enrolling an additional 132 subjects) enrolled subjects aged 18 to <24 months during the expanded-enrollment phase. The total study duration for subjects completing only Stage 1 will be approximately 18 months.

Stage 2 includes only those subjects randomly assigned to bivalent rLP2086 (irrespective of dose level). The total study duration for subjects who complete Stage 2 will be approximately 4.5 years (54 months).

Bivalent rLP2086 was administered at Months 0, 2, and 6 (Visits 1, 4, and 6). Pediatric HAV vaccine was administered at Months 0 and 6 (Visits 1 and 6), and saline was administered at Month 2 (Visit 4) to maintain the study blind.

Discussion of Study Design, Including Choice of Control Groups

This 2-stage study evaluated the safety, tolerability, and immunogenicity of bivalent rLP2086 at 2 dose levels (60 μg and 120 μg) in healthy toddlers aged 12 to <24 months.

HAV vaccine (at Months 0 and 6) was chosen as the control in this study. In comparison to other recommended vaccines for this age group, HAV vaccine has a better tolerability profile. In addition, HAV vaccine confers a benefit to subjects who might be at increased risk for hepatitis A viral infection either during future travel or during other exposures. The generally recommended regimen for HAV vaccine is 2 doses at Months 0 and 6. In this study, saline was given at Month 2 to maintain the study blind.

Vaccines Administered

Bivalent rLP2086 (60 μg or 120 μg) was administered 3 times over the course of the study: the first vaccination at Visit 1 (Month 0), second vaccination at Visit 4 (Month 2), and third vaccination at Visit 6 (Month 6). Bivalent rLP2086 was administered as an intramuscular injection into either the deltoid muscle or anterolateral thigh muscle. HAV vaccine was administered twice over the course of the study: the first vaccination at Visit 1 (Month 0) and third vaccination at Visit 6 (Month 6). Saline was administered at the second vaccination (Month 2) time point. HAV vaccine/saline was administered as an intramuscular injection into either the deltoid muscle or anterolateral thigh muscle. Only a third-party unblinded

medically qualified member of the study staff administered the investigational product. If muscle mass in the deltoid was not adequate for intramuscular injection, then the thigh was the preferred injection site. Site of administration (eg, left/right arm/thigh) was noted in the source notes and on the CRF.

Identity of Investigational Product(s)

Bivalent rLP2086 (containing either 30 μg [60-μg dose level] or 60 μg [120-μg dose level] each of a purified subfamily A and subfamily B rLP2086 protein, adsorbed to aluminum in a sterile buffered isotonic suspension) was provided in a 0.5-mL dose for injection.

A licensed pediatric HAV vaccine was provided in a 0.5-mL dose for injection.

The placebo was sterile saline for injection (0.85% sodium chloride) supplied as a 0.5-mL dose.

The investigational products (bivalent rLP2086, HAV vaccine, and saline) were provided by the sponsor to each study site. Study vaccines were packed and labeled as investigational product in accordance with current guidelines and applicable local and legal regulatory requirements. Each investigational product was labeled with a unique kit number.

Selection of Vaccination Regimen

Bivalent rLP2086 (either 60 μg or 120 μg) was administered on a Month 0, 2, and 6 schedule. The control group of subjects received HAV vaccine at Month 0 and Month 6 and an injection of saline at Month 2 to maintain the study blind.

Bivalent rLP2086 Serum Bactericidal Assay – Primary Test Strains

For assessment of the immune response to bivalent rLP2086, functional antibodies were analyzed in hSBAs with meningococcal serogroup B strains. The hSBA measures antibodies in human sera that mediate complement-dependent killing of the target meningococcal strain. Four (4) primary MnB test strains, PMB80 (A22), PMB2001 (A56), PMB2948 (B24), and PMB2707 (B44), each expressing a factor H binding protein (fHBP) variant heterologous to the vaccine component antigens, were used in the hSBAs for determination of the immunogenicity endpoints in this study.

Due to serum volume limitations, 2 of the primary strains (PMB80 [A22] and PMB2948 [B24]) were tested at each blood sampling time point for half of the subjects (in both age strata), and the other 2 primary strains (PMB2001 [A56] and PMB2707 [B44]) were tested at each blood sampling time point for the remaining half of the subjects.

Once all subjects completed enrollment (Visit 1), the independent statistical center (ISC), a statistical team not involved in the conduct of the study, provided 2 subject listings (randomly selected, 50% of subjects to be tested for PMB80 [A22]/PMB2948 [B24] and the

remaining 50% of subjects to be tested for PMB2001 [A56]/PMB2707 [B44]) to the sponsor's sample management team. Both listings followed the same randomization ratio (2:1) and age-strata distribution as in the study design. The same strain pair (PMB80 [A22]/PMB2948 [B24] or PMB2001 [A56]/PMB2707 [B44]) was tested across all visits for the same subjects.

Additional Assays

Once testing for the primary analyses was completed, and if sufficient volume of sera was available, additional testing to assess the immune response to bivalent rLP2086 could be considered as follows: PMB80 (A22) and PMB2948 (B24) could be tested in serum samples from the 50% of subjects who received bivalent rLP2086 and whose serum samples were originally tested for PMB2001 (A56) and PMB2707 (B44). Conversely, PMB2001 (A56) and PMB2707 (B44) could be tested in serum samples from the 50% of subjects who received bivalent rLP2086 and were originally tested for PMB80 (A22) and PMB2948 (B24). Testing for secondary strains could be performed.

Immunogenicity Analysis

Comparisons of Interest and Endpoints– Primary Immunogenicity Endpoints

The primary immunogenicity endpoints were:

- Proportions of subjects achieving an hSBA titer ≥ lower limit of quantitation(LLOQ) 1 month after the third vaccination, for each of the 4 primary MnB test strains in healthy toddlers 12 to <18 months of age at study entry.
- Proportions of subjects achieving an hSBA titer ≥ LLOQ 1 month after the third vaccination, for each of the 4 primary MnB test strains in healthy toddlers 18 to <24 months of age at study entry.

Comparisons of Interest and Endpoints– Secondary Immunogenicity Endpoints

All secondary immunogenicity endpoints for the entire study are described here but this Example will present results for the immunogenicity endpoints applicable to Visits 1 to 7 (Vaccination 1 to 3 months after Vaccination 3) only.

The following endpoint applied to results in healthy subjects 12 months to <24 months of age (ie, both age strata combined) at study entry:

- Proportion of subjects with hSBA titers ≥ LLOQ for each of the 4 primary MnB test strains 1 month after the third vaccination with bivalent rLP2086 and 6, 12, 24, 36, and 48 months after the third vaccination with bivalent rLP2016.

The following endpoints applied to results in healthy subjects 12 to <18 months of age and 18 to <24 months of age at study entry, and in both age strata combined:

- Proportions of subjects with hSBA titers ≥ LLOQ for each of the 4 primary MnB test strains at 1 month after the second vaccination with bivalent rLP2086.
- Proportions of subjects with hSBA titers ≥ LLOQ, ≥1:4, ≥1:8, ≥1:16, ≥1:32, ≥1:64, and ≥1:128 for each of the 4 primary MnB strains at each applicable blood sampling visit.
- hSBA geometric mean titers (GMTs) for each of the 4 primary MnB test strains at each applicable blood sampling visit.

Exploratory Immunogenicity Endpoints

All of the exploratory endpoints specified below applied to hSBA results from all healthy subjects 12 to <18 months of age or 18 to <24 months of age at study entry, and in both age strata combined, who received bivalent rLP2086 and were tested for the appropriate strain at the appropriate time point:

- Proportions of subjects with hSBA titers ≥ LLOQ, ≥1:4, ≥1:8, ≥1:16, ≥1:32, ≥1:64, and ≥1:128 at each applicable blood sampling time point.
- hSBA GMTs for each of the 4 primary MnB strains at each applicable blood sampling visit.
- Proportions of subjects achieving at least a 4-fold increase in hSBA titer from baseline to 1 month after the third vaccination with bivalent rLP2086 for each of the 4 primary test strains:
  - For subjects with a baseline hSBA titer below the limit of detection (LOD) or an hSBA titer of <1:4, a 4-fold response was defined as an hSBA titer of ≥1:16.
  - For subjects with a baseline hSBA titer of ≥ LOD (ie, hSBA titer of ≥1:4) and < LLOQ, a 4-fold response was defined as an hSBA titer of ≥4 times the LLOQ.
  - For subjects with a baseline hSBA titer of ≥ LLOQ, a 4-fold response was defined as an hSBA titer of ≥4 times the baseline titer.

The following endpoints were considered if there had been sufficient sera available to test each subject for all 4 primary strains and/or to test subjects for the secondary strains.

- Proportions of subjects achieving an hSBA titer ≥ LLOQ for all 4 primary test strains (PMB80 [A22], PMB2948 [B24], PMB2001 [A56], and PMB2707 [B44]) combined, 1 month after the third vaccination with bivalent rLP2086. This applied only to those subjects who had all 4 primary strains tested.
- Additional exploratory assays to test hSBA on the secondary MnB strains as follows:
  - hSBA GMTs to each secondary MnB strain tested, at 1 month after the second and third vaccinations and/or at each blood sampling time point thereafter.

- Proportions of subjects with an hSBA titer ≥ LLOQ, to each secondary MnB strain at 1 month after the second and third vaccinations and/or at each blood sampling time point thereafter.

Analysis Populations

Modified Intent-to-Treat Population

All randomized subjects who had at least 1 valid and determinate assay result related to a proposed analysis were included in the modified intent-to-treat (mITT) population. This analysis set was for the immunogenicity analysis. Subjects were analyzed according to the investigational product to which they were randomized in the analysis of the mITT population.

Methods of Analysis

The control group and each dose-level group from different cohorts within the same age stratum were pooled for analysis. All of the immunogenicity analyses were summarized for each age stratum separately, as well as for the overall population.

This was not a hypothesis-testing study; thus, an estimation approach was used to assess the primary, secondary, and exploratory objectives in this study.

The LLOQ was 1:16 for PMB80 (A22), 1:8 for PMB2001 (A56), 1:8 for PMB2707 (B44), and 1:8 for PMB2948 (B24).

For the calculation of GMTs, hSBA results below the LLOQ were set to 0.5 × LLOQ for the primary analysis.

Analysis of Primary Endpoints

The primary analysis for the primary objectives was the proportion of subjects with an hSBA titer ≥ LLOQ 1 month after the third vaccination, for each of the 4 primary MnB test strains in healthy toddlers aged 12 to <18 months, and 18 to <24 months, at study entry respectively. The evaluable immunogenicity population was used for this summary and both percentages and confidence intervals (CIs) are displayed.

Analysis of Secondary Endpoints

All of the analyses performed on the mITT population were considered as secondary analyses.

Analyses for secondary endpoints also included percent of subjects with hSBA titers ≥ LLOQ for each of the 4 primary MnB test strains 1 month following the third vaccination for both the evaluable immunogenicity population and for the mITT population.

For this Example, the percentage of subjects with hSBA titers ≥ LLOQ for each of the 4 primary MnB test strains at 1 month after Vaccination 2 and 1 month after Vaccination 3

were analyzed using both the evaluable immunogenicity population and the mITT population and presented in this report. The same analysis is planned for subsequent time points (6, 12, 24, 36, and 48 months after the third vaccination).

The percentage of subjects with hSBA titers ≥ LLOQ, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128 for each of the 4 primary MnB test strains at 1 month after Vaccination 2 and 1 month after Vaccination 3 were analyzed using both the evaluable immunogenicity population and the mITT population and presented in this report. The same analysis is planned for subsequent time points (6, 12, 24, 36, and 48 months after Vaccination 3).

The GMTs for each of the primary MnB test strains at 1 month following the second vaccination and 1 month after the third vaccination were summarized for the evaluable immunogenicity population and the mITT population and presented in this report. The same analysis is planned for subsequent time points (6, 12, 24, 36, and 48 months after Vaccination 3).

Analysis of Exploratory Endpoints

The proportion of subjects achieving at least a 4-fold increase in hSBA titer from baseline to 1 month after the second and third vaccinations with bivalent rLP2086 was summarized for the evaluable immunogenicity population and the mITT population.

Reverse Cumulative Distribution Curves

The empirical reverse cumulative distribution curves (RCDCs) were also assessed for each of the 4 primary MnB test strains and at 1 month after Vaccination 2 and 1 month after Vaccination 3 for the evaluable immunogenicity population.

IMMUNOGENICITY EVALUATION

Populations Analyzed

The evaluable immunogenicity population was the primary analysis population for the immunogenicity analyses. The mITT population was used as a supportive immunogenicity population for the immunogenicity analyses.

A total of 348 (87.9%) subjects were included in the evaluable immunogenicity population, and 48 (12.1%) subjects were excluded from the evaluable immunogenicity population. Subjects could have been excluded from the immunogenicity populations for more than 1 reason. A total of 31 (7.8%) subjects were excluded from the evaluable immunogenicity population because they did not have scheduled prevaccination or postvaccination blood drawn (includes subjects who did not have samples taken and subjects with samples taken outside of the protocol-specified window), 13 (3.3%) subjects were not eligible or became ineligible for the study before or at the 1-month post-Vaccination 3 visit, 11 (2.8%) subjects

did not receive vaccine as randomized at all vaccination visits, and 16 (4.0%) subjects received prohibited vaccines or treatments. A total of 84.3% of subjects 12 to <18 months of age and 91.4% of subjects 18 to <24 months of age were included in the evaluable immunogenicity population.

Immunogenicity Results

The primary analysis results including all of the immunogenicity data through 1 month after Vaccination 3 (Visit 7) are provided in the following sections. The analysis of immunogenicity endpoints was based on hSBA results for strains PMB2001 (A56) and PMB2707 (B44) for half of the subjects, and strains PMB80 (A22) and PMB2948 (B24) for the remaining half.

Primary and Secondary Endpoints

Proportion of Subjects Achieving an hSBA Titer ≥ LLOQ

The primary immunogenicity endpoints were the proportions of subjects 12 to <18 months of age (at study entry), and 18 to <24 months of age (at study entry), achieving an hSBA titer ≥ LLOQ for each of the 4 primary MnB test strains 1 month after the third vaccination with bivalent rLP2086. The proportion of all subjects in the combined age stratum achieving an hSBA titer ≥ LLOQ for each of the 4 primary MnB test strains 1 month after the third vaccination with bivalent rLP2086, along with the proportion of subjects in the individual and combined age strata achieving an hSBA titer ≥ LLOQ for each of the 4 primary MnB test strains 1 month after the second vaccination with bivalent rLP2086, were secondary endpoints.

The proportion of subjects in each age stratum achieving an hSBA titer ≥ LLOQ for each of the 4 primary MnB test strains is presented in Table for the evaluable immunogenicity population.

For the combined age stratum the proportion of subjects achieving an hSBA titer ≥ LLOQ at baseline was 0.0% and 3.1% for PMB80 (A22); 0.0% and 1.1% for PMB2001 (A56); 4.8% and 2.1% for PMB2948 (B24); and 0.0% and 1.1% for PMB2707 (B44) for the 60-μg and 120-μg groups, respectively.

At 1 month after Vaccination 2, the proportion of subjects achieving an hSBA titer ≥ LLOQ for the 12 to <18 months age stratum was 90.0% and 64.4% for PMB80 (A22); 100.0% and 100.0% for PMB2001 (A56); 70.0% and 23.8% for PMB2948 (B24); and 77.8% and 72.3% for PMB2707 (B44) for the 60-μg and 120-μg groups, respectively. For the 18 to <24 months age stratum, the proportion of subjects achieving an hSBA titer ≥ LLOQ was

66.7% and 84.0% for PMB80 (A22); 90.0% and 100.0% for PMB2001 (A56); 44.4% and 43.2% for PMB2948 (B24); and 60.0% and 63.8% for PMB2707 (B44) for the 60-μg and 120-μg groups, respectively. Overall, for the combined age stratum the proportion of subjects achieving an hSBA titer ≥ LLOQ at 1 month after Vaccination 2 was 78.9% and 74.7% for PMB80 (A22); 94.7% and 100.0% for PMB2001 (A56); 57.9% and 33.7% for PMB2948 (B24); and 68.4% and 68.1% for PMB2707 (B44) for the 60-μg and 120-μg groups, respectively.

At 1 month after Vaccination 3, the proportion of subjects achieving an hSBA titer ≥ LLOQ for the 12 to <18 months age stratum was 88.9% and 91.1% for PMB80 (A22); 100.0% and 100.0% for PMB2001 (A56); 88.9% and 71.1% for PMB2948 (B24); and 88.9% and 87.2% for PMB2707 (B44) for the 60-μg and 120-μg groups, respectively. In the 18 to <24 months age stratum, the proportion of subjects achieving an hSBA titer ≥ LLOQ was 90.9% and 88.2% for PMB80 (A22); 100.0% and 100.0% for PMB2001 (A56); 81.8% and 72.0% for PMB2948 (B24); and 90.0% and 85.1% for PMB2707 (B44) for the 60-μg and 120-μg groups, respectively. Overall, for the combined age stratum the proportion of subjects achieving an hSBA titer ≥ LLOQ at 1 month after Vaccination 3 was 90.0% and 89.6% for PMB80 (A22); 100.0% and 100.0% for PMB2001 (A56); 85.0% and 71.6% for PMB2948 (B24); and 89.5% and 86.2% for PMB2707 (B44) for the 60-μg and 120-μg groups, respectively.

In general, the proportion of subjects in the HAV/saline group achieving an hSBA titer ≥ LLOQ did not change over time compared to baseline (Table 25).

Results for the mITT population were similar to those of the evaluable immunogenicity population.

Subgroup analyses of the proportion of subjects achieving an hSBA titer ≥ LLOQ for each of the 4 primary MnB test strains were assessed for the evaluable immunogenicity population by sex and country. There were no clinically important differences observed in the subgroup analyses performed.

Table 25. Subjects With hSBA Titer ≥ LLOQ for Primary Strains – Evaluable Immunogenicity Population

Strain (Variant) Sampling Time Point Age Strata	Vaccine Group (as Randomized) 60 µg rLP2086 N[a]	n[b]	(%)	(95% CI)[c]	120 µg rLP2086 N[a]	n[b]	(%)	(95% CI)[c]	HAV/Saline N[a]	n[b]	(%)	(95% CI)[c]
PMB80 (A22)												
Before Vaccination 1												
12 to <24 Months	20	0	(0.0)	(0.0, 16.8)	97	3	(3.1)	(0.6, 8.8)	61	1	(1.6)	(0.0, 8.8)
12 to <18 Months	9	0	(0.0)	(0.0, 33.6)	46	1	(2.2)	(0.1, 11.5)	31	0	(0.0)	(0.0, 11.2)
18 to <24 Months	11	0	(0.0)	(0.0, 28.5)	51	2	(3.9)	(0.5, 13.5)	30	1	(3.3)	(0.1, 17.2)
1 Month after Vaccination 2												
12 to <24 Months	19	15	(78.9)	(54.4, 93.9)	95	71	(74.7)	(64.8, 83.1)	59	1	(1.7)	(0.0, 9.1)
12 to <18 Months	10	9	(90.0)	(55.5, 99.7)	45	29	(64.4)	(48.8, 78.1)	30	0	(0.0)	(0.0, 11.6)
18 to <24 Months	9	6	(66.7)	(29.9, 92.5)	50	42	(84.0)	(70.9, 92.8)	29	1	(3.4)	(0.1, 17.8)
1 Month after Vaccination 3												
12 to <24 Months	20	18	(90.0)	(68.3, 98.8)	96	86	(89.6)	(81.7, 94.9)	60	3	(5.0)	(1.0, 13.9)
12 to <18 Months	9	8	(88.9)	(51.8, 99.7)	45	41	(91.1)	(78.8, 97.5)	31	1	(3.2)	(0.1, 16.7)
18 to <24 Months	11	10	(90.9)	(58.7, 99.8)	51	45	(88.2)	(76.1, 95.6)	29	2	(6.9)	(0.8, 22.8)
PMB2001 (A56)												
Before Vaccination 1												
12 to <24 Months	19	0	(0.0)	(0.0, 17.6)	95	1	(1.1)	(0.0, 5.7)	53	0	(0.0)	(0.0, 6.7)
12 to <18 Months	9	0	(0.0)	(0.0, 33.6)	46	0	(0.0)	(0.0, 7.7)	24	0	(0.0)	(0.0, 14.2)
18 to <24 Months	10	0	(0.0)	(0.0, 30.8)	49	1	(2.0)	(0.1, 10.9)	29	0	(0.0)	(0.0, 11.9)
1 Month after Vaccination 2												
12 to <24 Months	19	18	(94.7)	(74.0, 99.9)	95	95	(100.0)	(96.2, 100.0)	52	0	(0.0)	(0.0, 6.8)
12 to <18 Months	9	9	(100.0)	(66.4, 100.0)	47	47	(100.0)	(92.5, 100.0)	23	0	(0.0)	(0.0, 14.8)
18 to <24 Months	10	9	(90.0)	(55.5, 99.7)	48	48	(100.0)	(92.6, 100.0)	29	0	(0.0)	(0.0, 11.9)
1 Month after Vaccination 3												
12 to <24 Months	19	19	(100.0)	(82.4, 100.0)	95	95	(100.0)	(96.2, 100.0)	54	1	(1.9)	(0.0, 9.9)
12 to <18 Months	9	9	(100.0)	(66.4, 100.0)	47	47	(100.0)	(92.5, 100.0)	24	0	(0.0)	(0.0, 14.2)
18 to <24 Months	10	10	(100.0)	(69.2, 100.0)	48	48	(100.0)	(92.6, 100.0)	30	1	(3.3)	(0.1, 17.2)
PMB2948 (B24)												
Before Vaccination 1												
12 to <24 Months	21	1	(4.8)	(0.1, 23.8)	97	2	(2.1)	(0.3, 7.3)	61	1	(1.6)	(0.0, 8.8)

Table 25. Subjects With hSBA Titer ≥ LLOQ for Primary Strains – Evaluable Immunogenicity Population

Strain (Variant) / Sampling Time Point / Age Strata	Vaccine Group (as Randomized) 60 µg rLP2086 N[a]	n[b]	(%)	(95% CI)[c]	120 µg rLP2086 N[a]	n[b]	(%)	(95% CI)[c]	HAV/Saline N[a]	n[b]	(%)	(95% CI)[c]
12 to <18 Months	10	0	(0.0)	(0.0, 30.8)	46	1	(2.2)	(0.1, 11.5)	31	0	(0.0)	(0.0, 11.2)
18 to <24 Months	11	1	(9.1)	(0.2, 41.3)	51	1	(2.0)	(0.0, 10.4)	30	1	(3.3)	(0.1, 17.2)
1 Month after Vaccination 2												
12 to <24 Months	19	11	(57.9)	(33.5, 79.7)	86	29	(33.7)	(23.9, 44.7)	59	1	(1.7)	(0.0, 9.1)
12 to <18 Months	10	7	(70.0)	(34.8, 93.3)	42	10	(23.8)	(12.1, 39.5)	30	0	(0.0)	(0.0, 11.6)
18 to <24 Months	9	4	(44.4)	(13.7, 78.8)	44	19	(43.2)	(28.3, 59.0)	29	1	(3.4)	(0.1, 17.8)
1 Month after Vaccination 3												
12 to <24 Months	20	17	(85.0)	(62.1, 96.8)	95	68	(71.6)	(61.4, 80.4)	60	3	(5.0)	(1.0, 13.9)
12 to <18 Months	9	8	(88.9)	(51.8, 99.7)	45	32	(71.1)	(55.7, 83.6)	31	1	(3.2)	(0.1, 16.7)
18 to <24 Months	11	9	(81.8)	(48.2, 97.7)	50	36	(72.0)	(57.5, 83.8)	29	2	(6.9)	(0.8, 22.8)
PMB2707 (B44)												
Before Vaccination 1												
12 to <24 Months	19	0	(0.0)	(0.0, 17.6)	95	1	(1.1)	(0.0, 5.7)	54	0	(0.0)	(0.0, 6.6)
12 to <18 Months	9	0	(0.0)	(0.0, 33.6)	46	1	(2.2)	(0.1, 11.5)	24	0	(0.0)	(0.0, 14.2)
18 to <24 Months	10	0	(0.0)	(0.0, 30.8)	49	0	(0.0)	(0.0, 7.3)	30	0	(0.0)	(0.0, 11.6)
1 Month after Vaccination 2												
12 to <24 Months	19	13	(68.4)	(43.4, 87.4)	94	64	(68.1)	(57.7, 77.3)	52	0	(0.0)	(0.0, 6.8)
12 to <18 Months	9	7	(77.8)	(40.0, 97.2)	47	34	(72.3)	(57.4, 84.4)	23	0	(0.0)	(0.0, 14.8)
18 to <24 Months	10	6	(60.0)	(26.2, 87.8)	47	30	(63.8)	(48.5, 77.3)	29	0	(0.0)	(0.0, 11.9)
1 Month after Vaccination 3												
12 to <24 Months	19	17	(89.5)	(66.9, 98.7)	94	81	(86.2)	(77.5, 92.4)	54	0	(0.0)	(0.0, 6.6)
12 to <18 Months	9	8	(88.9)	(51.8, 99.7)	47	41	(87.2)	(74.3, 95.2)	24	0	(0.0)	(0.0, 14.2)
18 to <24 Months	10	9	(90.0)	(55.5, 99.7)	47	40	(85.1)	(71.7, 93.8)	30	0	(0.0)	(0.0, 11.6)

Abbreviations: hSBA = serum bactericidal assay using human complement; LLOQ = lower limit of quantitation.
Note: LLOQ = 1:16 for A22; 1:8 for A56, B24, and B44.
a. N = number of subjects with valid and determinate hSBA titers for the given strain.
b. n = Number of subjects with observed hSBA titer ≥ LLOQ for the given strain at the given time point.
c. Exact 2-sided CI based upon observed proportion of subjects, using the Clopper and Pearson method.

hSBA GMTs

The hSBA GMTs for each of the 4 primary MnB test strains for subjects 12 to <18 months of age, 18 to <24 months of age, and for the combined age stratum, at baseline, 1 month after the second vaccination, and 1 month after the third vaccination with bivalent rLP2086 was a secondary endpoint. Table 26 provides hSBA GMTs for the 4 primary MnB strains for the evaluable immunogenicity population.

For the combined age stratum, the hSBA GMTs at baseline were 8.0 and 8.4 for PMB80 (A22); 4.0 and 4.1 for PMB2001 (A56); 4.4 and 4.1 for PMB2948 (B24); and 4.0 and 4.0 for PMB2707 (B44) for the 60-μg and 120-μg groups, respectively.

At 1 month after Vaccination 2, the hSBA GMTs for the 12 to <18 months age stratum were 42.2 and 24.6 for PMB80 (A22); 101.6 and 117.2 for PMB2001 (A56); 10.6 and 6.0 for PMB2948 (B24); and 23.5 and 22.1 for PMB2707 (B44) for the 60-μg and 120-μg groups, respectively. For the 18 to <24 months age stratum, the hSBA GMTs were 23.5 and 36.8 for PMB80 (A22); 68.6 and 104.6 for PMB2001 (A56); 6.9 and 8.5 for PMB2948 (B24); and 21.1 and 17.0 for PMB2707 (B44) for the 60-μg and 120-μg groups, respectively. Overall, at 1 month after Vaccination 2 the hSBA GMTs for the combined age stratum were 32.0 and 30.4 for PMB80 (A22); 82.6 and 110.6 for PMB2001 (A56); 8.6 and 7.2 for PMB2948 (B24); and 22.2 and 19.4 for PMB2707 (B44) for the 60-μg and 120-μg groups, respectively.

At 1 month after Vaccination 3, the hSBA GMTs for the 12 to <18 months age stratum were 80.6 and 63.0 for PMB80 (A22); 109.7 and 190.6 for PMB2001 (A56); 20.2 and 15.8 for PMB2948 (B24); and 29.6 and 46.3 for PMB2707 (B44) for the 60-μg and 120-μg groups, respectively. For the 18 to <24 months age stratum, the hSBA GMTs were 82.3 and 71.4 for PMB80 (A22); 181.0 and 154.4 for PMB2001 (A56); 17.0 and 14.5 for PMB2948 (B24); and 34.3 and 44.9 for PMB2707 (B44) for the 60-μg and 120-μg groups, respectively. Overall, at 1 month after Vaccination 3, the hSBA GMTs for the combined age stratum were 81.6 and 67.3 for PMB80 (A22); 142.8 and 171.4 for PMB2001 (A56); 18.4 and 15.1 for PMB2948 (B24); and 32.0 and 45.6 for PMB2707 (B44) for the 60-μg and 120-μg groups, respectively.

In general, the hSBA GMTs for subjects in the HAV/saline group did not change over time compared to baseline.

Table 26. hSBA GMTs for Primary Strains – Evaluable Immunogenicity Population

Strain (Variant) Sampling Time Point Age Strata	Vaccine Group (as Randomized) 60 µg rLP2086 N[a]	GMT[b]	(95% CI)[c]	120 µg rLP2086 N[a]	GMT[b]	(95% CI)[c]	HAV/Saline N[a]	GMT[b]	(95% CI)[c]
PMB80 (A22)									
Before Vaccination 1									
12 to <24 Months	20	8.0	(NE, NE)	97	8.4	(7.9, 9.0)	61	8.1	(7.9, 8.3)
12 to <18 Months	9	8.0	(NE, NE)	46	8.5	(7.5, 9.6)	31	8.0	(NE, NE)
18 to <24 Months	11	8.0	(NE, NE)	51	8.3	(7.8, 8.9)	30	8.2	(7.8, 8.6)
1 Month after Vaccination 2									
12 to <24 Months	19	32.0	(19.7, 52.0)	95	30.4	(24.3, 38.1)	59	8.3	(7.7, 8.9)
12 to <18 Months	10	42.2	(22.6, 79.1)	45	24.6	(17.8, 34.2)	30	8.0	(NE, NE)
18 to <24 Months	9	23.5	(10.1, 54.9)	50	36.8	(26.9, 50.3)	29	8.6	(7.4, 10.0)
1 Month after Vaccination 3									
12 to <24 Months	20	81.6	(46.6, 142.8)	96	67.3	(53.7, 84.3)	60	8.6	(7.9, 9.3)
12 to <18 Months	9	80.6	(30.9, 210.7)	45	63.0	(44.5, 89.3)	31	8.6	(7.5, 9.8)
18 to <24 Months	11	82.3	(36.5, 185.8)	51	71.4	(52.7, 96.6)	29	8.6	(7.7, 9.6)
PMB2001 (A56)									
Before Vaccination 1									
12 to <24 Months	19	4.0	(NE, NE)	95	4.1	(3.9, 4.3)	53	4.0	(NE, NE)
12 to <18 Months	9	4.0	(NE, NE)	46	4.0	(NE, NE)	24	4.0	(NE, NE)
18 to <24 Months	10	4.0	(NE, NE)	49	4.2	(3.8, 4.5)	29	4.0	(NE, NE)
1 Month after Vaccination 2									
12 to <24 Months	19	82.6	(51.4, 132.9)	95	110.6	(92.0, 133.0)	52	4.0	(NE, NE)
12 to <18 Months	9	101.6	(64.0, 161.2)	47	117.2	(89.7, 153.0)	23	4.0	(NE, NE)
18 to <24 Months	10	68.6	(28.2, 166.8)	48	104.6	(80.4, 136.0)	29	4.0	(NE, NE)
1 Month after Vaccination 3									
12 to <24 Months	19	142.8	(85.5, 238.6)	95	171.4	(141.6, 207.4)	54	4.2	(3.8, 4.5)
12 to <18 Months	9	109.7	(70.4, 171.1)	47	190.6	(146.9, 247.4)	24	4.0	(NE, NE)
18 to <24 Months	10	181.0	(68.6, 477.9)	48	154.4	(116.3, 205.1)	30	4.3	(3.7, 4.9)
PMB2948 (B24)									
Before Vaccination 1									
12 to <24 Months	21	4.4	(3.6, 5.4)	97	4.1	(4.0, 4.3)	61	4.2	(3.8, 4.6)

Table 26. hSBA GMTs for Primary Strains – Evaluable Immunogenicity Population

Strain (Variant) Sampling Time Point Age Strata	Vaccine Group (as Randomized) 60 µg rLP2086 N[a]	GMT[b]	(95% CI)[c]	120 µg rLP2086 N[a]	GMT[b]	(95% CI)[c]	HAV/Saline N[a]	GMT[b]	(95% CI)[c]
12 to <18 Months	10	4.0	(NE, NE)	46	4.1	(3.9, 4.4)	31	4.0	(NE, NE)
18 to <24 Months	11	4.8	(3.2, 7.4)	51	4.1	(3.9, 4.3)	30	4.4	(3.6, 5.3)
1 Month after Vaccination 2									
12 to <24 Months	19	8.6	(6.1, 12.2)	86	7.2	(5.9, 8.7)	59	4.1	(3.9, 4.4)
12 to <18 Months	10	10.6	(6.2, 18.0)	42	6.0	(4.7, 7.8)	30	4.0	(NE, NE)
18 to <24 Months	9	6.9	(4.1, 11.5)	44	8.5	(6.4, 11.3)	29	4.3	(3.7, 5.0)
1 Month after Vaccination 3									
12 to <24 Months	20	18.4	(11.8, 28.6)	95	15.1	(12.3, 18.6)	60	4.3	(3.9, 4.8)
12 to <18 Months	9	20.2	(11.1, 36.6)	45	15.8	(11.4, 21.8)	31	4.3	(3.7, 4.9)
18 to <24 Months	11	17.0	(8.2, 35.5)	50	14.5	(11.1, 19.1)	29	4.4	(3.8, 5.0)
PMB2707 (B44)									
Before Vaccination 1									
12 to <24 Months	19	4.0	(NE, NE)	95	4.0	(4.0, 4.1)	54	4.0	(NE, NE)
12 to <18 Months	9	4.0	(NE, NE)	46	4.1	(3.9, 4.2)	24	4.0	(NE, NE)
18 to <24 Months	10	4.0	(NE, NE)	49	4.0	(NE, NE)	30	4.0	(NE, NE)
1 Month after Vaccination 2									
12 to <24 Months	19	22.2	(11.2, 43.9)	94	19.4	(15.1, 24.9)	52	4.0	(NE, NE)
12 to <18 Months	9	23.5	(9.3, 59.4)	47	22.1	(15.5, 31.6)	23	4.0	(NE, NE)
18 to <24 Months	10	21.1	(6.5, 68.3)	47	17.0	(11.8, 24.4)	29	4.0	(NE, NE)
1 Month after Vaccination 3									
12 to <24 Months	19	32.0	(18.3, 55.8)	94	45.6	(35.2, 59.0)	54	4.0	(NE, NE)
12 to <18 Months	9	29.6	(11.6, 75.8)	47	46.3	(31.6, 67.8)	24	4.0	(NE, NE)
18 to <24 Months	10	34.3	(15.0, 78.2)	47	44.9	(31.3, 64.5)	30	4.0	(NE, NE)

Abbreviations: GMT = geometric mean titer; hSBA = serum bactericidal assay using human complement; LLOQ = lower limit of quantitation; NE = not estimable.

Note: LLOQ = 1:16 for A22; 1:8 for A56, B24, and B44. Titers below the LLOQ were set to 0.5 × LLOQ for analysis.

a. N = number of subjects with valid and determinate hSBA titers for the given strain.

b. GMTs were calculated using all subjects with valid and determinate hSBA titers at the given time point.

c. CIs are back transformations of confidence levels based on the Student t distribution for the mean logarithm of the hSBA titers.

Results for the mITT population were similar to those of the evaluable immunogenicity population.

Subgroup analyses of hSBA GMTs for each of the 4 primary MnB test strains were assessed for the evaluable immunogenicity population by sex and country. There were no clinically important differences observed in the subgroup analyses performed.

Defined hSBA Titers

The proportions of subjects 12 to <18 months of age, 18 to <24 months of age, and for the combined age stratum, achieving hSBA titers ≥1:4, ≥1:8, ≥1:16, ≥1:32, ≥1:64, and ≥1:128 for each of the 4 primary MnB test strains at baseline, 1 month after the second vaccination, and 1 month after the third vaccination with bivalent rLP2086 was a secondary immunogenicity endpoint.

The proportion of subjects achieving defined hSBA titers for the 4 primary MnB strains is presented in Table 27 for the evaluable immunogenicity population.

The results for subjects receiving bivalent rLP2086 who achieved an hSBA titer ≥1:4 and ≥1:16 are described below. An hSBA titer of ≥1:4 is widely recognized as the correlate of protection against IMD; however, a more conservative hSBA titer of ≥1:16 has been considered a level indicative of a 4-fold vaccine effect for subjects seronegative before vaccination.

In general, the proportion of subjects in the HAV/saline group achieving defined hSBA titers did not change over time compared to baseline.

Results for the mITT population were similar to those of the evaluable immunogenicity population.

hSBA Titer ≥1:4

At 1 month after Vaccination 2, the proportion of subjects 12 to <18 months of age achieving an hSBA titer ≥1:4 was 90.0% and 64.4% for PMB80 (A22); 100.0% and 100.0% for PMB2001 (A56); 70.0% and 28.6% for PMB2948 (B24); and 77.8% and 72.3% for PMB2707 (B44) for the 60-μg and 120-μg groups, respectively. The proportion of subjects 18 to <24 months of age achieving an hSBA titer ≥1:4 was 66.7% and 86.0% for PMB80 (A22); 100.0% and 100.0% for PMB2001 (A56); 44.4% and 43.2% for PMB2948 (B24); and 70.0% and 63.8% for PMB2707 (B44) for the 60-μg and 120-μg groups, respectively. Overall for the combined age stratum, the proportion of subjects achieving an hSBA titer ≥1:4 at 1 month after Vaccination 2 was 78.9% and 75.8% for PMB80 (A22); 100.0% and 100.0% for PMB2001 (A56); 57.9% and 36.0% for PMB2948 (B24); and 73.7% and 68.1% for PMB2707 (B44) for the 60-μg and 120-μg groups, respectively.

At 1 month after Vaccination 3, the proportion of subjects 12 to <18 months of age achieving an hSBA titer ≥1:4 was 88.9% and 91.1% for PMB80 (A22); 100.0% and 100.0% for

PMB2001 (A56); 88.9% and 71.1% for PMB2948 (B24); and 88.9% and 87.2% for PMB2707 (B44) for the 60-μg and 120-μg groups, respectively. The proportion of subjects 18 to <24 months of age achieving an hSBA titer ≥1:4 was 90.9% and 88.2% for PMB80 (A22); 100.0% and 100.0% for PMB2001 (A56); 81.8% and 72.0% for PMB2948 (B24); and 90.0% and 87.2% for PMB2707 (B44) for the 60-μg and 120-μg groups, respectively. Overall for the combined age stratum, the proportion of subjects achieving an hSBA titer ≥1:4 at 1 month after Vaccination 3 was 90.0% and 89.6% for PMB80 (A22); 100.0% and 100.0% for PMB2001 (A56); 85.0% and 71.6% for PMB2948 (B24); and 89.5% and 87.2% for PMB2707 (B44) for the 60-μg and 120-μg groups, respectively.

hSBA Titer ≥1:16

At 1 month after Vaccination 2, the proportion of subjects 12 to <18 months of age achieving an hSBA titer ≥1:16 was 90.0% and 64.4% for PMB80 (A22); 100.0% and 100.0% for PMB2001 (A56); 60.0% and 21.4% for PMB2948 (B24); and 77.8% and 72.3% for PMB2707 (B44) for the 60-μg and 120-μg groups, respectively. The proportion of subjects 18 to <24 months of age achieving an hSBA titer ≥1:16 was 66.7% and 86.0% for PMB80 (A22); 90.0% and 100.0% for PMB2001 (A56); 33.3% and 43.2% for PMB2948 (B24); and 60.0% and 61.7% for PMB2707 (B44) for the 60-μg and 120-μg groups, respectively. Overall for the combined age stratum, the proportion of subjects achieving an hSBA titer ≥1:16 at 1 month after Vaccination 2 was 78.9% and 74.7% for PMB80 (A22); 94.7% and 100.0% for PMB2001 (A56); 47.4% and 32.6% for PMB2948 (B24); and 68.4% and 67.0% for PMB2707 (B44) for the 60-μg and 120-μg groups, respectively.

At 1 month after Vaccination 3, the proportion of subjects 12 to <18 months of age achieving an hSBA titer ≥1:16 was 88.9% and 91.1% for PMB80 (A22); 100.0% and 97.9% for PMB2001 (A56); 88.9% and 66.7% for PMB2948 (B24); and 77.8% and 87.2% for PMB2707 (B44) for the 60-μg and 120-μg groups, respectively. The proportion of subjects 18 to <24 months of age achieving an hSBA titer ≥1:16 was 90.9% and 88.2% for PMB80 (A22); 100.0% and 100.0% for PMB2001 (A56); 63.6% and 68.0% for PMB2948 (B24); and 90.0% and 85.1% for PMB2707 (B44) for the 60-μg and 120-μg groups, respectively. Overall for the combined age stratum, the proportion of subjects achieving an hSBA titer ≥1:16 at 1 month after Vaccination 3 was 90.0% and 89.6% for PMB80 (A22); 100.0% and 98.9% for PMB2001 (A56); 75.0% and 67.4% for PMB2948 (B24); and 84.2% and 86.2% for PMB2707 (B44) for the 60-μg and 120-μg groups, respectively.

Table 27. Subjects Achieving Defined hSBA Titers for Primary Strains – Evaluable Immunogenicity Population

		Vaccine Group (as Randomized)											
		60 µg rLP2086				120 µg rLP2086				HAV/Saline			
Strain (Variant) Sampling Time Point Age Strata	Titer	N[a]	n[b]	%	(95% CI)[c]	N[a]	n[b]	%	(95% CI)[c]	N[a]	n[b]	%	(95% CI)[c]
PMB80 (A22)													
Before Vaccination 1													
12 to <24 Months	4	20	0	0.0	(0.0, 16.8)	97	4	4.1	(1.1, 10.2)	61	1	1.6	(0.0, 8.8)
	8	20	0	0.0	(0.0, 16.8)	97	3	3.1	(0.6, 8.8)	61	1	1.6	(0.0, 8.8)
	16	20	0	0.0	(0.0, 16.8)	97	3	3.1	(0.6, 8.8)	61	1	1.6	(0.0, 8.8)
	32	20	0	0.0	(0.0, 16.8)	97	2	2.1	(0.3, 7.3)	61	0	0.0	(0.0, 5.9)
	64	20	0	0.0	(0.0, 16.8)	97	1	1.0	(0.0, 5.6)	61	0	0.0	(0.0, 5.9)
	128	20	0	0.0	(0.0, 16.8)	97	1	1.0	(0.0, 5.6)	61	0	0.0	(0.0, 5.9)
12 to <18 Months	4	9	0	0.0	(0.0, 33.6)	46	2	4.3	(0.5, 14.8)	31	0	0.0	(0.0, 11.2)
	8	9	0	0.0	(0.0, 33.6)	46	1	2.2	(0.1, 11.5)	31	0	0.0	(0.0, 11.2)
	16	9	0	0.0	(0.0, 33.6)	46	1	2.2	(0.1, 11.5)	31	0	0.0	(0.0, 11.2)
	32	9	0	0.0	(0.0, 33.6)	46	1	2.2	(0.1, 11.5)	31	0	0.0	(0.0, 11.2)
	64	9	0	0.0	(0.0, 33.6)	46	1	2.2	(0.1, 11.5)	31	0	0.0	(0.0, 11.2)
	128	9	0	0.0	(0.0, 33.6)	46	1	2.2	(0.1, 11.5)	31	0	0.0	(0.0, 11.2)
18 to <24 Months	4	11	0	0.0	(0.0, 28.5)	51	2	3.9	(0.5, 13.5)	30	1	3.3	(0.1, 17.2)
	8	11	0	0.0	(0.0, 28.5)	51	2	3.9	(0.5, 13.5)	30	1	3.3	(0.1, 17.2)
	16	11	0	0.0	(0.0, 28.5)	51	2	3.9	(0.5, 13.5)	30	1	3.3	(0.1, 17.2)
	32	11	0	0.0	(0.0, 28.5)	51	1	2.0	(0.0, 10.4)	30	0	0.0	(0.0, 11.6)
	64	11	0	0.0	(0.0, 28.5)	51	0	0.0	(0.0, 7.0)	30	0	0.0	(0.0, 11.6)
	128	11	0	0.0	(0.0, 28.5)	51	0	0.0	(0.0, 7.0)	30	0	0.0	(0.0, 11.6)
1 Month after Vaccination 2													
12 to <24 Months	4	19	15	78.9	(54.4, 93.9)	95	72	75.8	(65.9, 84.0)	59	1	1.7	(0.0, 9.1)
	8	19	15	78.9	(54.4, 93.9)	95	72	75.8	(65.9, 84.0)	59	1	1.7	(0.0, 9.1)
	16	19	15	78.9	(54.4, 93.9)	95	71	74.7	(64.8, 83.1)	59	1	1.7	(0.0, 9.1)
	32	19	12	63.2	(38.4, 83.7)	95	56	58.9	(48.4, 68.9)	59	1	1.7	(0.0, 9.1)
	64	19	7	36.8	(16.3, 61.6)	95	33	34.7	(25.3, 45.2)	59	1	1.7	(0.0, 9.1)
	128	19	4	21.1	(6.1, 45.6)	95	13	13.7	(7.5, 22.3)	59	0	0.0	(0.0, 6.1)
12 to <18 Months	4	10	9	90.0	(55.5, 99.7)	45	29	64.4	(48.8, 78.1)	30	0	0.0	(0.0, 11.6)
	8	10	9	90.0	(55.5, 99.7)	45	29	64.4	(48.8, 78.1)	30	0	0.0	(0.0, 11.6)
	16	10	9	90.0	(55.5, 99.7)	45	29	64.4	(48.8, 78.1)	30	0	0.0	(0.0, 11.6)
	32	10	8	80.0	(44.4, 97.5)	45	23	51.1	(35.8, 66.3)	30	0	0.0	(0.0, 11.6)
	64	10	5	50.0	(18.7, 81.3)	45	13	28.9	(16.4, 44.3)	30	0	0.0	(0.0, 11.6)
	128	10	2	20.0	(2.5, 55.6)	45	5	11.1	(3.7, 24.1)	30	0	0.0	(0.0, 11.6)
18 to <24 Months	4	9	6	66.7	(29.9, 92.5)	50	43	86.0	(73.3, 94.2)	29	1	3.4	(0.1, 17.8)
	8	9	6	66.7	(29.9, 92.5)	50	43	86.0	(73.3, 94.2)	29	1	3.4	(0.1, 17.8)
	16	9	6	66.7	(29.9, 92.5)	50	42	84.0	(70.9, 92.8)	29	1	3.4	(0.1, 17.8)
	32	9	4	44.4	(13.7, 78.8)	50	33	66.0	(51.2, 78.8)	29	1	3.4	(0.1, 17.8)
	64	9	2	22.2	(2.8, 60.0)	50	20	40.0	(26.4, 54.8)	29	1	3.4	(0.1, 17.8)
	128	9	2	22.2	(2.8, 60.0)	50	8	16.0	(7.2, 29.1)	29	0	0.0	(0.0, 11.9)
1 Month after Vaccination 3													
12 to <24 Months	4	20	18	90.0	(68.3, 98.8)	96	86	89.6	(81.7, 94.9)	60	4	6.7	(1.8, 16.2)
	8	20	18	90.0	(68.3, 98.8)	96	86	89.6	(81.7, 94.9)	60	4	6.7	(1.8, 16.2)
	16	20	18	90.0	(68.3, 98.8)	96	86	89.6	(81.7, 94.9)	60	3	5.0	(1.0, 13.9)
	32	20	17	85.0	(62.1, 96.8)	96	81	84.4	(75.5, 91.0)	60	2	3.3	(0.4, 11.5)
	64	20	14	70.0	(45.7, 88.1)	96	64	66.7	(56.3, 76.0)	60	1	1.7	(0.0, 8.9)

Table 27. Subjects Achieving Defined hSBA Titers for Primary Strains – Evaluable Immunogenicity Population

Strain (Variant) / Sampling Time Point / Age Strata	Titer	Vaccine Group (as Randomized) 60 µg rLP2086 N[a]	n[b]	%	(95% CI)[c]	120 µg rLP2086 N[a]	n[b]	%	(95% CI)[c]	HAV/Saline N[a]	n[b]	%	(95% CI)[c]
	128	20	10	50.0	(27.2, 72.8)	96	42	43.8	(33.6, 54.3)	60	0	0.0	(0.0, 6.0)
12 to <18 Months	4	9	8	88.9	(51.8, 99.7)	45	41	91.1	(78.8, 97.5)	31	2	6.5	(0.8, 21.4)
	8	9	8	88.9	(51.8, 99.7)	45	41	91.1	(78.8, 97.5)	31	2	6.5	(0.8, 21.4)
	16	9	8	88.9	(51.8, 99.7)	45	41	91.1	(78.8, 97.5)	31	1	3.2	(0.1, 16.7)
	32	9	8	88.9	(51.8, 99.7)	45	37	82.2	(67.9, 92.0)	31	1	3.2	(0.1, 16.7)
	64	9	6	66.7	(29.9, 92.5)	45	27	60.0	(44.3, 74.3)	31	1	3.2	(0.1, 16.7)
	128	9	4	44.4	(13.7, 78.8)	45	17	37.8	(23.8, 53.5)	31	0	0.0	(0.0, 11.2)
18 to <24 Months	4	11	10	90.9	(58.7, 99.8)	51	45	88.2	(76.1, 95.6)	29	2	6.9	(0.8, 22.8)
	8	11	10	90.9	(58.7, 99.8)	51	45	88.2	(76.1, 95.6)	29	2	6.9	(0.8, 22.8)
	16	11	10	90.9	(58.7, 99.8)	51	45	88.2	(76.1, 95.6)	29	2	6.9	(0.8, 22.8)
	32	11	9	81.8	(48.2, 97.7)	51	44	86.3	(73.7, 94.3)	29	1	3.4	(0.1, 17.8)
	64	11	8	72.7	(39.0, 94.0)	51	37	72.5	(58.3, 84.1)	29	0	0.0	(0.0, 11.9)
	128	11	6	54.5	(23.4, 83.3)	51	25	49.0	(34.8, 63.4)	29	0	0.0	(0.0, 11.9)
PMB2001 (A56)													
Before Vaccination 1													
12 to <24 Months	4	19	0	0.0	(0.0, 17.6)	95	2	2.1	(0.3, 7.4)	53	1	1.9	(0.0, 10.1)
	8	19	0	0.0	(0.0, 17.6)	95	1	1.1	(0.0, 5.7)	53	0	0.0	(0.0, 6.7)
	16	19	0	0.0	(0.0, 17.6)	95	1	1.1	(0.0, 5.7)	53	0	0.0	(0.0, 6.7)
	32	19	0	0.0	(0.0, 17.6)	95	1	1.1	(0.0, 5.7)	53	0	0.0	(0.0, 6.7)
	64	19	0	0.0	(0.0, 17.6)	95	0	0.0	(0.0, 3.8)	53	0	0.0	(0.0, 6.7)
	128	19	0	0.0	(0.0, 17.6)	95	0	0.0	(0.0, 3.8)	53	0	0.0	(0.0, 6.7)
12 to <18 Months	4	9	0	0.0	(0.0, 33.6)	46	0	0.0	(0.0, 7.7)	24	1	4.2	(0.1, 21.1)
	8	9	0	0.0	(0.0, 33.6)	46	0	0.0	(0.0, 7.7)	24	0	0.0	(0.0, 14.2)
	16	9	0	0.0	(0.0, 33.6)	46	0	0.0	(0.0, 7.7)	24	0	0.0	(0.0, 14.2)
	32	9	0	0.0	(0.0, 33.6)	46	0	0.0	(0.0, 7.7)	24	0	0.0	(0.0, 14.2)
	64	9	0	0.0	(0.0, 33.6)	46	0	0.0	(0.0, 7.7)	24	0	0.0	(0.0, 14.2)
	128	9	0	0.0	(0.0, 33.6)	46	0	0.0	(0.0, 7.7)	24	0	0.0	(0.0, 14.2)
18 to <24 Months	4	10	0	0.0	(0.0, 30.8)	49	2	4.1	(0.5, 14.0)	29	0	0.0	(0.0, 11.9)
	8	10	0	0.0	(0.0, 30.8)	49	1	2.0	(0.1, 10.9)	29	0	0.0	(0.0, 11.9)
	16	10	0	0.0	(0.0, 30.8)	49	1	2.0	(0.1, 10.9)	29	0	0.0	(0.0, 11.9)
	32	10	0	0.0	(0.0, 30.8)	49	1	2.0	(0.1, 10.9)	29	0	0.0	(0.0, 11.9)
	64	10	0	0.0	(0.0, 30.8)	49	0	0.0	(0.0, 7.3)	29	0	0.0	(0.0, 11.9)
	128	10	0	0.0	(0.0, 30.8)	49	0	0.0	(0.0, 7.3)	29	0	0.0	(0.0, 11.9)
1 Month after Vaccination 2													
12 to <24 Months	4	19	19	100.0	(82.4, 100.0)	95	95	100.0	(96.2, 100.0)	52	1	1.9	(0.0, 10.3)
	8	19	18	94.7	(74.0, 99.9)	95	95	100.0	(96.2, 100.0)	52	0	0.0	(0.0, 6.8)
	16	19	18	94.7	(74.0, 99.9)	95	95	100.0	(96.2, 100.0)	52	0	0.0	(0.0, 6.8)
	32	19	18	94.7	(74.0, 99.9)	95	91	95.8	(89.6, 98.8)	52	0	0.0	(0.0, 6.8)
	64	19	16	84.2	(60.4, 96.6)	95	82	86.3	(77.7, 92.5)	52	0	0.0	(0.0, 6.8)
	128	19	9	47.4	(24.4, 71.1)	95	54	56.8	(46.3, 67.0)	52	0	0.0	(0.0, 6.8)
12 to <18 Months	4	9	9	100.0	(66.4, 100.0)	47	47	100.0	(92.5, 100.0)	23	1	4.3	(0.1, 21.9)
	8	9	9	100.0	(66.4, 100.0)	47	47	100.0	(92.5, 100.0)	23	0	0.0	(0.0, 14.8)
	16	9	9	100.0	(66.4, 100.0)	47	47	100.0	(92.5, 100.0)	23	0	0.0	(0.0, 14.8)
	32	9	9	100.0	(66.4, 100.0)	47	45	95.7	(85.5, 99.5)	23	0	0.0	(0.0, 14.8)
	64	9	9	100.0	(66.4, 100.0)	47	41	87.2	(74.3, 95.2)	23	0	0.0	(0.0, 14.8)
	128	9	4	44.4	(13.7, 78.8)	47	28	59.6	(44.3, 73.6)	23	0	0.0	(0.0, 14.8)

Table 27. Subjects Achieving Defined hSBA Titers for Primary Strains – Evaluable Immunogenicity Population

Strain (Variant) / Sampling Time Point / Age Strata	Titer	Vaccine Group (as Randomized) 60 µg rLP2086 N[a]	n[b]	%	(95% CI)[c]	120 µg rLP2086 N[a]	n[b]	%	(95% CI)[c]	HAV/Saline N[a]	n[b]	%	(95% CI)[c]
18 to <24 Months	4	10	10	100.0	(69.2, 100.0)	48	48	100.0	(92.6, 100.0)	29	0	0.0	(0.0, 11.9)
	8	10	9	90.0	(55.5, 99.7)	48	48	100.0	(92.6, 100.0)	29	0	0.0	(0.0, 11.9)
	16	10	9	90.0	(55.5, 99.7)	48	48	100.0	(92.6, 100.0)	29	0	0.0	(0.0, 11.9)
	32	10	9	90.0	(55.5, 99.7)	48	46	95.8	(85.7, 99.5)	29	0	0.0	(0.0, 11.9)
	64	10	7	70.0	(34.8, 93.3)	48	41	85.4	(72.2, 93.9)	29	0	0.0	(0.0, 11.9)
	128	10	5	50.0	(18.7, 81.3)	48	26	54.2	(39.2, 68.6)	29	0	0.0	(0.0, 11.9)
1 Month after Vaccination 3													
12 to <24 Months	4	19	19	100.0	(82.4, 100.0)	95	95	100.0	(96.2, 100.0)	54	5	9.3	(3.1, 20.3)
	8	19	19	100.0	(82.4, 100.0)	95	95	100.0	(96.2, 100.0)	54	1	1.9	(0.0, 9.9)
	16	19	19	100.0	(82.4, 100.0)	95	94	98.9	(94.3, 100.0)	54	1	1.9	(0.0, 9.9)
	32	19	18	94.7	(74.0, 99.9)	95	91	95.8	(89.6, 98.8)	54	1	1.9	(0.0, 9.9)
	64	19	17	89.5	(66.9, 98.7)	95	85	89.5	(81.5, 94.8)	54	0	0.0	(0.0, 6.6)
	128	19	13	68.4	(43.4, 87.4)	95	79	83.2	(74.1, 90.1)	54	0	0.0	(0.0, 6.6)
12 to <18 Months	4	9	9	100.0	(66.4, 100.0)	47	47	100.0	(92.5, 100.0)	24	2	8.3	(1.0, 27.0)
	8	9	9	100.0	(66.4, 100.0)	47	47	100.0	(92.5, 100.0)	24	0	0.0	(0.0, 14.2)
	16	9	9	100.0	(66.4, 100.0)	47	46	97.9	(88.7, 99.9)	24	0	0.0	(0.0, 14.2)
	32	9	9	100.0	(66.4, 100.0)	47	46	97.9	(88.7, 99.9)	24	0	0.0	(0.0, 14.2)
	64	9	9	100.0	(66.4, 100.0)	47	44	93.6	(82.5, 98.7)	24	0	0.0	(0.0, 14.2)
	128	9	5	55.6	(21.2, 86.3)	47	42	89.4	(76.9, 96.5)	24	0	0.0	(0.0, 14.2)
18 to <24 Months	4	10	10	100.0	(69.2, 100.0)	48	48	100.0	(92.6, 100.0)	30	3	10.0	(2.1, 26.5)
	8	10	10	100.0	(69.2, 100.0)	48	48	100.0	(92.6, 100.0)	30	1	3.3	(0.1, 17.2)
	16	10	10	100.0	(69.2, 100.0)	48	48	100.0	(92.6, 100.0)	30	1	3.3	(0.1, 17.2)
	32	10	9	90.0	(55.5, 99.7)	48	45	93.8	(82.8, 98.7)	30	1	3.3	(0.1, 17.2)
	64	10	8	80.0	(44.4, 97.5)	48	41	85.4	(72.2, 93.9)	30	0	0.0	(0.0, 11.6)
	128	10	8	80.0	(44.4, 97.5)	48	37	77.1	(62.7, 88.0)	30	0	0.0	(0.0, 11.6)
PMB2948 (B24)													
Before Vaccination 1													
12 to <24 Months	4	21	1	4.8	(0.1, 23.8)	97	2	2.1	(0.3, 7.3)	61	1	1.6	(0.0, 8.8)
	8	21	1	4.8	(0.1, 23.8)	97	2	2.1	(0.3, 7.3)	61	1	1.6	(0.0, 8.8)
	16	21	1	4.8	(0.1, 23.8)	97	2	2.1	(0.3, 7.3)	61	1	1.6	(0.0, 8.8)
	32	21	1	4.8	(0.1, 23.8)	97	0	0.0	(0.0, 3.7)	61	1	1.6	(0.0, 8.8)
	64	21	0	0.0	(0.0, 16.1)	97	0	0.0	(0.0, 3.7)	61	1	1.6	(0.0, 8.8)
	128	21	0	0.0	(0.0, 16.1)	97	0	0.0	(0.0, 3.7)	61	0	0.0	(0.0, 5.9)
12 to <18 Months	4	10	0	0.0	(0.0, 30.8)	46	1	2.2	(0.1, 11.5)	31	0	0.0	(0.0, 11.2)
	8	10	0	0.0	(0.0, 30.8)	46	1	2.2	(0.1, 11.5)	31	0	0.0	(0.0, 11.2)
	16	10	0	0.0	(0.0, 30.8)	46	1	2.2	(0.1, 11.5)	31	0	0.0	(0.0, 11.2)
	32	10	0	0.0	(0.0, 30.8)	46	0	0.0	(0.0, 7.7)	31	0	0.0	(0.0, 11.2)
	64	10	0	0.0	(0.0, 30.8)	46	0	0.0	(0.0, 7.7)	31	0	0.0	(0.0, 11.2)
	128	10	0	0.0	(0.0, 30.8)	46	0	0.0	(0.0, 7.7)	31	0	0.0	(0.0, 11.2)
18 to <24 Months	4	11	1	9.1	(0.2, 41.3)	51	1	2.0	(0.0, 10.4)	30	1	3.3	(0.1, 17.2)
	8	11	1	9.1	(0.2, 41.3)	51	1	2.0	(0.0, 10.4)	30	1	3.3	(0.1, 17.2)
	16	11	1	9.1	(0.2, 41.3)	51	1	2.0	(0.0, 10.4)	30	1	3.3	(0.1, 17.2)
	32	11	1	9.1	(0.2, 41.3)	51	0	0.0	(0.0, 7.0)	30	1	3.3	(0.1, 17.2)
	64	11	0	0.0	(0.0, 28.5)	51	0	0.0	(0.0, 7.0)	30	1	3.3	(0.1, 17.2)
	128	11	0	0.0	(0.0, 28.5)	51	0	0.0	(0.0, 7.0)	30	0	0.0	(0.0, 11.6)
1 Month after Vaccination 2													

Table 27. Subjects Achieving Defined hSBA Titers for Primary Strains – Evaluable Immunogenicity Population

		Vaccine Group (as Randomized)											
		60 µg rLP2086				120 µg rLP2086				HAV/Saline			
Strain (Variant) Sampling Time Point Age Strata	Titer	N[a]	n[b]	%	(95% CI)[c]	N[a]	n[b]	%	(95% CI)[c]	N[a]	n[b]	%	(95% CI)[c]
12 to <24 Months	4	19	11	57.9	(33.5, 79.7)	86	31	36.0	(26.0, 47.1)	59	1	1.7	(0.0, 9.1)
	8	19	11	57.9	(33.5, 79.7)	86	29	33.7	(23.9, 44.7)	59	1	1.7	(0.0, 9.1)
	16	19	9	47.4	(24.4, 71.1)	86	28	32.6	(22.8, 43.5)	59	1	1.7	(0.0, 9.1)
	32	19	1	5.3	(0.1, 26.0)	86	12	14.0	(7.4, 23.1)	59	1	1.7	(0.0, 9.1)
	64	19	0	0.0	(0.0, 17.6)	86	3	3.5	(0.7, 9.9)	59	0	0.0	(0.0, 6.1)
	128	19	0	0.0	(0.0, 17.6)	86	1	1.2	(0.0, 6.3)	59	0	0.0	(0.0, 6.1)
12 to <18 Months	4	10	7	70.0	(34.8, 93.3)	42	12	28.6	(15.7, 44.6)	30	0	0.0	(0.0, 11.6)
	8	10	7	70.0	(34.8, 93.3)	42	10	23.8	(12.1, 39.5)	30	0	0.0	(0.0, 11.6)
	16	10	6	60.0	(26.2, 87.8)	42	9	21.4	(10.3, 36.8)	30	0	0.0	(0.0, 11.6)
	32	10	1	10.0	(0.3, 44.5)	42	4	9.5	(2.7, 22.6)	30	0	0.0	(0.0, 11.6)
	64	10	0	0.0	(0.0, 30.8)	42	1	2.4	(0.1, 12.6)	30	0	0.0	(0.0, 11.6)
	128	10	0	0.0	(0.0, 30.8)	42	1	2.4	(0.1, 12.6)	30	0	0.0	(0.0, 11.6)
18 to <24 Months	4	9	4	44.4	(13.7, 78.8)	44	19	43.2	(28.3, 59.0)	29	1	3.4	(0.1, 17.8)
	8	9	4	44.4	(13.7, 78.8)	44	19	43.2	(28.3, 59.0)	29	1	3.4	(0.1, 17.8)
	16	9	3	33.3	(7.5, 70.1)	44	19	43.2	(28.3, 59.0)	29	1	3.4	(0.1, 17.8)
	32	9	0	0.0	(0.0, 33.6)	44	8	18.2	(8.2, 32.7)	29	1	3.4	(0.1, 17.8)
	64	9	0	0.0	(0.0, 33.6)	44	2	4.5	(0.6, 15.5)	29	0	0.0	(0.0, 11.9)
	128	9	0	0.0	(0.0, 33.6)	44	0	0.0	(0.0, 8.0)	29	0	0.0	(0.0, 11.9)
1 Month after Vaccination 3													
12 to <24 Months	4	20	17	85.0	(62.1, 96.8)	95	68	71.6	(61.4, 80.4)	60	3	5.0	(1.0, 13.9)
	8	20	17	85.0	(62.1, 96.8)	95	68	71.6	(61.4, 80.4)	60	3	5.0	(1.0, 13.9)
	16	20	15	75.0	(50.9, 91.3)	95	64	67.4	(57.0, 76.6)	60	3	5.0	(1.0, 13.9)
	32	20	8	40.0	(19.1, 63.9)	95	34	35.8	(26.2, 46.3)	60	1	1.7	(0.0, 8.9)
	64	20	3	15.0	(3.2, 37.9)	95	13	13.7	(7.5, 22.3)	60	0	0.0	(0.0, 6.0)
	128	20	1	5.0	(0.1, 24.9)	95	2	2.1	(0.3, 7.4)	60	0	0.0	(0.0, 6.0)
12 to <18 Months	4	9	8	88.9	(51.8, 99.7)	45	32	71.1	(55.7, 83.6)	31	1	3.2	(0.1, 16.7)
	8	9	8	88.9	(51.8, 99.7)	45	32	71.1	(55.7, 83.6)	31	1	3.2	(0.1, 16.7)
	16	9	8	88.9	(51.8, 99.7)	45	30	66.7	(51.0, 80.0)	31	1	3.2	(0.1, 16.7)
	32	9	4	44.4	(13.7, 78.8)	45	17	37.8	(23.8, 53.5)	31	1	3.2	(0.1, 16.7)
	64	9	1	11.1	(0.3, 48.2)	45	8	17.8	(8.0, 32.1)	31	0	0.0	(0.0, 11.2)
	128	9	0	0.0	(0.0, 33.6)	45	1	2.2	(0.1, 11.8)	31	0	0.0	(0.0, 11.2)
18 to <24 Months	4	11	9	81.8	(48.2, 97.7)	50	36	72.0	(57.5, 83.8)	29	2	6.9	(0.8, 22.8)
	8	11	9	81.8	(48.2, 97.7)	50	36	72.0	(57.5, 83.8)	29	2	6.9	(0.8, 22.8)
	16	11	7	63.6	(30.8, 89.1)	50	34	68.0	(53.3, 80.5)	29	2	6.9	(0.8, 22.8)
	32	11	4	36.4	(10.9, 69.2)	50	17	34.0	(21.2, 48.8)	29	0	0.0	(0.0, 11.9)
	64	11	2	18.2	(2.3, 51.8)	50	5	10.0	(3.3, 21.8)	29	0	0.0	(0.0, 11.9)
	128	11	1	9.1	(0.2, 41.3)	50	1	2.0	(0.1, 10.6)	29	0	0.0	(0.0, 11.9)
PMB2707 (B44)													
Before Vaccination 1													
12 to <24 Months	4	19	0	0.0	(0.0, 17.6)	95	1	1.1	(0.0, 5.7)	54	0	0.0	(0.0, 6.6)
	8	19	0	0.0	(0.0, 17.6)	95	1	1.1	(0.0, 5.7)	54	0	0.0	(0.0, 6.6)
	16	19	0	0.0	(0.0, 17.6)	95	0	0.0	(0.0, 3.8)	54	0	0.0	(0.0, 6.6)
	32	19	0	0.0	(0.0, 17.6)	95	0	0.0	(0.0, 3.8)	54	0	0.0	(0.0, 6.6)
	64	19	0	0.0	(0.0, 17.6)	95	0	0.0	(0.0, 3.8)	54	0	0.0	(0.0, 6.6)
	128	19	0	0.0	(0.0, 17.6)	95	0	0.0	(0.0, 3.8)	54	0	0.0	(0.0, 6.6)
12 to <18 Months	4	9	0	0.0	(0.0, 33.6)	46	1	2.2	(0.1, 11.5)	24	0	0.0	(0.0, 14.2)

Table 27. Subjects Achieving Defined hSBA Titers for Primary Strains – Evaluable Immunogenicity Population

Strain (Variant) / Sampling Time Point / Age Strata	Titer	Vaccine Group (as Randomized) 60 µg rLP2086 N[a]	n[b]	%	(95% CI)[c]	120 µg rLP2086 N[a]	n[b]	%	(95% CI)[c]	HAV/Saline N[a]	n[b]	%	(95% CI)[c]
	8	9	0	0.0	(0.0, 33.6)	46	1	2.2	(0.1, 11.5)	24	0	0.0	(0.0, 14.2)
	16	9	0	0.0	(0.0, 33.6)	46	0	0.0	(0.0, 7.7)	24	0	0.0	(0.0, 14.2)
	32	9	0	0.0	(0.0, 33.6)	46	0	0.0	(0.0, 7.7)	24	0	0.0	(0.0, 14.2)
	64	9	0	0.0	(0.0, 33.6)	46	0	0.0	(0.0, 7.7)	24	0	0.0	(0.0, 14.2)
	128	9	0	0.0	(0.0, 33.6)	46	0	0.0	(0.0, 7.7)	24	0	0.0	(0.0, 14.2)
18 to <24 Months	4	10	0	0.0	(0.0, 30.8)	49	0	0.0	(0.0, 7.3)	30	0	0.0	(0.0, 11.6)
	8	10	0	0.0	(0.0, 30.8)	49	0	0.0	(0.0, 7.3)	30	0	0.0	(0.0, 11.6)
	16	10	0	0.0	(0.0, 30.8)	49	0	0.0	(0.0, 7.3)	30	0	0.0	(0.0, 11.6)
	32	10	0	0.0	(0.0, 30.8)	49	0	0.0	(0.0, 7.3)	30	0	0.0	(0.0, 11.6)
	64	10	0	0.0	(0.0, 30.8)	49	0	0.0	(0.0, 7.3)	30	0	0.0	(0.0, 11.6)
	128	10	0	0.0	(0.0, 30.8)	49	0	0.0	(0.0, 7.3)	30	0	0.0	(0.0, 11.6)
1 Month after Vaccination 2													
12 to <24 Months	4	19	14	73.7	(48.8, 90.9)	94	64	68.1	(57.7, 77.3)	52	0	0.0	(0.0, 6.8)
	8	19	13	68.4	(43.4, 87.4)	94	64	68.1	(57.7, 77.3)	52	0	0.0	(0.0, 6.8)
	16	19	13	68.4	(43.4, 87.4)	94	63	67.0	(56.6, 76.4)	52	0	0.0	(0.0, 6.8)
	32	19	11	57.9	(33.5, 79.7)	94	53	56.4	(45.8, 66.6)	52	0	0.0	(0.0, 6.8)
	64	19	6	31.6	(12.6, 56.6)	94	23	24.5	(16.2, 34.4)	52	0	0.0	(0.0, 6.8)
	128	19	2	10.5	(1.3, 33.1)	94	10	10.6	(5.2, 18.7)	52	0	0.0	(0.0, 6.8)
12 to <18 Months	4	9	7	77.8	(40.0, 97.2)	47	34	72.3	(57.4, 84.4)	23	0	0.0	(0.0, 14.8)
	8	9	7	77.8	(40.0, 97.2)	47	34	72.3	(57.4, 84.4)	23	0	0.0	(0.0, 14.8)
	16	9	7	77.8	(40.0, 97.2)	47	34	72.3	(57.4, 84.4)	23	0	0.0	(0.0, 14.8)
	32	9	5	55.6	(21.2, 86.3)	47	29	61.7	(46.4, 75.5)	23	0	0.0	(0.0, 14.8)
	64	9	3	33.3	(7.5, 70.1)	47	13	27.7	(15.6, 42.6)	23	0	0.0	(0.0, 14.8)
	128	9	1	11.1	(0.3, 48.2)	47	5	10.6	(3.5, 23.1)	23	0	0.0	(0.0, 14.8)
18 to <24 Months	4	10	7	70.0	(34.8, 93.3)	47	30	63.8	(48.5, 77.3)	29	0	0.0	(0.0, 11.9)
	8	10	6	60.0	(26.2, 87.8)	47	30	63.8	(48.5, 77.3)	29	0	0.0	(0.0, 11.9)
	16	10	6	60.0	(26.2, 87.8)	47	29	61.7	(46.4, 75.5)	29	0	0.0	(0.0, 11.9)
	32	10	6	60.0	(26.2, 87.8)	47	24	51.1	(36.1, 65.9)	29	0	0.0	(0.0, 11.9)
	64	10	3	30.0	(6.7, 65.2)	47	10	21.3	(10.7, 35.7)	29	0	0.0	(0.0, 11.9)
	128	10	1	10.0	(0.3, 44.5)	47	5	10.6	(3.5, 23.1)	29	0	0.0	(0.0, 11.9)
1 Month after Vaccination 3													
12 to <24 Months	4	19	17	89.5	(66.9, 98.7)	94	82	87.2	(78.8, 93.2)	54	0	0.0	(0.0, 6.6)
	8	19	17	89.5	(66.9, 98.7)	94	81	86.2	(77.5, 92.4)	54	0	0.0	(0.0, 6.6)
	16	19	16	84.2	(60.4, 96.6)	94	81	86.2	(77.5, 92.4)	54	0	0.0	(0.0, 6.6)
	32	19	12	63.2	(38.4, 83.7)	94	72	76.6	(66.7, 84.7)	54	0	0.0	(0.0, 6.6)
	64	19	7	36.8	(16.3, 61.6)	94	55	58.5	(47.9, 68.6)	54	0	0.0	(0.0, 6.6)
	128	19	4	21.1	(6.1, 45.6)	94	30	31.9	(22.7, 42.3)	54	0	0.0	(0.0, 6.6)
12 to <18 Months	4	9	8	88.9	(51.8, 99.7)	47	41	87.2	(74.3, 95.2)	24	0	0.0	(0.0, 14.2)
	8	9	8	88.9	(51.8, 99.7)	47	41	87.2	(74.3, 95.2)	24	0	0.0	(0.0, 14.2)
	16	9	7	77.8	(40.0, 97.2)	47	41	87.2	(74.3, 95.2)	24	0	0.0	(0.0, 14.2)
	32	9	5	55.6	(21.2, 86.3)	47	35	74.5	(59.7, 86.1)	24	0	0.0	(0.0, 14.2)
	64	9	4	44.4	(13.7, 78.8)	47	27	57.4	(42.2, 71.7)	24	0	0.0	(0.0, 14.2)
	128	9	2	22.2	(2.8, 60.0)	47	14	29.8	(17.3, 44.9)	24	0	0.0	(0.0, 14.2)
18 to <24 Months	4	10	9	90.0	(55.5, 99.7)	47	41	87.2	(74.3, 95.2)	30	0	0.0	(0.0, 11.6)
	8	10	9	90.0	(55.5, 99.7)	47	40	85.1	(71.7, 93.8)	30	0	0.0	(0.0, 11.6)
	16	10	9	90.0	(55.5, 99.7)	47	40	85.1	(71.7, 93.8)	30	0	0.0	(0.0, 11.6)
	32	10	7	70.0	(34.8, 93.3)	47	37	78.7	(64.3, 89.3)	30	0	0.0	(0.0, 11.6)

Table 27. Subjects Achieving Defined hSBA Titers for Primary Strains – Evaluable Immunogenicity Population

		Vaccine Group (as Randomized)										
		60 µg rLP2086				120 µg rLP2086				HAV/Saline		
Strain (Variant) Sampling Time Point Age Strata	Titer	N[a]	n[b]	%	(95% CI)[c]	N[a]	n[b]	%	(95% CI)[c]	N[a]	n[b]	%	(95% CI)[c]
	64	10	3	30.0	(6.7, 65.2)	47	28	59.6	(44.3, 73.6)	30	0	0.0	(0.0, 11.6)
	128	10	2	20.0	(2.5, 55.6)	47	16	34.0	(20.9, 49.3)	30	0	0.0	(0.0, 11.6)

Abbreviations: hSBA = serum bactericidal assay using human complement.

a. N = number of subjects with valid and determinate hSBA titers for the given strain.

b. n = Number of subjects with observed hSBA titer ≥ the defined titer for the given strain at the given time point.

c. Exact 2-sided CI based upon observed proportion of subjects, using the Clopper and Pearson method.

Exploratory Immunogenicity Endpoints

hSBA Titer ≥4-Fold Increase from Baseline

Table 28 presents the proportion of subjects with hSBA titers with a ≥4-fold rise from baseline for the 4 primary MnB test strains.

At 1 month after Vaccination 2, the proportion of subjects 12 to <18 months of age achieving a ≥4-fold rise in hSBA titer from baseline was 80.0% and 62.2% for PMB80 (A22); 100.0% and 97.9% for PMB2001 (A56); 60.0% and 19.0% for PMB2948 (B24); and 77.8% and 70.2% for PMB2707 (B44) for the 60-μg and 120-μg groups, respectively. For the 18 to <24 months age stratum, the proportion of subjects achieving a ≥4-fold rise in hSBA titer from baseline was 66.7% and 80.0% for PMB80 (A22); 90.0% and 100.0% for PMB2001 (A56); 33.3% and 40.9% for PMB2948 (B24); and 60.0% and 61.7% for PMB2707 (B44) for the 60-μg and 120-μg groups, respectively. Overall, for the combined age stratum, the proportion of subjects achieving a ≥4-fold rise in hSBA titer from baseline at 1 month after Vaccination 2 was 73.7% and 71.6% for PMB80 (A22); 94.7% and 98.9% for PMB2001 (A56); 47.4% and 30.2% for PMB2948 (B24); and 68.4% and 66.0% for PMB2707 (B44) for the 60-μg and 120-μg groups, respectively.

At 1 month after Vaccination 3, the proportion of subjects 12 to <18 months of age achieving a ≥4-fold rise in hSBA titer from baseline was 77.8% and 86.7% for PMB80 (A22); 100.0% and 95.7% for PMB2001 (A56); 88.9% and 66.7% for PMB2948 (B24); and 77.8% and 85.1% for PMB2707 (B44) for the 60-μg and 120-μg groups, respectively. For the 18 to <24 months age stratum, the proportion of subjects achieving a ≥4-fold rise in hSBA titer from baseline was 90.9% and 88.2% for PMB80 (A22); 100.0% and 100.0% for PMB2001 (A56); 63.6% and 68.0% for PMB2948 (B24); and 90.0% and 85.1% for PMB2707 (B44) for the 60-μg and 120-μg groups, respectively. Overall, for the combined age stratum, the proportion of subjects achieving a ≥4-fold rise in hSBA titer from baseline at 1 month after Vaccination 3 was 85.0% and 87.5% for PMB80 (A22); 100.0% and 97.9% for PMB2001 (A56); 75.0% and 67.4% for PMB2948 (B24); and 84.2% and 85.1% for PMB2707 (B44) for the 60-μg and 120-μg groups, respectively.

The proportion of subjects in the HAV/saline group (combined age stratum) achieving a ≥4-fold rise in hSBA titer at 1 month after Vaccination 3 was 5.0% for PMB80 (A22), 1.9% for PMB2001 (A56), 3.3% for PMB2948 (B24), and 0.0% for PMB2707 (B44).

Table 28. Subjects Achieving ≥4-Fold Rise in hSBA Titer for Primary Strains – Evaluable Immunogenicity Population

Strain (Variant) Sampling Time Point Age Strata	Vaccine Group (as Randomized) 60 µg rLP2086 N[a]	n[b] (%)	(%)	(95% CI)[c]	120 µg rLP2086 N[a]	n[b] (%)	(%)	(95% CI)[c]	HAV/Saline N[a]	n[b] (%)	(%)	(95% CI)[c]
PMB80 (A22)												
1 Month after Vaccination 2												
12 to <24 Months	19	14	(73.7)	(48.8, 90.9)	95	68	(71.6)	(61.4, 80.4)	59	1	(1.7)	(0.0, 9.1)
12 to <18 Months	10	8	(80.0)	(44.4, 97.5)	45	28	(62.2)	(46.5, 76.2)	30	0	(0.0)	(0.0, 11.6)
18 to <24 Months	9	6	(66.7)	(29.9, 92.5)	50	40	(80.0)	(66.3, 90.0)	29	1	(3.4)	(0.1, 17.8)
1 Month after Vaccination 3												
12 to <24 Months	20	17	(85.0)	(62.1, 96.8)	96	84	(87.5)	(79.2, 93.4)	60	3	(5.0)	(1.0, 13.9)
12 to <18 Months	9	7	(77.8)	(40.0, 97.2)	45	39	(86.7)	(73.2, 94.9)	31	1	(3.2)	(0.1, 16.7)
18 to <24 Months	11	10	(90.9)	(58.7, 99.8)	51	45	(88.2)	(76.1, 95.6)	29	2	(6.9)	(0.8, 22.8)
PMB2001 (A56)												
1 Month after Vaccination 2												
12 to <24 Months	19	18	(94.7)	(74.0, 99.9)	95	94	(98.9)	(94.3, 100.0)	52	0	(0.0)	(0.0, 6.8)
12 to <18 Months	9	9	(100.0)	(66.4, 100.0)	47	46	(97.9)	(88.7, 99.9)	23	0	(0.0)	(0.0, 14.8)
18 to <24 Months	10	9	(90.0)	(55.5, 99.7)	48	48	(100.0)	(92.6, 100.0)	29	0	(0.0)	(0.0, 11.9)
1 Month after Vaccination 3												
12 to <24 Months	19	19	(100.0)	(82.4, 100.0)	95	93	(97.9)	(92.6, 99.7)	54	1	(1.9)	(0.0, 9.9)
12 to <18 Months	9	9	(100.0)	(66.4, 100.0)	47	45	(95.7)	(85.5, 99.5)	24	0	(0.0)	(0.0, 14.2)
18 to <24 Months	10	10	(100.0)	(69.2, 100.0)	48	48	(100.0)	(92.6, 100.0)	30	1	(3.3)	(0.1, 17.2)
PMB2948 (B24)												
1 Month after Vaccination 2												
12 to <24 Months	19	9	(47.4)	(24.4, 71.1)	86	26	(30.2)	(20.8, 41.1)	59	0	(0.0)	(0.0, 6.1)
12 to <18 Months	10	6	(60.0)	(26.2, 87.8)	42	8	(19.0)	(8.6, 34.1)	30	0	(0.0)	(0.0, 11.6)
18 to <24 Months	9	3	(33.3)	(7.5, 70.1)	44	18	(40.9)	(26.3, 56.8)	29	0	(0.0)	(0.0, 11.9)
1 Month after Vaccination 3												
12 to <24 Months	20	15	(75.0)	(50.9, 91.3)	95	64	(67.4)	(57.0, 76.6)	60	2	(3.3)	(0.4, 11.5)
12 to <18 Months	9	8	(88.9)	(51.8, 99.7)	45	30	(66.7)	(51.0, 80.0)	31	1	(3.2)	(0.1, 16.7)
18 to <24 Months	11	7	(63.6)	(30.8, 89.1)	50	34	(68.0)	(53.3, 80.5)	29	1	(3.4)	(0.1, 17.8)

Table 28. Subjects Achieving ≥4-Fold Rise in hSBA Titer for Primary Strains – Evaluable Immunogenicity Population

Strain (Variant) Sampling Time Point Age Strata	Vaccine Group (as Randomized) 60 µg rLP2086 N[a]	n[b] (%)	(%)	(95% CI)[c]	120 µg rLP2086 N[a]	n[b] (%)	(%)	(95% CI)[c]	HAV/Saline N[a]	n[b] (%)	(%)	(95% CI)[c]
PMB2707 (B44)												
1 Month after Vaccination 2												
12 to <24 Months	19	13	(68.4)	(43.4, 87.4)	94	62	(66.0)	(55.5, 75.4)	52	0	(0.0)	(0.0, 6.8)
12 to <18 Months	9	7	(77.8)	(40.0, 97.2)	47	33	(70.2)	(55.1, 82.7)	23	0	(0.0)	(0.0, 14.8)
18 to <24 Months	10	6	(60.0)	(26.2, 87.8)	47	29	(61.7)	(46.4, 75.5)	29	0	(0.0)	(0.0, 11.9)
1 Month after Vaccination 3												
12 to <24 Months	19	16	(84.2)	(60.4, 96.6)	94	80	(85.1)	(76.3, 91.6)	54	0	(0.0)	(0.0, 6.6)
12 to <18 Months	9	7	(77.8)	(40.0, 97.2)	47	40	(85.1)	(71.7, 93.8)	24	0	(0.0)	(0.0, 14.2)
18 to <24 Months	10	9	(90.0)	(55.5, 99.7)	47	40	(85.1)	(71.7, 93.8)	30	0	(0.0)	(0.0, 11.6)

Abbreviations: hSBA = serum bactericidal assay using human complement; LLOQ = lower limit of quantitation; LOD = limit of detection;
Note: LLOQ = 1:16 for A22; 1:8 for A56, B24, and B44.
Note: The 4-fold increase is defined as follows: (1) For subjects with a baseline hSBA titer below the LOD (hSBA titer <1:4), a response is defined as an hSBA titer
≥1:16 or the LLOQ (whichever titer is higher). (2) For subjects with a baseline hSBA titer ≥ LOD and < LLOQ, a response is defined as an hSBA titer ≥4 times the
LLOQ. (3) For subjects with a baseline hSBA titer ≥ LLOQ, a response is defined as an hSBA titer ≥4 times the baseline titer.
a. For hSBA titer fold rise ≥4 from baseline, N = number of subjects with valid and determinate hSBA titers for the given strain at both the specified time point and baseline.
b. For hSBA titer fold rise ≥4 from baseline, n = number of subjects who achieved hSBA titer fold rise ≥4 from baseline for the given strain.
c. Exact 2-sided CI based upon observed proportion of subjects, using the Clopper and Pearson method.

Similar results were observed for the mITT population.

Subgroup analyses of the proportion of subjects achieving a ≥4-fold rise in hSBA titer for each of the 4 primary MnB test strains were assessed for the evaluable immunogenicity population by sex and country. There were no clinically important differences observed in the subgroup analyses performed.

Reverse Cumulative Distribution Curves for the Primary MnB Test Strains

The RCDCs of the proportions of subjects exhibiting an hSBA response (≥ LLOQ) for each of the 4 primary MnB test strains and at each sampling time point, for the combined age stratum were assessed for PMB80 [A22], PMB2001 [A56], PMB2948 [B24], and PMB2707 [B44]. The RCDCs showed that the immune responses were higher after Vaccination 2 and Vaccination 3 for the bivalent rLP2086 groups versus the HAV/saline group. The immune responses for the bivalent rLP2086 groups increased with each vaccination.

Immunogenicity Conclusions

The primary objectives of this study were to describe the immune response to bivalent rLP2086 as measured by hSBA against 4 primary MnB test strains, 2 expressing an LP2086 subfamily A protein and 2 expressing an LP2086 subfamily B protein, measured 1 month after the third vaccination in healthy subjects 12 to <18 months of age and 18 to <24 months of age. The description of immune responses for the combined age stratum (12 to <24 months) was a secondary objective. The primary endpoints for the primary objectives were the proportions of subjects in each age stratum achieving hSBA titers ≥ LLOQ for each of the 4 primary MnB strains 1 month after the third vaccination.

A robust immune response was observed at both dose levels for toddlers 12 to <18 months of age and for toddlers 18 to <24 months of age, as well as for the combined age stratum (12 to <24 months) 1 month after the third dose of bivalent rLP2086, as confirmed by the proportion of subjects achieving an hSBA titer ≥ LLOQ (1:8 for A56, B24 and B44; 1:16 for A22) for each of the 4 primary MnB test strains. For the 60-μg group, the proportion of subjects achieving an hSBA titer ≥ LLOQ ranged from 88.9% to 100.0% for the younger toddlers (12 to <18 months) and from 81.8% to 100.0% for the older toddlers (18 to <24 months) after 3 doses. For the 120-μg group, the proportion of subjects achieving an hSBA titer ≥ LLOQ ranged from 71.1% to 100.0% for toddlers 12 to <18 months of age and from 72.0% to 100.0% for toddlers 18 to <24 months of age after 3 doses. For the combined age stratum the proportion of subjects achieving an hSBA titer ≥ LLOQ for each of the 4 primary MnB test strains 1 month after the third vaccination ranged from 85.0% to 100.0% for the 60-μg group and from 71.6% to 100.0% for the 120-μg group. These findings are further supported by increases in GMTs (range from 4.0 to 8.5 at baseline to 15.1 to 171.4 at 1 month after Vaccination 3) and in the proportion of subjects achieving an hSBA titer ≥1:4 (71.1% to 100.0%) or ≥1:16 (63.6% to 100.0%) against each of the 4 primary MnB test strains after 3 doses of bivalent rLP2086 compared to baseline

across both age strata and dose levels. Additionally, the proportion of subjects for the combined age stratum achieving an hSBA fold rise ≥4 from baseline to 1 month after the third vaccination for each of the 4 primary MnB test strains ranged from 67.4% to 100.0% for both dose levels. In conclusion, 3 doses of either 60 μg or 120 μg of bivalent rLP2086 administered on a 0-, 2-, 6-month schedule, induced robust immune responses in toddlers 12 to <24 months of age (both individual and combined age strata).

The secondary objective of the study was to describe immune responses 1 month after the second dose of bivalent rLP2086, as assessed by ≥ LLOQ responses, defined hSBA titers, and hSBA GMTs for the 2 age strata and the combined age stratum. For the combined age stratum, the proportion of subjects achieving an hSBA titer ≥ LLOQ after the second dose of bivalent rLP2086 (administered 2 months after the first dose) ranged from 57.9% to 94.7% for subjects in the 60-μg group and from 33.7% to 100.0% for subjects in the 120-μg group. Similar results were obtained for the 2 individual age strata with no clinically meaningful differences between the younger and older age strata. These findings are supported by increases in GMTs (range 7.2 to 110.6) over baseline and in the proportion of subjects achieving an hSBA titer ≥1:4 (36.0% to 100.0%) or ≥1:16 (32.6% to 100%) against each of the 4 primary MnB test strains after 2 doses of bivalent rLP2086 compared to baseline across both dose levels for the combined age stratum. Similar results were obtained for the 2 individual age strata.

Additionally, the proportion of subjects for the combined age stratum achieving an hSBA fold rise ≥4 from baseline to 1 month after the second vaccination for each of the 4 primary MnB test strains ranged from 30.2% to 98.9%. In conclusion, 2 doses of either 60 μg or 120 μg of bivalent rLP2086 administered 2 months apart induced immune responses in toddlers 12 to <24 months of age (both individual and combined age strata).

In summary, at both the 60-μg and 120-μg dose levels, bivalent rLP2086 given as 3 doses on a 0-, 2-, and 6-month schedule elicits a robust immune response among toddlers 12 to <24 months of age with protective antibody titers achieved as measured by hSBA in a high proportion of subjects after the third dose.

DISCUSSION AND OVERALL CONCLUSIONS

Immunogenicity Discussion. Immunogenicity results from this Phase 2 study of a 3-dose regimen (0-, 2-, and 6-month schedule) of bivalent rLP2086 (at 2 dose levels) given to toddlers 12 to <24 months of age are consistent with previous studies in adolescents and young adults at the 120-μg dose level.

Immunogenicity responses to bivalent rLP2086 vaccination were measured in validated hSBAs using 4 primary MnB test strains, each expressing fHBP variants heterologous to the vaccine component antigens, using criteria more stringent than the accepted correlate of protection (hSBA titer ≥1:4). Based on an hSBA titer ≥ LLOQ for the 4 primary MnB test strains 1 month after Vaccination 3, the toddlers participating in this study (at either dose level) had similar

immune responses compared to adolescents (10 years to <19 years) participating in Study B1971009 and toddlers and children participating in Study B1971017 (≥24 months to <10 years), with proportions of subjects achieving an hSBA titer ≥ LLOQ after the third vaccination (0-, 2-, 6-month schedule) ranging from 71.6% to 100.0% for the 120-μg group (12 to <24 months age) in this study, 87.1% to 99.5% in Study B1971009, and 79.1% to 100.0% in Study B1971017. Clinically meaningful differences in the proportion of subjects achieving an hSBA titer ≥ LLOQ for the 4 primary MnB test strains 1 month after Vaccination 3 between these 3 studies are not apparent, despite the fact that Study B1971009 had a much higher proportion of adolescent subjects with a prevaccination hSBA titer ≥ LLOQ compared to the toddlers in this study. Bivalent rLP2086 appears to be highly immunogenic in the 12 to <24 months age population and is likely to offer protection against MnB infection similarly to that expected for adolescents based on the hSBA correlate of protection. After 3 doses for the individual age strata and for both age strata combined, responses to the 60-μg dose level were not meaningfully different than responses to the 120-μg dose level.

With regard to the secondary objectives, immune responses in this study for the combined age stratum (12 to <24 months) 1 month after the second dose of bivalent rLP2086 (either dose level), the proportion of subjects achieving an hSBA titer ≥ LLOQ ranged from 33.7% to 100.0% compared to adolescents (10 years to <19 years) participating in Study B1971009 receiving 2 doses of bivalent rLP2086 given 2 months apart, which ranged from 64.2% to 99.1% and toddlers and children (≥24 months to <10 years) participating in Study B1971017 which ranged from 48.5% to 100.0%. For both age strata and the combined age stratum, immune responses after 2 doses of 60 μg bivalent rLP2086 were not meaningfully different than responses following 2 doses of 120 μg bivalent rLP2086. It should be noted that the smaller sample size in the 60-μg group make definitive conclusions difficult when comparing response rates between 60-μg and 120-μg dose levels.

In summary, at both the 60-μg and 120-μg dose levels, bivalent rLP2086 administered on a 0-, 2-,and 6-month schedule is highly immunogenic among toddlers 12 to <24 months of age with protective immune responses achieved as measured by hSBA in a high proportion of subjects after the third dose. Immune responses, as measured in this study, appear to be similar to that observed in prior studies among adolescents and children 1 month after the third dose. The 3-dose regimen appears to provide high rates of protective immunity in toddlers 12 to <24 months of age.

Overall Conclusions. In conclusion, the 60-μg and 120-μg dose levels of bivalent rLP2086 when administered to toddlers 12 to <24 months of age on a 0-, 2-, and 6-month schedule elicit protective antibody titers after the third dose as measured by hSBAs. The vaccine, as administered in this study, was safe and well tolerated with an acceptable safety profile for toddlers 12 to <24 months of age.

Example 22: Assessment of the *Neisseria meningitidis* Serogroup B Immunogenicity of Mn Pentavalent and Trumenba® Vaccines in CBA/J Mice

The immune response to *Neisseria meningitidis* serogroup B fHBP following vaccination with either bivalent Mn B fHBP vaccine, Trumenba, or the bivalent Mn B fHBP vaccine formulated with quadrivalent ACWY polysaccharide conjugate vaccine (Mn Pentavalent ABCWY) was evaluated in CBA/J mice. Groups of CBA/J mice were immunized with 3 different vaccines: Pentavalent (ABCYW), Trumenba® (MnB) and Nimenrix® (ACYW) (Table 29).

Table 29 - Study Design: Dose Levels for each Vaccine

	Dose Levels, µg /0.25 mL Dose			
Dilution Factor	**Mn Pentavalent (ABCWY)**	**TRUMENBA® (B)**	**NIMENRIX® (ACWY)**	**$AlPO_4$ (diluent)**
1	8 + 1.33	8	1.33	125
2	4 + 0.67	4	0.67	125
4	2 + 0.33	2	0.33	125
8	1 + 0.17	1	0.17	125

For each arm, CBA/J mice (25/group) were subcutaneously immunized in the scruff of the neck using 2-fold dilution dose levels of the respective vaccine (Table 29). Mice were primed with the vaccine at time 0 and boosted at week 2. Sera were collected PD2 at week 3 for testing using two different serum bactericidal assays that utilized human complement (hSBA). One hSBA used an fHBP subfamily A expressing strain (M98250771) and the other an fHBP subfamily B expressing strain (CDC1127).

The hSBA measures antibody-dependent, complement mediated bactericidal activity against *N meningitidis* serogroup B strains. Briefly, test sera at the appropriate dilution were mixed in 96-well microtiter assay plates with freshly prepared bacterial cultures of the *N meningitidis* B strains (subfamily A or B) and human complement. Assay plates were placed on an orbital shaker and mixed for 30 min in a humidified incubator ($37^{o}C/5\%\ CO_2$). Subsequently, aliquots of the assay reaction from each well were transferred to 96-well filter plates for enumeration of surviving bacteria.

Response rates to vaccination were calculated as the percentage of mice in each dosing group (n=25) that respond in hSBAs. When tested at a predetermined dilution level, mouse serum samples that kill $\geq$ 50% of the T_{30} control meningococcal bacteria

are considered responders. The T_{30} control wells contain bacteria and complement but no test serum and are counted at the end of the 30 minute assay incubation.

Table 30 and Table 31 show comparable dose-dependent response rates induced by either TRUMENBA® or Mn Pentavalent for both subfamily A and subfamily B of the *N meningitidis* serotype B strains. As expected, NIMENRIX™ did not induce a functional immune response to Mn B strains.

Table 30 - Subfamily A hSBA responses (% responders)

Dilution Factor [a]	TRUMENBA	NIMENRIX	Penta
8	24%	0%	8%
4	40%	0%	16%
2	52%	0%	56%
1	80%	0%	92%

[a] **See corresponding dose levels in Table 29**

Table 31 - Subfamily B hSBA responses (% responders)

Dilution Factor [a]	TRUMENBA	NIMENRIX	Penta
8	28%	0%	36%
4	56%	0%	60%
2	60%	0%	76%
1	72%	0%	76%

[a] **See corresponding dose levels in Table 29**

The following clauses describe additional embodiments of the invention:

C1. A composition comprising a polypeptide and a *Neisseria meningitidis* serogroup A (MenA) capsular saccharide conjugate; a *Neisseria meningitidis* serogroup C (MenC) capsular saccharide conjugate; a *Neisseria meningitidis* serogroup W_{135} (MenW) capsular saccharide conjugate; and a *Neisseria meningitidis* serogroup Y (MenY) capsular saccharide conjugate.

C2. The composition of clause C1, wherein the MenA capsular saccharide is conjugated to a carrier protein; the MenC capsular saccharide is conjugated to a carrier protein; the MenW capsular saccharide is conjugated to a carrier protein; and the MenY capsular saccharide is conjugated to a carrier protein.

C3. The composition of clause C1, wherein the composition further includes a *Neisseria meningitidis* serogroup X (MenX) capsular saccharide conjugate.

C4. The composition of clause C1, wherein the polypeptide is a factor H binding protein (fHBP).

C5. The composition of clause C1, wherein the polypeptide comprises an amino acid sequence having at least 70% identity to the amino acid sequence set forth in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, and SEQ ID NO: 62.

C6. A composition comprising

a. a first lipidated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 1;

b. a second lipidated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2; and

c. a *Neisseria meningitidis* serogroup A (MenA) capsular saccharide conjugated to a carrier protein.

C7. A composition comprising

a. a first lipidated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 1;

b. a second lipidated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2; and

c. a *Neisseria meningitidis* serogroup C (MenC) capsular saccharide conjugated to a carrier protein.

C8. A composition comprising

a. a first lipidated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 1;

b. a second lipidated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2; and

c. a *Neisseria meningitidis* serogroup W-135 (MenW) capsular saccharide conjugated to a carrier protein.

C9. A composition comprising

a. a first lipidated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 1;

b. a second lipidated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2; and

c. a *Neisseria meningitidis* serogroup Y (MenY) capsular saccharide conjugated to a carrier protein.

C10. A composition comprising

a. a first lipidated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 1;

b. a second lipidated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2;

c. a *Neisseria meningitidis* serogroup A (MenA) capsular saccharide conjugated to a carrier protein;

d. a *Neisseria meningitidis* serogroup C (MenC) capsular saccharide conjugated to a carrier protein;

e. a *Neisseria meningitidis* serogroup W_{135} (MenW) capsular saccharide conjugated to a carrier protein; and

f. a *Neisseria meningitidis* serogroup Y (MenY) capsular saccharide conjugated to a carrier protein.

C11. A composition comprising

a. a first lipidated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 1;

b. a second lipidated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2;

c. a *Neisseria meningitidis* serogroup A (MenA) capsular saccharide conjugated to tetanus toxoid carrier protein (TT);

d. a *Neisseria meningitidis* serogroup C (MenC) capsular saccharide conjugated to tetanus toxoid carrier protein (TT);

e. a *Neisseria meningitidis* serogroup W_{135} (MenW) capsular saccharide conjugated to tetanus toxoid carrier protein (TT); and

f. a *Neisseria meningitidis* serogroup Y (MenY) capsular saccharide conjugated to tetanus toxoid carrier protein (TT).

C12. The composition according to clause C1, wherein the MenA capsular saccharide is conjugated to an adipic acid dihydrazide (ADH) linker by 1-cyano-4-dimethylamino pyridinium tetrafluoroborate chemistry, and wherein the linker is conjugated to tetanus toxoid carrier protein (TT) by carbodiimide chemistry ($MenA_{AH}$-TT conjugate).

C13. The composition according to clause C1, wherein the MenC capsular saccharide is conjugated to an ADH linker by 1-cyano-4-dimethylamino pyridinium tetrafluoroborate chemistry, and wherein the linker is conjugated to tetanus toxoid carrier protein (TT) by carbodiimide chemistry ($MenC_{AH}$-TT conjugate).

C14. The composition according to clause C1, wherein the MenW capsular saccharide is directly conjugated to tetanus toxoid carrier protein (TT) by 1-cyano-4-dimethylamino pyridinium tetrafluoroborate chemistry, in the absence of a linker (MenW-TT conjugate).

C15. The composition according to clause C1, wherein the MenY capsular saccharide is directly conjugated to tetanus toxoid carrier protein (TT) by 1-cyano-4-dimethylamino pyridinium tetrafluoroborate chemistry, in the absence of a linker (MenY-TT conjugate).

C16. The composition according to clause C1, further comprising Tris-HCl.

C17. The composition according to clause C1, further comprising sodium chloride.

C18. The composition according to clause C1, further comprising sucrose.

C19. The composition according to clause C1, further comprising Histidine.

C20. The composition according to clause C1, further comprising polysorbate 80.

C21. The composition according to clause C1, further comprising aluminum.

C22. The composition according to clause C1, further comprising aluminum phosphate.

C23. The composition according to clause C1, wherein the composition comprises about 120 µg/ml of the first polypeptide; about 120 µg/ml of the second polypeptide; about 0.5 mg/ml aluminum as aluminum phosphate; about 0.02 mg polysorbate-80; about 10 mM histidine; and about 150 mM sodium chloride.

C24. The composition according to clause C1, wherein the composition comprises about 60 µg of the first polypeptide; about 60 µg of the second polypeptide; about 5 µg of the MenA capsular saccharide conjugated to about 7.5 µg TT; about 5 µg of the MenC capsular saccharide conjugated to about 7.5 µg TT; about 5 µg of the MenW capsular saccharide conjugated to about 3.75 µg TT; about 5 µg of the MenY capsular saccharide conjugated to about 3.25 µg TT; about 97 µg Tris-HCl, pH 6.8 ± 0.3; 4.69-4.71 mg of sodium chloride; about 28 mg of sucrose; about 0.78 mg of L-Histidine; about 0.02 mg polysorbate-80; about 0.25 mg aluminum; and further comprising 0.5 mL water, per dose.

C25. The composition according to clause C1, wherein the composition is suitable for use in a patient aged 12 to <18 Months or 18 to <24 Months.

C26. The composition according to clause C1, wherein the composition is suitable for use in a patient aged 18 to <24 Months.

C27. The composition according to clause C1, wherein the composition is suitable for use in a patient aged ≥24 Months to <10 Years.

C28. The composition according to clause C20, wherein the composition comprises at least 0.010 mg polysorbate-80 and at most 0.018 mg polysorbate-80.

C29. The composition according to clause C20, wherein the composition comprises at least 0.01 mg polysorbate-80 and at most 0.02 mg polysorbate-80.

C30. The composition according to clause C1, wherein the composition does not further comprise a polypeptide having less than 100% sequence identity to SEQ ID NO: 1.

C31. The composition according to clause C1, wherein the first polypeptide has a total of 258 amino acids.

C32. The composition according to clause C1, wherein the composition does not further comprise a polypeptide having less than 100% sequence identity to SEQ ID NO: 2.

C33. The composition according to clause C1, wherein the second polypeptide has a total of 261 amino acids.

C34. The composition according to clause C1, wherein the composition comprises at most two lipidated polypeptides.

C35. The composition according to clause C1, wherein the composition does not comprise a hybrid protein.

C36. The composition according to clause C1, wherein the composition does not comprise a chimeric protein.

C37. The composition according to clause C1, wherein the composition does not comprise a fusion protein.

C38. The composition according to clause C1, wherein the composition is not lyophilized.

C39. The composition according to clause C1, wherein the composition does not comprise formaldehyde.

C40. The composition according to clause C1, wherein the composition does not comprise diphtheria toxoid or CRM.

C41. The composition according to clause C1, wherein the composition does not comprise a MenA capsular saccharide in the absence of an adipic acid dihydrazide (ADH) linker.

C42. The composition according to clause C1, wherein the composition does not comprise a MenC capsular saccharide in the absence of an adipic acid dihydrazide (ADH) linker.

C43. The composition according to clause C1, wherein the composition is a liquid composition.

C44. The composition according to clause C1, wherein the composition does not further comprise any one of the following immunogenic compositions: MENACTRA(R), MENVEO(R), ADACEL(R), HAVRIX(R), GARDASIL(R), REPEVAX, or any combination thereof.

C45. The composition according to clause C1, wherein the composition does not further comprise a meningococcal A, C, Y and W-135 polysaccharide conjugate (MCV4) composition, wherein the carrier protein is diphtheria toxoid.

C46. The composition according to clause C1, wherein the composition does not further comprise a meningococcal A, C, Y and W-135 polysaccharide conjugate (MCV4) composition, wherein the carrier protein is CRM_{197}.

C47. The composition according to clause C1, wherein the composition does not further comprise a NIMENRIX vaccine.

C48. The composition according to clause C1, wherein the composition does not further comprise a NIMENRIX vaccine, wherein NIMENRIX comprises of a diluent consisting of sodium chloride and water.

C49. A kit comprising (a) a first composition comprising a lipidated MenB rLP2086 subfamily A polypeptide and a lipidated MenB rLP2086 subfamily B polypeptide; and (b) a second composition comprising a *Neisseria meningitidis* serogroup A (MenA) capsular saccharide conjugated to tetanus toxoid carrier protein (TT); a *Neisseria meningitidis* serogroup C (MenC) capsular saccharide conjugated to tetanus toxoid carrier protein (TT); a *Neisseria meningitidis* serogroup W_{135} (MenW) capsular saccharide conjugated to tetanus toxoid carrier protein (TT); and a *Neisseria meningitidis* serogroup Y (MenY) capsular saccharide conjugated to tetanus toxoid carrier protein (TT).

C50. The kit according to clause C49, wherein the first composition is a liquid composition and the second composition is a lyophilized composition.

C51. The kit according to clause C49, wherein the lyophilized composition does not comprise polysorbate 80.

C52. The kit according to clause C49, wherein the kit does not further comprise any one of the following immunogenic compositions: MENACTRA(R), MENVEO(R), ADACEL(R), HAVRIX(R), GARDASIL(R), REPEVAX, or any combination thereof.

C53. The kit according to clause C49, wherein the kit does not further comprise a meningococcal A, C, Y and W-135 polysaccharide conjugate (MCV4) composition, wherein the carrier protein is diphtheria toxoid.

C54. The kit according to clause C49, wherein the kit does not further comprise a meningococcal A, C, Y and W-135 polysaccharide conjugate (MCV4) composition, wherein the carrier protein is CRM_{197}.

C55. The kit according to clause C49, wherein the kit does not further comprise a NIMENRIX vaccine.

C56. The kit according to clause C49, wherein the kit does not further comprise a NIMENRIX(R) vaccine, wherein NIMENRIX(R) comprises of a diluent consisting of sodium chloride and water.

C57. A kit comprising:

a. a liquid composition comprising
   i. a first lipidated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 1; and
   ii. a second lipidated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2; and

b. a lyophilized composition comprising
   i. a *Neisseria meningitidis* serogroup A (MenA) capsular saccharide conjugated to an adipic acid dihydrazide (ADH) linker by 1-cyano-4-dimethylamino pyridinium tetrafluoroborate chemistry, wherein the linker is conjugated to tetanus toxoid carrier protein (TT) by carbodiimide chemistry ($MenA_{AH}$-TT conjugate);
   ii. a *Neisseria meningitidis* serogroup C (MenC) capsular saccharide conjugated to an ADH linker by 1-cyano-4-dimethylamino pyridinium tetrafluoroborate chemistry, wherein the linker is conjugated to tetanus toxoid carrier protein (TT) by carbodiimide chemistry ($MenC_{AH}$-TT conjugate);
   iii. a *Neisseria meningitidis* serogroup W_{135} (MenW) capsular saccharide directly conjugated to tetanus toxoid carrier protein (TT) by 1-cyano-4-dimethylamino pyridinium tetrafluoroborate chemistry, in the absence of a linker (MenW-TT conjugate); and
   iv. a *Neisseria meningitidis* serogroup Y (MenY) capsular saccharide directly conjugated to tetanus toxoid carrier protein (TT) by 1-cyano-4-dimethylamino pyridinium tetrafluoroborate chemistry, in the absence of a linker (MenY-TT conjugate).

C58. The kit according to clause C57, wherein the liquid composition further comprises sodium chloride.

C59. The kit according to clause C57, wherein the liquid composition further comprises L-Histidine.

C60. The kit according to clause C57, wherein the liquid composition further comprises polysorbate 80.

C61. The kit according to clause C57, wherein the liquid composition further comprises aluminum phosphate.

C62. The kit according to clause C57, wherein the liquid composition does not further comprise Tris-HCl.

C63. The kit according to clause C57, wherein the liquid composition does not further comprise sucrose.

C64. The kit according to clause C57, wherein the lyophilized composition further comprises sodium chloride.

C65. The kit according to clause C57, wherein the lyophilized composition does not comprise polysorbate-80.

C66. The kit according to clause C60, wherein the liquid composition comprises at least 0.010 mg polysorbate-80 and at most 0.018 mg polysorbate-80.

C67. The kit according to clause C60, wherein the liquid composition comprises at least 0.010 mg polysorbate-80 and at most 0.02 mg polysorbate-80.

C68. An immunogenic composition comprising:

a. a liquid composition comprising (i) a first lipidated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 1; and (ii) a second lipidated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2; and

b. a lyophilized composition comprising

i. a *Neisseria meningitidis* serogroup A (MenA) capsular saccharide conjugated to an adipic acid dihydrazide (ADH) linker by 1-cyano-4-dimethylamino pyridinium tetrafluoroborate chemistry, wherein the linker is conjugated to tetanus toxoid carrier protein (TT) by carbodiimide chemistry ($MenA_{AH}$-TT conjugate);

ii. a *Neisseria meningitidis* serogroup C (MenC) capsular saccharide conjugated to an ADH linker by 1-cyano-4-dimethylamino pyridinium tetrafluoroborate chemistry, wherein the linker is conjugated to tetanus toxoid carrier protein (TT) by carbodiimide chemistry ($MenC_{AH}$-TT conjugate);

iii. a *Neisseria meningitidis* serogroup W_{135} (MenW) capsular saccharide directly conjugated to tetanus toxoid carrier protein (TT) by 1-cyano-4-dimethylamino pyridinium tetrafluoroborate chemistry, in the absence of a linker (MenW-TT conjugate); and

iv. a *Neisseria meningitidis* serogroup Y (MenY) capsular saccharide directly conjugated to tetanus toxoid carrier protein (TT) by 1-cyano-4-dimethylamino pyridinium tetrafluoroborate chemistry, in the absence of a linker (MenY-TT conjugate).

C69. The immunogenic composition according to clause C68, wherein the lyophilized composition is reconstituted with the liquid composition.

C70. The immunogenic composition according to clause C68, wherein the liquid composition further comprises histidine.

C71. The immunogenic composition according to clause C68, wherein the liquid composition further comprises polysorbate-80.

C72. The immunogenic composition according to clause C68, wherein the liquid composition further comprises aluminum phosphate.

C73. The immunogenic composition according to clause C68, wherein the liquid composition further comprises sodium chloride.

C74. The immunogenic composition according to clause C68, wherein the composition is suitable for use in a patient aged 12 to <18 Months or 18 to <24 Months.

C75. The immunogenic composition according to clause C68, wherein the composition is suitable for use in a patient aged 18 to <24 Months.

C76. The immunogenic composition according to clause C68, wherein the composition is suitable for use in a patient aged ≥24 Months to <10 Years.

C77. An immunogenic composition comprising:

a. a liquid composition comprising (i) a first lipidated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 1; and (ii) a second lipidated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2; and

b. a lyophilized composition comprising

i. a *Neisseria meningitidis* serogroup A (MenA) capsular saccharide conjugated to an adipic acid dihydrazide (ADH) linker by 1-cyano-4-dimethylamino pyridinium tetrafluoroborate chemistry, wherein the linker is conjugated to tetanus toxoid carrier protein (TT) by carbodiimide chemistry ($MenA_{AH}$-TT conjugate);

ii. a *Neisseria meningitidis* serogroup C (MenC) capsular saccharide conjugated to an ADH linker by 1-cyano-4-dimethylamino pyridinium tetrafluoroborate chemistry, wherein the linker is conjugated to tetanus toxoid carrier protein (TT) by carbodiimide chemistry ($MenC_{AH}$-TT conjugate);

iii. a *Neisseria meningitidis* serogroup W_{135} (MenW) capsular saccharide directly conjugated to tetanus toxoid carrier protein (TT) by 1-cyano-4-dimethylamino pyridinium tetrafluoroborate chemistry, in the absence of a linker (MenW-TT conjugate); and

iv. a *Neisseria meningitidis* serogroup Y (MenY) capsular saccharide directly conjugated to tetanus toxoid carrier protein (TT) by 1-cyano-4-dimethylamino pyridinium tetrafluoroborate chemistry, in the absence of a linker (MenY-TT conjugate)

wherein the lyophilized composition is reconstituted with the liquid composition to produce the immunogenic composition.

C78. The immunogenic composition according to clause C77, wherein the liquid composition further comprises aluminum.

C79. The immunogenic composition according to clause C77, wherein the liquid composition further comprises aluminum phosphate.

C80. The immunogenic composition according to clause C77, wherein the lyophilized composition further comprises sodium chloride.

C81. The immunogenic composition according to clause C77, wherein the immunogenic composition comprises at least 0.010 mg polysorbate-80 and at most 0.018 mg polysorbate-80.

C82. The immunogenic composition according to clause C77, wherein the immunogenic composition comprises at least 0.01 mg polysorbate-80 and at most 0.02 mg polysorbate-80.

C83. The immunogenic composition according to clause C77, wherein the lyophilized composition does not contain aluminum.

C84. The immunogenic composition according to clause C78, wherein the first polypeptide and the second polypeptide are bound to the aluminum.

C85. The immunogenic composition according to clause C78, wherein the first polypeptide and the second polypeptide are bound to the aluminum in the immunogenic composition.

C86. The immunogenic composition according to clause C78, wherein the concentration of polypeptides bound to the aluminum in the immunogenic composition is not decreased after 24 hours, as compared to the concentration of polypeptides bound to the aluminum in the liquid composition prior to reconstituting the lyophilized composition.

C87. The immunogenic composition according to clause C77, wherein the concentration of $MenA_{AH}$-TT conjugate in the immunogenic composition is not decreased after 24 hours, as compared to the concentration of the $MenA_{AH}$-TT conjugate in the lyophilized composition.

C88. The immunogenic composition according to clause C77, wherein the concentration of $MenC_{AH}$-TT conjugate in the immunogenic composition is not decreased after 24 hours, as compared to the concentration of the $MenC_{AH}$-TT conjugate in the lyophilized composition.

C89. The immunogenic composition according to clause C77, wherein the concentration of MenW-TT conjugate in the immunogenic composition is not decreased after 24 hours, as compared to the concentration of the MenW-TT conjugate in the lyophilized composition.

C90. The immunogenic composition according to clause C77, wherein the concentration of MenY-TT conjugate in the immunogenic composition is not decreased after 24 hours, as compared to the concentration of the MenY-TT conjugate in the lyophilized composition.

C91. The immunogenic composition according to clause C86, wherein the concentration is decreased by at most 1% after 24 hours, as compared to the respective concentration in the liquid composition prior to reconsititution.

C92. The immunogenic composition according to clause C86, wherein the concentration is decreased by at most 5% after 24 hours, as compared to the respective concentration in the liquid composition prior to reconsititution.

C93. The immunogenic composition according to clause C86, wherein the concentration is decreased by at most 10% after 24 hours, as compared to the respective concentration in the liquid composition prior to reconsititution.

C94. The immunogenic composition according to clause C87-C90, wherein the concentration is decreased by at most 1% after 24 hours, as compared to the respective concentration in the lyophilized composition prior to reconsititution.

C95. The immunogenic composition according to clause C87-C90, wherein the concentration is decreased by at most 5% after 24 hours, as compared to the respective concentration in the lyophilized composition prior to reconsititution.

C96. The immunogenic composition according to clause C87-C90, wherein the concentration is decreased by at most 10% after 24 hours, as compared to the respective concentration in the lyophilized composition prior to reconsititution.

C97. The immunogenic composition according to clause C68 or clause C77, wherein the pH of the reconstituted immunogenic composition is less than the pH of the lyophilized composition, when reconstituted with sodium chloride.

C98. A composition comprising a) a first lipidated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 1, and b) a second lipidated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2.

C99. The composition according to clause C98, wherein the composition further comprises polysorbate-80, aluminum, histidine, and sodium chloride.

C100. The composition according to clause C98, wherein the composition comprises 60 µg of the first lipidated polypeptide and 60 µg of the second lipidated polypeptide.

C101. A method of inducing a bactericidal immune response against a *Neisseria meningitidis* serogroup B subfamily A strain and against a *Neisseria meningitidis* serogroup B subfamily B strain in human, comprising administering to the human an effective amount of the composition according to clause C1.

C102. A method of inducing a bactericidal immune response against a *Neisseria meningitidis* serogroup B subfamily A strain and against a *Neisseria meningitidis* serogroup B subfamily B strain in human, comprising administering to the human an effective amount of the composition according to clause C68.

C103. A method of inducing a bactericidal immune response against a *Neisseria meningitidis* serogroup B subfamily A strain and against a *Neisseria meningitidis* serogroup B subfamily B strain in human, comprising administering to the human an effective amount of the composition according to clause C77.

C104. A method of inducing a bactericidal immune response against a *Neisseria meningitidis* serogroup A, a *Neisseria meningitidis* serogroup C, a *Neisseria meningitidis* serogroup W, and/or a *Neisseria meningitidis* serogroup Y strain in a human, comprising administering to the human an effective amount of the composition according to clause C1.

C105. A method of inducing a bactericidal immune response against a *Neisseria meningitidis* serogroup A, a *Neisseria meningitidis* serogroup C, a *Neisseria meningitidis* serogroup W, and/or a *Neisseria meningitidis* serogroup Y strain in a human, comprising administering to the human an effective amount of the composition according to clause C68.

C106. A method of inducing a bactericidal immune response against a *Neisseria meningitidis* serogroup A, a *Neisseria meningitidis* serogroup C, a *Neisseria meningitidis* serogroup W, and/or a *Neisseria meningitidis* serogroup Y strain in a human, comprising administering to the human an effective amount of the composition according to clause C77.

C107. A method of inducing a bactericidal immune response against a *Neisseria meningitidis* serogroup A, *Neisseria meningitidis* serogroup B, a *Neisseria meningitidis* serogroup C, a *Neisseria meningitidis* serogroup W, and/or a *Neisseria meningitidis* serogroup Y strain in a human, comprising administering to the human an effective amount of the composition according to clause C1.

C108. A method of inducing a bactericidal immune response against a *Neisseria meningitidis* serogroup A, *Neisseria meningitidis* serogroup B, a *Neisseria meningitidis* serogroup C, a *Neisseria meningitidis* serogroup W, and/or a *Neisseria meningitidis* serogroup Y strain in a human, comprising administering to the human an effective amount of the composition according to clause C68.

C109. A method of inducing a bactericidal immune response against a *Neisseria meningitidis* serogroup A, *Neisseria meningitidis* serogroup B, a *Neisseria meningitidis* serogroup C, a *Neisseria meningitidis* serogroup W, and/or a *Neisseria meningitidis* serogroup Y strain in a human, comprising administering to the human an effective amount of the composition according to clause C77.

C110. A method of inducing a bactericidal immune response against a *Neisseria meningitidis* serogroup A, *Neisseria meningitidis* serogroup B, a *Neisseria meningitidis* serogroup C, a *Neisseria meningitidis* serogroup W, a *Neisseria meningitidis* serogroup Y strain, and a *Neisseria meningitidis* serogroup X strain in a human, comprising administering to the human an effective amount of the composition according to clause C1.

C111. A method of inducing a bactericidal immune response against a *Neisseria meningitidis* serogroup A, *Neisseria meningitidis* serogroup B, a *Neisseria meningitidis* serogroup C, a *Neisseria meningitidis* serogroup W, a *Neisseria meningitidis* serogroup Y strain, and a *Neisseria meningitidis* serogroup X strain in a human, comprising administering to the human an effective amount of the composition according to clause C68.

C112. A method of inducing a bactericidal immune response against a *Neisseria meningitidis* serogroup A, *Neisseria meningitidis* serogroup B, a *Neisseria meningitidis* serogroup C, a *Neisseria meningitidis* serogroup W, a *Neisseria meningitidis* serogroup Y strain, and a *Neisseria meningitidis* serogroup X strain in a human, comprising administering to the human an effective amount of the composition according to clause C77.

C113. A method of inducing a bactericidal immune response against a *Neisseria meningitidis* serogroup A, *Neisseria meningitidis* serogroup B, a *Neisseria meningitidis* serogroup C, a *Neisseria meningitidis* serogroup W, a *Neisseria meningitidis* serogroup Y strain, and a *Neisseria meningitidis* serogroup X strain in a human, comprising administering to the human an effective amount of the composition according to clause C98.

C114. A method of inducing a bactericidal immune response against *N. meningitidis* serogroup C strain expressing factor H binding protein A10 in a human, the method comprising administering to the human a composition comprising a first lipidated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 1; a second lipidated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2.

C115. A method of inducing a bactericidal immune response against *N. meningitidis* serogroup W strain expressing factor H binding protein A10 in a human, comprising administering to the human a composition comprising a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 1.

C116. A method of inducing a bactericidal immune response against *N. meningitidis* serogroup W strain expressing factor H binding protein A19 in a human, comprising administering to the human a composition comprising a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 1.

C117. A method of inducing a bactericidal immune response against *N. meningitidis* serogroup A strain expressing factor H binding protein B16 in a human, comprising administering to the human a composition comprising a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2.

C118. A method of inducing a bactericidal immune response against *N. meningitidis* serogroup Y strain expressing factor H binding protein B47 in a human, comprising administering to the human a composition comprising a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2.

C119. A method of inducing a bactericidal immune response against *N. meningitidis* serogroup C strain expressing factor H binding protein A10 in a human, the method comprising administering to the human a composition comprising a first lipidated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 1; a second lipidated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2.

C120. A method of inducing a bactericidal immune response against *N. meningitidis* serogroup W strain expressing factor H binding protein A10 in a human, comprising administering to the human a composition comprising a first lipidated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 1; a second lipidated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2.

C121. A method of inducing a bactericidal immune response against *N. meningitidis* serogroup W strain expressing factor H binding protein A19 in a human, comprising administering to

the human a composition comprising a first lipidated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 1; a second lipidated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2.

C122. A method of inducing a bactericidal immune response against *N. meningitidis* serogroup A strain expressing factor H binding protein B16 in a human, comprising administering to the human a composition comprising a first lipidated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 1; a second lipidated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2.

C123. A method of inducing a bactericidal immune response against *N. meningitidis* serogroup Y strain expressing factor H binding protein B47 in a human, comprising administering to the human a composition comprising a first lipidated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 1; a second lipidated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2

C124. A method of inducing a bactericidal immune response against a *Neisseria meningitidis* serogroup X strain in a human, comprising administering to the human an effective amount of a polypeptide having at least 70% identity to the amino acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, and SEQ ID NO: 62.

C125. A method of inducing a bactericidal immune response against a *Neisseria meningitidis* serogroup X strain in a human, comprising administering to the human an effective amount of the composition according to clause C1.

C126. A method of inducing a bactericidal immune response against a *Neisseria meningitidis* serogroup X strain in a human, comprising administering to the human an effective amount of the composition according to clause C68.

C127. A method of inducing a bactericidal immune response against a *Neisseria meningitidis* serogroup X strain in a human, comprising administering to the human an effective amount of the composition according to clause C77.

C128. A method of inducing a bactericidal immune response against a *Neisseria meningitidis* serogroup X strain in a human, comprising administering to the human an effective amount of the composition according to clause C98.

C129. A method of inducing a bactericidal immune response against *N. meningitidis* serogroup X strain expressing factor H binding protein B49 in a human, comprising administering to the human a composition according to clause C98.

C130. A method of inducing a bactericidal immune response against *N. meningitidis* serogroup X strain expressing factor H binding protein B49 in a human, comprising administering to the human a composition comprising a first lipidated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 1; a second lipidated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2.

C131. The method according to any one of clauses C101-C130, wherein the composition induces a bactericidal titer of serum immunoglobulin that is at least 2-fold higher in the human after receiving the first dose than a bactericidal titer of serum immunoglobulin in the human prior to receiving the first dose, when measured under identical conditions in a serum bactericidal assay using human complement.

C132. The method according to any one of clauses C101-C130, wherein the composition induces a bactericidal titer of serum immunoglobulin that is at least 4-fold higher in the human after receiving the first dose than a bactericidal titer of serum immunoglobulin in the human prior to receiving the first dose, when measured under identical conditions in a serum bactericidal assay using human complement.

C133. The method according to any one of clauses C101-C132, wherein the composition induces a bactericidal titer of serum immunoglobulin that is at least 8-fold higher in the human after receiving the first dose than a bactericidal titer of serum immunoglobulin in the human prior to receiving the first dose, when measured under identical conditions in a serum bactericidal assay using human complement.

C134. The method according to any one of clauses C101-C132, wherein the patient is aged 12 to <18 Months or 18 to <24 Months.

C135. The method according to any one of clauses C101-C132, wherein the patient is aged 18 to <24 Months.

C136. The method according to any one of clauses C101-C132, wherein the patient is aged ≥24 Months to <10 Years.

C137. A method for eliciting an immune response in a patient, comprising: (a) administering an immunogenic composition to the patient when the patient is aged between 12 and 18 months; wherein the immunogenic composition comprises a first lipidated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 1; and a second lipidated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2.

C138. A method for eliciting an immune response in a patient, comprising: (a) administering an immunogenic composition to the patient when the patient is aged between 18 and 24 months; wherein the immunogenic composition comprises a first lipidated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 1; and a second lipidated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2.

C139. A method for eliciting an immune response in a patient, comprising: (a) administering an immunogenic composition to the patient when the patient is aged between 24 months and 10 years; wherein the immunogenic composition comprises a first lipidated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 1; and a second lipidated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2.

C140. The method according to any one of clauses C137-C139, wherein the composition further comprises polysorbate-80, aluminum, histidine, and sodium chloride.

WHAT IS CLAIMED IS:

1. A composition comprising a factor H binding protein (fHBP) and a *Neisseria meningitidis* serogroup A (MenA) capsular saccharide conjugated to a carrier protein; a *Neisseria meningitidis* serogroup C (MenC) capsular saccharide conjugated to a carrier protein; a *Neisseria meningitidis* serogroup W_{135} (MenW) capsular saccharide conjugated to a carrier protein; and a *Neisseria meningitidis* serogroup Y (MenY) capsular saccharide conjugated to a carrier protein.

2. The composition according to claim 1, wherein the composition comprises a first fHBP polypeptide and a second fHBP polypeptide.

3. The composition according to claim 1, wherein the composition further comprises aluminum phosphate.

4. The composition according to claim 2, wherein at least 90% of the first polypeptide is bound to aluminum in the composition.

5. The composition according to claim 2, wherein at least 90% of the second polypeptide is bound to aluminum in the composition.

6. The composition according to claim 1, wherein the composition further comprises polysorbate-80.

7. The composition according to claim 2, wherein the composition comprises about 120 µg/ml of the first polypeptide; about 120 µg/ml of the second polypeptide; about 0.5 mg/ml aluminum as aluminum phosphate; about 0.02 mg polysorbate-80; about 10 mM histidine; and about 150 mM sodium chloride.

8. The composition according to claim 2, wherein the composition comprises about 60 µg of the first polypeptide; about 60 µg of the second polypeptide; about 5 µg of the MenA capsular saccharide conjugated to about 7.5 µg TT; about 5 µg of the MenC capsular saccharide conjugated to about 7.5 µg TT; about 5 µg of the MenW capsular saccharide conjugated to about 3.75 µg TT; about 5 µg of the MenY capsular saccharide conjugated to about 3.25 µg TT; about 97 µg Tris-HCl, pH 6.8 $\pm$ 0.3; 4.69-4.71 mg of sodium chloride; about 28 mg of sucrose; about 0.78 mg of L-Histidine; about 0.02 mg polysorbate-80; about 0.25 mg aluminum; and further comprising 0.5 mL water, per dose.

9. The composition according to claim 2, wherein the first polypeptide consists of the amino acid sequence set forth in SEQ ID NO: 1.

10. The composition according to claim 2, wherein the second polypeptide consists of the amino acid sequence set forth in SEQ ID NO: 2.

11. The composition according to claim 1, wherein the composition does not comprise a hybrid protein.

12. The composition according to claim 1, wherein the composition does not comprise a fusion protein.

13. The composition according to claim 1, wherein the composition is not lyophilized.

14. The composition according to claim 1, wherein the composition does not comprise formaldehyde.

15. The composition according to claim 1, wherein the composition does not comprise diphtheria toxoid or CRM.

16. The composition according to claim 1, wherein the composition does not comprise a MenA capsular saccharide in the absence of an adipic acid dihydrazide (ADH) linker.

17. The composition according to claim 2, wherein the first polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 1; the second polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 2; the *Neisseria meningitidis* serogroup A (MenA) capsular saccharide is conjugated to an adipic acid dihydrazide (ADH) linker by 1-cyano-4-dimethylamino pyridinium tetrafluoroborate chemistry, wherein the linker is conjugated to tetanus toxoid carrier protein (TT) by carbodiimide chemistry ($MenA_{AH}$-TT conjugate); the *Neisseria meningitidis* serogroup C (MenC) capsular saccharide is conjugated to an ADH linker by 1-cyano-4-dimethylamino pyridinium tetrafluoroborate chemistry, wherein the linker is conjugated to tetanus toxoid carrier protein (TT) by carbodiimide chemistry ($MenC_{AH}$-TT conjugate); the *Neisseria meningitidis* serogroup W_{135} (MenW) capsular saccharide is directly conjugated to tetanus toxoid carrier protein (TT) by 1-cyano-4-dimethylamino pyridinium tetrafluoroborate chemistry, in the absence of a linker (MenW-TT conjugate); and the *Neisseria meningitidis* serogroup Y (MenY) capsular saccharide is directly conjugated to tetanus toxoid carrier protein (TT) by 1-cyano-4-dimethylamino pyridinium tetrafluoroborate chemistry, in the absence of a linker (MenY-TT conjugate).

18. The composition according to claim 17, wherein the composition further comprises Tris-HCl; sodium chloride; sucrose; L-Histidine; polysorbate 80; and aluminum phosphate.

19. A method of inducing an immune response against a *Neisseria meningitidis* serogroup B subfamily A strain and against a *Neisseria meningitidis* serogroup B subfamily B strain in human, comprising administering to the human an effective amount of the composition according to claim 1.

20. A method of inducing an immune response against a *Neisseria meningitidis* serogroup A, a *Neisseria meningitidis* serogroup C, a *Neisseria meningitidis* serogroup W, and/or a *Neisseria meningitidis* serogroup Y strain in a human, comprising administering to the human an effective amount of the composition according to claim 1.

21. A method of inducing an immune response against a *Neisseria meningitidis* serogroup A, *Neisseria meningitidis* serogroup B, a *Neisseria meningitidis* serogroup C, a *Neisseria meningitidis* serogroup W, and/or a *Neisseria meningitidis* serogroup Y strain in a human, comprising administering to the human an effective amount of the composition according to claim 1.

22. A method of inducing an immune response against a *Neisseria meningitidis* serogroup A, *Neisseria meningitidis* serogroup B, a *Neisseria meningitidis* serogroup C, a *Neisseria meningitidis* serogroup W, a *Neisseria meningitidis* serogroup Y strain, and/or a *Neisseria meningitidis* serogroup X strain in a human, comprising administering to the human an effective amount of the composition according to claim 1.

23. The method according to any one of claims 25-27, wherein the patient is aged 12 to <18 Months or 18 to <24 Months.

24. The method according to any one of claims 25-27, wherein the patient is aged 18 to <24 Months.

25. The method according to any one of claims 25-27, wherein the patient is aged ≥24 Months to <10 Years.

FIG. 1

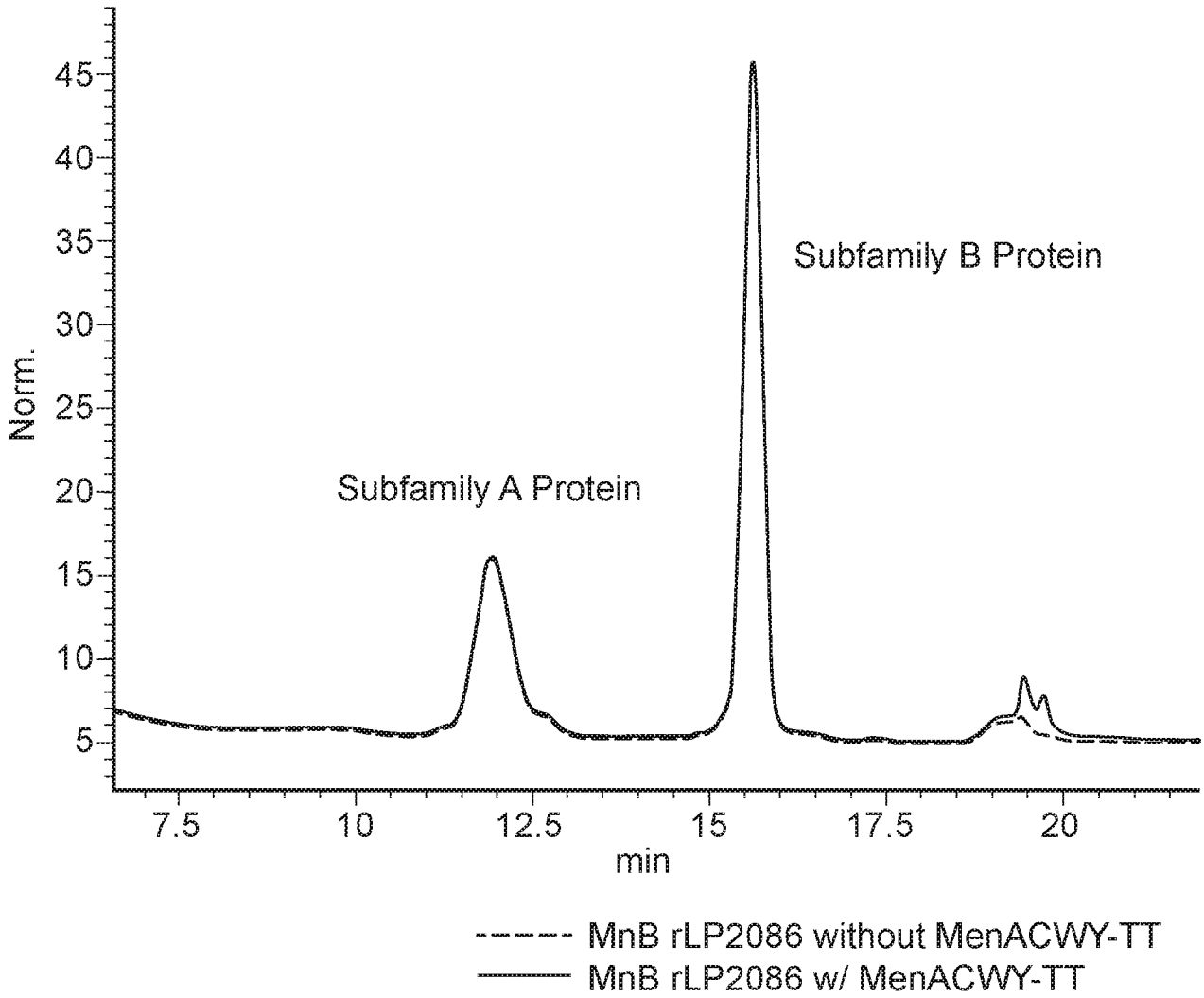

Norm.
45
40
35
30
25
20
15
10
5
Subfamily A Protein
Subfamily B Protein
7.5
10
12.5
15
17.5
20
min
MnB rLP2086 without MenACWY-TT
MnB rLP2086 w/ MenACWY-TT

FIG. 2

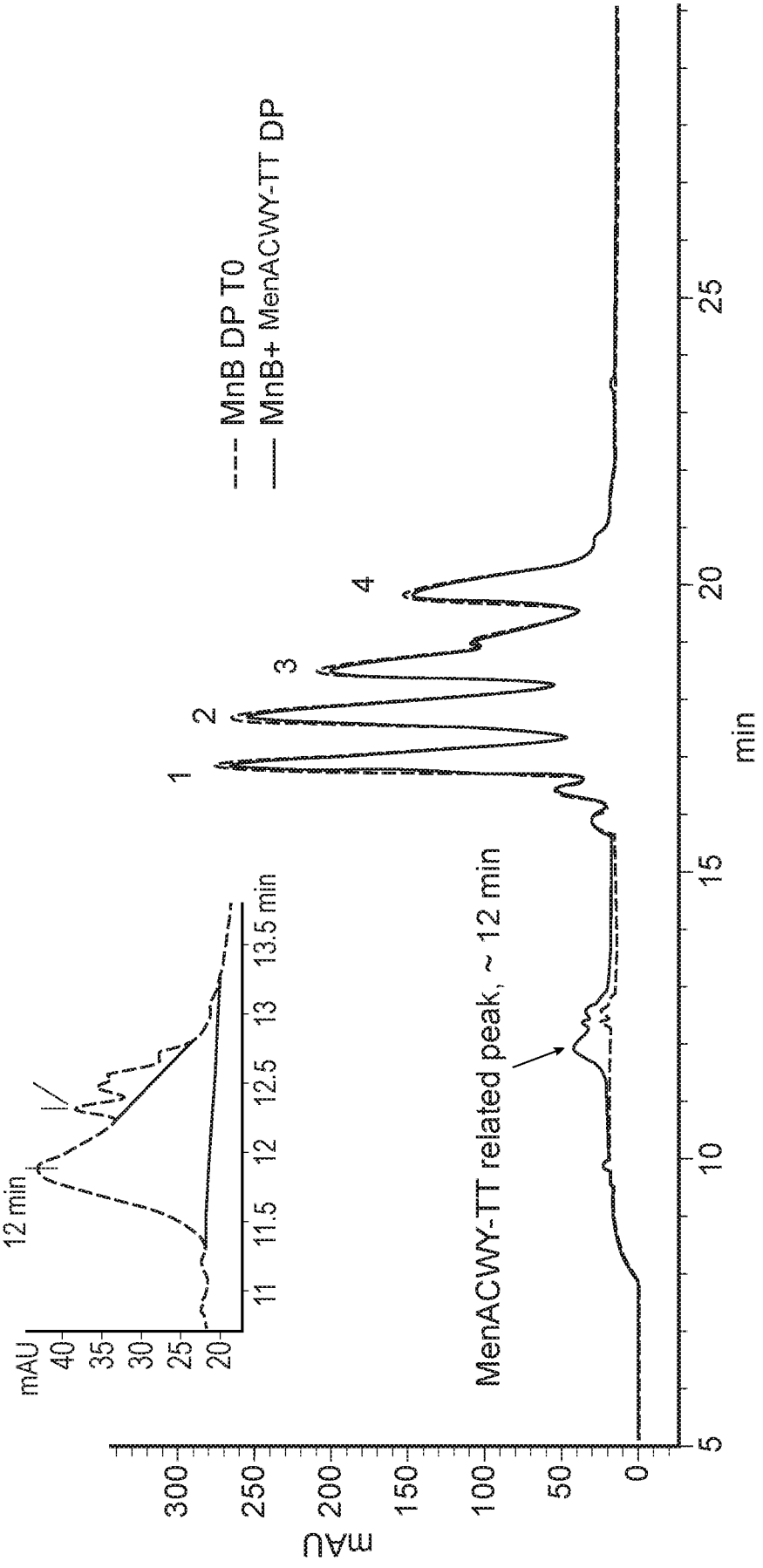

MnB DP T0
MnB+ MenACWY-TT DP
1
2
3
4
MenACWY-TT related peak, ~ 12 min
12 min
mAU
min
mAU
5
10
15
20
25
0
50
100
150
200
250
300
11
11.5
12
12.5
13
13.5 min
20
25
30
35
40

FIG. 3

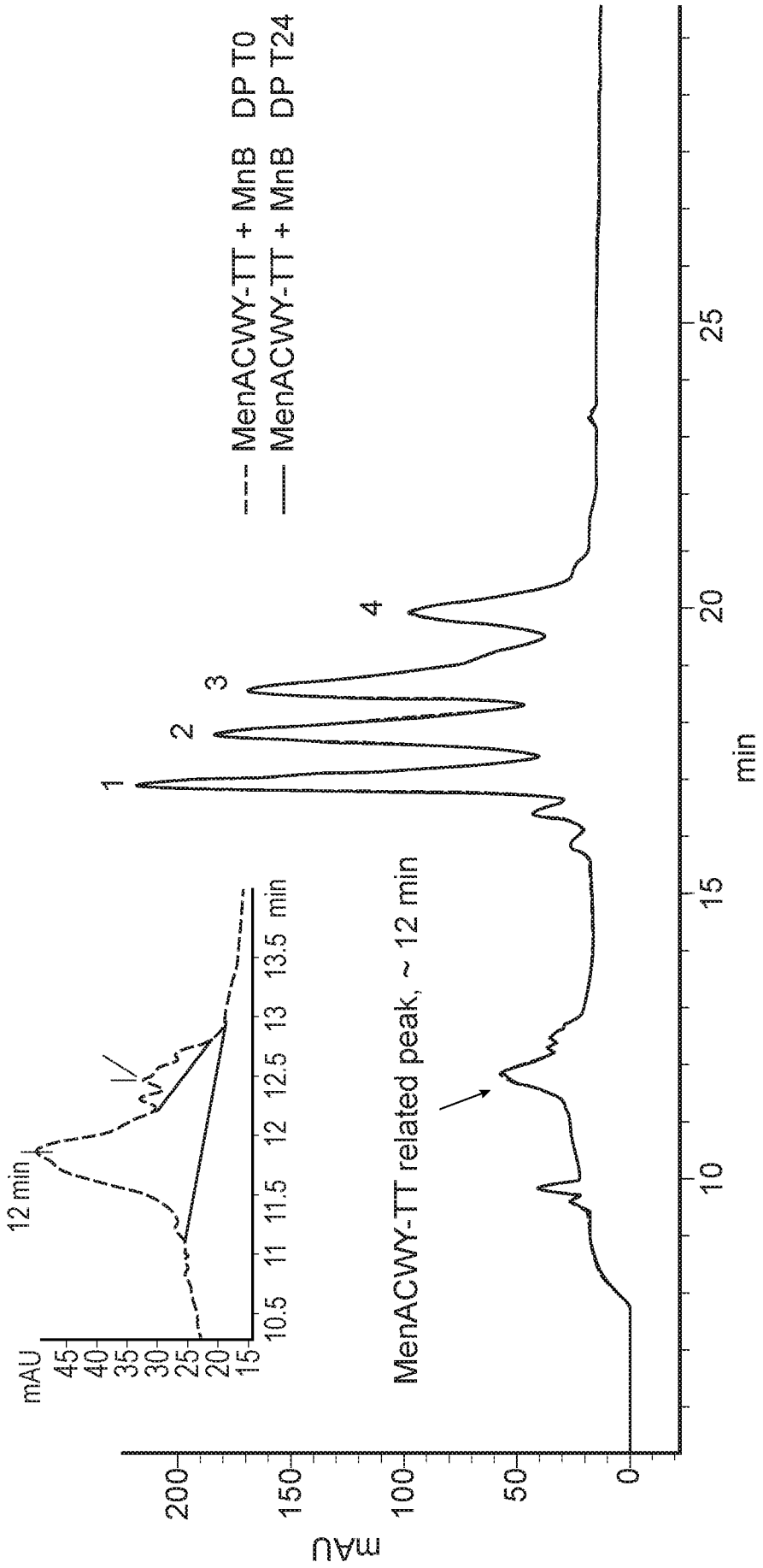

MenACWY-TT + MnB DP T0
MenACWY-TT + MnB DP T24
1
2
3
4
MenACWY-TT related peak, ~ 12 min
12 min
mAU
mAU
min
0
50
100
150
200
5
10
15
20
25
10.5
11
11.5
12
12.5
13
13.5

FIG. 4A

```
  1 CGSSGGGGVA ADIGTGLADA LTAPLDHKDK GLKSLTLEDS ISQNGTLTLS
 51 AQGAEKTFKV GDKDNSLNTG KLKNDKISRF DFVQKIEVDG QTITLASGEF
101 QIYKQDHSAV VALQIEKINN PDKIDSLINQ RSFLVSGLGG EHTAFNQLPS
151 GKAEYHGKAF SSDDAGGKLT YTIDFAAKQG HGKIEHLKTP EQNVELASAE
201 LKADEKSHAV ILGDTRYGSE EKGTYHLALF GDRAQEIAGS ATVKIREKVH
251 EIGIAGKQ (SEQ ID NO: 1)
```

FIG. 4B

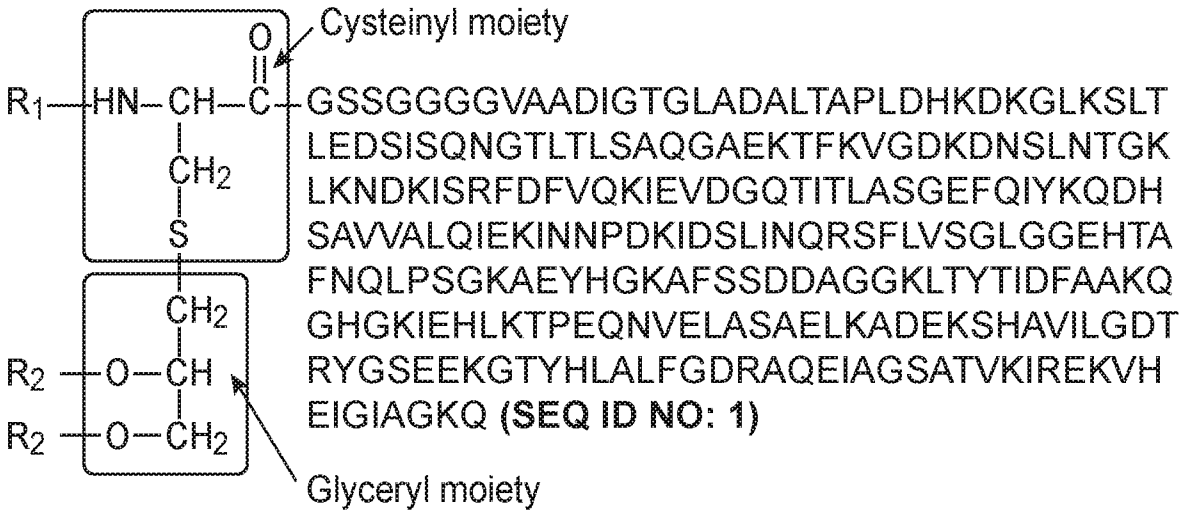


FIG. 5A

```
  1 CGSSGGGGSG GGGVTADIGT GLADALTAPL DHKDKGLKSL TLEDSISQNG
 51 TLTLSAQGAE KTYGNGDSLN TGKLKNDKVS RFDFIRQIEV DGQLITLESG
101 EFQVYKQSHS ALTALQTEQE QDPEHSEKMV AKRRFRIGDI AGEHTSFDKL
151 PKDVMATYRG TAFGSDDAGG KLTYTIDFAA KQGHGKIEHL KSPELNVDLA
201 VAYIKPDEKH HAVISGSVLY NQDEKGSYSL GIFGEKAQEV AGSAEVETAN
251 GIHHIGLAAK Q (SEQ ID NO: 2)
```

FIG. 5B

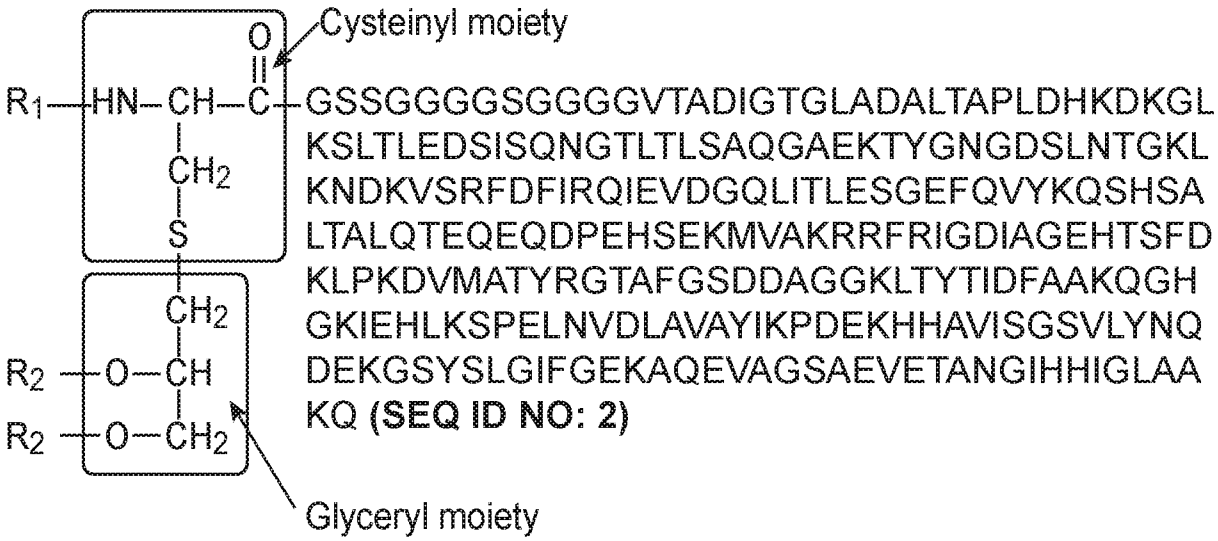


FIG. 6A

CSSGGGGVAA DIGAGLADAL TAPLDHKDKS LQSLTLDQSV RKNEKLKLAA
QGAEKTYGNG DSLNTGKLKN DKVSRFDFIR QIEVDGQLIT LESGEFQVYK
QSHSALTALQ TEQVQDSEHS GKMVAKRQFR IGDIAGEHTS FDKLPEGGRA
TYRGTAFGSD DASGKLTYTI DFAAKQGHGK IEHLKSPELN VDLAASDIKP
DKKRHAVISG SVLYNQAEKG SYSLGIFGGQ AQEVAGSAEV ETANGIRHIG
LAAKQ **(SEQ ID NO: 26)**

FIG. 6B

CSSGGGGVAA DIGAGLADAL TAPLDHKDKS LQSLTLDQSV RKNEKLKLAA
QGAEKTYGNG DSLNTGKLKN DKVSRFDFIR QIEVDGQLIT LESGEFQIYK
QDHSAVVALQ IEKINNPDKI DSLINQRSFL VSGLGGEHTA FNQLPSGKAE
YHGKAFSSDD PNGRLHYSID FTKKQGYGRI EHLKTPEQNV ELASAELKAD
EKSHAVILGD TRYGGEEKGT YHLALFGDRA QEIAGSATVK IREKVHEIGI
AGKQ **(SEQ ID NO: 27)**

FIG. 6C

CSSGGGGVAA DIGAGLADAL TAPLDHKDKS LQSLTLDQSV RKNEKLKLAA
QGAEKTYGNG DSLNTGKLKN DKVSRFDFIR QIEVDGQLIT LESGEFQIYK
QDHSAVVALQ IEKINNPDKI DSLINQRSFL VSGLGGEHTA FNQLPDGKAE
YHGKAFSSDD AGGKLTYTID FAAKQGHGKI EHLKTPEQNV ELAAAELKAD
EKSHAVILGD TRYGSEEKGT YHLALFGDRA QEIAGSATVK IGEKVHEIGI
AGKQ **(SEQ ID NO: 28)**

FIG. 6D

CSSGGGGVAA DIGAGLADAL TAPLDHKDKS LQSLTLDQSV RKNEKLKLAA
QGAEKTYGNG DSLNTGKLKN DKVSRFDFIR QIEVDGQLIT LESGEFQIYK
QDHSAVVALQ IEKINNPDKI DSLINQRSFL VSGLGGEHTA FNQLPSGKAE
YHGKAFSSDD PNGRLHYSID FTKKQGYGRI EHLKTPEQNV ELASAELKAD
EKSHAVILGD TRYGGEEKGT YHLALFGDRA QEIAGSATVK IREKVHEIGI
AGKQ **(SEQ ID NO: 27)**

FIG. 6E

CSSGGGGSGG GGVAADIGTG LADALTAPLD HKDKGLQSLM LDQSVRKNEK
LKLSAQGAEK TYGNGDSLNT GKLKNDKISR FDFIHQIEVD GQLITLESGE
FQVYKQSHSA LTALQTEQVQ DSEHSEKMVA KRRFKIGDIA GEHTSFDKLP
KDVMATYRGT AFGSDDAGGK LTYTIDFAAK QGHGKIEHLK SPELNVELAA
AYIKPDEKRH AVISGSVLYN QDEKGSYSLG IFGGQAQEVA GSAEVETANG
IHHIGLAAKQ **(SEQ ID NO: 29)**

FIG. 6F

CSSGGGGVAA DIGAGLADAL TAPLDHKDKG LQSLTLDQSV RKNEKLKLAA
QGAEKTYGNG DSLNTGKLKN DKVSRFDFIR QIEVDGQLIT LESGEFQVYK
QSHSALTALQ TEQEQDPEHS GKMVAKRRFK IGDIAGEHTS FDKLPKDVMA
TYRGTAFGSD DAGGKLTYTI DFAAKQGHGK IEHLKSPELN VDLAVAYIKP
DEKHHAVISG SVLYNQDEKG SYSLGIFGEK AQEVAGSAEV KTANGIHHIG
LAAKQ **(SEQ ID NO: 30)**

FIG. 7

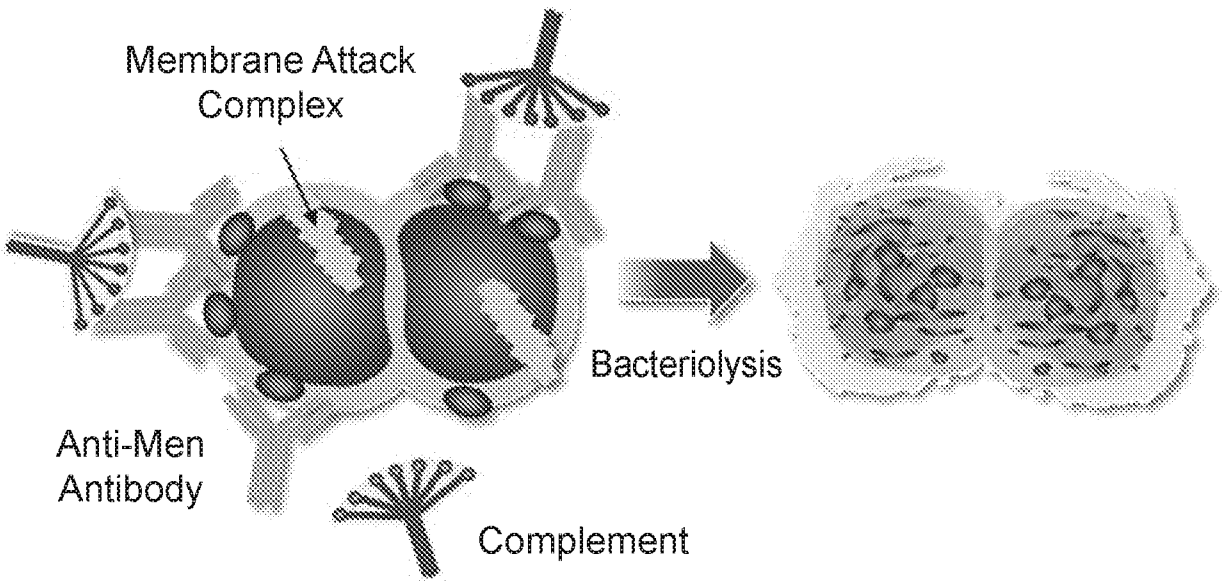


FIG. 8

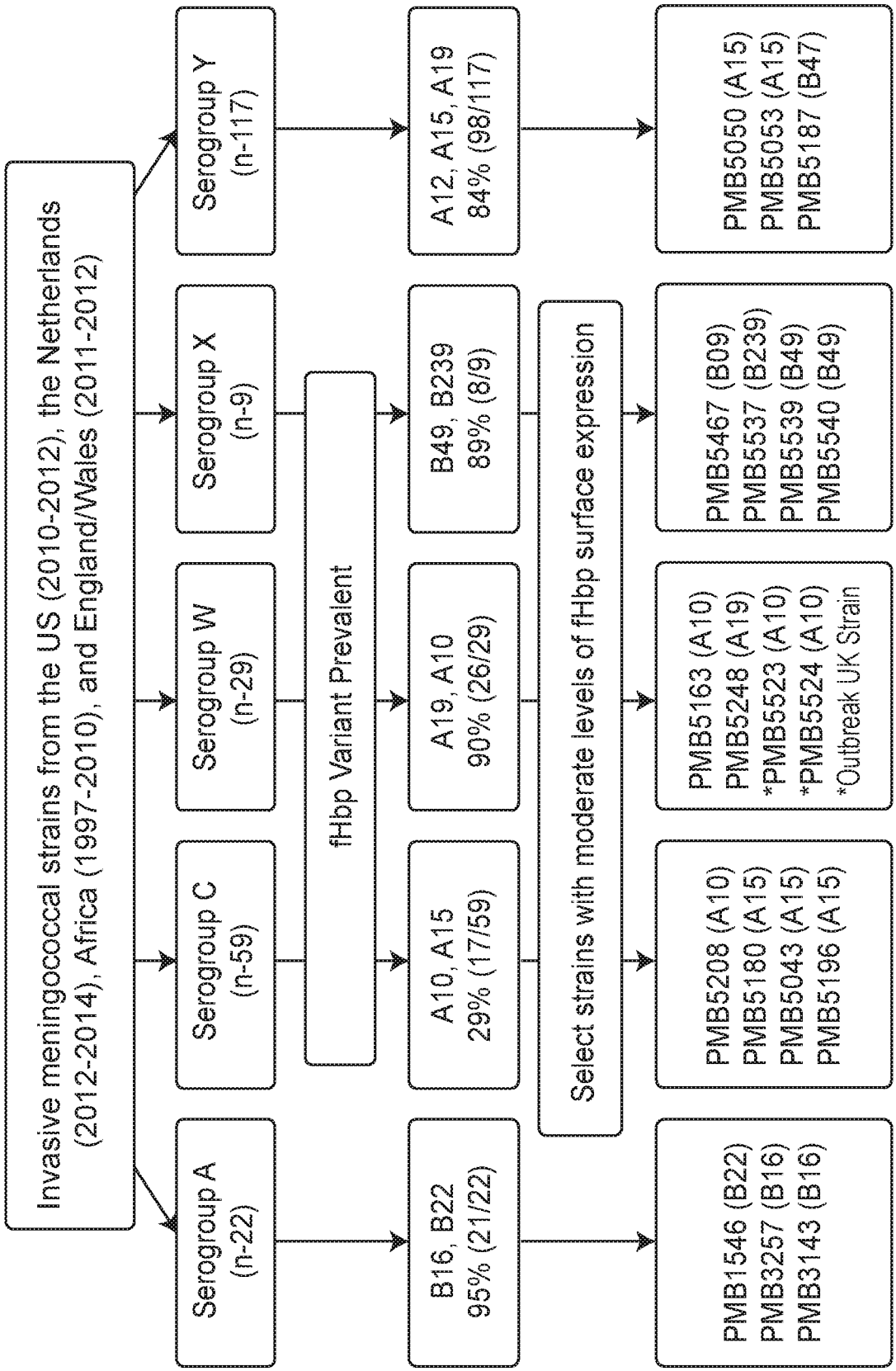

Invasive meningococcal strains from the US (2010-2012), the Netherlands (2012-2014), Africa (1997-2010), and England/Wales (2011-2012)
Serogroup A (n-22)
Serogroup C (n-59)
Serogroup W (n-29)
Serogroup X (n-9)
Serogroup Y (n-117)
fHbp Variant Prevalent
B16, B22 95% (21/22)
A10, A15 29% (17/59)
A19, A10 90% (26/29)
B49, B239 89% (8/9)
A12, A15, A19 84% (98/117)
Select strains with moderate levels of fHbp surface expression
PMB1546 (B22)
PMB3257 (B16)
PMB3143 (B16)
PMB5208 (A10)
PMB5180 (A15)
PMB5043 (A15)
PMB5196 (A15)
PMB5163 (A10)
PMB5248 (A19)
*PMB5523 (A10)
*PMB5524 (A10)
*Outbreak UK Strain
PMB5467 (B09)
PMB5537 (B239)
PMB5539 (B49)
PMB5540 (B49)
PMB5050 (A15)
PMB5053 (A15)
PMB5187 (B47)

FIG. 9

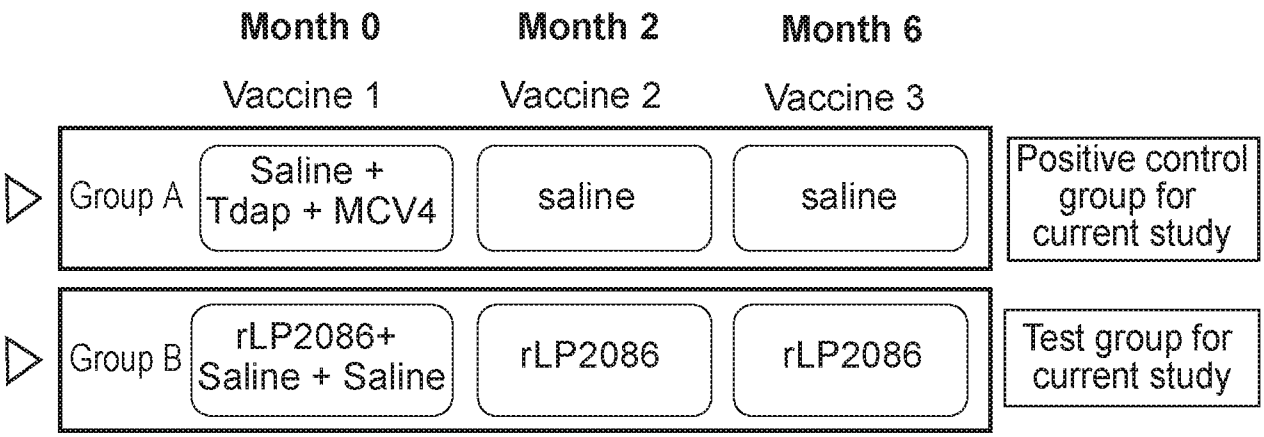

Month 0
Month 2
Month 6
Vaccine 1
Vaccine 2
Vaccine 3
Group A
Saline + Tdap + MCV4
saline
saline
Positive control group for current study
Group B
rLP2086+ Saline + Saline
rLP2086
rLP2086
Test group for current study

FIG. 10

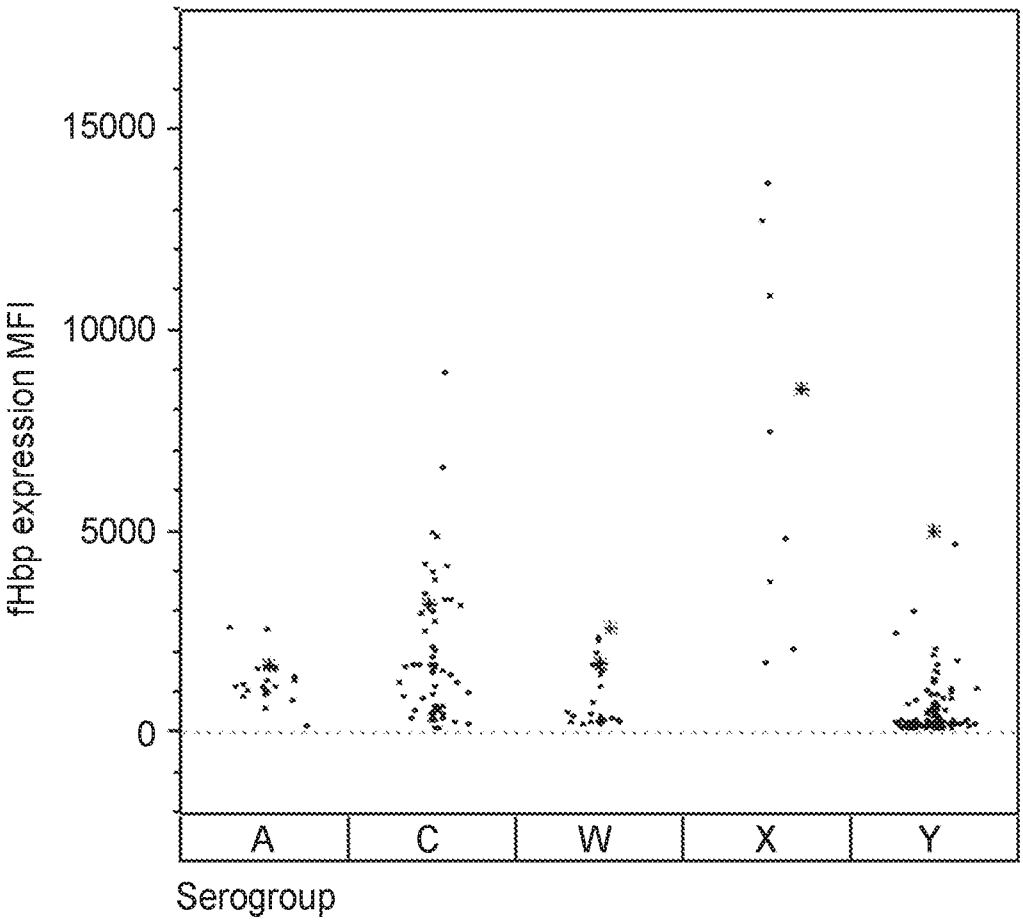
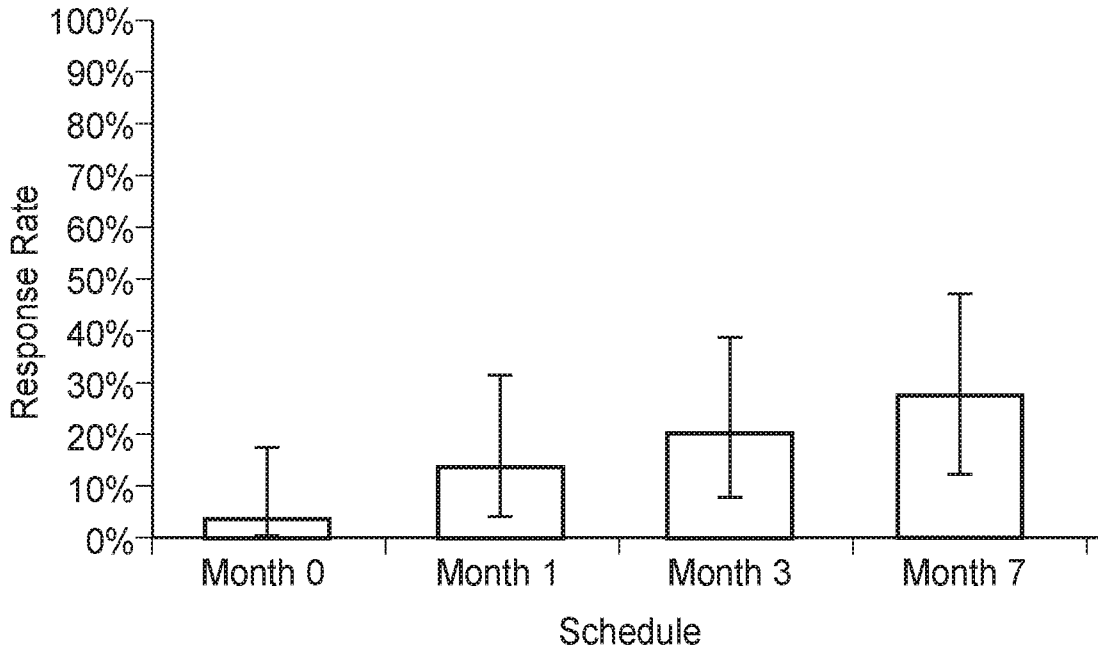


FIG. 11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Response Rate
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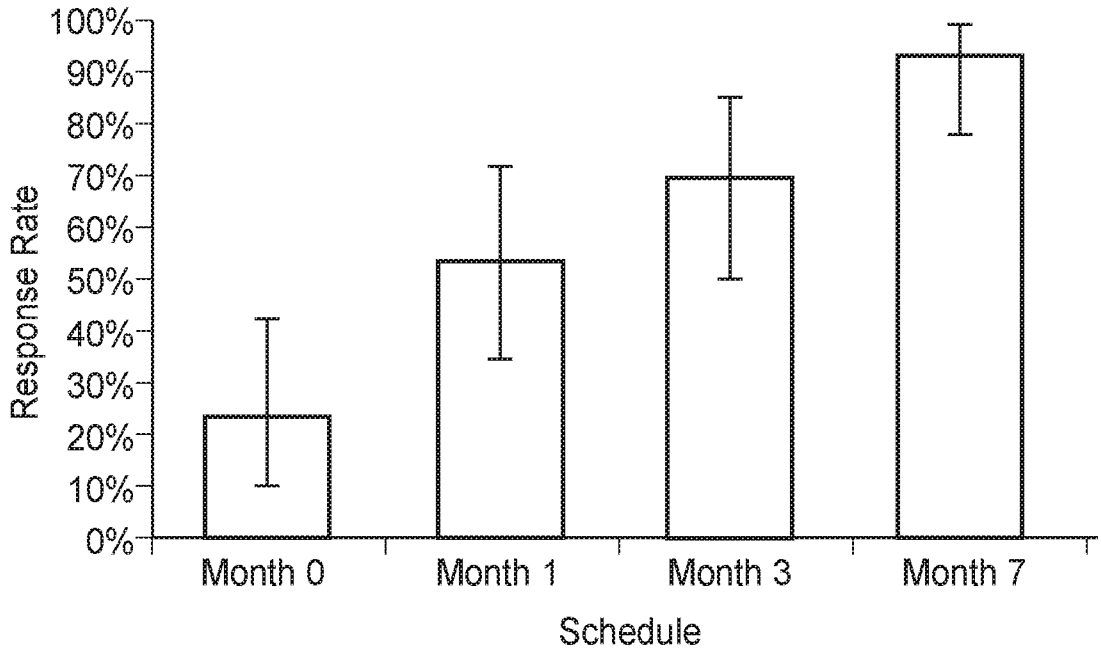
Month 3
Month 7
Schedule

FIG. 12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Response Rate
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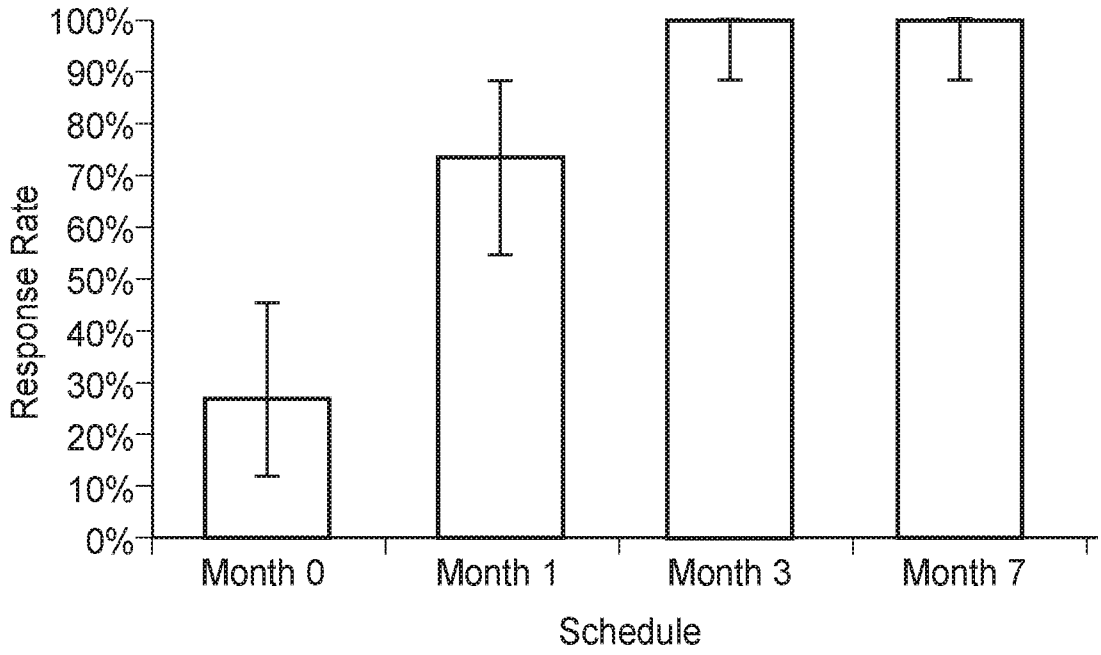
Schedule

FIG. 13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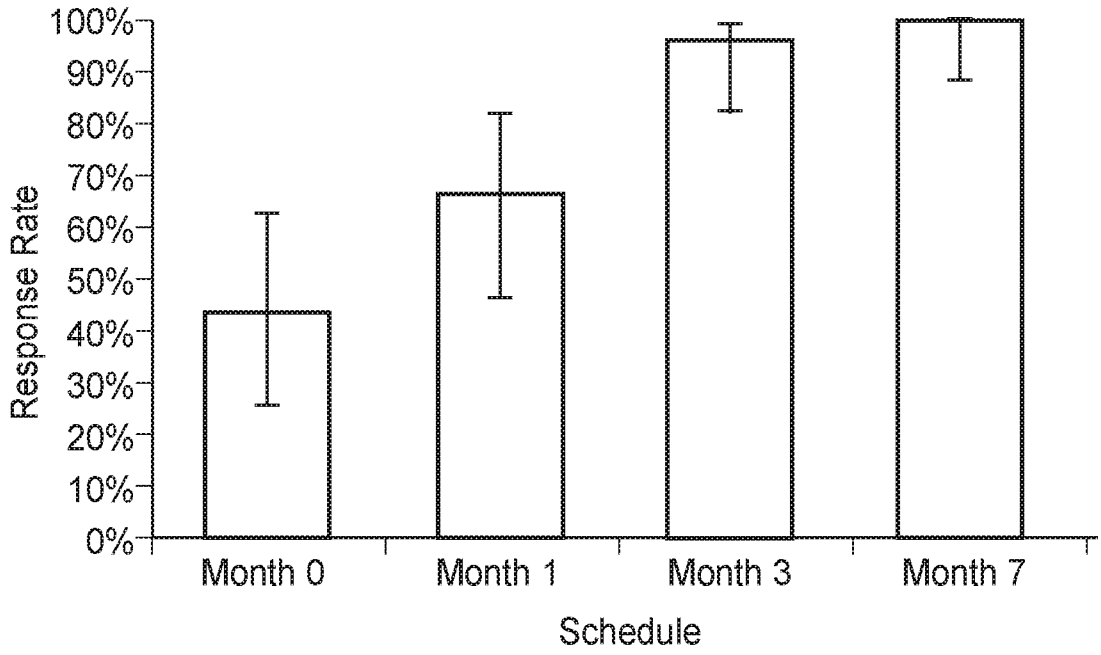
Schedule

FIG. 14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Response Rate
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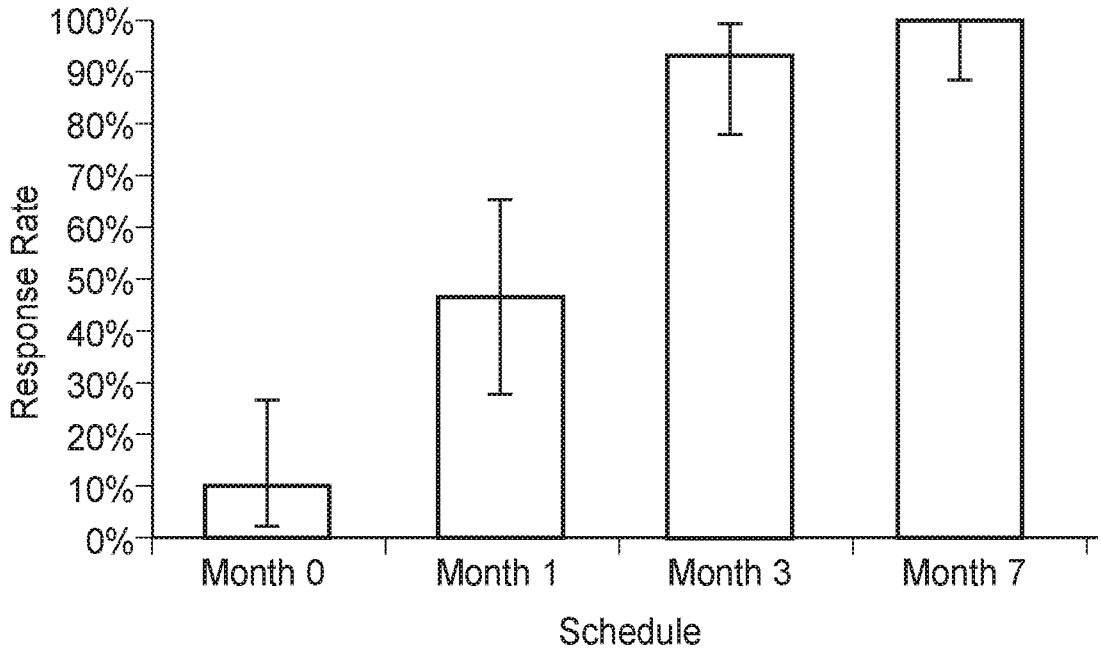
Month 7
Schedule

FIG. 15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Response Rate
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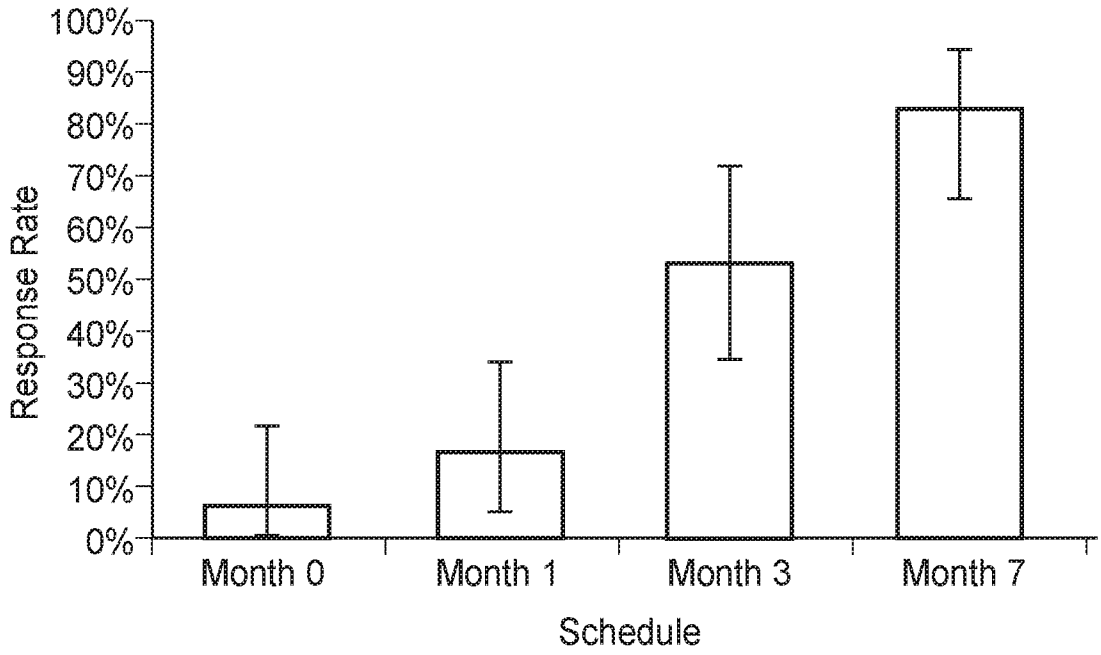
Month 3
Month 7
Schedule

FIG. 16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Response Rate
Month 0
Month 1
Month 3
Month 7
Schedule