737. The pharmaceutical composition of claim 734, wherein the at least one additional serotype is selected from the group consisting of 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 24F, 33F, and 35B.

738. The pharmaceutical composition of claim 734, wherein the at least one additional serotype is selected from the group consisting of 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B.

739. The pharmaceutical composition of claim 734, wherein the at least one additional serotype is selected from the group consisting of 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B, and 38.

740. The pharmaceutical composition of claim 734, wherein the at least one additional serotype consists of 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.

741. The pharmaceutical composition of claim 734, wherein the at least one additional serotype consists of 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B.

742. The pharmaceutical composition of claim 734, wherein the at least one additional serotype consists of 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B.

743. The pharmaceutical composition of claim 734, wherein the at least one additional serotype consists of 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B and 38.

744. The pharmaceutical composition of claim 734, wherein the at least one additional serotype consists of 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.

745. The pharmaceutical composition of claim 734, wherein the at least one additional serotype consists of 3, 4, 7F, 8, 9N, 11A, 12F, 15A, 15B, 15C, 16F, 19A, 22F, 23A, 23B, and 35B.

746. The pharmaceutical composition of claim 734, wherein the at least one additional serotype consists of 1, 3, 4, 6A, 7F, 8, 9N, 10A, 11A, 12F, 14, 19A, 19F, 22F, 23A, 23B, 23F, 24F, and 33F.

747. The pharmaceutical composition of claim 734, wherein the at least one additional serotype consists of 3, 4, 6B, 10A, 15A, 15B, 19A, 19F, 22F, 23F, 24F, and 35B.

748. The pharmaceutical composition of claim 721-747, wherein the immunogenic saccharide-polypeptide conjugate comprises the capsular polysaccharide.

749. The pharmaceutical composition of claim 721-748 wherein the immunogenic saccharide-polypeptide conjugate comprises a fragment of the capsular polysaccharide.

Petitioner Merck Ex-1004, 0435

750. The pharmaceutical composition of claim 749, wherein the fragment of the capsular polysaccharide comprises a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, or a hexasaccharide.

751. The pharmaceutical composition of any one of claims 721-750, wherein the polypeptide is an oligopeptide.

752. The pharmaceutical composition of any one of claims 721-751, wherein the polypeptide comprises CRM197, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, non-typable H. influenzae P4 protein, non-typable H. influenzae P6 protein, M catarrhalis uspA, keyhole limpet haemocyanin (KLH), OMPC from N. meningitidis, the purified protein derivative of tuberculin(PPD), and protein D from H. influenzae, any fragment thereof, or any combination thereof.

753. The pharmaceutical composition of any one of claims 721-752, wherein a toxin activity of a polypeptide is at least partly mitigated.

754. The pharmaceutical composition of any one of claims 721-753, further comprising an adjuvant.

755. The pharmaceutical composition of claim 754, wherein the adjuvant is an aluminum-cation comprising adjuvant.

756. The pharmaceutical composition of claim 755, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide.

757. The pharmaceutical composition of any one of claims 721-756, wherein the pharmaceutical composition is in a form of an intramuscularly injectable composition, intradermally injectable composition, subcutaneously injectable composition, or an intranasally administrable composition.

758. The pharmaceutical composition of any one of claims 721-757, further comprising a chelating agent.

759. The pharmaceutical composition of claim 758, wherein the chelating agent is ethylenediainetetraacetic acid (EDTA).

760. The pharmaceutical composition of any one of claims 721-759, wherein the pharmaceutical composition is in the form of a suspension.

Petitioner Merck Ex-1004, 0436

761. The pharmaceutical composition of any one of claims 721-760, wherein the pharmaceutical composition is in the form of a homogenous mixture or a heterogeneous mixture.

762. The pharmaceutical composition of claim 758, wherein the pharmaceutical composition is in the form of a homogenous mixture.

763. The pharmaceutical composition of any one of claims 721 or 734-747, which is contained in a pre-filled syringe.

764. The pharmaceutical composition of any one of claims 721-763, further comprising a surfactant , an emulsifier, or a combination thereof.

765. The pharmaceutical composition of claim 764, comprising the surfactant wherein the surfactant is polysorbate, polymer glycol, a sorbitan ester, or any combination thereof.

766. The pharmaceutical composition of claim 765, comprising the polysorbate wherein the polysorbate is polysorbate 80.

767. The pharmaceutical composition of claim 766, comprising the polymer glycol wherein the polymer glycol is polyethylene glycol.

768. The pharmaceutical composition of claim of any one of claims 721-767, further comprising a buffering agent.

769. The pharmaceutical composition of claim 768, wherein the buffering agent is a succinate buffer agent, a tris-acetate- ethylenediainetetraacetic acid (TAE) buffering agent, a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffering agent, a phosphate buffering agent, a sodium phosphate buffering agent, a potassium phosphate buffering agent, or any combination thereof.

770. The pharmaceutical composition of any one of claims 721-769, further comprising sterile water.

771. The pharmaceutical composition of any one of claim 721-770, wherein when the pharmaceutical composition is administered to a plurality of humans, the administration elicits at least one of the following side effects in less than about 10% of the plurality of humans: fatigue, headache, muscle pain, joint pain, decreased appetite, chills, or rash.

772. The pharmaceutical composition of any one of claims 721-771, wherein when the pharmaceutical composition is administered intramuscularly to a human, the pharmaceutical composition elicits an immune response that lasts from about 1 week to about 30 years.

Petitioner Merck Ex-1004, 0437

773. The pharmaceutical composition of any one of claims 721-771, biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C and the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure.

774. The pharmaceutical composition of any of claims 721-773 in unit dose form.

775. A kit comprising of a pharmaceutical composition of any of claims 566-606 or 721-744 contained in a container.

776. The kit of claim 775, further comprising instructions for use.

777. The kit of any one of claims 775-776, wherein the container is a syringe.

778. The kit of any one of claims 775-777, wherein the container is a vial comprising a cap, wherein the cap comprises a rubber septum.

779. The composition of, or composition employed in the method of, any claim, further comprising a preservative.

780. The composition of, or composition employed in the method of, any claim, further comprising an anti-fungal compound or a salt thereof.

781. The composition of, or composition employed in the method of, any claim, further comprising a salt.

782. The composition of, or composition employed in the method of, claim 781, wherein the salt is an inorganic salt.

783. The composition of, or composition employed in the method of, any claim, wherein the immunogenic saccharide-polypeptide conjugate is isolated and purified.

784. The composition of, or composition employed in the method of, any claim, which is a vaccine.

785. The method of any claim which is a method of vaccinating a subject.

786. The method of any claim, wherein the subject is a human.

787. The method of any claim, wherein the subject or human is a subject or human in need thereof.

788. The composition of, or composition employed in the method of, any claim, wherein the immunogenic saccharide-polypeptide conjugate(s) are individually, respectively, or collectively present in an amount of about: 0.001, 0.01, 0.1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, or 100%, by weight, based on the weight of the composition.

Petitioner Merck Ex-1004, 0438

789. The method of any claim, which when conducted, does not result in more than about: 50%, 40%, 30%, 20%, 10%, 5%, 4%, 3%, 2%, or 1% suppression of an immunity conveyed by a first vaccine containing an immunogenic saccharide-polypeptide conjugate, as measured by antibody response to the saccharide of the immunogenic saccharide-polypeptide conjugate, wherein the antibody response is measured by measuring antibody titers.

790. The composition of, or composition employed in the method of, any claim, wherein the immunogenic saccharide-polypeptide conjugate is produced using a bacteria.

791. The composition of, or composition employed in the method of, any claim, wherein the immunogenic saccharide-polypeptide conjugate is produced using E. coli.

792. The composition of, or composition employed in the method of, any claim, wherein the immunogenic saccharide-polypeptide comprises the capsular polysaccharide at least partially embedded in the polypeptide.

793. The composition of, or composition employed in the method of, any claim, wherein the immunogenic saccharide-polypeptide comprises the capsular polysaccharide chemically cross-linked to the polypeptide.

794. The composition of, or composition employed in the method of, any claim, wherein the immunogenic saccharide-polypeptide comprises the capsular polysaccharide at least partially chemically cross-linked to the polypeptide.

795. A pharmaceutical composition comprising immunogenic saccharide-polypeptide conjugates comprising capsular polysaccharides, fragments thereof, or combinations thereof comprising at least four serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 35F, and 38 of Streptococcus pneumoniae; each conjugated to a polypeptide; each conjugated to a polypeptide.

796. The pharmaceutical composition of claim 795, wherein immunogenic saccharide-polypeptide conjugates comprise the capsular polysaccharides.

797. The pharmaceutical composition of claim 795, wherein immunogenic saccharide-polypeptide conjugates comprise fragments of the capsular polysaccharide.

798. The pharmaceutical composition of claim 797, wherein fragments of the capsular polysaccharide comprise a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, or a hexasaccharide.

Petitioner Merck Ex-1004, 0439

799. The pharmaceutical composition of any one of claims 795-798, wherein at least one polypeptide is an oligopeptide.

800. The pharmaceutical composition of any one of claims 795-799, wherein at least one polypeptide comprises CRM197, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable H. influenzae P4 protein, a non-typable H. influenzae P6 protein, M catarrhalis uspA, keyhole limpet haemocyanin (KLH), OMPC from N. meningitidis, a purified protein derivative of tuberculin(PPD), protein D from H. influenzae, any fragment thereof, or any combination thereof.

801. The pharmaceutical composition of any one of claims 795-800, wherein a toxin activity of at least one of the polypeptide is at least partly mitigated.

802. The pharmaceutical composition of any one of claims 795-800, comprising at least 9 immunogenic saccharide-polypeptide conjugates each comprising a serotype selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 35F, and 38.

803. The pharmaceutical composition of any one of claims 795-800, comprising at least 10 immunogenic saccharide-polypeptide conjugates each comprising a serotype selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 33F, and 38.

804. The pharmaceutical composition of any one of claims 795-800, comprising at least 13 immunogenic saccharide-polypeptide conjugates each comprising a serotype selected from the group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.

805. The pharmaceutical composition of any one of claims 795-800, comprising at least 14 immunogenic saccharide-polypeptide conjugates each comprising a serotype selected from the group consisting of 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 24F, 33F and 35B.

806. The pharmaceutical composition of any one of claims 795-800, comprising at least 13 immunogenic saccharide-polypeptide conjugates each comprising a serotype selected from the group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B.

Petitioner Merck Ex-1004, 0440

807. The pharmaceutical composition of any one of claims 795-800, comprising at least 12 immunogenic saccharide-polypeptide conjugates each comprising a serotype selected from the group consisting of 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B, and 38.

808. The pharmaceutical composition of any one of claims 795-800, comprising at least 13 immunogenic saccharide-polypeptide conjugates each comprising a serotype selected from the group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.

809. The pharmaceutical composition of any one of claims 795-800, comprising at least 13 immunogenic saccharide-polypeptide conjugates each comprising a serotype selected from the group consisting of 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B.

810. The pharmaceutical composition of any one of claims 795-800, comprising at least 13 immunogenic saccharide-polypeptide conjugates each comprising a serotype selected from the group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B.

811. The pharmaceutical composition of any one of claims 795-800, comprising at least 12 immunogenic saccharide-polypeptide conjugates each comprising a serotype selected from the group consisting of 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B and 38.

812. The pharmaceutical composition of any one of claims 795-800, comprising at least 11 immunogenic saccharide-polypeptide conjugates each comprising a serotype selected from the group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.

813. The pharmaceutical composition of any one of claims 795-800, comprising at least 11 immunogenic saccharide-polypeptide conjugates each comprising a serotype selected from the group consisting of 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B.

814. The pharmaceutical composition of any one of claims 795-813, further comprising an adjuvant.

815. The pharmaceutical composition of claim 814, wherein the adjuvant is an aluminum-cation comprising adjuvant.

816. The pharmaceutical composition of claim 814, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide.

817. The pharmaceutical composition of any one of claims 795-816, wherein the pharmaceutical composition is in the form of an intramuscularly injectable composition, intradermally injectable composition, subcutaneously injectable composition, or an intranasally administrable composition.

Petitioner Merck Ex-1004, 0441

818. The pharmaceutical composition of any one of claims 795-817, further comprising a chelating agent.

819. The pharmaceutical composition of claim 818, wherein the chelating agent comprises ethylenediainetetraacetic acid (EDTA).

820. The pharmaceutical composition of any one of claims 795-819, which is in a form of a suspension.

821. The pharmaceutical composition of any one of claims 795-820, which is in the form of a homogenous mixture or a heterogeneous mixture.

822. The pharmaceutical composition of claim 821, wherein the pharmaceutical composition is in a form of a homogenous mixture.

823. The pharmaceutical composition of any one of claims 795-822, which is contained in a pre-filled syringe.

824. The pharmaceutical composition of any one of claims 795-823, further comprising a surfactant , an emulsifier, or a combination thereof.

825. The pharmaceutical composition of claim 824, comprising the surfactant, wherein the surfactant is a polysorbate, a polymer glycol, a sorbitan ester, or any combination thereof.

826. The pharmaceutical composition of claim 825, comprising the polysorbate, wherein the polysorbate is polysorbate 80.

827. The pharmaceutical composition of claim 825, comprising the polymer glycol, wherein the polymer glycol is polyethylene glycol.

828. The pharmaceutical composition of any one of claims 795-827, further comprising a buffering agent.

829. The pharmaceutical composition of claim 828, wherein the buffering agent is a succinate buffer agent, a tris-acetate- ethylenediainetetraacetic acid (TAE) buffering agent, a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffering agent, a phosphate buffering agent, a sodium phosphate buffering agent, a potassium phosphate buffering agent, or any combination thereof.

830. The pharmaceutical composition of any one of claims 795-829, further comprising sterile water.

831. The pharmaceutical composition of any one of claims 795-830, wherein when the pharmaceutical composition is administered to a plurality of humans, the administration

Petitioner Merck Ex-1004, 0442

elicits at least one of the following side effects in less than about 10% of the plurality of humans: fatigue, headache, muscle pain, joint pain, decreased appetite, chills, or rash.

832. The pharmaceutical composition of any one of claims 795-831, wherein when the pharmaceutical composition is administered intramuscularly to a human, the pharmaceutical composition elicits an immune response that lasts from about 1 week to about 30 years.

833. The pharmaceutical composition form of any one of claims 795-832, wherein the pharmaceutical composition retains at least about 80% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C and the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure.

834. The pharmaceutical composition of any one of claims 795-833, wherein the serotype is not any of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F.

835. The pharmaceutical composition of any one of claims 795-834 in unit dose form.

836. A method comprising administering to a subject a first composition, wherein the first composition is the pharmaceutical composition of any one of claims 795-835.

837. The method of claim 836, wherein the administering to the subject the first composition occurs at least about four weeks before or at least about four weeks after an administration of a second composition comprising a second polypeptide to the subject, wherein the second polypeptide has at least about 70% length homology, at least about 70% sequence homology, or a combination thereof, to the polypeptide of the first composition.

838. The method any one of claims 836-837, wherein the second polypeptide comprises CRM_{197}, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable H. influenzae P4 protein, a non-typable H. influenzae P6 protein, M catarrhalis uspA, keyhole limpet haemocyanin (KLH), OMPC from N. meningitidis, a purified protein derivative of tuberculin(PPD), protein D from H. influenzae, any fragment thereof, or any combination thereof.

839. The method of any one of claims 836-838, further comprising an adjuvant, an emulsifier, a surfactant, a chelating agent, or a combination thereof.

Petitioner Merck Ex-1004, 0443

840. A pharmaceutical composition comprising immunogenic saccharide-polypeptide conjugates comprising capsular polysaccharides, fragments thereof, or combinations thereof comprising at least 13 serotype selected from the group consisting of 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, and 38 of S. pneumoniae; each conjugated to a polypeptide.

841. The pharmaceutical composition of claim 840, wherein the polypeptide comprises CRM_{197}, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable H. influenzae P4 protein, a non-typable H. influenzae P6 protein, M catarrhalis uspA, keyhole limpet haemocyanin (KLH), OMPC from N. meningitidis, a purified protein derivative of tuberculin(PPD), protein D from H. influenzae, or any fragment thereof.

842. The pharmaceutical composition of any one of claims 840-841, wherein a toxin activity of the polypeptide is at least partly mitigated.

843. The pharmaceutical composition of any one of claims 840-842, wherein the polypeptide comprises a mixture of polypeptides.

844. The pharmaceutical composition of claim 843, wherein the mixture of polypeptides comprises at least two polypeptides selected from the group consisting of CRM_{197}, tetanus toxoid, a diphtheria toxoid, cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable H. influenzae P4 protein, a non-typable H. influenzae P6 protein, M catarrhalis uspA, keyhole limpet haemocyanin (KLH), OMPC from N. meningitidis, a purified protein derivative of tuberculin(PPD), protein D from H. influenzae, any fragment thereof, any combination thereof.

845. The pharmaceutical composition of any one of claims 840-844, further comprising an adjuvant, an emulsifier, a surfactant, a chelating agent, or a combination thereof.

846. The pharmaceutical composition of any one of claims 840-845 in unit dose form.

Petitioner Merck Ex-1004, 0444

847. A kit comprising of a pharmaceutical composition of any of claims 795-825 or 840-846 contained in a container.

848. The kit of claim 847, further comprising instructions for use.

849. The kit of any one of claims 847-848, wherein the container is a syringe.

850. The kit of any one of claims 847-849, wherein the container is a vial comprising a cap, wherein the cap comprises a rubber septum.

851. The composition of, or composition employed in the method of, any claim, wherein the immunogenic saccharide-polypeptide conjugate(s) are individually, respectively, or collectively present in an amount of about: 0.001, 0.01, 0.1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, or 100%, by weight, based on the weight of the composition.

Petitioner Merck Ex-1004, 0445

ABSTRACT OF THE DISCLOSURE

Saccharide-polypeptide conjugates, compositions containing these, methods of making and using the conjugates and compositions, and kits containing these, are disclosed.

Petitioner Merck Ex-1004, 0446

PTO/AIA/14 (11-15)
Approved for use through 04/30/2017. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	50308-701.104
	Application Number	
Title of Invention	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

Secrecy Order 37 CFR 5.2:

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Bruce	D.	FORREST	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	South Nyack	State/Province	NY	Country of Residence	US

Mailing Address of Inventor:

Address 1	135 Piermont Avenue
Address 2	

City	South Nyack	State/Province	NY
Postal Code	10960	Country	US

Inventor 2 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Jack	D.	LOVE	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	Tampa	State/Province	FL	Country of Residence	US

Mailing Address of Inventor:

Address 1	4111 W. Inman Avenue
Address 2	

City	Tampa	State/Province	FL
Postal Code	33609	Country	US

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below.
For further information see 37 CFR 1.33(a).

PTO/AIA/14 (11-15)
Approved for use through 04/30/2017. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	50308-701.104
	Application Number	
Title of Invention	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF	

☐ **An Address is being provided for the correspondence Information of this application.**			
Customer Number	21971		
Email Address	uspatentdocket@wsgr.com	Add Email	Remove Email

Application Information:

Title of the Invention	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF		
Attorney Docket Number	50308-701.104	**Small Entity Status Claimed**	☒
Application Type	Provisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)		**Suggested Figure for Publication (if any)**	

Filing By Reference:

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32). Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	◉ Customer Number	○ US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)
Customer Number	21971		

PTO/AIA/14 (11-15)
Approved for use through 04/30/2017. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	50308-701.104
	Application Number	
Title of Invention	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF	

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.
When referring to the current application, please leave the "Application Number" field blank.

Prior Application Status			Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add**button.

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX), the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

			Remove
Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Access Code (if applicable)

Additional Foreign Priority Data may be generated within this form by selecting the **Add**button.

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

PTO/AIA/14 (11-15)
Approved for use through 04/30/2017. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	50308-701.104
	Application Number	
Title of Invention	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF	

Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

B. Search Results from U.S. Application to EPO- Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

PTO/AIA/14 (11-15)
Approved for use through 04/30/2017. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	50308-701.104
	Application Number	
Title of Invention	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF	

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Applicant 1

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

(●) Assignee	( ) Legal Representative under 35 U.S.C. 117	( ) Joint Inventor
( ) Person to whom the inventor is obligated to assign.	( ) Person who shows sufficient proprietary interest	

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor:

If the Applicant is an Organization check here. [x]

Organization Name	LIFFEY BIOTECH LIMITED		
Mailing Address Information For Applicant:			
Address 1	c/o Mathesons		
Address 2	70 Sir John Rogerson's Quay		
City	Dublin 2	**State/Province**	
Country	IR	Postal Code	
Phone Number		Fax Number	
Email Address			

Additional Applicant Data may be generated within this form by selecting the Add button.

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

PTO/AIA/14 (11-15)
Approved for use through 04/30/2017. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	50308-701.104
	Application Number	
Title of Invention	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF	

Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

If the Assignee or Non-Applicant Assignee is an Organization check here. ☐

Prefix	**Given Name**	Middle Name	**Family Name**	Suffix

Mailing Address Information For Assignee including Non-Applicant Assignee:

Address 1			
Address 2			
City		**State/Province**	
Country[i]		Postal Code	
Phone Number		Fax Number	
Email Address			

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button.

Signature:

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). **However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).** This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants. See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/Keli Holzapfel/			Date (YYYY-MM-DD)	2016-08-11
First Name	Keli	Last Name	Holzapfel	Registration Number	65254

Additional Signature may be generated within this form by selecting the Add button.

PTO/AIA/14 (11-15)
Approved for use through 04/30/2017. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	50308-701.104
	Application Number	
Title of Invention	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF	

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Electronic Acknowledgement Receipt	
EFS ID:	26620740
Application Number:	62373807
International Application Number:	
Confirmation Number:	8821
Title of Invention:	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF
First Named Inventor/Applicant Name:	Bruce D. Forrest
Customer Number:	21971
Filer:	Keli Holzapfel/William Tronvig
Filer Authorized By:	Keli Holzapfel
Attorney Docket Number:	50308-701.104
Receipt Date:	11-AUG-2016
Filing Date:	
Time Stamp:	18:17:42
Application Type:	Provisional

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$130
RAM confirmation Number	4904
Deposit Account	232415
Authorized User	WILSON, SONSINI, GOODRICH & ROSATI
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: Charge any Additional Fees required under 37 CFR 1.16 (National application filing, search, and examination fees) Charge any Additional Fees required under 37 CFR 1.17 (Patent application and reexamination processing fees)	

Charge any Additional Fees required under 37 CFR 1.19 (Document supply fees)

Charge any Additional Fees required under 37 CFR 1.20 (Post Issuance fees)

Charge any Additional Fees required under 37 CFR 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		2016_08_11_PROV_APP_50308_701_104.pdf	1030209 14d6c42fd399789deab172b7bb67268ef325743d	yes	155

Multipart Description/PDF files in .zip description

Document Description	Start	End
Provisional Cover Sheet (SB16)	1	2
Miscellaneous Incoming Letter	3	3
Specification	4	63
Claims	64	147
Abstract	148	148
Abstract	149	155

Warnings:

Information:

2	Fee Worksheet (SB06)	fee-info.pdf	30300 a053af20a39f361c1e2eb3b4eb106925cf7340cf	no	2

Warnings:

Information:

Total Files Size (in bytes):	1060509

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/SB/16 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995 no persons are required to respond to a collection of information unless it displays a valid OMB control number

PROVISIONAL APPLICATION FOR PATENT COVER SHEET – Page 1 of 2

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. VIA EFS WEB

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Bruce D.	FORREST	South Nyack, NY
Jack D.	LOVE	Tampa, FL

Additional inventors are being named on the _____ separately numbered sheets attached hereto.

TITLE OF THE INVENTION (500 characters max):

SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

☑ The address corresponding to Customer Number: 21971

OR

☐ Firm or Individual Name

Address

City	State	Zip
Country	Telephone	Email

ENCLOSED APPLICATION PARTS (*check all that apply*)

☑ Application Data Sheet. See 37 CFR 1.76.
☐ CD(s), Number of CDs _____
☐ Drawing(s) *Number of Sheets* _____
☑ Other (specify) Specification Cover Sheet
☑ Specification (e.g., description of the invention) *Number of Pages* 60

Fees Due: Filing Fee of $260 ($130 for small entity) ($65 for micro entity). If the specification and drawings exceed 100 sheets of paper, an application size fee is also due, which is $400 ($200 for small entity) ($100 for micro entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

METHOD OF PAYMENT OF THE FILING FEE AND APPLICATION SIZE FEE FOR THIS PROVISIONAL APPLICATION FOR PATENT

☑ Applicant asserts small entity status. See 37 CFR 1.27.
☐ Applicant certifies micro entity status. See 37 CFR 1.29. Applicant must attach form PTO/SB/15A or B or equivalent.
☐ A check or money order made payable to the *Director of the United States Patent and Trademark Office* is enclosed to cover the filing fee and application size fee (if applicable).
☐ Payment by credit card. Form PTO-2038 is attached.
☑ The Director is hereby authorized to charge the filing fee and application size fee (if applicable) or credit any overpayment to Deposit Account Number: 23-2415.

130.00
TOTAL FEE AMOUNT ($)

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 10 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

PTO/SB/16 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995 no persons are required to respond to a collection of information unless it displays a valid OMB control number

PROVISIONAL APPLICATION FOR PATENT COVER SHEET – Page 2 of 2

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☑ No.

☐ Yes, the invention was made by an agency of the U.S. Government. The U.S. Government agency name is: _____

☐ Yes, the invention was made under a contract with an agency of the U.S. Government. The name of the U.S. Government agency and Government contract number are: _____

WARNING:

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

SIGNATURE /Keli Holzapfel/ DATE 2016-08-11

TYPED OR PRINTED NAME Keli L. Holzapfel, Ph.D. REGISTRATION NO. 65,254 (*if appropriate*)

TELEPHONE 206-883-2639 DOCKET NUMBER 50308-701.104

WIPO
WORLD
INTELLECTUAL PROPERTY
ORGANIZATION

DOCUMENT MADE AVAILABLE UNDER THE PATENT COOPERATION TREATY (PCT)

International application number:		**PCT/US2017/025621**
International filing date:		**31 March 2017 (31.03.2017)**
Document type:		**Certified copy of priority document**
Document details:	Country/Office:	**US**
	Number:	**62/373,807**
	Filing date:	**11 August 2016 (11.08.2016)**
Date of receipt at the International Bureau:		**15 April 2017 (15.04.2017)**

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a),(b) or (b-*bis*)

34, chemin des Colombettes
1211 Geneva 20, Switzerland
www.wipo.int

PCT Recordation of Search History

Case/PCT Application Number: PCT/US17/25621

CLIN Number/Technical Field of PCT Application: 0007 BIOTECHNOLOGY

Date During Which the Search was Conducted: 22 August 2017

Date of Completion of Recordation of Search History Form: 22 August 2017

Research Analyst Initials: DKK

Search Approval Official (SAO) Initials: DGF

Field of Search/Classification Information:

IPC(8) Classifications: A61K 39/09, 39/02, 39/385, 39/095, 47/48, 39/00; C12N 1/21; A61P 31/04; C07K 16/12, 14/315 (2017.01)

CPC Classifications: A61K 39/092, 39/02, 47/646, 39/385, 2039/6068, 2039/70, 39/095, 2039/6037; C07K 16/1271, 14/3156

Databases Searched (Patent and Non-Patent Literature (NPL), Including Sub-Databases and Files Searched) and Search Terms Used:

PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, INPADOC Data); Google Scholar; Pubmed; EBSCO

Keywords: pneumococcal, polysaccharide, polypeptide, conjugate, immune response, vaccine

Database Search String Recordation, Including Dates of Searches:

Patent Database Search Strategy/Results:

Patseer: 22 August 2017

ID	Search String	#
L20	tacd: (streptococcus pneumoniae or S_pnemoniae or PNPS) and (capsul* w3 polysaccharide) and (conjugat*) and (polypeptide) and (serotype*) and ((first and second) w5 immuniz*) and inv: (malley or lu or zhang or mccool or harding or greenspoan or schreiber or kolkman or zeijst or nujiten)	19
L19	tacd: (streptococcus pneumoniae or S_pnemoniae or PNPS) and (capsul* w3 polysaccharide) and (conjugat*) and (polypeptide) and (serotype*) and ((first and second) w5 immuniz*) and cpc: (A61K39/092 or A61K39/02 or A61K47/646 or A61K39/385 or A61K2039/6068 or A61K2039/70 or A61K39/095 or A61K2039/6037 or C07K16/1271 or C07K14/3156)	670
L18	tacd: (streptococcus pneumoniae or S_pnemoniae or PNPS) and (capsul* w3 polysaccharide) and (conjugat*) and (polypeptide) and (serotype*) and ((first and second) w5 immuniz*) and icr: (A61K39/09 or A61K39/02 or A61K39/385 or A61K39/095 or A61K47/48 or A61K39/00 or C12N1/21 or A61P31/04 or C07K16/12 or C07K14/315)	891
L17	tacd: (streptococcus pneumoniae or S_pnemoniae or PNPS) and (capsul* w3 polysaccharide) and (conjugat*) and (polypeptide) and (serotype*) and ((first and second) w5 immuniz*)	1068
L16	tac: (streptococcus pneumoniae or S_pnemoniae or PNPS) and (capsul* w3 polysaccharide) and (conjugat*) and (polypeptide) and (serotype*) and ((first and second) w5 immuniz*)	6
L15	ta: (streptococcus pneumoniae or S_pnemoniae or PNPS) and (capsul* w3 polysaccharide) and (conjugat*) and (polypeptide) and (serotype*) and ((first and second) w5 immuniz*)	0
L14	tacd: (streptococcus pneumoniae or S_pnemoniae or PNPS) and (capsul* w3 polysaccharide) and (conjugat*) and (polypeptide) and (serotype*) and icr: (A61K* or C07K*)	4895
L13	tacd: (streptococcus pneumoniae or S_pnemoniae or PNPS) and (capsul* w3 polysaccharide) and (conjugat*) and (polypeptide) and (serotype*) and cpc: (A61K* or C07K*)	4692
L12	tacd: (streptococcus pneumoniae or S_pnemoniae or PNPS) and (capsul* w3 polysaccharide) and (conjugat*) and (polypeptide) and (serotype*) and aasn: (liffey biotech)	0
L11	tacd: (streptococcus pneumoniae or S_pnemoniae or PNPS) and (capsul* w3 polysaccharide) and (conjugat*) and (polypeptide) and (serotype*) and inv: (forrest or love)	0
L10	tacd: (streptococcus pneumoniae or S_pnemoniae or PNPS) and (capsul* w3 polysaccharide) and (conjugat*) and (polypeptide) and (serotype*)	5237
L9	tac: (streptococcus pneumoniae or S_pnemoniae or PNPS) and (capsul* w3 polysaccharide) and (conjugat*) and (polypeptide) and (serotype*)	72
L8	ta: (streptococcus pneumoniae or S_pnemoniae or PNPS) and (capsul* w3 polysaccharide) and (conjugat*) and (polypeptide) and (serotype*)	3
L7	tacD: (streptococcus pneumoniae or S_pnemoniae or PNPS) and (capsul* w3 polysaccharide) and (conjugat*) and (polypeptide) and cpc: (A61K* or C07K*)	6100
L6	tacD: (streptococcus pneumoniae or S_pnemoniae or PNPS) and (capsul* w3 polysaccharide) and (conjugat*) and (polypeptide) and icr: (A61K* or C07K*)	6311
L5	tacD: (streptococcus pneumoniae or S_pnemoniae or PNPS) and (capsul* w3 polysaccharide) and (conjugat*) and (polypeptide) and aasn: (liffey biotech)	0
L4	tacD: (streptococcus pneumoniae or S_pnemoniae or PNPS) and (capsul* w3	0

	polysaccharide) and (conjugat*) and (polypeptide) and inv: (forrest or love)	
L3	tacd: (streptococcus pneumoniae or S_pnemoniae or PNPS) and (capsul* w3 polysaccharide) and (conjugat*) and (polypeptide)	6931
L2	tac: (streptococcus pneumoniae or S_pnemoniae or PNPS) and (capsul* w3 polysaccharide) and (conjugat*) and (polypeptide)	123
L1	ta: (streptococcus pneumoniae or S_pnemoniae or PNPS) and (capsul* w3 polysaccharide) and (conjugat*) and (polypeptide)	3

Non-Patent Literature (NPL) Search Strategy/Results:

Google Scholar: 22 August 2017

Search terms	#
immunogenic pneumococcal polysaccharide-polypeptide conjugate	42
immunogenic pneumococcal capsular polysaccharide fragments	5830
immunogenic pneumococcal capsular monosaccharides	1320

Pubmed: 22 August 2017

Search terms	#
immunogenic pneumococcal polysaccharide-polypeptide conjugate	0
immunogenic pneumococcal capsular polysaccharide fragments	3
immunogenic pneumococcal capsular monosaccharides	0

EBSCO: 22 August 2017

Search terms	#
immunogenic pneumococcal polysaccharide-polypeptide conjugate	122
immunogenic pneumococcal capsular polysaccharide fragments	0997
immunogenic pneumococcal capsular monosaccharides	1906

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92*bis*.1 and Administrative Instructions, Section 422)

To:

HOLZAPFEL, Keli L.
Wilson Sonsini Goodrich & Rosati
650 Page Mill Road
Palo Alto, California 94304
ÉTATS-UNIS D'AMÉRIQUE

Date of mailing *(day/month/year)*
25 June 2018 (25.06.2018)

Applicant's or agent's file reference
503087-01601

IMPORTANT NOTIFICATION

International application No.
PCT/US2017/025621

International filing date *(day/month/year)*
31 March 2017 (31.03.2017)

1. The following indications appeared on record concerning:

☒ the applicant ☒ the inventor ☐ the agent ☐ the common representative

Name and Address
FORREST, Bruce D.
LOVE, Jack D.

State of Nationality | State of Residence

Telephone No.

Facsimile No.

E-mail address

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person ☐ the name ☐ the address ☐ the nationality ☐ the residence

Name and Address
See Box 3.

State of Nationality | State of Residence

Telephone No.

Facsimile No.

E-mail address
☐ Notifications by e-mail authorized

3. Further observations, if necessary:
The persons identified in Box 1 are now inventors only.

4. A copy of this notification has been sent to:

☒ the receiving Office
☐ the International Searching Authority
☐ the Authority(ies) specified for supplementary search
☐ the International Preliminary Examining Authority
☒ the designated Offices concerned
☐ the elected Offices concerned
☐ other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. +41 22 338 89 70

Authorized officer
Zandrini Anita-Eva
e-mail pct.team6@wipo.int
Telephone No. +41 22 338 74 06

Form PCT/IB/306 (January 2009) 1/PV2ZT3UQHCQGC0

PATENT ASSIGNMENT COVER SHEET

Electronic Version v1.1
Stylesheet Version v1.2

EPAS ID: PAT5622519

SUBMISSION TYPE:	NEW ASSIGNMENT
NATURE OF CONVEYANCE:	ASSIGNMENT

CONVEYING PARTY DATA

Name	Execution Date
LIFFEY BIOTECH LIMITED	07/16/2019

RECEIVING PARTY DATA

Name:	POGONA, LLC
Street Address:	PO BOX 444
City:	NYACK
State/Country:	NEW YORK
Postal Code:	10960

PROPERTY NUMBERS Total: 1

Property Type	Number
PCT Number:	US2017025621

CORRESPONDENCE DATA

Fax Number:

Correspondence will be sent to the e-mail address first; if that is unsuccessful, it will be sent using a fax number, if provided; if that is unsuccessful, it will be sent via US Mail.

Phone: 845-353-4353
Email: bforrest@liffeybio.com
Correspondent Name: BRUCE FORREST
Address Line 1: PO BOX 444
Address Line 4: NYACK, NEW YORK 10960

NAME OF SUBMITTER:	BRUCE FORREST
SIGNATURE:	/Bruce Forrest/
DATE SIGNED:	07/17/2019
	This document serves as an Oath/Declaration (37 CFR 1.63).

Total Attachments: 3
source=Patent 025621 Assign to Pogona - Executed#page1.tif
source=Patent 025621 Assign to Pogona - Executed#page2.tif
source=Patent 025621 Assign to Pogona - Executed#page3.tif

Copied from PCT/US17/25621 on 03/19/2020

WHEREAS, LIFFEY BIOTECH LIMITED (hereinafter "Assignor"), having a mailing address at 70 Sir John Rogerson's Quay, Dublin 2, Ireland, owns the entire right, title and interest in and to the inventions disclosed in the Application(s), and in and to all embodiments of the inventions, heretofore conceived, made or discovered (collectively hereinafter referred to as "Inventions") entitled:

SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF

for which application serial number PCT/US2017/025621 was filed on March 31st, 2017, in the United States Patent and Trademark Office; WHEREAS, Pogona, LLC. a limited liability company, having a mailing address at PO Box 444, Nyack. New York 10960, United States of America (hereinafter "Assignee"), is desirous of acquiring the entire right, title and interest in and to said Application(s), and the inventions disclosed therein, and in and to all embodiments of the inventions, heretofore conceived, made or discovered, whether jointly or severally (hereinafter collectively referred to as "Inventions"), and in and to any and all patents, inventor's certificates and other forms of protection thereon granted in the United States, foreign countries, or under any international convention, agreement, protocol, or treaty, including those filed under the Paris Convention for the Protection of Industrial Property, The Patent Cooperation Treaty or otherwise (hereinafter "Patents)").

NOW, THEREFORE, in consideration of good and valuable consideration acknowledged by said Assignor to have been received in full from said Assignee:

1. Said Assignor does hereby sell, assign, transfer and convey unto said Assignee the entire right, tide and interest (a) in and to said Inventions; (b) in and to said Applications, including the right to claim priority to and from said Application(s); (c) in and to each and every application that is a divisional, substitution, continuation, or continuation-in-part of any of said Application(s); (d) in and to said Patent(s) and each and every patent issuing or reissuing from any of the foregoing; (e) in and to each and every reissue, reexamination, renewal or extension of any kind of any of the foregoing; (f) in and to each and every patent and application filed outside the United States and corresponding to any of the foregoing; and (g) in and to all claims for past, present and future infringement of the Patent(s), including all rights to sue for and to receive and recover for Assignee's own use all past, present, and future lost profits, royalties, and damages of whatever nature recoverable from an infringement of the Patents).

2. Said Assignor hereby covenants and agrees to cooperate with said Assignee to enable said Assignee to enjoy to the fullest extent the right, tide and interest herein conveyed in the United States, foreign countries, or under any international convention, agreement, protocol, or treaty. Such cooperation by the Assignor shall include prompt production of pertinent facts and documents, giving of testimony, execution of petitions, oaths, specifications, declarations or other papers, and other assistance all to the extent deemed necessary or desirable by the parties (a) for perfecting in said Assignee the right, tide and interest herein conveyed; (b) for prosecuting any of said applications covering said Inventions; (c) for filing and prosecuting substitute, divisional, continuing or additional applications covering said Inventions; (d) for filing and prosecuting applications for reissuance of any said Patent(s); (e) for interference or other priority proceedings involving said Inventions; and (f) for legal proceedings involving said Inventions and any applications therefor and any Patent(s) granted thereon, including without limitation reissues and reexaminations, opposition proceedings, cancellation proceedings, priority contests, public use proceedings, infringement actions and court actions.

3. The terms and covenants of this assignment shall inure to the benefit of said Assignee, its successors, assigns and other legal representatives, and shall be binding upon the Assignor, its successors, assigns and other legal representatives.

4. Said Assignor hereby warrants, represents and covenants that said Assignor has not entered and will not enter into any assignment, contract, or understanding in conflict herewith.

5. Said Assignor hereby request that any Patent(s) issuing in the United States, foreign countries, or under any international convention, agreement, protocol, or treaty, be issued in the name of the Assignee, or its successors and assigns, for the sole use of said Assignee, its successors, legal representatives and assigns.

Copied from PCT/US17/25621 on 03/19/2020

6. This instrument will be interpreted and construed in accordance with the laws of the State of New York, without regard to conflict of law principles. If any provision of this instrument is found to be illegal or unenforceable, the other provisions shall remain effective and enforceable to the greatest extent permitted by law. This instrument may be executed in counterparts, each of which is deemed an original, but all of which together constitute one and the same agreement.

IN WITNESS WHEREOF, said Assignor has executed and delivered this instrument to said Assignee as of the date written below.

Date: July 15, 2019

By: Name: Jack Love, Director

Title: 7111 W. Fowler Ave
Tampa, FL 33609

A notary public or otter officer completing this certificate verifies only the identity of the individual who signed the document to which this certificate is attached, and not the truthfulness, accuracy, or validity of that document.

State of Florida

County of Hillsborough

On 7/15/19 (Date) before me, W. Jared Salansky (Here Insert Name and Title of the Officer), Notary Public

Personally appeared Jack Love (Name(s) of Signer(s))

who proved to me on the basis of satisfactory evidence to be the person(s) whose name(s) is/are subscribed to the within instrument and acknowledged to me that he/she/they executed the same in his/her/their authorized capacities), and that by his/her/their signature(s) on the instrument the person(s), or the entity upon behalf of which the person(s) acted, executed the instrument.

WILLIAM JARED SALANSKY
Notary Public - State of Florida
Commission # GG 304507
My Comm. Expires Feb 21, 2023

Place Notary Seal Above

I certify under PENALTY OF PERJURY under the laws of the State of Florida the foregoing paragraph is true and correct.

WITNESS my hand and official seal.

Signature _____
Signature of Notary Public

Copied from PCT/US17/25621 on 03/19/2020

RECEIVED AND AGREED TO BY ASSIGNEE:

Date: 07/16/2019 By: _____

Name: BRUCE D. FORREST

Title: DIRECTOR

A notary public or other officer completing this certificate verifies only the identity of the individual who signed the document to which this certificate is attached, and not the truthfulness, accuracy, or validity of that document.

State of NEW YORK

County of ROCKLAND

On July 16, 2019 before me, BARBARA B KUNAR, Notary Public
Here Insert Name and Title of the Officer

personally appeared BRUCE D FORREST
Name(s) of Signer(s)

who proved to me on the basis of satisfactory evidence to be the person(s) whose name(s) is/are subscribed to the within instrument and acknowledged to me that he/she/they executed the some in his/her/their authorized capacity(ies), and that by his/her/their signature(s) on the instrument the person(s), or the entity upon behalf of which the person(s) acted, executed the instrument.

I certify under PENALTY OF PERJURY under the laws of the State of NEW YORK the foregoing paragraph is true and correct.

WITNESS my hand and official seal.

Signature _____
Signature of Notary Public

Place Notary Seal Above

BARBARA B. KUNAR
Notary Public, State of New York
No. 01KU6061272
Qualified in Rockland County
Commission Expires July 16, 2023

Copied from PCT/US17/25621 on 03/19/2020

PTO/AIA/80 (07-17)
Approved for use through 03/31/2021. OMB 0651-0035
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no person is required to respond to a collection of information unless it displays a valid OMB control number

POWER OF ATTORNEY TO PROSECUTE APPLICATIONS BEFORE THE USPTO

I hereby revoke all previous powers of attorney given in the application identified in the attached statement under 37 CFR 3.73(c).

I hereby appoint:

[X] Practitioners associated with Customer Number: 23628

OR

[ ] Practitioner(s) named below (if more than ten patent practitioners are to be named, then a customer number must be used):

Name	Registration Number	Name	Registration Number

As attorney(s) or agent(s) to represent the undersigned before the United States Patent and Trademark Office (USPTO) in connection with any and all patent applications assigned only to the undersigned according to the USPTO assignment records or assignments documents attached to this form in accordance with 37 CFR 3.73(c).

Please change the correspondence address for the application identified in the attached statement under 37 CFR 3.73(c) to:

[X] The address associated with Customer Number: 23628

OR

[ ]

Firm or individual name		
Address		
City	State	Zip
Country		
Telephone	Email	

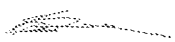
Assignee name and address:

Pogona, LLC
135 Piermont Avenue
South Nyack, NY 10960

A copy of this form, together with a statement under 37 CFR 3.73(c) (Form PTO/AIA/96 or equivalent) is required to be filed in each application in which this form is used. The statement under 37 CFR 3.73(c) may be completed by one of the practitioners appointed in this form, and must identify the application in which this Power of Attorney is to be filed.

SIGNATURE of Assignee of Record

The individual whose signature and title is supplied below is authorized to act on behalf of the assignee.

Signature 	Date: 10 Dec 2019
Name Bruce D. Forrest	Telephone +1 845 353 4353
Title Manager Pogona, LLC	

PTO/AIA/96 (08-12)
Approved for use through 01/31/2013. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

STATEMENT UNDER 37 CFR 3.73(c)

Applicant/Patent Owner: Pogona, LLC

Application No./Patent No.: 16/089,040 Filed/Issue Date: September 27, 2018

Titled: SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF

Pogona, LLC (Name of Assignee) , a Limited Liability Company (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that, for the patent application/patent identified above, it is (choose **one** of options 1, 2, 3 or 4 below):

1. [x] The assignee of the entire right, title, and interest.

2. [ ] An assignee of less than the entire right, title, and interest (check applicable box):

   [ ] (The extent (by percentage) of its ownership interest is _____ %). Additional Statement(s) by the owners holding the balance of the interest must be submitted to account for 100% of the ownership interest.

   [ ] There are unspecified percentages of ownership. The other parties, including inventors, who together own the entire right, title and interest are:

   Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

3. [ ] The assignee of an undivided interest in the entirety (a complete assignment from one of the joint inventors was made). The other parties, including inventors, who together own the entire right, title, and interest are:

   Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

4. [ ] The recipient, via a court proceeding or the like (*e.g.*, bankruptcy, probate), of an undivided interest in the entirety (a complete transfer of ownership interest was made). The certified document(s) showing the transfer is attached.

The interest identified in option 1, 2, or 3 above (not option 4) is evidence by either (choose **one** of options A or B below):

A. [ ] An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel _____ , Frame _____ , or for which a copy thereof is attached.

B. [x] A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

   1. From: Bruce D. Forrest To: Liffey Biotech Limited
      The document was recorded in the United States Patent and Trademark Office at Reel 051137 , Frame 0658 , or for which a copy thereof is attached.

   2. From: Jack D. Love To: Liffey Biotech Limited
      The document was recorded in the United States Patent and Trademark Office at Reel 051137 , Frame 0658 , or for which a copy thereof is attached.

Petitioner Merck Ex-1004, 0469

PTO/AIA/96 (08-12)
Approved for use through 01/31/2013. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

STATEMENT UNDER 37 CFR 3.73(c)

3. From: Liffey Biotech Limited To: Pogona, LLC
The document was recorded in the United States Patent and Trademark Office at
Reel 049777 , Frame 0788 , or for which a copy thereof is attached.

4. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____ , Frame _____ , or for which a copy thereof is attached.

5. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____ , Frame _____ , or for which a copy thereof is attached.

6. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____ , Frame _____ , or for which a copy thereof is attached.

☐ Additional documents in the chain of title are listed on a supplemental sheet(s).

☒ As required by 37 CFR 3.73(c)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (i.e., a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

Signature	10 December 2019 Date
Bruce D. Forrest Name	Manager Title

Petitioner Merck Ex-1004, 0470

Corrected Application Data Sheet

Inventor Information

Inventor Number::	1
Given Name::	Bruce
Middle Name::	D.
Family Name::	Forrest
City of Residence::	South Nyack
State or Province of Residence::	NY
Country of Residence::	US
Street of mailing address::	135 Piermont Avenue
City of mailing address::	South Nyack
State or Province of mailing address::	NY
Country of mailing address::	US
Postal or Zip Code of mailing address::	10960

Inventor Number::	2
Given Name::	Jack
Middle Name::	D.
Family Name::	Love
City of Residence::	Tampa
State or Province of Residence::	FL
Country of Residence::	US
Street of mailing address::	4111 W. Inman Avenue

Petitioner Merck Ex-1004, 0471

City of mailing address::	Tampa
State or Province of mailing address::	FL
Country of mailing address::	US
Postal or Zip Code of mailing address::	33609

Correspondence Information

Correspondence Customer Number::	~~021971~~ 23628

Application Information

Application Number::	16/089,040
Filing Date::	09/27/18
Application Type::	Regular
Subject Matter::	Utility
CD-ROM or CD-R?::	None
Sequence submission?::	None
Computer Readable Form (CRF)?::	No
Title::	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF
Attorney Docket Number::	~~50308-701.831~~ P1143.70000US04
Request for Early Publication?::	No
Request for Non-Publication?::	No

Petitioner Merck Ex-1004, 0472

Small Entity?::	Yes
Petition included?::	No
Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2::	No

Representative Information

Representative Customer Number::	~~021971~~
	23628

Domestic Priority Information

Application::	Continuity Type::	Parent Application::	Parent Filing Date::	Prior Appl Status::
This Application	a 371 of international	PCT/US2017/025621	03/31/17	
PCT/US2017/025621	Claims benefit of provisional	62/373807	08/11/16	Expired
PCT/US2017/025621	Claims benefit of provisional	62/372263	08/08/16	Expired
PCT/US2017/025621	Claims benefit of provisional	62/330245	05/02/16	Expired
PCT/US2017/025621	Claims benefit of provisional	62/316555	03/31/16	Expired

Applicant Information

~~Applicant Number::~~	~~1~~
~~Organization Name::~~	~~Liffey Biotech Limited~~
~~Street of mailing address::~~	~~c/o Mathesons~~
	~~70 Sir John Rogersons Quay~~

Petitioner Merck Ex-1004, 0473

~~City of mailing address::~~	~~Dublin~~
~~State or Province of mailing address::~~	~~IE~~
~~Country of mailing address::~~	~~IE~~
~~Postal or Zip Code of mailing address::~~	~~D02 R296~~

Applicant Number::	1
Organization Name::	Pogona, LLC
Street of mailing address::	135 Piermont Avenue
City of mailing address::	South Nyack
State or Province of mailing address::	NY
Country of mailing address::	US
Postal or Zip Code of mailing address::	10960

Assignee Information Including Non-Applicant Assignee Information

Assignee Number::	1
Organization Name::	Pogona, LLC
Street of mailing address::	135 Piermont Avenue
City of mailing address::	South Nyack
State or Province of mailing address::	NY
Country of mailing address::	US
Postal or Zip Code of mailing address::	10960

Petitioner Merck Ex-1004, 0474

Signature:

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). **However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).** This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants. See 37 CFR 1.4(d) for the manner of making signatures and certifications.			
Signature	/C. Hunter Baker/	Date (YYYY-MM-DD)	2019-12-11
Name	C. Hunter Baker, MD, PhD	Registration Number	46,533

Petitioner Merck Ex-1004, 0475

Electronic Acknowledgement Receipt

EFS ID:	37995992
Application Number:	16089040
International Application Number:	
Confirmation Number:	8702
Title of Invention:	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF
First Named Inventor/Applicant Name:	Bruce D. FORREST
Customer Number:	21971
Filer:	Charles Baker/Theressa Bresnahan
Filer Authorized By:	Charles Baker
Attorney Docket Number:	50308-701.831
Receipt Date:	11-DEC-2019
Filing Date:	
Time Stamp:	12:45:57
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Miscellaneous Incoming Letter	P114370000US04-TRN-CHB.pdf	76056 655188d474f65b0470b986f8a00b15c7cb1e81d3	no	1

Warnings:

Information:					
2	Power of Attorney	P114370000US04-POA-CHB.pdf	79725 c009a37c94d92c48e41208a635d8b09667ebaba1	no	1
Warnings:					
Information:					
3	Assignee showing of ownership per 37 CFR 3.73	P114370000US04-373-CHB.pdf	96270 bbeb4a2b272d529b875efae53f89eefff07c33dd	no	2
Warnings:					
Information:					
4	Req to correct/update/change appl name until pay issue fee	P114370000US04-ADS-CHB.pdf	64194 b9ffd3f4f911daf076e5bce9a48fd460db1ef6ea	no	5
Warnings:					
Information:					
		Total Files Size (in bytes):	316245		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

<u>New Applications Under 35 U.S.C. 111</u>
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.
<u>National Stage of an International Application under 35 U.S.C. 371</u>
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.
<u>New International Application Filed with the USPTO as a Receiving Office</u>
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Doc Code: TRAN.LET
Document Description: Transmittal Letter

USED IN LIEU OF PTO/SB/21 (07-09)

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission	

Application Number	16/089,040-Conf. #8702
Filing Date	September 27, 2018
First Named Inventor	Bruce D. Forrest
Art Unit	Not Yet Assigned
Examiner Name	Not Yet Assigned
Attorney Docket Number	P1143.70000US04

ENCLOSURES (*Check all that apply*)

- [ ] Fee Transmittal Form
  - [ ] Fee Attached
- [ ] Amendment/Reply
  - [ ] After Final
  - [ ] Affidavits/declaration(s)
- [ ] Extension of Time Request
- [ ] Express Abandonment Request
- [ ] Information Disclosure Statement
- [ ] Certified Copy of Priority Document(s)
- [ ] Reply to Missing Parts/ Incomplete Application
  - [ ] Reply to Missing Parts under 37 CFR 1.52 or 1.53
- [ ] Drawing(s)
- [ ] Licensing-related Papers
- [ ] Petition
- [ ] Petition to Convert to a Provisional Application
- [x] Power of Attorney, Revocation Change of Correspondence Address
- [ ] Terminal Disclaimer
- [ ] Request for Refund
- [ ] CD, Number of CD(s) _____
  - [ ] Landscape Table on CD
- [ ] After Allowance Communication to TC
- [ ] Appeal Communication to Board of Appeals and Interferences
- [ ] Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief)**
- [ ] Proprietary Information
- [ ] Status Letter
- [x] Other Enclosure(s) (please Identify below):

Statement under 37 CFR 3.73(c) and Corrected Application Data Sheet

Remarks

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	WOLF, GREENFIELD & SACKS, P.C.		
Signature	/C. Hunter Baker/		
Printed name	C. Hunter Baker, MD, PhD		
Date	December 11, 2019	Reg. No.	46,533

Certificate of Electronic Filing under 37 C.F.R. § 1.8

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office's electronic filing system in accordance with 37 C.F.R. § 1.6(a)(4).

Dated: December 11, 2019 Signature: /Theresa A. Bresnahan/ (Theresa A. Bresnahan)

7770613.1

MULTIPLE DEPENDENT CLAIM FEE CALCULATION SHEET

Substitute for Form PTO-1360
(For use with Form PTO/SB/06)

Application Number	Filing Date
16089040	

Applicant(s) Bruce FORREST

* May be used for additional claims or amendments

CLAIMS	AS FILED		AFTER FIRST AMENDMENT		AFTER SECOND AMENDMENT	
	Indep	Depend	Indep	Depend	Indep	Depend
1	1					
2		1				
3		1				
4		1				
5		1				
6		1				
7		1				
8		1				
9		1				
10		1				
11		1				
12		1				
13		1				
14		1				
15		1				
16		1				
17		1				
18		1				
19		1				
20		1				
21		1				
22		1				
23		22				
24	1					
25		1				
26		1				
27		1				
28		1				
29	1					
30		1				
31		1				
32		1				
33		1				
34		1				
35		1				
36		1				
37		1				
38		1				
39		1				
40		1				
41		1				
42		1				
43		1				
44		1				
45		1				
46		1				
47		1				
48		1				
49		1				
50		1				

	*		*		*	
	Indep	Depend	Indep	Depend	Indep	Depend
51		1				
52		1				
53		1				
54		1				
55		1				
56		1				
57		1				
58		1				
59		1				
60		1				
61		1				
62	1					
63		1				
64		1				
65		1				
66		1				
67	1					
68		1				
69		1				
70		1				
71		(1)				
72		(1)				
73		(1)				
74		(1)				
75		(1)				
76		(1)				
77		(1)				
78		(1)				
79		(1)				
80		(1)				
81		(1)				
82		(1)				
83		(1)				
84		(1)				
85		(1)				
86		(1)				
87		(1)				
88		(1)				
89		(1)				
90		(1)				
91		(1)				
92		(1)				
93		(1)				
94		(1)				
95		(1)				
96		(1)				
97		(1)				
98		(1)				
99		(1)				
100		(1)				

MULTIPLE DEPENDENT CLAIM FEE CALCULATION SHEET

Substitute for Form PTO-1360
(For use with Form PTO/SB/06)

Application Number	Filing Date
16089040	

Applicant(s) Bruce FORREST

* May be used for additional claims or amendments

CLAIMS	AS FILED		AFTER FIRST AMENDMENT		AFTER SECOND AMENDMENT	
	Indep	Depend	Indep	Depend	Indep	Depend
101		(1)				
102		(1)				
103		(1)				
104		(1)				
105		(1)				
106		(1)				
107		(1)				
108		(1)				
109		(1)				
110		(1)				
111		(1)				
112		(1)				
113		(1)				
114		(1)				
115		(1)				
116		(1)				
117		(1)				
118		(1)				
119		(1)				
120		(1)				
121		(1)				
122		(1)				
123		(1)				
124		(1)				
125		(1)				
126		(1)				
127		(1)				
128		(1)				
129		(1)				
130		(1)				
131		(1)				
132		(1)				
133		(1)				
134		(1)				
135		(1)				
136		(1)				
137		(1)				
138		(1)				
139		(1)				
140		(1)				
141		(1)				
142		(1)				
143		(1)				
144		(1)				
145		(1)				
146		(1)				
147		(1)				
148		(1)				
149		(1)				
150		(1)				

	*		*		*	
	Indep	Depend	Indep	Depend	Indep	Depend
151		(1)				
152		(1)				
153		(1)				
154		(1)				
155		(1)				
156		(1)				
157		(1)				
158		(1)				
159		(1)				
160		(1)				
161		(1)				
162		(1)				
163	1					
164		1				
165		1				
166		1				
167		4				
168		4				
169		(1)				
170		(1)				
171		(1)				
172		(1)				
173		(1)				
174		(1)				
175		(1)				
176		(1)				
177		(1)				
178		(1)				
179		(1)				
180		(1)				
181		(1)				
182		(1)				
183		(1)				
184		(1)				
185		(1)				
186		(1)				
187		(1)				
188		(1)				
189		(1)				
190		(1)				
191		(1)				
192		(1)				
193		(1)				
194		(1)				
195		(1)				
196		(1)				
197		(1)				
198		(1)				
199		(1)				
200		(1)				

MULTIPLE DEPENDENT CLAIM FEE CALCULATION SHEET

Substitute for Form PTO-1360
(For use with Form PTO/SB/06)

Application Number	Filing Date
16089040	

Applicant(s) Bruce FORREST

* May be used for additional claims or amendments

CLAIMS	AS FILED		AFTER FIRST AMENDMENT		AFTER SECOND AMENDMENT	
	Indep	Depend	Indep	Depend	Indep	Depend
201		(1)				
202		(1)				
203		(1)				
204		(1)				
205		(1)				
206		(1)				
207		(1)				
208		(1)				
209		(1)				
210		(1)				
211		(1)				
212		(1)				
213		(1)				
214		(1)				
215		(1)				
216		(1)				
217		(1)				
218		(1)				
219		(1)				
220		(1)				
221		(1)				
222		(1)				
223		(1)				
224		(1)				
225		(1)				
226		(1)				
227		(1)				
228		(1)				
229		(1)				
230		(1)				
231		(1)				
232		(1)				
233		(1)				
234		(1)				
235		(1)				
236		(1)				
237		(1)				
238		(1)				
239		(1)				
240		(1)				
241		(1)				
242		(1)				
243		(1)				
244		(1)				
245		(1)				
246		(1)				
247		(1)				
248		(1)				
249		(1)				
250		(1)				
Total Indep	6		0		0	
Total Depend	316	↵	0	↵	0	↵
Total Claims	322		0		0	

	*		*		*	
	Indep	Depend	Indep	Depend	Indep	Depend
251		(1)				
252		(1)				
253		(1)				
254		(1)				
255		(1)				
256		(1)				
257		(1)				
258		(1)				
259		(1)				
260		(1)				
261		(1)				
262		(1)				
263		(1)				
264		(1)				
265		(1)				
266		(1)				
267		(1)				
268		(1)				
269		(1)				
270		(1)				
271		(1)				
272		(1)				
273		(1)				
274		(1)				
275		(1)				
276		(1)				
277		(1)				
278		(1)				
279		(1)				
280		(1)				
281		(1)				
282		(1)				
283		(1)				
284		(1)				
285		(1)				
286		(1)				
287		(1)				
288		(1)				
289		(1)				
290		(1)				
291		(1)				
292		(1)				
293		(1)				
294		(1)				
295		(1)				
296						
297						
298						
299						
300						

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

U.S. APPLICATION NO.	FIRST NAMED INVENTOR	ATTY. DOCKET NO.
16/089,040	Bruce D. FORREST	P1143.70000US04

23628
WOLF GREENFIELD & SACKS, P.C.
600 ATLANTIC AVENUE
BOSTON, MA 02210-2206

INTERNATIONAL APPLICATION NO.	
PCT/US17/25621	
I.A. FILING DATE	PRIORITY DATE
03/31/2017	03/31/2016

CONFIRMATION NO. 8702
371 FORMALITIES LETTER



Date Mailed: 01/10/2020

Notification of Insufficiency Under 37 CFR 1.492 and/or 1.495 (DO/EO/US)

The following items have been submitted by the applicant or the International Bureau to the United States Patent and Trademark Office as a Designated / Elected Office (37 CFR 1.495).

- Indication of Small Entity Status
- Priority Document
- Copy of the International Application filed on 09/27/2018
- Copy of the International Search Report filed on 09/27/2018
- U.S. Basic National Fees filed on 09/27/2018
- Authorize Access to Search Results filed on 09/27/2018
- Power of Attorney filed on 12/11/2019
- Authorization to Permit Access filed on 09/27/2018
- Application Data Sheet (37 CFR 1.76) filed on 09/27/2018

The following items **MUST** be furnished within the period set forth below:

- Additional claim fees of $**16200** as a small entity, including any required multiple dependent claim fee, are required. Applicant must submit the additional claim fees or cancel the additional claims for which fees are due.

SUMMARY OF FEES DUE:

Total additional fees required for this application is $**16200** for a Small Entity:

- Total additional claim fee(s) for this application is $**16200**
  - $**690** for **3** independent claims over 3.
  - $**15100** for **302** total claims over 20.
  - $**410** for multiple dependent claim surcharge.

ALL OF THE ITEMS SET FORTH ABOVE MUST BE SUBMITTED WITHIN TWO (2) MONTHS FROM THE DATE OF THIS NOTICE. FAILURE TO PROPERLY RESPOND WILL RESULT IN ABANDONMENT.

The time period set above may be extended by filing a petition and fee for extension of time under the provisions of 37 CFR 1.136(a).

Applicant is reminded that any communications to the United States Patent and Trademark Office must be mailed to the address given in the heading and include the U.S. application no. shown above (37 CFR 1.5)

FORM PCT/DO/EO/923 (371 Formalities Notice)

Petitioner Merck Ex-1004, 0482

Registered users of EFS-Web may alternatively submit their reply to this notice via EFS-Web.
https://sportal.uspto.gov/authenticate/AuthenticateUserLocalEPF.html

For more information about EFS-Web please call the USPTO Electronic Business Center at **1-866-217-9197** or visit our website at http://www.uspto.gov/ebc.

If you are not using EFS-Web to submit your reply, you must include a copy of this notice.

TONI M HOOD

Telephone: (571) 272-3654

FORM PCT/DO/EO/923 (371 Formalities Notice)

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
16/089,040	09/27/2018		600	P1143.70000US04	295	6

CONFIRMATION NO. 8702

23628
WOLF GREENFIELD & SACKS, P.C.
600 ATLANTIC AVENUE
BOSTON, MA 02210-2206

FILING RECEIPT

OC000000113818330

Date Mailed: 01/10/2020

Receipt is acknowledged of this non-provisional utility patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF FIRST INVENTOR, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection.

Please verify the accuracy of the data presented on this receipt. If an error is noted on this Filing Receipt, please submit a written request for a corrected Filing Receipt, including a properly marked-up ADS showing the changes with strike-through for deletions and underlining for additions. If you received a "Notice to File Missing Parts" or other Notice requiring a response for this application, please submit any request for correction to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections provided that the request is grantable.

Inventor(s)

Bruce D. FORREST, South Nyack, NY;
Jack D. LOVE, Tampa, FL;

Applicant(s)

Pogona, LLC, South Nyack, NY;

Assignment For Published Patent Application

Pogona, LLC, South Nyack, NY

Power of Attorney: The patent practitioners associated with Customer Number 23628

Domestic Priority data as claimed by applicant

This application is a 371 of PCT/US17/25621 03/31/2017
which claims benefit of 62/316,555 03/31/2016
and claims benefit of 62/330,245 05/02/2016
and claims benefit of 62/372,263 08/08/2016
and claims benefit of 62/373,807 08/11/2016

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

Permission to Access Application via Priority Document Exchange: Yes

Petitioner Merck Ex-1004, 0484

Permission to Access Search Results: Yes

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

If Required, Foreign Filing License Granted: 01/08/2020
The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 16/089,040**
Projected Publication Date: 04/16/2020
Non-Publication Request: No
Early Publication Request: No
**** SMALL ENTITY ****
Title

SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF

Preliminary Class

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative,

this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

SelectUSA

The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The U.S. offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to promote and facilitate business investment. SelectUSA provides information assistance to the international investor community; serves as an ombudsman for existing and potential investors; advocates on behalf of U.S. cities, states, and regions competing for global investment; and counsels U.S. economic development organizations on investment attraction best practices. To learn more about why the United States is the best country in the world to develop

technology, manufacture products, deliver services, and grow your business, visit http://www.SelectUSA.gov or call +1-202-482-6800.

Petitioner Merck Ex-1004, 0487

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 16/089,040

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	150		N/A	
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	70		N/A	
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	380		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	322 minus 20 =	* 302	x 50 =	15100	OR		
INDEPENDENT CLAIMS (37 CFR 1.16(h))	6 minus 3 =	* 3	x 230 =	690			
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			0.00			
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				410			
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	16800		TOTAL	

APPLICATION AS AMENDED - PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

FORM PTO-1449/A and B (modified PTO/SB/08)	APPLICATION NO.: 16/089,040	ATTY. DOCKET NO.: P1143.70000US04
INFORMATION DISCLOSURE STATEMENT BY APPLICANT	FILING DATE: September 27, 2018	CONFIRMATION NO.: 8702
	FIRST NAMED INVENTOR: Bruce D. Forrest	
Sheet 1 of 2	GROUP ART UNIT: 1645	EXAMINER: Not Yet Assigned

U.S. PATENT DOCUMENTS

Examiner's Initials #	Cite No.	U.S. Patent Document Number	U.S. Patent Document Kind Code	Name of Patentee or Applicant of Cited Document	Date of Publication or Issue of Cited Document MM-DD-YYYY
		4,221,906		Querry et al.	09-09-1980
		4,242,501		Cano et al.	12-30-1980
		4,686,102		Ritchey et al.	08-11-1987
		5,623,057		Marburg et al.	04-22-1997
		5,847,112		Kniskern et al.	12-08-1998
		8,753,645		Biemans et al.	06-17-2014
		8,808,707		Siber et al.	08-19-2014
		9,764,039		Thanos et al.	09-19-2017
		9,359,400		Usera et al.	06-07-2016
		2015-0374811	A1	Malley et al.	12-31-2015

FOREIGN PATENT DOCUMENTS

Examiner's Initials #	Cite No.	Foreign Patent Document Office/ Country	Foreign Patent Document Number	Foreign Patent Document Kind Code	Name of Patentee or Applicant of Cited Document	Date of Publication of Cited Document MM-DD-YYYY	Translation (Y/N)
		CN	104069488	A	Sinovac Res & Dev Co. Ltd.	10-01-2014	Y – Abstract Only
		EP	2865392	A1	SK Chemicals Co., Ltd.	04-29-2015	
		WO	2008/143709	A2	Wyeth	11-27-2008	
		WO	2011/100151	A1	Merck Sharp & Dohme Corp.	08-18-2011	
		WO	2016/207905	A2	Biological E Limited	12-19-2016	
		WO	2018/064444	A1	Biological E Limited	04-05-2018	
		WO	2019/070994	A1	Liffey Biotech Limited	04-11-2019	

OTHER ART – NON PATENT LITERATURE DOCUMENTS

Examiner's Initials #	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	Translation (Y/N)
		Extended European Search Report, mailed November 4, 2019, in connection with Application No. EP 17776881 (P1143.70000EP00).	
		International Search Report and Written Opinion, mailed September 13, 2017, in connection with Application No. PCT/US2017/025621 (P1143.70000WO00).	

EXAMINER:	DATE CONSIDERED:

EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to Applicant.

7915412.1

FORM PTO-1449/A and B (modified PTO/SB/08) **INFORMATION DISCLOSURE STATEMENT BY APPLICANT**				APPLICATION NO.: 16/089,040	ATTY. DOCKET NO.: P1143.70000US04
				FILING DATE: September 27, 2018	CONFIRMATION NO.: 8702
				FIRST NAMED INVENTOR: Bruce D. Forrest	
Sheet	2	of	2	GROUP ART UNIT: 1645	EXAMINER: Not Yet Assigned

		International Preliminary Report on Patentability, mailed October 11, 2018, in connection with Application No. PCT/US2017/025621 (P1143.70000WO00).	
		BENTLEY et al., Genetic Analysis of the Capsular Biosynthetic Locus from All 90 Pneumococcal Serotypes. PLoS Genet. 2006 Mar; 2(3): e31. Epub 2006 Mar 10. doi: 10.1371/journal.pgen.0020031.	
		DURANDO et al., Experience with pneumococcal polysaccharide conjugate vaccine (conjugated to CRM197 carrier protein) in children and adults. Clin Microbiol Infect. 2013;19 Suppl 1:1–9. doi:10.1111/1469-0691.12320.	
		HU et al., Towards the next generation of biomedicines by site-selective conjugation. Chem Soc Rev. 2016;45(6):1691–1719. doi:10.1039/c4cs00388h.	
		JEFFERIES et al., 13-valent pneumococcal conjugate vaccine (PCV13). Hum Vaccin. 2011;7(10):1012–1018. doi:10.4161/hv.7.10.16794.	
		KOLKMAN et al., Functional analysis of glycosyltransferases encoded by the capsular polysaccharide biosynthesis locus of Streptococcus pneumoniae serotype 14. J Biol Chem. 1997;272(31):19502–19508. doi:10.1074/jbc.272.31.19502.	
		SKINNER et al., Pre-clinical evaluation of a 15-valent pneumococcal conjugate vaccine (PCV15-CRM197) in an infant-rhesus monkey immunogenicity model. Vaccine. 2011;29(48):8870–8876. doi:10.1016/j.vaccine.2011.09.078.	

[NOTE – No copies of U.S. patents, published U.S. patent applications, or pending, unpublished patent applications stored in the USPTO's Image File Wrapper (IFW) system, are included. See 37 CFR § 1.98 and 1287OG163. Copies of all other patent(s), publication(s), unpublished, pending U.S. patent applications, or other information listed are provided as required by 37 CFR § 1.98 unless 1) such copies were provided in an IDS in an earlier application that complies with 37 CFR § 1.98, and 2) the earlier application is relied upon for an earlier filing date under 35 U.S.C. § 120.]

EXAMINER:	DATE CONSIDERED:

[#] EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to Applicant.

7915412.1

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To: Keli L. Holzapfel
Wilson Sonsini Goodrich & Rosati
650 Page Mill Road
Palo Alto, California 94304
United States of America

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

	Date of mailing *(day/month/year)* 13 SEP 2017
Applicant's or agent's file reference 503087-01601	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/US17/25621	International filing date *(day/month/year)* 31 March 2017 (31.03.2017)
Applicant LIFFEY BIOTECH LIMITED	

1. ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

   Filing of amendments and statement under Article 19:
   The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46).

   When? The time limit for filing such amendments is normally twononths from the date of transmittal of the international search report.

   How? Directly to the International Bureau of WIPO preferably through ePCT or on paper to, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Facsimile No.: +41 22 338 82 70

   For more detailed instructions, see *PCT Applicant's Guide*, International Phase, paragraphs 9.004 – 9.011.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. ☐ **With regard to any protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

   ☐ the protest together with the decision thereon has been transmitted to the International Bureau together with any request to forward the texts of both the protest and the decision thereon to the designated Offices.

   ☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

   The applicant may **submit comments on an informal basis on the written opinion of the International Searching Authority** to the International Bureau. These comments will be made available to the public after international publication. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established.

   Shortly after the expiration of **18 months from the priority date, the international application will be published** by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau before the completion of the technical preparation for international publication (Rules 90*bis*.1 and 90*bis*.3).

   Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for **entry into the national phase** before those designated Offices. In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months. For details about the applicable time limits, Office by Office, see www.wipo.int/pct/en/texts/time_limits.html and the *PCT Applicant's Guide*, National Chapters.

   Within **19 months from the priority date, the applicant may request that a supplementary international search be carried out** by a different International Searching Authority that offers this service (Rule 45 *bis*.1). The procedure for requesting supplementary international search is described in the *PCT Applicant's Guide*, International Phase, paragraphs 8.006-8.032.

Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas Telephone No. PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/220 (July 2014)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/25621

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 39/09, 39/02, 39/385; C07K 14/315 (2017.01)

CPC - A61K 39/092, 39/02, 47/646, 39/385; C07K 14/3156, 16/1271

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

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

See Search History document

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2015/0374811 A1 (CHILDREN'S MEDICAL CENTER CORPORATION) 31 December, 2015; paragraphs [0055], [0134], [0136], [0232]-[0233], [0242], [0266], [0419]	1-3, 23/1-3, 24, 67, 70 --- 163-166, 167/163-166
Y	(DURANDO, P et al.) Experience with Pneumococcal Polysaccharide Conjugate Vaccine (Conjugated to CRM197 Carrier Protein) in Children and Adults. Clinical Microbiology and Infection. October, 2013; Vol. 19, Supplement 1; pages 1-9; page 7, column 1, paragraph 2; DOI: 10.1111/1469-0691.12320	163-166, 167/163-166
Y	(KOLKMAN, MAB et al.) Functional Analysis of Glycosyltransferases Encoded by the Capsular Polysaccharide Biosynthesis Locus of Streptococcus pneumoniae Serotype 14. Journal of Biological Chemistry. 01 August 1997; Vol. 272, No. 31; pages 19502-19508; abstract; page 19502, column 2, paragraph 4	165-166, 167/165-166

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

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 August 2017 (22.08.2017)	13 SEP 2017
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/25621

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 71-162, 168-295
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

This International Searching Authority found multiple inventions in this international application, as follows:
-Please See Supplemental Page-*-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Group I: claims 1 (in-part), 2 (in-part), 3 (in-part), 23 (in-part), 24 (in-part), 67 (in-part), 70 (in-part) and 163-167

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Petitioner Merck Ex-1004, 0493

-***-Continued from Box No. III: Observations where unity of invention is lacking-***-

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+, Claims 1-70, 163-167, and S. pneumoniae serotype 2 are directed toward pharmaceutical compositions comprising a plurality of immunogenic saccharide-polypeptide conjugates; and a method comprising administering a second composition at least 4 weeks after a first composition.

The compositions, method of making and administration method will be searched to the extent they encompass a S. pneumoniae serotype encompassing serotype 2 (first exemplary serotype). Applicant is invited to elect additional serotype(s) to be searched. Additional serotype(s) will be searched upon the payment of additional fees. It is believed that claims 1 (in-part), 2 (in-part), 3 (in-part), 23 (in-part), 24 (in-part), 67 (in-part), 70 (in-part) and 163-167 encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass serotype 2 (serotype). Applicants must specify the claims that encompass any additionally elected serotype(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be a serotype encompassing serotype 6C (first exemplary elected serotype).

No technical features are shared between the S. penumoniae serotypes of Groups I+ and, accordingly, these groups lack unity a priori.

Groups I+ share the technical features including: a pharmaceutical composition comprising at least 2 immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide; and a method comprising: administering a second immunogenic saccharide-polypeptide conjugate composition to a subject at least four weeks after a first immunogenic saccharide-polypeptide conjugate composition was administered; wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a first polypeptide that has at least about 70% length homology, at least about 70% sequence homology, or a combination thereof, to a second polypeptide of the second immunogenic saccharide-polypeptide conjugate composition; and wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a first serotype; wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a second serotype, and wherein the first serotype and the second serotype are different.

However, these shared technical features are previously disclosed by US 2015/0374811 A1 to Children's Medical Center Corporation (hereinafter 'CMCC').

CMCC discloses a pharmaceutical composition (a pharmaceutical composition; paragraph [0026]) comprising at least 2 immunogenic saccharide-polypeptide conjugates (comprising at least 2 immunogenic saccharide-polypeptide conjugates; paragraphs [0134], [0197]) each comprising individually a capsular polysaccharide conjugated to a polypeptide (each comprising individually a capsular polysaccharide conjugated to a polypeptide; paragraph [0134]); and a method comprising: administering a second immunogenic saccharide-polypeptide conjugate composition to a subject after a first immunogenic saccharide-polypeptide conjugate composition was administered (administering a second immunogenic saccharide-polypeptide conjugate composition to a subject after a first immunogenic saccharide-polypeptide conjugate composition was administered; paragraphs [0134], [0162]); wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a first polypeptide that has at least about 70% length homology, at least about 70% sequence homology, to a second polypeptide of the second immunogenic saccharide-polypeptide conjugate composition (wherein different capsular polysaccharides are conjugated to a single antigen polypeptide (wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a first polypeptide that has at least about 70% length homology, at least about 70% sequence homology, to a second polypeptide of the second immunogenic saccharide-polypeptide conjugate composition); paragraph [1034]); and wherein the immunogenic saccharide-polypeptide conjugate composition comprises an immunogen from a first serotype; and the same immunogen from a second serotype, and wherein the first serotype and the second serotype are different, based on seasonal variation (wherein the immunogenic saccharide-polypeptide conjugate composition comprises an immunogen from a first serotype; and the same immunogen from a second serotype, and wherein the first serotype and the second serotype are different, based on seasonal variation; paragraph [0197]). CMCC further discloses wherein the second immunogenic composition differs from the first immunogenic composition, including comprising a different subset of antigens, or a different antigen substance (wherein the second immunogenic composition differs from the first immunogenic composition, including comprising a different subset of antigens, or a different antigen substance; paragraph [0162]).

CMCC does not disclose: at least four weeks after a first immunogenic saccharide-polypeptide conjugate composition was administered; and wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a first serotype; wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a second serotype, and wherein the first serotype and the second serotype are different. However, it would have been obvious to a person of ordinary skill in the art at the time of the invention was made to have modified the disclosure of CMCC to have included a specific period of time between administration of a first dose of an immunogenic composition and a second dose of a related immunogenic composition in order to ensure the production of an immune response therebetween, including a period of from 3 weeks to about 6 weeks, such as 4 weeks. It further would have been obvious to a person of ordinary skill in the art at the time of the invention was made to have modified the disclosure of CMCC to have provided a booster administration of an immunogenic composition comprising antigens from a different serotype of S. pneumoniae, based on the disclosure of CMCC, in order to ensure the production of effective immune responses to one or more seasonal variants of S. pneumoniae, thereby effectively producing year-round immunity in a subject.

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the CMCC reference, unity of invention is lacking.

Petitioner Merck Ex-1004, 0494

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: Keli L. Holzapfel
Wilson Sonsini Goodrich & Rosati
650 Page Mill Road
Palo Alto, California 94304
United States of America

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	13 SEP 2017
Applicant's or agent's file reference 503087-01601	FOR FURTHER ACTION See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US17/25621	31 March 2017 (31.03.2017)	31 March 2016 (31.03.2016)

International Patent Classification (IPC) or both national classification and IPC
IPC - A61K 39/09, 39/02, 39/385; C07K 14/315 (2017.01)
CPC - A61K 39/092, 39/02, 47/646, 39/385; C07K 14/3156, 16/1271

Applicant LIFFEY BIOTECH LIMITED

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	22 August 2017 (22.08.2017)	Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (January 2015)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US17/25621

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a)).

3. ☒ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing:

a. ☐ forming part of the international application as filed:

☐ in the form of an Annex C/ST.25 text file.

☐ on paper or in the form of an image file.

b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. ☒ furnished subsequent to the international filing date for the purposes of international search only:

☒ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

4. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US17/25621

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 71-162, 168-295

because:

☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search *(specify)*:

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 71-162, 168-295 are so unclear that no meaningful opinion could be formed *(specify)*:

Claims 71-162, 168-295 are all dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

☒ no international search report has been established for said claims Nos. 71-162, 168-295

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

☐ furnish a sequence listing in the form of an Annex C/ST.25 text file, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.

☐ furnish a sequence listing on paper or in the form of an image file complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.

☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13*ter*.1(a) or (b).

☐ See Supplemental Box for further details.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US17/25621

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the applicable time limit:

   ☐ paid additional fees.

   ☐ paid additional fees under protest and, where applicable, the protest fee.

   ☐ paid additional fees under protest but the applicable protest fee was not paid.

   ☒ not paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+, Claims 1-70, 163-167, and S. pneumoniae serotype 2 are directed toward pharmaceutical compositions comprising a plurality of immunogenic saccharide-polypeptide conjugates; and a method comprising administering a second composition at least 4 weeks after a first composition.

The compositions, method of making and administration method will be searched to the extent they encompass a S. pneumoniae serotype encompassing serotype 2 (first exemplary serotype). Applicant is invited to elect additional serotype(s) to be searched. Additional serotype(s) will be searched upon the payment of additional fees. It is believed that claims 1 (in-part), 2 (in-part), 3 (in-part), 23 (in-part), 24 (in-part), 67 (in-part), 70 (in-part) and 163-167 encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass serotype 2 (serotype). Applicants must specify the claims that encompass any additionally elected serotype(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be a serotype encompassing serotype 6C (first exemplary elected serotype).

No technical features are shared between the S. penumoniae serotypes of Groups I+ and, accordingly, these groups lack unity a priori.

Groups I+ share the technical features including: a pharmaceutical composition comprising at least 2 immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide; and a method comprising: administering a second immunogenic saccharide-polypeptide conjugate composition to a subject at least four weeks after a first immunogenic saccharide-polypeptide conjugate composition was administered; wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a first polypeptide that has at least about 70% length homology, at least about 70% sequence homology, or a combination thereof, to a second polypeptide of the second immunogenic saccharide-polypeptide conjugate composition; and wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a first serotype; wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a second serotype, and wherein the first serotype and the second serotype are different.

However, these shared technical features are previously disclosed by US 2015/0374811 A1 to Children's Medical Center Corporation (hereinafter 'CMCC').

-***-Continued Within the Next Supplemental Box-***-

4. Consequently, this opinion has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-3, 23-24, 67, 70, 163-167

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US17/25621

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	23/1-3, 163-166, 167/163-166	YES
	Claims	1-3, 24, 67, 70	NO
Inventive step (IS)	Claims	NONE	YES
	Claims	1-3, 23/1-3, 24, 67, 70, 163-166, 167/163-166	NO
Industrial applicability (IA)	Claims	1-3, 23/1-3, 24, 67, 70, 163-166, 167/163-166	YES
	Claims	NONE	NO

2. Citations and explanations:

Claims 1-3, 24, 67 and 70 lack novelty under PCT Article 33(2) as being anticipated by US 2015/0374811 A1 (CHILDREN'S MEDICAL CENTER CORPORATION) (hereinafter 'Children').

As per claim 1, Children discloses a pharmaceutical composition (pharmaceutical immunogenic composition; paragraph [0136]) comprising a plurality of at least eight unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide conjugated to a polypeptide (plurality of at least eight unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide conjugated to a pneumococcal antigen polypeptide; paragraph [0134]), wherein the capsular polysaccharide (capsular polysaccharide; paragraph [0134]), is from a unique Streptococcus pneumoniae serotype selected from a group consisting of 2 (paragraph [0134]), 8 (paragraph [0134]), 9N (paragraph [0134]), 10A (paragraph [0134]), 11A (paragraph [0134]), 12F (paragraph [0134]), 15B (paragraph [0134]), 17F (paragraph [0134]), 22F (paragraph [0134]), 23A (paragraph [0134]), 24F (paragraph [0134]), 33F (paragraph [0134]) and 34 (paragraph [0134]).

As per claim 2, Children discloses the pharmaceutical composition of claim 1, and Children further discloses comprising a plurality of at least 9 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide conjugated to a polypeptide (plurality of at least 9 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide conjugated to a pneumococcal antigen polypeptide; paragraph [0134]), wherein the capsular polysaccharide (capsular polysaccharide; paragraph [0134]), is from a unique Streptococcus pneumoniae serotype selected from a group consisting of 2 (paragraph [0134]), 8 (paragraph [0134]), 9N (paragraph [0134]), 10A (paragraph [0134]), 11A (paragraph [0134]), 12F (paragraph [0134]), 15B (paragraph [0134]), 17F (paragraph [0134]), 22F (paragraph [0134]), 23A (paragraph [0134]), 24F (paragraph [0134]), 33F (paragraph [0134]) and 34 (paragraph [0134]).

As per claim 3, Children discloses the pharmaceutical composition of claim 1, and Children further discloses comprising a plurality of at least 10 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide conjugated to a polypeptide (plurality of at least 10 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide conjugated to a pneumococcal antigen polypeptide; paragraph [0134]), wherein the capsular polysaccharide (capsular polysaccharide; paragraph [0134]), is from a unique Streptococcus pneumoniae serotype selected from a group consisting of 2 (paragraph [0134]), 8 (paragraph [0134]), 9N (paragraph [0134]), 10A (paragraph [0134]), 11A (paragraph [0134]), 12F (paragraph [0134]), 15B (paragraph [0134]), 17F (paragraph [0134]), 22F (paragraph [0134]), 23A (paragraph [0134]), 24F (paragraph [0134]), 33F (paragraph [0134]) and 34 (paragraph [0134]).

As per claim 24, Children discloses a pharmaceutical composition (pharmaceutical immunogenic composition; paragraph [0136]) comprising a plurality of at least 28 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide conjugated to a polypeptide (plurality of at least 28 unique (pathogenic) immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide conjugated to a pneumococcal antigen polypeptide; paragraphs [0055], [0134]), wherein the capsular polysaccharide (capsular polysaccharide; paragraph [0134]), is from a unique Streptococcus pneumoniae serotype selected from a group consisting of 2 (paragraph [0134]), 8 (paragraph [0134]), 9N (paragraph [0134]), 10A (paragraph [0134]), 11A (paragraph [0134]), 12F (paragraph [0134]), 15B (paragraph [0134]), 17F (paragraph [0134]), 22F (paragraph [0134]), 23A (paragraph [0134]), 24F (paragraph [0134]), 33F (paragraph [0134]) and 34 (paragraph [0134]).

As per claim 67, Children discloses a pharmaceutical composition (pharmaceutical immunogenic composition; paragraph [0136]) comprising an immunogenic saccharide-polypeptide conjugate (unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide conjugated to a pneumococcal antigen polypeptide; paragraph [0134]) comprising, individually, at least two unique capsular polysaccharides conjugated to a polypeptide (unique immunogenic saccharide-polypeptide conjugates each comprising one or more capsular polysaccharides conjugated to a pneumococcal antigen polypeptide; paragraph [0134]), wherein the at least two unique capsular polysaccharides are from a Streptococcus pneumoniae serotype selected from a group consisting of 1 (paragraph [0134]), 2 (paragraph [0134]), 3 (paragraph [0134]), 4 (paragraph [0134]), 5 (paragraph [0134]), 6B (paragraph [0134]), 7F (paragraph [0134]), 9V (paragraph [0134]), 14 (paragraph [0134]), 18C (paragraph [0134]), 19A (paragraph [0134]), 19F (paragraph [0134]), 23F (paragraph [0134]), 8 (paragraph [0134]), 9N (paragraph [0134]), 10A (paragraph [0134]), 11A (paragraph [0134]), 12F (paragraph [0134]), 15B (paragraph [0134]), 17F (paragraph [0134]), 22F (paragraph [0134]), 23A (paragraph [0134]), 24F (paragraph [0134]), 33F (paragraph [0134]) and 34 (paragraph [0134]).

-***-Continued Within the Next Supplemental Box-***-

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US17/25621

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

-***-Continued from Box V: Citations and Explanations-***-

As per claim 70, Children discloses the pharmaceutical composition of claim 67, and Children further discloses comprising at least one unique immunogenic saccharide-polypeptide conjugate each comprising individually a capsular polysaccharide conjugated to a polypeptide (plurality of unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide conjugated to a pneumococcal antigen polypeptide; paragraph [0134]), wherein the capsular polysaccharide is from a unique Streptococcus pneumoniae serotype 14 (capsular polysaccharide is from a unique Streptococcus pneumoniae serotype 14; paragraph [0134]).

Claims 23/1-3 lack an inventive step under PCT Article 33(3) as being obvious over Children.

As per claims 23/1-3, Children discloses the pharmaceutical composition of claims 1-3, and Children further discloses wherein the unique Streptococcus pneumoniae serotype is not any of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F (unique Streptococcus pneumoniae serotype can be 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 22F, 23A, 24F, 33F and 34; paragraph [0134]). It would have been obvious to one of ordinary skill in the art at the time of invention to have modified the disclosure of Children, by including that unique Streptococcus pneumoniae serotype is not any of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F, based on the previous disclosure of Children that any S. pneumoniae capsular polysaccharide can be used in the immunogenic composition (Children; paragraph [0134]).

Claims 163-164 and 167/163-164 lack an inventive step under PCT Article 33(3) as being obvious over Children in view of the publication entitled 'Experience with Pneumococcal Polysaccharide Conjugate Vaccine (Conjugated to CRM197 Carrier Protein) in Children and Adults' by Durando et al. (hereinafter 'Durando').

As per claim 163, Children discloses a method comprising: administering a second immunogenic saccharide-polypeptide conjugate composition to a subject after a first immunogenic saccharide-polypeptide conjugate composition was administered (secondary immunization with the immunogenic composition after the first immunization; paragraph [0419]); wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a first polypeptide that has at least about 70% length homology to a second polypeptide of the second immunogenic saccharide-polypeptide conjugate composition (unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide conjugated to a pneumococcal antigen polypeptide, where each conjugate can have 75-99 percent homology; paragraphs [0134], [0232]-[0233]); and wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a first serotype (first immunogenic saccharide-polypeptide conjugate composition can comprise a first serotype picked from any of the listed 28 different serotypes; paragraphs [0055], [0134], [0266]); wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a second serotype (second immunogenic saccharide-polypeptide conjugate composition comprises a second serotype specifically 6B, 14F or 19F; paragraphs [0134], [0266]), and wherein the first serotype and the second serotype are different (first and second serotypes can be different; paragraphs [0134], [0266]). Children does not disclose administering a second immunogenic saccharide-polypeptide conjugate composition to a subject at least four weeks after a first immunogenic saccharide-polypeptide conjugate composition was administered. Durando discloses administering a second immunogenic saccharide-polypeptide conjugate composition to a subject at least four weeks after a first immunogenic saccharide-polypeptide conjugate composition was administered (patient receives 2 different doses of immunogenic saccharide-polypeptide conjugates 4 weeks apart; page 7, column 1, paragraph 2). It would have been obvious to one of ordinary skill in the art at the time of invention to have modified the disclosure of Children, by including the time interval between 2 doses, as disclosed by Durando, for providing the advantage of priming and boosting the immune response using different antigens.

As per claim 164, Children and Durando, in combination, disclose the method of claim 163, and Children further discloses wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a capsular polysaccharide (plurality of unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide conjugated to a pneumococcal antigen polypeptide; paragraph [0134]).

-***-Continued Within the Next Supplemental Box-***-

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US17/25621

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

-***-Continued from Previous Supplemental Box-***-

As per claims 167/163-164, Children and Durando, in combination, disclose the method of claims 163-164, and Children further discloses wherein the first polypeptide is an oligopeptide (oligopeptide can be a fragment of the pneumococcal polypeptide; paragraph [0242]).

Claims 165-166 and 167/165-166 lack an inventive step under PCT Article 33(3) as being obvious over Children and Durando in view of the publication entitled 'Functional Analysis of Glycosyltransferases Encoded by the Capsular Polysaccharide Biosynthesis Locus of Streptococcus pneumoniae Serotype 14' by Kolkman et al. (hereinafter 'Kolkman').

As per claim 165, Children and Durando, in combination, disclose the method of claim 164, and Children further discloses wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a capsular polysaccharide (plurality of unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide conjugated to a pneumococcal antigen polypeptide; paragraph [0134]). Children does not disclose wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a fragment of the capsular polysaccharide. Kolkman discloses wherein the immunogenic saccharide-polypeptide conjugate composition comprises a fragment of the capsular polysaccharide (pneumococcal capsular monosaccharides (fragments) from serotype 14; abstract; page 19502, column 2, paragraph 4). It would have been obvious to one of ordinary skill in the art at the time of invention to have modified the disclosure of Children, by including a fragment of the capsular polysaccharide, as disclosed by Kolkman, for providing the advantage of generating conjugate vaccines to elicit immune response against S.pneumoniae infection.

As per claim 166, Children, Durando and Kolkman, in combination, disclose the method of claim 165. Children does not disclose wherein the fragment of the capsular polysaccharide comprises a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, or a hexasaccharide. Kolkman discloses wherein the fragment of the capsular polysaccharide comprises a monosaccharide (pneumococcal capsular monosaccharides (fragments) from serotype 14; abstract; page 19502, column 2, paragraph 4). It would have been obvious to one of ordinary skill in the art at the time of invention to have modified the disclosure of Children, by including a fragment of the capsular polysaccharide, as disclosed by Kolkman, for providing the advantage of generating conjugate vaccines to elicit immune response against S.pneumoniae infection.

As per claims 167/165-166, Children, Durando and Kolkman, in combination, disclose the method of claims 165-166, and Children further discloses wherein the first polypeptide is an oligopeptide (oligopeptide can be a fragment of the pneumococcal polypeptide; paragraph [0242]).

Claims 1-3, 23/1-3, 24, 67, 70, 163-166 and 167/163-166 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US17/25621

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

-***-Continued from Box IV: Lack of Unity of Invention-***-

CMCC discloses a pharmaceutical composition (a pharmaceutical composition; paragraph [0026]) comprising at least 2 immunogenic saccharide-polypeptide conjugates (comprising at least 2 immunogenic saccharide-polypeptide conjugates; paragraphs [0134], [0197]) each comprising individually a capsular polysaccharide conjugated to a polypeptide (each comprising individually a capsular polysaccharide conjugated to a polypeptide; paragraph [0134]); and a method comprising: administering a second immunogenic saccharide-polypeptide conjugate composition to a subject after a first immunogenic saccharide-polypeptide conjugate composition was administered (administering a second immunogenic saccharide-polypeptide conjugate composition to a subject after a first immunogenic saccharide-polypeptide conjugate composition was administered; paragraphs [0134], [0162]), wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a first polypeptide that has at least about 70% length homology, at least about 70% sequence homology, to a second polypeptide of the second immunogenic saccharide-polypeptide conjugate composition (wherein different capsular polysaccharides are conjugated to a single antigen polypeptide (wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a first polypeptide that has at least about 70% length homology, at least about 70% sequence homology, to a second polypeptide of the second immunogenic saccharide-polypeptide conjugate composition); paragraph [1034]); and wherein the immunogenic saccharide-polypeptide conjugate composition comprises an immunogen from a first serotype; and the same immunogen from a second serotype, and wherein the first serotype and the second serotype are different, based on seasonal variation (wherein the immunogenic saccharide-polypeptide conjugate composition comprises an immunogen from a first serotype; and the same immunogen from a second serotype, and wherein the first serotype and the second serotype are different, based on seasonal variation; paragraph [0197]). CMCC further discloses wherein the second immunogenic composition differs from the first immunogenic composition, including comprising a different subset of antigens, or a different antigen substance (wherein the second immunogenic composition differs from the first immunogenic composition, including comprising a different subset of antigens, or a different antigen substance; paragraph [0162]).

CMCC does not disclose: at least four weeks after a first immunogenic saccharide-polypeptide conjugate composition was administered; and wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a first serotype; wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a second serotype, and wherein the first serotype and the second serotype are different. However, it would have been obvious to a person of ordinary skill in the art at the time of the invention was made to have modified the disclosure of CMCC to have included a specific period of time between administration of a first dose of an immunogenic composition and a second dose of a related immunogenic composition in order to ensure the production of an immune response therebetween, including a period of from 3 weeks to about 6 weeks, such as 4 weeks. It further would have been obvious to a person of ordinary skill in the art at the time of the invention was made to have modified the disclosure of CMCC to have provided a booster administration of an immunogenic composition comprising antigens from a different serotype of S. pneumoniae, based on the disclosure of CMCC, in order to ensure the production of effective immune responses to one or more seasonal variants of S. pneumoniae, thereby effectively producing year-round immunity in a subject.

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the CMCC reference, unity of invention is lacking.

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING TRANSMITTAL OF COPY OF INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY (CHAPTER I OF THE PATENT COOPERATION TREATY)
(PCT Rule 44bis.1(c))

To:

HOLZAPFEL, Keli L.
Wilson Sonsini Goodrich & Rosati
650 Page Mill Road
Palo Alto, California 94304
ETATS-UNIS D'AMERIQUE

Date of mailing *(day/month/year)*
11 October 2018 (11.10.2018)

Applicant's or agent's file reference
503087-01601

IMPORTANT NOTICE

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2017/025621	31 March 2017 (31.03.2017)	31 March 2016 (31.03.2016)

Applicant
LIFFEY BIOTECH LIMITED

The International Bureau transmits herewith a copy of the international preliminary report on patentability (Chapter I of the Patent Cooperation Treaty)

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. +41 22 338 82 70

Authorized officer
Agnès Wittmann-Regis

e-mail: pct.team6@wipo.int

Form PCT/IB/326 (January 2004)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference 503087-01601	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/US2017/025621	International filing date *(day/month/year)* 31 March 2017 (31.03.2017)	Priority date *(day/month/year)* 31 March 2016 (31.03.2016)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant LIFFEY BIOTECH LIMITED		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

	Date of issuance of this report 02 October 2018 (02.10.2018)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Agnès Wittmann-Regis e-mail: pct.team6@wipo.int

Form PCT/IB/373 (January 2004)

Petitioner Merck Ex-1004, 0504

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: Keli L. Holzapfel
Wilson Sonsini Goodrich & Rosati
650 Page Mill Road
Palo Alto, California 94304
United States of America

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	13 SEP 2017

Applicant's or agent's file reference	FOR FURTHER ACTION
503087-01601	See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US17/25621	31 March 2017 (31.03.2017)	31 March 2016 (31.03.2016)

International Patent Classification (IPC) or both national classification and IPC
IPC - A61K 39/09, 39/02, 39/385; C07K 14/315 (2017.01)
CPC - A61K 39/092, 39/02, 47/646, 39/385; C07K 14/3156, 16/1271

Applicant LIFFEY BIOTECH LIMITED

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	22 August 2017 (22.08.2017)	Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (January 2015)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US17/25621

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a)).

3. ☒ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing:

   a. ☐ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☒ furnished subsequent to the international filing date for the purposes of international search only:

      ☒ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

4. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US17/25621

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 71-162, 168-295

because:

☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search *(specify)*:

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 71-162, 168-295 are so unclear that no meaningful opinion could be formed *(specify)*:

Claims 71-162, 168-295 are all dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

☒ no international search report has been established for said claims Nos. 71-162, 168-295

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

- ☐ furnish a sequence listing in the form of an Annex C/ST.25 text file, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.
- ☐ furnish a sequence listing on paper or in the form of an image file complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13*ter*.1(a) or (b).

☐ See Supplemental Box for further details.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US17/25621

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the applicable time limit:

   ☐ paid additional fees.

   ☐ paid additional fees under protest and, where applicable, the protest fee.

   ☐ paid additional fees under protest but the applicable protest fee was not paid.

   ☒ not paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+, Claims 1-70, 163-167, and S. pneumoniae serotype 2 are directed toward pharmaceutical compositions comprising a plurality of immunogenic saccharide-polypeptide conjugates; and a method comprising administering a second composition at least 4 weeks after a first composition.

The compositions, method of making and administration method will be searched to the extent they encompass a S. pneumoniae serotype encompassing serotype 2 (first exemplary serotype). Applicant is invited to elect additional serotype(s) to be searched. Additional serotype(s) will be searched upon the payment of additional fees. It is believed that claims 1 (in-part), 2 (in-part), 3 (in-part), 23 (in-part), 24 (in-part), 67 (in-part), 70 (in-part) and 163-167 encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass serotype 2 (serotype). Applicants must specify the claims that encompass any additionally elected serotype(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be a serotype encompassing serotype 6C (first exemplary elected serotype).

No technical features are shared between the S. penumoniae serotypes of Groups I+ and, accordingly, these groups lack unity a priori.

Groups I+ share the technical features including: a pharmaceutical composition comprising at least 2 immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide; and a method comprising: administering a second immunogenic saccharide-polypeptide conjugate composition to a subject at least four weeks after a first immunogenic saccharide-polypeptide conjugate composition was administered; wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a first polypeptide that has at least about 70% length homology, at least about 70% sequence homology, or a combination thereof, to a second polypeptide of the second immunogenic saccharide-polypeptide conjugate composition; and wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a first serotype; wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a second serotype, and wherein the first serotype and the second serotype are different.

However, these shared technical features are previously disclosed by US 2015/0374811 A1 to Children's Medical Center Corporation (hereinafter 'CMCC').

-***-Continued Within the Next Supplemental Box-***-

4. Consequently, this opinion has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-3, 23-24, 67, 70, 163-167

Petitioner Merck Ex-1004, 0508

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US17/25621

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	23/1-3, 163-166, 167/163-166	YES
	Claims	1-3, 24, 67, 70	NO
Inventive step (IS)	Claims	NONE	YES
	Claims	1-3, 23/1-3, 24, 67, 70, 163-166, 167/163-166	NO
Industrial applicability (IA)	Claims	1-3, 23/1-3, 24, 67, 70, 163-166, 167/163-166	YES
	Claims	NONE	NO

2. Citations and explanations:

Claims 1-3, 24, 67 and 70 lack novelty under PCT Article 33(2) as being anticipated by US 2015/0374811 A1 (CHILDREN'S MEDICAL CENTER CORPORATION) (hereinafter 'Children').

As per claim 1, Children discloses a pharmaceutical composition (pharmaceutical immunogenic composition; paragraph [0136]) comprising a plurality of at least eight unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide conjugated to a polypeptide (plurality of at least eight unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide conjugated to a pneumococcal antigen polypeptide; paragraph [0134]), wherein the capsular polysaccharide (capsular polysaccharide; paragraph [0134]), is from a unique Streptococcus pneumoniae serotype selected from a group consisting of 2 (paragraph [0134]), 8 (paragraph [0134]), 9N (paragraph [0134]), 10A (paragraph [0134]), 11A (paragraph [0134]), 12F (paragraph [0134]), 15B (paragraph [0134]), 17F (paragraph [0134]), 22F (paragraph [0134]), 23A (paragraph [0134]), 24F (paragraph [0134]), 33F (paragraph [0134]) and 34 (paragraph [0134]).

As per claim 2, Children discloses the pharmaceutical composition of claim 1, and Children further discloses comprising a plurality of at least 9 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide conjugated to a polypeptide (plurality of at least 9 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide conjugated to a pneumococcal antigen polypeptide; paragraph [0134]), wherein the capsular polysaccharide (capsular polysaccharide; paragraph [0134]), is from a unique Streptococcus pneumoniae serotype selected from a group consisting of 2 (paragraph [0134]), 8 (paragraph [0134]), 9N (paragraph [0134]), 10A (paragraph [0134]), 11A (paragraph [0134]), 12F (paragraph [0134]), 15B (paragraph [0134]), 17F (paragraph [0134]), 22F (paragraph [0134]), 23A (paragraph [0134]), 24F (paragraph [0134]), 33F (paragraph [0134]) and 34 (paragraph [0134]).

As per claim 3, Children discloses the pharmaceutical composition of claim 1, and Children further discloses comprising a plurality of at least 10 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide conjugated to a polypeptide (plurality of at least 10 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide conjugated to a pneumococcal antigen polypeptide; paragraph [0134]), wherein the capsular polysaccharide (capsular polysaccharide; paragraph [0134]), is from a unique Streptococcus pneumoniae serotype selected from a group consisting of 2 (paragraph [0134]), 8 (paragraph [0134]), 9N (paragraph [0134]), 10A (paragraph [0134]), 11A (paragraph [0134]), 12F (paragraph [0134]), 15B (paragraph [0134]), 17F (paragraph [0134]), 22F (paragraph [0134]), 23A (paragraph [0134]), 24F (paragraph [0134]), 33F (paragraph [0134]) and 34 (paragraph [0134])

As per claim 24, Children discloses a pharmaceutical composition (pharmaceutical immunogenic composition; paragraph [0136]) comprising a plurality of at least 28 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide conjugated to a polypeptide (plurality of at least 28 unique (pathogenic) immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide conjugated to a pneumococcal antigen polypeptide; paragraphs [0055], [0134]), wherein the capsular polysaccharide (capsular polysaccharide; paragraph [0134]), is from a unique Streptococcus pneumoniae serotype selected from a group consisting of 2 (paragraph [0134]), 8 (paragraph [0134]), 9N (paragraph [0134]), 10A (paragraph [0134]), 11A (paragraph [0134]), 12F (paragraph [0134]), 15B (paragraph [0134]), 17F (paragraph [0134]), 22F (paragraph [0134]), 23A (paragraph [0134]), 24F (paragraph [0134]), 33F (paragraph [0134]) and 34 (paragraph [0134]).

As per claim 67, Children discloses a pharmaceutical composition (pharmaceutical immunogenic composition; paragraph [0136]) comprising an immunogenic saccharide-polypeptide conjugate (unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide conjugated to a pneumococcal antigen polypeptide; paragraph [0134]) comprising, individually, at least two unique capsular polysaccharides conjugated to a polypeptide (unique immunogenic saccharide-polypeptide conjugates each comprising one or more capsular polysaccharides conjugated to a pneumococcal antigen polypeptide; paragraph [0134]), wherein the at least two unique capsular polysaccharides are from a Streptococcus pneumoniae serotype selected from a group consisting of 1 (paragraph [0134]), 2 (paragraph [0134]), 3 (paragraph [0134]), 4 (paragraph [0134]), 5 (paragraph [0134]), 6B (paragraph [0134]), 7F (paragraph [0134]), 9V (paragraph [0134]), 14 (paragraph [0134]), 18C (paragraph [0134]), 19A (paragraph [0134]), 19F (paragraph [0134]), 23F (paragraph [0134]), 8 (paragraph [0134]), 9N (paragraph [0134]), 10A (paragraph [0134]), 11A (paragraph [0134]), 12F (paragraph [0134]), 15B (paragraph [0134]), 17F (paragraph [0134]), 22F (paragraph [0134]), 23A (paragraph [0134]), 24F (paragraph [0134]), 33F (paragraph [0134]) and 34 (paragraph [0134]).

-***-Continued Within the Next Supplemental Box-***-

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US17/25621

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

-***-Continued from Box V: Citations and Explanations-***-

As per claim 70, Children discloses the pharmaceutical composition of claim 67, and Children further discloses comprising at least one unique immunogenic saccharide-polypeptide conjugate each comprising individually a capsular polysaccharide conjugated to a polypeptide (plurality of unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide conjugated to a pneumococcal antigen polypeptide; paragraph [0134]), wherein the capsular polysaccharide is from a unique Streptococcus pneumoniae serotype 14 (capsular polysaccharide is from a unique Streptococcus pneumoniae serotype 14; paragraph [0134]).

Claims 23/1-3 lack an inventive step under PCT Article 33(3) as being obvious over Children.

As per claims 23/1-3, Children discloses the pharmaceutical composition of claims 1-3, and Children further discloses wherein the unique Streptococcus pneumoniae serotype is not any of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F (unique Streptococcus pneumoniae serotype can be 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 22F, 23A, 24F, 33F and 34; paragraph [0134]). It would have been obvious to one of ordinary skill in the art at the time of invention to have modified the disclosure of Children, by including that unique Streptococcus pneumoniae serotype is not any of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F, based on the previous disclosure of Children that any S. pneumoniae capsular polysaccharide can be used in the immunogenic composition (Children; paragraph [0134]).

Claims 163-164 and 167/163-164 lack an inventive step under PCT Article 33(3) as being obvious over Children in view of the publication entitled 'Experience with Pneumococcal Polysaccharide Conjugate Vaccine (Conjugated to CRM197 Carrier Protein) in Children and Adults' by Durando et al. (hereinafter 'Durando').

As per claim 163, Children discloses a method comprising: administering a second immunogenic saccharide-polypeptide conjugate composition to a subject after a first immunogenic saccharide-polypeptide conjugate composition was administered (secondary immunization with the immunogenic composition after the first immunization; paragraph [0419]); wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a first polypeptide that has at least about 70% length homology to a second polypeptide of the second immunogenic saccharide-polypeptide conjugate composition (unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide conjugated to a pneumococcal antigen polypeptide, where each conjugate can have 75-99 percent homology; paragraphs [0134], [0232]-[0233]); and wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a first serotype (first immunogenic saccharide-polypeptide conjugate composition can comprise a first serotype picked from any of the listed 28 different serotypes; paragraphs [0055], [0134], [0266]); wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a second serotype (second immunogenic saccharide-polypeptide conjugate composition comprises a second serotype specifically 6B, 14F or 19F; paragraphs [0134], [0266]), and wherein the first serotype and the second serotype are different (first and second serotypes can be different; paragraphs [0134], [0266]). Children does not disclose administering a second immunogenic saccharide-polypeptide conjugate composition to a subject at least four weeks after a first immunogenic saccharide-polypeptide conjugate composition was administered. Durando discloses administering a second immunogenic saccharide-polypeptide conjugate composition to a subject at least four weeks after a first immunogenic saccharide-polypeptide conjugate composition was administered (patient receives 2 different doses of immunogenic saccharide-polypeptide conjugates 4 weeks apart; page 7, column 1, paragraph 2). It would have been obvious to one of ordinary skill in the art at the time of invention to have modified the disclosure of Children, by including the time interval between 2 doses, as disclosed by Durando, for providing the advantage of priming and boosting the immune response using different antigens.

As per claim 164, Children and Durando, in combination, disclose the method of claim 163, and Children further discloses wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a capsular polysaccharide (plurality of unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide conjugated to a pneumococcal antigen polypeptide; paragraph [0134]).

-***-Continued Within the Next Supplemental Box-***-

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US17/25621

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

-***-Continued from Previous Supplemental Box-***-

As per claims 167/163-164, Children and Durando, in combination, disclose the method of claims 163-164, and Children further discloses wherein the first polypeptide is an oligopeptide (oligopeptide can be a fragment of the pneumococcal polypeptide; paragraph [0242]).

Claims 165-166 and 167/165-166 lack an inventive step under PCT Article 33(3) as being obvious over Children and Durando in view of the publication entitled 'Functional Analysis of Glycosyltransferases Encoded by the Capsular Polysaccharide Biosynthesis Locus of Streptococcus pneumoniae Serotype 14' by Kolkman et al. (hereinafter 'Kolkman').

As per claim 165, Children and Durando, in combination, disclose the method of claim 164, and Children further discloses wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a capsular polysaccharide (plurality of unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide conjugated to a pneumococcal antigen polypeptide; paragraph [0134]). Children does not disclose wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a fragment of the capsular polysaccharide. Kolkman discloses wherein the immunogenic saccharide-polypeptide conjugate composition comprises a fragment of the capsular polysaccharide (pneumococcal capsular monosaccharides (fragments) from serotype 14; abstract; page 19502, column 2, paragraph 4). It would have been obvious to one of ordinary skill in the art at the time of invention to have modified the disclosure of Children, by including a fragment of the capsular polysaccharide, as disclosed by Kolkman, for providing the advantage of generating conjugate vaccines to elicit immune response against S.pneumoniae infection.

As per claim 166, Children, Durando and Kolkman, in combination, disclose the method of claim 165. Children does not disclose wherein the fragment of the capsular polysaccharide comprises a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, or a hexasaccharide. Kolkman discloses wherein the fragment of the capsular polysaccharide comprises a monosaccharide (pneumococcal capsular monosaccharides (fragments) from serotype 14; abstract; page 19502, column 2, paragraph 4). It would have been obvious to one of ordinary skill in the art at the time of invention to have modified the disclosure of Children, by including a fragment of the capsular polysaccharide, as disclosed by Kolkman, for providing the advantage of generating conjugate vaccines to elicit immune response against S.pneumoniae infection.

As per claims 167/165-166, Children, Durando and Kolkman, in combination, disclose the method of claims 165-166, and Children further discloses wherein the first polypeptide is an oligopeptide (oligopeptide can be a fragment of the pneumococcal polypeptide; paragraph [0242]).

Claims 1-3, 23/1-3, 24, 67, 70, 163-166 and 167/163-166 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US17/25621

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

-***-Continued from Box IV: Lack of Unity of Invention-***-

CMCC discloses a pharmaceutical composition (a pharmaceutical composition; paragraph [0026]) comprising at least 2 immunogenic saccharide-polypeptide conjugates (comprising at least 2 immunogenic saccharide-polypeptide conjugates; paragraphs [0134], [0197]) each comprising individually a capsular polysaccharide conjugated to a polypeptide (each comprising individually a capsular polysaccharide conjugated to a polypeptide; paragraph [0134]); and a method comprising: administering a second immunogenic saccharide-polypeptide conjugate composition to a subject after a first immunogenic saccharide-polypeptide conjugate composition was administered (administering a second immunogenic saccharide-polypeptide conjugate composition to a subject after a first immunogenic saccharide-polypeptide conjugate composition was administered; paragraphs [0134], [0162]); wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a first polypeptide that has at least about 70% length homology, at least about 70% sequence homology, to a second polypeptide of the second immunogenic saccharide-polypeptide conjugate composition (wherein different capsular polysaccharides are conjugated to a single antigen polypeptide (wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a first polypeptide that has at least about 70% length homology, at least about 70% sequence homology, to a second polypeptide of the second immunogenic saccharide-polypeptide conjugate composition); paragraph [1034]); and wherein the immunogenic saccharide-polypeptide conjugate composition comprises an immunogen from a first serotype; and the same immunogen from a second serotype, and wherein the first serotype and the second serotype are different, based on seasonal variation (wherein the immunogenic saccharide-polypeptide conjugate composition comprises an immunogen from a first serotype; and the same immunogen from a second serotype, and wherein the first serotype and the second serotype are different, based on seasonal variation; paragraph [0197]). CMCC further discloses wherein the second immunogenic composition differs from the first immunogenic composition, including comprising a different subset of antigens, or a different antigen substance (wherein the second immunogenic composition differs from the first immunogenic composition, including comprising a different subset of antigens, or a different antigen substance; paragraph [0162]).

CMCC does not disclose: at least four weeks after a first immunogenic saccharide-polypeptide conjugate composition was administered; and wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a first serotype; wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a second serotype, and wherein the first serotype and the second serotype are different. However, it would have been obvious to a person of ordinary skill in the art at the time of the invention was made to have modified the disclosure of CMCC to have included a specific period of time between administration of a first dose of an immunogenic composition and a second dose of a related immunogenic composition in order to ensure the production of an immune response therebetween, including a period of from 3 weeks to about 6 weeks, such as 4 weeks. It further would have been obvious to a person of ordinary skill in the art at the time of the invention was made to have modified the disclosure of CMCC to have provided a booster administration of an immunogenic composition comprising antigens from a different serotype of S. pneumoniae, based on the disclosure of CMCC, in order to ensure the production of effective immune responses to one or more seasonal variants of S. pneumoniae, thereby effectively producing year-round immunity in a subject.

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the CMCC reference, unity of invention is lacking.

Espacenet

Bibliographic data: CN104069488 (A) — 2014-10-01

Multivalent pneumococcus capsular polysaccharide-protein conjugated composition and preparation method thereof

Inventor(s): GAO QIANG; DONG SHANSHAN; WANG XINLI; CAI FANG; GE XIAOQIN; YIN WEIDONG ± (GAO QIANG, ; DONG SHANSHAN, ; WANG XINLI, ; CAI FANG, ; GE XIAOQIN, ; YIN WEIDONG)

Applicant(s): SINOVAC RES & DEV CO LTD ± (SINOVAC RESEARCH & DEVELOPMENT CO., LTD)

Classification: **- international:** ***A61K39/09; A61K47/48; A61P31/04; C08B37/00***
- cooperative:

Application number: CN201310109045 20130329 Global Dossier

Priority number(s): CN201310109045 20130329

Abstract of CN104069488 (A)

The invention provides a multivalent pneumococcus capsular polysaccharide-protein conjugated composition and a preparation method thereof. The conjugated composition is formed by covalent linkage of multivalent pneumococcus capsular polysaccharides of 14 different serotypes and carrier protein, wherein the 14 serotypes include 1, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F. The conjugated composition has good adsorption effect and good stability, has multiple immunogenicity and protective performance against invasion of the pneumococcus of 14 serotypes, is superior to on-sale low-valent pneumonia compositions, and the immune response of the conjugated composition disclosed by the invention is higher than that of an uncombined composition. The inoculating injection frequency can be reduced by using the multivalent pneumococcus capsular polysaccharide conjugate vaccine containing the conjugated composition, the immune process can be simplified, and diseases of human and animals caused by the 14 serotypes of pneumococcal bacteria can be effectively prevented. The conjugated composition has wider coverage and better immune effect.

Petitioner Merck Ex-1004, 0513

(19)中华人民共和国国家知识产权局

(12)发明专利申请

(10)申请公布号 CN 104069488 A
(43)申请公布日 2014.10.01

(21)申请号 201310109045.8

(22)申请日 2013.03.29

(71)申请人 北京科兴中维生物技术有限公司
地址 100085 北京市海淀区上地西路 39 号北大生物城

(72)发明人 高强 董珊珊 王新立 蔡芳 戈小琴 尹卫东

(74)专利代理机构 北京路浩知识产权代理有限公司 11002
代理人 王朋飞 张庆敏

(51)Int.Cl.
A61K 39/09(2006.01)
A61K 47/48(2006.01)
C08B 37/00(2006.01)
A61P 31/04(2006.01)

权利要求书1页 说明书8页

(54)发明名称

多价肺炎球菌荚膜多糖-蛋白质共轭组合物及其制备方法

(57)摘要

本发明提供了一种多价肺炎球菌荚膜多糖-蛋白质共轭组合物及其制备方法，该共轭组合物是包括 14 种不同血清型的肺炎链球菌荚膜多糖和载体蛋白质共价连接形成的共轭组合物，其中，所述 14 种不同血清型包括 1、4、5、6A、6B、7F、9V、14、18C、19A、19F、22F、23F 和 33F。该共轭组合物吸附效果及稳定性好，具有针对上述 14 种血清型的肺炎球菌侵袭的多重免疫原性和保护性能，优于已上市的低价次肺炎组合物，且免疫应答高于未结合的组合物。接种含有该共轭组合物的多价肺炎球菌荚膜多糖结合疫苗可以减少接种针次，简化免疫程序，同时可有效预防人及动物因上述 14 种血清型肺炎球菌所致的疾病，覆盖更加广泛，免疫效果更好。

CN 104069488 A

Petitioner Merck Ex-1004, 0514

1. 多价肺炎球菌荚膜多糖－蛋白质共轭组合物，其特征在于，其是包括 14 种不同血清型肺炎链球菌（Streptococcus pneumoniae）荚膜多糖和载体蛋白质共价连接形成的共轭组合物；其中，所述 14 种不同血清型包括 1、4、5、6A、6B、7F、9V、14、18C、19A、19F、22F、23F 和 33F。

2. 根据权利要求 1 所述的共轭组合物，其特征在于，其是由 14 种不同血清型肺炎链球菌荚膜多糖和载体蛋白质共价连接形成的共轭组合物；其中，所述 14 种不同血清型为 1、4、5、6A、6B、7F、9V、14、18C、19A、19F、22F、23F 和 33F。

3. 根据权利要求 1 所述的共轭组合物，其特征在于，荚膜多糖与载体蛋白质的重量比为 1:2-2:1，各血清型荚膜多糖的质量为血清型 1：血清型 4：血清型 5：血清型 6A：血清型 7F：血清型 9V：血清型 14：血清型 18C：血清型 19A：血清型 19F：血清型 22F：血清型 23F：血清型 33F：血清型 6B=1:1:1:1:1:1:1:1:1:1:1:1:1:2。

4. 根据权利要求 1 所述的共轭组合物，其特征在于，所述载体蛋白质为白喉类毒素或破伤风类毒素。

5. 根据权利要求 4 所述的共轭组合物，其特征在于，所述载体蛋白质为白喉类毒素 CRM197。

6. 制备权利要求 1-5 任一项所述共轭组合物的方法，其特征在于，分别对 14 种不同血清型肺炎链球菌的荚膜多糖进行分离纯化，然后将各血清型荚膜多糖分别与载体蛋白质进行共价连接，形成单价共轭物，最后将各型单价共轭物混合，即得多价肺炎球菌荚膜多糖－蛋白质共轭组合物。

7. 根据权利要求 6 所述的方法，其特征在于，肺炎链球菌荚膜多糖分离纯化的步骤为：

1）建立肺炎球菌菌种库，采用工作种子进行肺炎球菌发酵；

2）向肺炎球菌发酵液中加入适量的去氧胆酸钠进行杀菌并剥离肺炎荚膜多糖；

3）对杀菌后的发酵液进行离心，去除菌体，收集离心上清，然后进行超滤浓缩；

4）向浓缩液中加入无水乙醇，使乙醇终浓度为 25% ～ 45%，搅拌后离心去除核酸；

5）向上清中加入乙醇使其终浓度达到 55% ～ 80%，搅拌后，离心，去除上清收集沉淀；

6）加水溶解沉淀，加入 1-3 倍溶液体积的苯酚进行抽提，去除蛋白；

7）向上清中加入乙醇，使其终浓度达到 55% ～ 80%，离心后收集沉淀，即得纯化的肺炎球菌荚膜多糖。

8. 权利要求 1-5 任一项所述共轭组合物在制备用于预防或治疗肺炎链球菌所致疾病的药物中的应用。

9. 多价肺炎球菌荚膜多糖结合疫苗，其含有权利要求 1-5 任一项所述的共轭组合物，以及赋形剂和 / 或任选基于铝的佐剂；其中，基于铝的佐剂其终浓度为 0.25-1.0mg/mL。

10. 根据权利要求 9 所述的结合疫苗，其特征在于，其为喷雾剂、注射剂、冻干剂、胶囊剂、片剂或丸剂。

Petitioner Merck Ex-1004, 0515

多价肺炎球菌荚膜多糖－蛋白质共轭组合物及其制备方法

技术领域

[0001] 本发明属于微生物学及免疫学领域，具体地说，涉及一种多价肺炎球菌荚膜多糖－蛋白质共轭组合物及其制备方法。

背景技术

[0002] 肺炎链球菌(Streptococcus pneumoniae)可引起脑膜炎、肺炎等侵入性疾病和中耳炎等非侵入性疾病。据联合国统计数据显示，每年有超过一百万的两岁以下儿童死于肺炎球菌引起的疾病。目前，针对肺炎链球菌的主要防御手段为多糖疫苗和结合疫苗，已经证实了前者在成年人中的保护效果，但是对于肺炎链球菌的主要易感人群，婴儿和幼儿中的保护力却很弱。结合疫苗因其可以在婴幼儿中引起有效保护和免疫记忆，自2000年首次问世以来，在全世界许多国家被纳入免疫计划或批准上市。

[0003] 目前我国仅有7价肺炎球菌结合疫苗Prevnar®被批准上市，仅能保护7个价次，保护范围有限，而象其他10价肺炎球菌结合疫苗Synflorix®，13价肺炎球菌结合疫苗Prevenar 13®，其血清型组合主要针对欧美发达国家流行情况而设计，对我国特有的血清型缺乏保护。

发明内容

[0004] 本发明的目的是提供一种多价肺炎球菌荚膜多糖－蛋白质共轭组合物、其制备方法及应用。

[0005] 本发明的另一目的是提供一种含有上述共轭组合物的多价肺炎球菌荚膜多糖结合疫苗。

[0006] 为了实现本发明目的，本发明的一种多价肺炎球菌荚膜多糖－蛋白质共轭组合物，其是包括14种不同血清型肺炎链球菌(Streptococcus pneumoniae)荚膜多糖和载体蛋白质共价连接形成的共轭组合物；其中，所述14种不同血清型包括1、4、5、6A、6B、7F、9V、14、18C、19A、19F、22F、23F和33F。

[0007] 本发明的一种多价肺炎球菌荚膜多糖－蛋白质共轭组合物，其是由14种不同血清型肺炎链球菌荚膜多糖和载体蛋白质共价连接形成的共轭组合物；其中，所述14种不同血清型为1、4、5、6A、6B、7F、9V、14、18C、19A、19F、22F、23F和33F。

[0008] 前述的多价肺炎球菌荚膜多糖－蛋白质共轭组合物，荚膜多糖与载体蛋白质的重量比为1:2-2:1，各血清型荚膜多糖的质量为血清型1：血清型4：血清型5：血清型6A：血清型7F：血清型9V：血清型14：血清型18C：血清型19A：血清型19F：血清型22F：血清型23F：血清型33F：血清型6B=1:1:1:1:1:1:1:1:1:1:1:1:1:2。

[0009] 前述的多价肺炎球菌荚膜多糖－蛋白质共轭组合物，所述载体蛋白质应为无毒性，且能与肺炎荚膜多糖结合并且能够引起多糖免疫原性显著升高的蛋白质，同时易于大量获得。本发明的载体蛋白质优选为白喉类毒素或破伤风毒素，更优选为白喉类毒素CRM197。上述蛋白质载体的获得，需经过菌体发酵、超滤、纯化、脱毒等步骤，均为本领域常

Petitioner Merck Ex-1004, 0516

规技术。

[0010] 本发明还提供制备所述多价肺炎球菌荚膜多糖－蛋白质共轭组合物的方法，分别对 14 种不同血清型（1、4、5、6A、6B、7F、9V、14、18C、19A、19F、22F、23F 和 33F）肺炎链球菌的荚膜多糖进行分离纯化，然后将各血清型荚膜多糖分别与载体蛋白质进行共价连接（例如，先将纯化的荚膜多糖化学活化，通过还原胺化法将活化荚膜多糖分别单独结合到载体蛋白上），形成单价共轭物，最后将各型单价共轭物混合，即得多价肺炎球菌荚膜多糖－蛋白质共轭组合物。

[0011] 其中，肺炎链球菌荚膜多糖分离纯化的步骤为：

[0012] 1）建立肺炎球菌菌种库，采用工作种子进行肺炎球菌发酵；

[0013] 2）向肺炎球菌发酵液中加入适量的去氧胆酸钠进行杀菌并剥离肺炎荚膜多糖；

[0014] 3）对杀菌后的发酵液进行离心，去除菌体，收集离心上清，然后进行超滤浓缩；

[0015] 4）向浓缩液中加入无水乙醇，使乙醇终浓度为 25% ～ 45%，搅拌后离心去除核酸；

[0016] 5）向上清中加入乙醇使其终浓度达到 55% ～ 80%，搅拌后，离心，去除上清收集沉淀；

[0017] 6）加水溶解沉淀，加入 1-3 倍溶液体积的苯酚进行多次抽提，去除蛋白；

[0018] 7）向上清中加入乙醇，使其终浓度达到 55% ～ 80%，离心后收集沉淀，即得纯化的肺炎球菌荚膜多糖。

[0019] 还可采用本领域熟知的其它纯化技术，如柱层析等方法对肺炎链球菌荚膜多糖进行纯化。

[0020] 本发明还提供所述多价肺炎球菌荚膜多糖－蛋白质共轭组合物在制备用于预防或治疗肺炎链球菌所致疾病的药物中的应用。

[0021] 本发明进一步提供一种多价肺炎球菌荚膜多糖结合疫苗，其含有所述多价肺炎球菌荚膜多糖－蛋白质共轭组合物，以及任选赋形剂和／或基于铝的佐剂。所述基于铝的佐剂，如氧化铝、氢氧化铝、磷酸铝、硫酸铝等。所述基于铝的佐剂可市售获得，也可以采用本领域常规方法进行制备，所述赋形剂优选氯化钠和／或琥珀酸等。

[0022] 前述的多价肺炎球菌荚膜多糖结合疫苗中，基于铝的佐剂其终浓度为 0.25-1.0mg/mL，优选 0.25-0.8mg/mL。

[0023] 前述的多价肺炎球菌荚膜多糖结合疫苗，其可以为喷雾剂、注射剂、冻干剂、胶囊剂、片剂或丸剂等。可通过以下方式进行免疫：皮肤、注射（包括皮下注射、肌肉注射、静脉注射）、口服、滴鼻或粘膜喷雾等。

[0024] 所述多价肺炎球菌荚膜多糖结合疫苗的制备方法包括：将各型单价共轭物分别采用铝佐剂吸附后混合；或各型单价共轭物混合后再采用铝佐剂吸附，即得 14 价肺炎球菌荚膜多糖结合疫苗（14vPnC）。其中，各血清型荚膜多糖含量：6B 为 4-16 μg/mL，其余血清型为 2-8 μg/mL，优选 6B 为 8 μg/mL，其余血清型 4 μg/mL；载体蛋白质为 30-90 μg/mL；铝含量终浓度为 0.25-1.0mg/mL，优选 0.5-0.7mg/mL。

[0025] 本发明的多价肺炎球菌荚膜多糖结合疫苗可以用于保护或者治疗由于肺炎球菌感染引发肺炎的病人，疫苗可以通过肌肉、皮下、皮内途径注射或经过粘膜给药。该多价结合疫苗适于接种 2、4、6 和 12-15 个月龄的婴幼儿，也更适合于儿童、青年和成人。

[0026] 本发明是针对我国特有血清型肺炎球菌的流行情况，创造性地选择了 1、4、5、6A、

Petitioner Merck Ex-1004, 0517

6B、7F、9V、14、18C、19A、19F、22F、23F 和 33F 的血清型组合，从而开发出针对我国特有血清型肺炎球菌的流行情况而设计的多价肺炎球菌荚膜多糖 - 蛋白质共轭组合物，在严格控制载体蛋白含量从而减少免疫抑制作用的前提下，提供最大限度的保护。

[0027] 采用本发明方法制备的多价肺炎球菌荚膜多糖 - 蛋白质共轭组合物，其吸附效果及稳定性较好。该共轭组合物具有针对上述 14 种血清型的肺炎球菌侵袭的多重免疫原性和保护性能，优于已上市的低价次肺炎组合物，且免疫应答高于未结合的组合物。接种本发明的多价肺炎球菌荚膜多糖结合疫苗可以减少接种针次，简化免疫程序，同时可有效预防人及动物因上述 14 种血清型肺炎球菌所致的疾病，覆盖更加广泛，免疫效果更好。另外，该多价结合疫苗免疫原性效果远高于同类多价次的多糖类疫苗，可有效激活 2 岁以下儿童 B 细胞，从而产生抗体，并激活记忆 B 细胞，诱发免疫记忆反应，可以起到更好的保护效果。

[0028] 本发明的多价肺炎球菌荚膜多糖结合疫苗可以由预防上述 14 种血清型肺炎球菌所致的侵袭性疾病，更加方便多效，成本更低。上述 14 种血清型荚膜多糖的生产，均采用发酵后纯化的方式进行制备，具有相似的生产工艺，便于工业化大规模生产，且通过灭活后提纯荚膜多糖，使疫苗具有更高的安全性及良好的免疫原性，同时，所述多价肺炎球菌荚膜多糖 - 蛋白质共轭组合物吸附完全，理化性质稳定，互无干扰。

[0029] 动物实验表明，本发明的多价肺炎球菌荚膜多糖结合疫苗可有效诱导机体产生针对上述 14 种血清型荚膜多糖的高滴度、特异性抗体，体外实验表明，由该多价结合疫苗所诱导的抗体具有明显的反应性与中和活性；特别是组合物中各血清型荚膜多糖的免疫效果不受另一组分的影响与干扰，吸附率大于 75%，免疫原性不低于单价共轭物。该多价结合疫苗具有与各血清型单价结合原液相同的免疫原性，组合接种可以在不影响疗效的基础上大大减少接种次数。

具体实施方式

[0030] 以下实施例用于说明本发明，但不用来限制本发明的范围。若未特别指明，实施例中所用的技术手段为本领域技术人员所熟知的常规手段，所用原料均为市售商品。

[0031] 实施例 1 肺炎球菌荚膜多糖原液制备

[0032] 1、4、5、6A、6B、7F、9V、14、18C、19A、19F、22F、23F 和 33F 血清型肺炎球菌 (Streptococcus pneumoniae) 菌株均来源于中国药品生物制品检定所。将上述菌种进行传代建立原始种子库、主种子库和工作种子库，代次分别为：原始种子批为第 1 代，主种子批为第 4 代，工作种子批为第 8 代。工作种子批启开后至接种发酵罐培养，传代应不超过 5 代。每代种子采用奶粉做冻干保护剂，冻干保藏。取工作种子在大豆蛋白胨固体培养基划线挑取菌种，接种于 5mL 的液体综合培养基，35℃ ±2℃培养后，转种到 500mL 的培养液后经过培养再扩增至 10L 培养液和 50L 培养液。于对数生长期的后期或静止期的前期中止培养，取样进行纯菌检查，纯菌检查合格后在收获的培养液中加入合适量的无菌的去氧胆酸钠至终浓度为 1% 杀菌两小时以裂解细菌细胞并释放荚膜多糖。将已杀菌的培养液离心后收集上清液，上清液用 100KD 截留分子量的超滤膜包超滤浓缩 3-10 倍。

[0033] 用 pH7.0 左右的磷酸盐缓冲液对超滤液采进行超滤换液，然后加入无水乙醇，使乙醇终浓度为 25% ～ 45%，充分搅拌后离心去除核酸。

[0034] 向上清中加入乙醇使其终浓度达到 55% ～ 80%，搅拌后，离心，去除上清收集沉淀。

Petitioner Merck Ex-1004, 0518

[0035] 加水溶解沉淀，加入 1-3 倍溶液体积的苯酚进行多次抽提，去除蛋白。

[0036] 向上清中加入乙醇，使其终浓度达到 55% ～ 80%，离心后收集沉淀，将沉淀采用注射水复溶，经过 0.2μm 的无菌滤器除菌过滤后，获得 1、4、5、6A、6B、7F、9V、14、18C、19A、19F、22F、23F 和 33F 共 14 种纯化血清型的肺炎球菌荚膜多糖。

[0037] 实施例 2 多价肺炎球菌荚膜多糖 - 蛋白质共轭组合物的制备

[0038] 将实施例 1 中制备的 14 种肺炎球菌荚膜多糖原液，采用盐酸进行酸化降解，然后在多糖中加入高碘酸钠氧化，氧化后检测多糖的活化度，然后按照多糖 :蛋白质 =1:2-2:1 的重量比加入纯化后的白喉类毒素 CRM197 蛋白质进行结合，结合后加入硼氢化钠终止结合反应，制备肺炎球菌荚膜多糖结合物，结合物采用超滤透析，去除未结合的蛋白质和多糖，纯化后采用 0.2μm 的滤器除菌过滤，即为肺炎球菌荚膜多糖 - 蛋白质单价共轭物。

[0039] 将上述制备的 1、4、5、6A、6B、7F、9V、14、18C、19A、19F、22F、23F 和 33F 血清型的肺炎球菌荚膜多糖 - 蛋白质单价共轭物按照多糖含量 4μg/mL，6B 结合物按照多糖含量 8μg/mL 混合，即得 14 价的肺炎球菌荚膜多糖 - 蛋白质共轭组合物。

[0040] 实施例 3 多价肺炎球菌荚膜多糖结合疫苗的制备

[0041] 将实施例 2 制备的 1、4、5、6A、6B、7F、9V、14、18C、19A、19F、22F、23F 和 33F 血清型的肺炎球菌荚膜多糖 - 蛋白质单价共轭物按照多糖终浓度 4μg/mL，6B 结合物按照多糖终浓度 8μg/mL 混合，加入磷酸铝佐剂，制成 14 价肺炎球菌荚膜多糖结合疫苗，铝含量为 0.25mg/mL。

[0042] 将上述制备的 14 价肺炎球菌荚膜多糖结合疫苗，采用低温干燥的方式制成冻干剂型，使用前采用生理盐水进行复溶。

[0043] 实施例 4 多价肺炎球菌荚膜多糖 - 蛋白质共轭组合物的铝吸附

[0044] 采用两种方式对多价肺炎球菌荚膜多糖 - 蛋白质共轭组合物进行吸附制备疫苗。

[0045] (1) 将纯化后的 1、4、5、6A、6B、7F、9V、14、18C、19A、19F、22F、23F 和 33F 血清型的肺炎球菌荚膜多糖 - 蛋白质单价共轭物先混合，然后与 0.5μg/mL 的磷酸铝佐剂混合，使多糖含量为 :6B 血清型 8μg/mL，1、4、5、6A、6B、7F、9V、14、18C、19A、19F、22F、23F 和 33F 血清型各为 4μg/mL，制成 14 价肺炎球菌荚膜多糖结合疫苗 (14vPnC)，铝佐剂终浓度为 0.25mg/mL。

[0046] (2) 将 1、4、5、6A、6B、7F、9V、14、18C、19A、19F、22F、23F 和 33F 血清型的肺炎球菌荚膜多糖 - 蛋白质单价共轭物分别与 0.5mg/mL 的磷酸铝佐剂混合，使多糖含量为 :6B 血清型 112μg/mL，其余血清型为 56μg/mL (其余各血清型按对比例混合)，铝佐剂浓度为 0.25mg/mL，然后将各吸附后的溶液等体积混合，制成终产物。

[0047] 检测上述吸附方式制备的 14vPnC 的吸附完全性、各型多糖含量回收率。14vPnC 各型多糖的吸附效果与各型多糖含量采用免疫浊度法进行检测。

[0048] 多糖吸附完全性与各型多糖回收率计算方法如下 :

[0049] 吸附完全性 :

[0050]

$$\text{Xn 型多糖吸附完全性} = [1 - \frac{\text{上清 Xn 型多糖含量}}{\text{Xn 型多糖理论含量}}] \times 100\%$$

Petitioner Merck Ex-1004, 0519

[0051] Xn 型多糖为 1、4、5、6A、6B、7F、9V、14、18C、19A、19F、22F、23F 或 33F 血清型。

[0052] 疫苗的吸附完全性反应疫苗中未被铝佐剂捕获的游离的各型多糖的含量占疫苗中总的理论各型多糖含量的比值，结果越高显示疫苗的吸附效果越好。

[0053] 疫苗各型多糖回收率：

[0054] 疫苗解离后分别检测各型多糖的含量，将检测值与该血清型多糖含量的理论值进行对比，要求回收率为理论值的 70%-130% 之间。先混合后吸附配比方式的检测结果见表 1，先分别吸附后混合配比方式的检测结果见表 2。

[0055] 表 114vPnC 各型多糖吸附完全性与各型多糖含量结果

[0056]

血清型	多糖理论含量 μg/mL	上清 μg/mL	吸附率 %	多糖含量 μg/mL	多糖回收率 %	是否合格
1	4	<1	>75	4.2	105	合格
4	4	<1	>75	3.5	87.5	合格
5	4	<1	>75	5	125	合格
6A	4	<1	>75	4.5	112.5	合格
6B	8	<1	>87.5	8.2	102.5	合格
7F	4	<1	>75	4.6	115	合格
9V	4	<1	>75	3.2	80	合格
14	4	<1	>75	5.1	127.5	合格
18C	4	<1	>75	4.8	120	合格
19A	4	<1	>75	3.3	82.5	合格
19F	4	<1	>75	5	125	合格
22F	4	<1	>75	5.2	130	合格
23F	4	<1	>75	3.9	97.5	合格
33F	4	<1	>75	4.6	115	合格

[0057] 表 214vPnC 各型多糖吸附完全性与各型多糖含量结果

Petitioner Merck Ex-1004, 0520

[0058]

血清型	多糖理论含量	上清 μg/mL	吸附率 %	多糖含量 μg/mL	多糖回收率	是否合格
1	4	<1	>75	5.2	130	合格
4	4	<1	>75	4.3	107.5	合格
5	4	<1	>75	3.4	85	合格
6A	4	<1	>75	4.5	112.5	合格
6B	8	<1	>87.5	7.4	92.5	合格
7F	4	<1	>75	5	125	合格
9V	4	<1	>75	4.4	110	合格
14	4	<1	>75	5	125	合格
18C	4	<1	>75	3.2	80	合格
19A	4	<1	>75	4.7	117.5	合格
19F	4	<1	>75	3.8	95	合格
22F	4	<1	>75	4.2	105	合格
23F	4	<1	>75	5.1	127.5	合格
33F	4	<1	>75	5	125	合格

[0059] 从表 1 和表 2 可以看出，采用上述方法制得的 14vPnC 组合物(疫苗)的吸附率效果较好，均超过 75%（检测限度）；疫苗解离后的各型多糖含量在 70%-130% 之间。表明疫苗的含量准确，吸附效率高。两种吸附方式无差异。

[0060] 采用氢氧化铝与硫酸铝，调整铝凝胶佐剂的铝含量终浓度至 0.25mg/mL、1.0mg/mL；制备多价肺炎球菌结合物组合物，亦得到相似的结果。

[0061] 实施例 5 采用不同铝佐剂对多价肺炎球菌荚膜多糖结合疫苗吸附效果的影响

[0062] 将 1、4、5、6A、6B、7F、9V、14、18C、19A、19F、22F、23F 和 33F 血清型的肺炎球菌荚膜多糖 - 蛋白质单价共轭物混合成 6B 多糖含量 16μg/mL，其余各血清型多糖含量 8μg/mL，然后分别与 0.5mg/mL 的磷酸铝、0.5mg/mL 的硫酸铝、0.5mg/mL 的氢氧化铝佐剂等体积混合吸附。吸附后，离心吸附产物，吸取上清，采用免疫浊度法检测上清中的各型多糖含量，检测各配比方式的吸附效果(表 3)。

[0063] 表 3 不同铝佐剂的吸附效果

Petitioner Merck Ex-1004, 0521

[0064]

血清型	多糖理论含量 μg/mL	磷酸铝佐剂上清多糖含量 μg/mL	硫酸铝佐剂上清多糖含量 μg/mL	氢氧化铝佐剂上清多糖含量 μg/mL
1	4	≤1	≤1	≤1
4	4	≤1	≤1	≤1
5	4	≤1	≤1	≤1
6A	4	≤1	≤1	≤1
6B	8	≤1	≤1	≤1
7F	4	≤1	≤1	≤1
9V	4	≤1	≤1	≤1
14	4	≤1	≤1	≤1
18C	4	≤1	≤1	≤1
19A	4	≤1	≤1	≤1
19F	4	≤1	≤1	≤1
22F	4	≤1	≤1	≤1
23F	4	≤1	≤1	≤1
33F	4	≤1	≤1	≤1

[0065] 从表3可以看出，14价肺炎球菌荚膜多糖-蛋白质共轭组合物，经过不同的铝佐剂吸附，在相同浓度时，吸附后未吸附的各型多糖含量均低于检测方法的检测下限。该结果表明，在该铝佐剂浓度下，三种佐剂对多价肺炎球菌荚膜多糖-蛋白质共轭组合物的吸附效果均较好，且三种佐剂的吸附效果未见差异。

[0066] 实施例6不同载体蛋白质对多价肺炎球菌荚膜多糖结合疫苗的影响

[0067] 将1、4、5、6A、6B、7F、9V、14、18C、19A、19F、22F、23F和33F血清型的肺炎球菌荚膜多糖分别与白喉类毒素CRM197、白喉毒素、破伤风毒素进行偶联，然后将各偶联方式获得的肺炎球菌荚膜多糖-蛋白质单价共轭物分别混合后，与0.5mg/mL的氢氧化铝佐剂进行混合，制备14价肺炎球菌荚膜多糖结合疫苗，使6B血清型肺炎球菌荚膜多糖含量为8μg/mL，其余血清型荚膜多糖含量各为4μg/mL，铝佐剂终浓度0.25mg/mL。检测不同载体蛋白质对于相同的吸附方式下的吸附效果，以及免疫原性。

[0068] 结果表明，采用不同的载体蛋白质偶联肺炎球菌荚膜多糖后，采用氢氧化铝吸附，吸附后离心吸附物质检测上清中各型多糖含量，检测结果均小于检测下限1μg/mL，表明不同的载体对于肺炎球菌荚膜多糖结合后的吸附效果较好，且无可见差异。

[0069] 实施例7多价肺炎球菌荚膜多糖结合疫苗免疫原性分析

[0070] 用实施例2制备的14价肺炎球菌荚膜多糖-蛋白质共轭组合物、实施例4制备的14价肺炎球菌荚膜多糖-蛋白质共轭组合物的铝吸附物，免疫18-22g的BALB/c小鼠，两组免疫物质的多糖含量均为6B8μg/mL，其余血清型各为4μg/mL。每组免疫小鼠10只。0、14天两针腹腔免疫，每次0.5mL，第28天采血，采用调理吞噬测定法(OPA)分别测定所有血

Petitioner Merck Ex-1004, 0522

清型肺炎球菌荚膜多糖的抗体功能，结果如表 4 所示。

[0071] 表 414vPnC 免疫 BALB/c 小鼠合并血清的肺炎链球菌 OPA 检测结果

[0072]

血清型	14vPnC			14vPnC+磷酸铝佐剂		
	第 0 天	第 28 天	比率 D28:D0	第 0 天	第 28 天	比率 D28:D0
1	<8	64	16	<8	128	32
4	<8	8	2	<8	64	16
5	<8	32	8	<8	256	64
6A	<8	128	32	<8	512	128
6B	<8	256	64	<8	1024	256
7F	8	256	32	<8	1024	256
9V	<8	128	32	<8	1024	256
14	<8	64	16	<8	256	64
18C	<8	128	32	<8	512	128
19A	16	128	8	8	1024	128
19F	<8	256	64	<8	1024	256
22F	<8	128	32	16	512	32
23F	<8	128	32	<8	512	128
33F	8	256	32	<8	1024	256

[0073]

[0074] 注：由各免疫组（n=10）每只小鼠等体积血清混合组成

[0075] 结果表明，14 价肺炎球菌荚膜多糖 - 蛋白质共轭组合物和 14 价肺炎球菌荚膜多糖 - 蛋白质共轭组合物的铝吸附物分别两针免疫小鼠后，两组小鼠血清的 OPA 功能均比阴性对照有显著性提高，同时，14 价肺炎球菌荚膜多糖 - 蛋白质共轭组合物的铝吸附物的 OPA 水平高于无佐剂的 14 价肺炎球菌荚膜多糖 - 蛋白质共轭组合物。因此，多糖在偶联蛋白质后可产生高水平的抗体，同时，吸附铝佐剂后抗体水平能够得到显著的提高。

[0076] 虽然，上文中已经用一般性说明及具体实施方案对本发明作了详尽的描述，但在本发明基础上，可以对之作一些修改或改进，这对本领域技术人员而言是显而易见的。因此，在不偏离本发明精神的基础上所做的这些修改或改进，均属于本发明要求保护的范围。

Petitioner Merck Ex-1004, 0523

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 2 865 392 A1

(12) **EUROPEAN PATENT APPLICATION**
published in accordance with Art. 153(4) EPC

(43) Date of publication:
29.04.2015 Bulletin 2015/18

(21) Application number: **13806263.3**

(22) Date of filing: **19.06.2013**

(51) Int Cl.:
A61K 47/48 *(2006.01)* ***A61K 31/70*** *(2006.01)*
A61K 38/00 *(2006.01)* ***A61P 31/00*** *(2006.01)*

(86) International application number:
PCT/KR2013/005392

(87) International publication number:
WO 2013/191459 (27.12.2013 Gazette 2013/52)

(84) Designated Contracting States:
AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR
Designated Extension States:
BA ME

(30) Priority: **20.06.2012 KR 20120065893**

(71) Applicant: **SK Chemicals Co., Ltd.**
Seongnam-si, Gyeonggi-do 463-400 (KR)

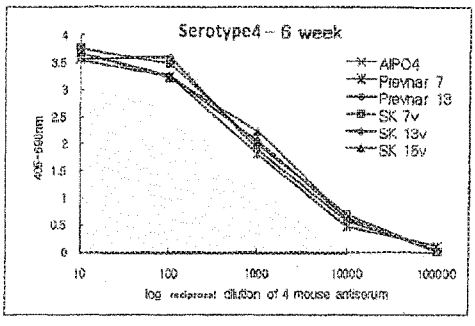
(72) Inventors:
- **SHIN, Jin-Hwan**
  Seoul 138-160 (KR)
- **YANG, Ji-Hye**
  Seoul 120-813 (KR)
- **HAM, Dong-Soo**
  Suwon-si
  Gyeonggi-do 442-754 (KR)
- **PARK, Mahn-Hoon**
  Yongin-si
  Gyeonggi-do 446-902 (KR)
- **KIM, Hun**
  Suwon-si
  Gyeonggi-do 442-754 (KR)
- **NOH, Myeong-Ju**
  Seoul 151-763 (KR)
- **PARK, Su-Jin**
  Seongnam-si
  Gyeonggi-do 463-856 (KR)
- **YANG, Seon-Young**
  Incheon 402-709 (KR)

(74) Representative: **Weickmann & Weickmann**
Postfach 860 820
81635 München (DE)

(54) POLYVALENT PNEUMOCOCCAL POLYSACCHARIDE-PROTEIN CONJUGATE COMPOSITION

(57) Provided is an immunogenic composition comprising 15 different polysaccharide-protein conjugates. Each of the conjugates comprises a capsular polysaccharide prepared from different serotype Streptococcus pneumoniae conjugated to a carrier protein, that is, serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9N, 9V, 14, 18C, 19A, 19F and 23F. An immunogenic composition formulated into a vaccine comprising an aluminum-based adjuvant increases application range with respect to pneumococcal diseases in infants and children.

[Fig. 1]



EP 2 865 392 A1

Printed by Jouve, 75001 PARIS (FR)

Petitioner Merck Ex-1004, 0524

Description

TECHNICAL FIELD

[0001] The present invention relates to a multivalent immunogenic composition comprising: 15 distinct polysaccharide-protein conjugates prepared by conjugating capsular polysaccharide derived from Streptococcus pneumoniae serotypes, 1, 2, 3, 4, 5, 6A, 6B, 7F, 9N, 9V, 14, 18C, 19A, 19F and 23F to a carrier protein such as CRM_{197}. The present invention relates generally to the field of medicine, and specifically to microbiology, immunology, vaccines and the prevention of pneumococcal disease in infants, children, and adults by immunization.

BACKGROUND ART

[0002] Streptococcus pneumoniae is a leading cause of pneumonia. According to 2010 Mortality Trend by Cause published by The National Statistical Office, pneumonia was one of the top 10 causes of death, with 14.9 deaths per 100,000 people, which is an 82.9% increase from 2000. The World Health Organization (WHO) also estimated in 2012 that globally, 476,000 HIV negative children younger than 5 years of age died from infection by Streptococcus pneumoniae, which accounts for 5% of all-cause child mortality for children under five.

[0003] In 1977, Dr. Robert Austrian developed a 14-valent pneumococcal polysaccharide vaccine in order to prevent pneumococcal disease and then the vaccine evolved to a 23-valent polysaccharide vaccine. The multivalent pneumococcal polysaccharide vaccines have proved valuable in preventing pneumococcal disease in elderly adults and high-risk patients. However, infants and young children respond poorly to most pneumococcal polysaccharides due to T-cell independent immune response. The 7-valent pneumococcal conjugate vaccine (7vPnC, Prevnar®) contains the capsular polysaccharides from the seven most prevalent serotypes 4, 6B, 9V, 14, 18C, 19F and 23F. Since approved in the U.S. in 2000, Prevnar has been demonstrated to be highly immunogenic and effective against invasive disease and otitis media in infants and young children. This vaccine is now approved in about 80 countries around the world. Prevnar covers approximately 80-90%, 60-80%, and 40-80% of invasive pneumococcal disease (IPD) in the US, Europe, and other regions of the world, respectively. As expected, surveillance data gathered in the years following Prevnar's introduction has clearly demonstrated a reduction of invasive pneumococcal disease caused by the serotypes covered by Prevnar in the US. However, the coverage of the serotypes was limited in some regions and invasive pneumococcal diseases caused by the serotypes that are not covered by Prevnar, in particular 19A, have increased.

[0004] The Advisory Committee on Immunization Practices (ACIP) announced in February, 2010 its recommendation of a newly approved 13-valent pneumococcal conjugate vaccine (PCV-13) for vaccination. PCV-13 is a pneumococcal conjugate vaccine comprising six additional serotypes (1, 3, 5, 6A, 7F, 19A) in addition to the seven serotypes (4, 6B, 9V, 14, 18C, 19F, 23F) comprised in Prevnar. According to US Active Bacterial Core surveillance (ABCs), a total of 64% in the IPD cases known as the pathogenic serotypes among children of younger than 5 years of age is covered by PCV-13. In 2007, only 70 cases among 4600 IPDs in children younger than 5 years of age were covered by PCV7, while 2900 cases were covered by PCV-13, which accounts for the majority. Now, a 15-valent pneumococcal conjugate vaccine is under development which covers additional serotypes whose incidence increases with serotype replacement.

DETAILED DESCRIPTION OF THE INVENTION

[0005] Accordingly, the present invention provides a multivalent immunogenic composition for the prevention of pneumococcal disease in infants, children and adults, comprising capsule polysaccharides derived from 15 pneumococcal serotypes including serotypes 2 and 9N. Specifically, the present invention provides a 15-valent pneumococcal conjugate (PCV-15) composition comprising serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9N, 9V, 14, 18C, 19A, 19F and 23F.

TECHNICAL SOLUTION

[0006] According to one aspect of the invention, provided is a multivalent immunogenic composition, comprising 15 distinctive polysaccharide-protein conjugates together with a physiologically acceptable vehicle, wherein each of the conjugates comprises a capsular polysaccharide derived from a different serotype of Streptococcus pneumoniae conjugated to a carrier protein, and the capsular polysaccharides are prepared from serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9N, 9V, 14, 18C, 19A, 19F and 23F.

[0007] In the multivalent immunogenic composition according to the present invention, the carrier protein may be CRM_{197}. The multivalent immunogenic composition according to the present invention may further comprise an adjuvant, for example, the adjuvant comprising an aluminum-based adjuvant. The adjuvant may be selected from the group consisting of aluminum phosphate, aluminum sulfate and aluminum hydroxide, and preferably, aluminum phosphate.

[0008] According to a further aspect of the invention, provided is a pharmaceutical composition for inducing an immune

Petitioner Merck Ex-1004, 0525

response to a Streptococcus pneumoniae capsular polysaccharide conjugate, comprising an immunologically effective amount of said immunogenic composition.

[0009] In one embodiment, the pharmaceutical composition may be an immunogenic composition formulated to contain: 2 μg of each saccharide, except for 6B at 4 μg; approximately 34 μg CRM_{197} carrier protein; 0.125 mg of elemental aluminum (0.5 mg aluminum phosphate) adjuvant; and sodium chloride and sodium succinate buffer as excipients.

TECHNICAL EFFECT

[0010] The multivalent-immunogenic composition comprises capsular polysaccharides derived from 15 distinctive pneumococcal serotypes including serotypes 2 and 9N, thereby leading to elevated serum IgG titer and functional antibody activity. Therefore, the multivalent immunogenic composition can be advantageously used for prevention of pneumococcal disease in infants, children and adults.

DESCRIPTION OF THE DRAWINGS

[0011] FIGS. 1 to 15 show the level of serotype-specific IgG measured 3 weeks after secondary injection (i.e., a total of 6 weeks) of the vaccine composition of the present invention and the comparative example (Prevnar 7 and Prevnar 13).

MODE FOR INVENTION

[0012] Serotype replacement has been made by some serotypes with antibiotic resistance and multiple drug resistance. Regional difference in serotype distribution has led to difference in coverage of Prevnar by region. (Harboe ZB, Benfield TL, Valentiner-Branth P, et al. Temporal Trends in Invasive Pneumococcal Disease and Pneumococcal Serotypes over 7 Decades. Clin Infect Dis 2010; 50:329-37). Thus, there is no reason to remove any of the serotypes in the existing pneumococcal conjugate vaccines. Rather, there is a need to further expand the coverage by addition of serotypes.

[0013] In 2008, the Pneumococcal Global Serotype Project (GSP) announced a report based on the IPD data selected from 1980 to 2007, showing that, following serotype 18C, serotype 2 was the 11th highest incidence serotype among the top 20 global serotypes. In addition, Samir K. Saha et al. reported that serotype 2 may become a threat because the serotype 2 has a high probability of causing pneumococcal meningitis in Bangladesh but is not included in any pneumococcal conjugate vaccine (Saha SK, Al Emran HM, Hossain B, Darmstadt GL, Saha S, et al. (2012) Streptococcus pneumoniae Serotype-2 Childhood Meningitis in Bangladesh: A Newly Recognized Pneumococcal Infection Threat. PLoS ONE 2012; 7(3): e32134). Thus, if serotype 2 is included, the number of pneumococcal diseases can be reduced and further be prepared for the serotype replacement that may occur with the vaccination by PCV-13.

[0014] Pneumococcal serotypes show different distribution patterns by age. In particular, serotype 9N has been found to be relatively important in infants aged 0 to 23 months, compared to children aged 24 to 59 months. Serotype 9N was the 14th most common following the 13 serotypes included in PCV-13. This indicates that inclusion of serotype 9N will contribute to reduction in pneumococcal diseases, in particular among infants.

[0015] The present invention provides a multivalent immunogenic composition comprising capsular polysaccharides derived from 15 pneumococcal serotypes including serotypes 2 and 9N. Specifically, the present invention provides a multivalent immunogenic composition, comprising: 15 distinct polysaccharide-protein conjugates, together with a physiologically acceptable vehicle, wherein each of the conjugates comprises a capsular polysaccharide from a different serotype of Streptococcus pneumoniae conjugated to a carrier protein, and the capsular polysaccharides are prepared from serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9N, 9V, 14, 18C, 19A, 19F and 23F.

[0016] Capsular polysaccharides may be prepared by standard techniques known to those skilled in the art. Capsular polysaccharides can be reduced in size in order to decrease the viscosity or increase the solubility of activated capsular polysaccharides. In the present invention, capsular polysaccharides are prepared from serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9N, 9V, 14, 18C, 19A, 19F and 23F of Streptococcus pneumoniae. These pneumococcal conjugates are prepared by separate processes and formulated into a single dosage formulation. For example, each pneumococcal polysaccharide serotype is grown in a soy-based medium and then is purified through centrifugation, precipitation, and ultra-filtration.

[0017] Carrier proteins are preferably proteins that are non-toxic and non-reactogenic and obtainable in sufficient amount and purity. Carrier proteins should be amenable to standard conjugation procedures. In the multivalent immunogenic composition of the present invention, the carrier protein may be CRM_{197} is. CRM_{197} is a non-toxic variant (i.e., toxoid) of diphteria toxin isolated from cultures of Corynebacterium diphteria strain C7 (β197) grown on casamino acids and yeast extract-based medium. CRM197 is purified through ultra-filtration, ammonium sulfate precipitation, and ion-exchange chromatography. Alternatively, CRM197 is prepared recombinantly in accordance with U.S. Patent No. 5,614,382.

[0018] Other diphteria toxoids are also suitable for use as carrier proteins. Other suitable carrier proteins include inactivated bacterial toxins such as tetanus toxoid, pertussis toxoid; cholera toxoid (WO2004/083251).E. coli LT, E. coli

Petitioner Merck Ex-1004, 0526

ST, and exotoxin A from Pseudomonas aeruginosa. Bacterial outer membrane proteins such as outer membrane complex c (OMPC), porins, transferrin binding proteins, pneumolysin, pneumococcal surface protein A (PspA), pneumococal adhesin protein (PsaA), C5a peptidase from Group A or Group B streptococcus, or Haermophilus influenzae protein D, can also be used. Other proteins, such as ovalbumin, keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA) or purified protein derivative of tuberculin (PPD) can also be used as carrier proteins. Diphtheria toxin variants such as CRM_{173}, CRM_{228}, and CRM_{45} can be used as a carrier protein.

[0019] In order to prepare polysaccharides for reaction with a carrier protein, purified polysaccharides are chemically activated. Once activated, each capsular polysaccharide is separately conjugated to a carrier protein to form a glycoconjugate. In one embodiment, each capsular polysaccharide is conjugated to the same carrier protein. The chemical activation of the polysaccharides and subsequent conjugation to the carrier protein are achieved by conventional means (for example, U.S. Pat. Nos. 4,673,574 and 4,902,506). Hydroxyl groups in the polysaccharides are oxidized to aldehyde groups by oxidizing agents such as periodates (including sodium periodate, potassium periodate, calcium periodate or periodic acid). The chemical activation leads to irregular oxidative degradation of adjacent hydroxyl groups. The conjugation is achieved by reductive amination. For example, the activated capsular polysaccharides and the carrier protein are reacted with a reducing agent such as sodium cyanoborohydride. Unreacted aldehyde groups can be removed by addition of a strong oxidizing agent.

[0020] After conjugation of the capsular polysaccharide to the carrier protein, the polysaccharide-protein conjugates are purified (enriched with respect to the amount of polysaccharide-protein conjugate) by a variety of techniques. These techniques include concentration/dialfiltration, column chromatography, and depth filtration. The purified polysaccharide-protein conjugates are compounded to formulate the immunogenic composition of the present invention, which can be used as a vaccine. Formulation of the immunogenic composition of the present invention can be accomplished using art-recognized methods. For instance, the 15 individual pneumococcal conjugates can be formulated with a physiologically acceptable vehicle to prepare the composition. Examples of such vehicles include, but are not limited to, water, buffered saline, polyols (e.g., glycerol, propylene glycol, liquid polyethylene glycol) and dextrose solutions.

[0021] In one embodiment, the immunogenic composition of the present invention may comprise one or more adjuvants. As defined herein, an "adjuvant" is a substance that serves to enhance the immunogenicity of an immunogenic composition of this invention. Thus, adjuvants are often given to boost the immune response and are well known to the skilled artisan. Suitable adjuvants to enhance effectiveness of the composition include, but are not limited to:

(1) aluminum salts (alum), such as aluminum hydroxide, aluminum phosphate, aluminum sulfate, etc.;

(2) oil-in-water emulsion formulations (with or without other specific immunostimulating agents such as muramyl peptides (defined below) or bacterial cell wall components), such as, for example, (a) MF59 (WO 90/14837), containing 5% Squalene, 0.5% Tween 80, and 0.5% Span 85 (optionally containing various amounts of MTP-PE (see below), although not required) formulated into submicron particles using a microfluidizer such as Model 110Y microfluidizer (Microfluidics, Newton, MA), (b) SAF, containing 10% Squalene, 0.4% Tween 80, 5% pluronic-blocked polymer L121, and thr-MDP (see below) either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion, and (c) Ribi™ adjuvant system (RAS), (Corixa, Hamilton, MT) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of 3-O-deaylated monophosphorylipid A (MPL™) described in U.S. Patent No. 4,912,094 (Corixa), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (Detox™);

(3) saponin adjuvants, such as Quil A or STIMULON™ QS-21 (Antigenics, Framingham, MA) (U.S. Patent No. 5,057,540) or particles generated therefrom such as ISCOMs (immunostimulating complexes);

(4) bacterial lipopolysaccharides, synthetic lipid A analogs such as aminoalkyl glucosamine phosphate compounds (AGP), or derivatives or analogs thereof, which are available from Corixa, and which are described in U.S. Patent No. 6,113,918; one such AGP is 2-[(R)-3-Tetradecanoyloxytetradecanoylamino]ethyl 2-Deoxy-4-0-phosphono-3-0-[(R)-3-tetradecanoyloxytetradecanoyl]-2-[(R)-3-tetr adecanoyloxytetradecanoylamino]-b-D-glucopyranoside, which is also known as 529 (formerly known as RC529), which is formulated as an aqueous form or as a stable emulsion,

(5) synthetic polynucleotides such as oligonucleotides containing CpG motif(s) (U.S. Patent No. 6,207,646);

(6) cytokines, such as interleukins (e.g., IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, IL-15, IL-18, etc.), interferons (e.g., gamma interferon), granulocyte macrophage colony stimulating factor (GM-CSF), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF), costimulatory molecules B7-1 and B7-2, etc.;

(7) detoxified mutants of a bacterial ADP-ribosylating toxin such as a cholera toxin (CT) either in a wild-type or mutant form, for example, where the glutamic acid at amino acid position 29 is replaced by another amino acid, preferably a histidine, in accordance with WO 00/18434 (see also WO 02/098368 and WO 02/098369), a pertussis toxin (PT), or an E. coli heat-labile toxin (LT), particularly LT-K63, LT-R72, CT-S109, PT-K9/G129 (see, e.g., WO 93/13302 and WO 92/19265); and

(8) complement components such as trimer of complement component C3d.

Petitioner Merck Ex-1004, 0527

[0022] Muramyl peptides include, but are not limited to, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanine-2-(1'-2' dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (MTP-PE), etc.

[0023] In a specific embodiment, an aluminum salt is used as an adjuvant. Aluminum salt adjuvant may be an alum-precipitated vaccine or alum-adsorbed vaccine. Aluminum salts include, but are not limited to, hydrated alumina, alumina trihydrate (ATH), aluminum hydrate, aluminum trihydrate, Alhydrogel, Superfos, Amphojel, aluminum hydroxide (III), aluminum hydroxyphosphate sulfate (Aluminum Phosphate Adjuvant (APA)), and amorphous alumina. APA is a suspension of aluminum hydroxyphosphate. If aluminum chloride and sodium phosphate are mixed in a ratio of 1:1, aluminum hydroxyphosphate sulfate is precipitated. The precipitates are sized to 2-8μm by using high shear mixer and dialyzed with physiological saline, followed by sterilization. In one embodiment, commercially available $Al(OH)_3$ (for example, Alhydrogel or Superfos) is used to adsorb proteins. 50-200 g of protein can be adsorbed per 1 mg of aluminum hydroxide, and this ratio is dependent on isoelectric point (pI) of proteins and pH of solvents. Proteins of low pI are strongly adsorbed compared to proteins of high pI. Aluminum salts form an antigen depot that slowly releases antigens over 2 to 3 weeks, nonspecifically activating phagocytes, complements and the congenital immune mechanism.

[0024] The present invention provides a pharmaceutical composition (for example, a vaccine formulation) for inducing an immune response to a Streptococcus pneumoniae capsular polysaccharide conjugate, comprising an immunologically effective amount of the immunogenic composition.

[0025] The vaccine formulations of the present invention can be used to protect or treat a human susceptible to pneumococcal infection, by administering the vaccine via a systemic or mucosal route. As defined herein, the term "effective dose" refers to the amount required to induce antibody to a level sufficient to significantly reduce the probability of Streptococcus pneumonia infection or severity thereof. These administrations can include injection via the intramuscular, intraperitoneal, intradermal or subcutaneous routes; or via mucosal administration to the oral/alimentary, respiratory or genitourinary tracts.

[0026] In one embodiment, intranasal administration is used for the treatment of pneumonia or otitis media since nasopharyngeal carriage of pneumococci can be more effectively prevented, thus attenuating infection at its earliest stage. The amount of conjugate in each vaccine dose is selected as an amount that induces an immunoprotective response without significant, adverse effects. Such an amount can vary depending upon the pneumococcal serotype. Generally, each dose will comprise 0.1 to 100 μg of polysaccharide, particularly 0.1 to 10 μg, and more particularly 1 to 5 μg. Optimal amounts of components for a particular vaccine can be ascertained by standard studies involving observation of appropriate immune responses in subjects. For example, the amount for vaccination of a human subject can be determined by extrapolating the animal test result. In addition, the dosage can be determined empirically.

[0027] In a particular embodiment of the present invention, the vaccine composition is a sterile liquid formulation of pneumococcal capsular polysaccharides of serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F individually conjugated to CRM197. Each 0.5 mL dose is formulated to contain: 2 μg of each saccharide, except for 6B at 4 μg; approximately 34 μg CRM197 carrier protein; 0.125 mg of elemental aluminum (0.5 mg aluminum phosphate) adjuvant; and sodium chloride and sodium succinate buffer as excipients. The liquid is filled into single dose syringes without a preservative. After shaking, the vaccine is a homogeneous, white suspension ready for intramuscular administration.

[0028] In a further embodiment, the composition of the present invention can be administered in a single injection. For example, the vaccine composition of the present invention can be administered 2, 3, 4, or more times at appropriately spaced intervals, such as, with a 1, 2, 3, 4, 5, or 6 month interval or combination thereof. The immunization schedule can follow that designated for the Prevnar vaccine. For example, the routine schedule for infants and toddlers against invasive disease caused by S. pneumoniae due to the serotypes included in the Prevnar vaccine is at 2, 4, 6 and 12-15 months of age. Thus, in this aspect, the composition is administered 4 times, i.e., at 2, 4, 6, and 12-15 months of age.

[0029] The compositions of the present invention may also include one or more proteins from Streptococcus pneumoniae. Examples of Streptococcus pneumoniae proteins suitable for inclusion include those identified in International Patent Application WO02/083855, as well as that described in International Patent Application WO02/053761.

[0030] The composition of the present invention can be administered to a subject via one or more administration routes known to one of ordinary skill in the art such as a parenteral, transdermal, or transmucosal, intranasal, intramuscular, intraperitoneal, intracutaneous, intravenous, or subcutaneous route and be formulated accordingly. In one embodiment, the composition of the present invention can be administered as a liquid formulation by intramuscular, intraperitoneal, subcutaneous, intravenous, intraarterial, or transdermal injection or respiratory mucosal injection. The liquid formulation for injection includes a solution or the like.

[0031] The composition of the present invention can be formulated in a form of a unit dose vial, multiple dose vial, or pre-filled syringe. A pharmaceutically acceptable carrier for a liquid formulation includes aqueous or nonaqueous solvent, suspension, emulsion or oil. Examples of a nonaqueous solvent include propylene glycol, polyethylene glycol, and ethyl oleate. Aqueous carriers include water, alcohol/aqueous solvent, emulsion, or suspension, physiological saline, buffer solution. Examples of oil include vegetable or animal oil, peanut oil, soybean oil, olive oil, sunflower oil, liver oil, synthetic oil such as marine oil, and lipids obtained from milk or eggs. The pharmaceutical composition may be isotonic, hypertonic or hypotonic. However, it is desirable that the pharmaceutical composition for infusion or injection is basically isotonic.

Petitioner Merck Ex-1004, 0528

Thus, isotonicity or hypertonicity may be advantageous for storage of the composition. When the pharmaceutical composition is hypertonic, the composition can be diluted to isotonicity before administration. Tonicity agent may be ionic tonicity agent such as salt or non-ionic tonicity agent such as carbohydrate. The ionic tonicity agent includes sodium chloride, calcium chloride, potassium chloride, and magnesium chloride, but is not limited thereto. Nonionic tonicity agent includes sorbitol and glycerol, but is not limited thereto. Preferably, at least one pharmaceutically acceptable buffer is included. For example, when the pharmaceutical composition is an infusion or injection, it is preferable to be formulated in a buffer with buffering capacity at pH 4 to 10, such as pH 5-9 or 6-8. The buffer can be selected from the group consisting of TRIS, acetate, glutamate, lactate, maleate, tartrate, phosphate, citrate, carbonate, glycinate, histidine, glycine, succinate, and triethanolamine buffer solution.

[0032] In particular, if the pharmaceutical composition is for parenteral administration, a buffer may be selected from those suitable for United States Pharmacopeia (USP). For example, the buffer may be selected from the group consisting of monobasic acid such as acetic acid, benzoic acid, gluconic acid, glyceric acid, and lactic acid; polybasic acid such as aconitic acid, adipic acid, ascorbic acid, carbonic acid, glutamic acid, malic acid, succinic acid, and tartaric acid; and base such as ammonia, diethanolamine, glycine, triethanolamine, and TRIS. For parenteral administration, vehicles (for subcutaneous, intravenous, intraarticular, and intramuscular injection) include sodium chloride solution, Ringer's dextrose solution, dextrose and sodium chloride, lactated Ringer's solution and fixed oils. Vehicles for intravenous administration include Ringer's dextrose solution or a similar dextrose based infusion solution, nutritional supplements and electrolyte supplements. The example includes a sterile liquid such as water and oil, with or without a surface active agent and pharmaceutically acceptable adjuvant. Generally, water, physiological saline, dextrose solution, related sugar solution, and glycols such as propylene glycol or polyethylene glycol, in particular, polysorbate 80 are suitable for an injection. Examples of oil include animal and vegetable oil, peanut oil, soybean oil, olive oil, sunflower oil, liver oil, synthetic oil such as marine oil and lipids from milk or eggs.

[0033] The formulation of the present invention may comprise surface active agents. Preferably, polyoxyethylene sorbitan ester (generally referred to as Tweens), in particular, polysorbate 20 and polysorbate 80; copolymers (such as DOWFAXTM) of ethylene oxide (EO), propylene oxide (PO), butylenes oxide (BO); oxtocynols with different repeats of ethoxy(oxy-1,2-ethanediyl) group, in particular, octoxynol-9(Triton-100); ethylphenoxypolyethoxyethanol (IGEPAL CA-630/NP-40); phospholipid such as lecithin; nonylphenol ethoxylate such as TergitolTM NP series; lauryl, cetyl, stearyl, oleyl alcohol-derived polyoxyethylene fatty ether (Brij surfactant), in particular, triethyleneglycol monolauryl ether (Brij 30); sorbitan ether known as SPAN, in particular, sorbitan trioleate (Span 85) and sorbitan monolaurate but without limitation thereto. Tween 80 is preferably comprised in an emulsion.

[0034] Mixtures of surface active agents such as Tween 80/Span 85 can be used. A combination of polyoxyethylene sorbitan ester such as Tween 80 and octocynol such as Triton X-100 is also suitable. A combination of Laureth 9 and Tween and/or octocynol is also advantageous. Preferably, the amount of polyoxyethylene sorbitan ester (such as Tween 80) included is 0.01% to 1% (w/v), in particular 0.1%; the amount of octylphenoxy polyoxyethanol or nonylphenoxy polyoxyethanol (such as Triton X-100) included is 0.001% to 0.1% (w/v), in particular 0.005% to 0.02%; and the amount of polyoxyethylene ether (such as laureth 9) included is 0.1% to 20% (w/v), possibly 0.1% to 10%, in particular 0.1% to 1% or about 0.5%. In one embodiment, the pharmaceutical composition is delivered via a release control system. For example, intravenous infusion, transdermal patch, liposome, or other routes can be used for administration. In one aspect, macromolecules such as microsphere or implant can be used.

[0035] The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples. These examples are described solely for the purpose of illustration and are not intended to limit the scope of the invention.

EXAMPLES

Example 1. Preparation of S. pneumoniae Capsular Polysaccharide

[0036] Cultivation of S. pnemoniae and purification of capsular polysaccharides were conducted as known to one of ordinary skill in the art. S. pneumoniae serotypes were obtained from the American Type Culture Collection (ATCC). S. pneumoniae were characterized by capsules and immobility, Gram-positive lancet-shaped diplococcus and alpha hemolysis in a blood agar medium. Serotypes were identified by Quelling test using specific anti-sera (US Patent No. 5,847,112).

Preparation of Cell Banks

[0037] Several generations of seed stocks were created in order to expand the strain and remove components of animal origin (generations F1, F2, and F3). Two additional generations of seed stocks were produced. The first additional generation was made from an F3 vial, and the subsequent generation was made from a vial of the first additional generation. Seed vials were stored frozen (≤-70°C) with synthetic glycerol as a cryopreservative. For cell bank preparation,

Petitioner Merck Ex-1004, 0529

all cultures were grown in a soy-based medium. Prior to freezing, cells were concentrated by centrifugation, spent medium was removed, and cell pellets were re-suspended in a fresh medium containing a cryopreservative (such as synthetic glycerol).

Inoculation

[0038] Cultures from the working cell bank were used to inoculate seed bottles containing a soy-based medium. The seed bottle was used to inoculate a seed fermentor containing a soy-based medium.

Seed Fermentation

[0039] Seed fermentation was conducted in a seed fermentor with the temperature and pH controlled. After the target optical density was reached, the seed fermentor was used to inoculate the production fermentor containing the soy-based medium.

Production Fermentation

[0040] Production fermentation is the last step in fermentation. Temperature, pH and agitation speed were controlled.

Inactivation

[0041] The fermentation was terminated after cessation of growth by addition of an inactivator. After inactivation, the contents in the fermentor were cooled and the pH of the lysed culture broth was adjusted.

Purification

[0042] Broth from the fermentor was centrifuged and filtered to remove bacterial cell debris. Several concentration/diafiltration operations, precipitation/elution, and depth filtration steps were used to remove contaminants and purify capsular polysaccharides.

Example 2. Preparation of S. pneumoniae Capsular Polysaccharide-CRM_{197} Conjugate

[0043] Polysaccharides of different serotypes were activated following different pathways and then conjugated to CRM_{197}. The activation process comprises reduction of the size of capsular polysaccharides to the target molecular weights, chemical activation and buffer exchange via ultrafiltration. Purified CRM_{197} is conjugated to activated capsular polysaccharides, and the conjugates are purified using ultrafiltration and finally filtered through 0.22 μm filter. The process parameters such as pH, temperature, concentration and time are as follows.

(1) Activation

Step 1

[0044] Each serotype polysaccharide was diluted with water for injection, sodium acetate and sodium phosphate to a final concentration in a range of 1.0 to 2.0 mg/mL. For serotype 1, sodium hydroxide (0.05M final base concentration) was added and the solution was incubated at 50°C±2°C Then, the solution was cooled to 21 to 25°C and the hydrolysis was stopped by adding 1 M HCl until a target pH of 6.0±0.1 was reached. For serotype 3, HCl (0.01 M final acid concentration) was added and the solution was incubated at 50°C±2°C Then, the solution was cooled to 21 to 25°C and the hydrolysis was stopped by adding 1 M sodium phosphate until a target pH of 6.0±0.1 was reached. For serotype 4, HCl (0.1M final acid concentration) was added and the solution was incubated at 45°C ±2°C. Then, the solution was cooled to 21 to 25°C and the hydrolysis was stopped by adding 1M sodium phosphate until a target pH of 6.0±0.1 was reached. For serotype 6A, glacial acetic acid (0.2M final acid concentration) was added and the solution was incubated at 60°C ±2°C. Then, the solution was cooled to 21 to 25°C and the hydrolysis was stopped by adding 1M sodium hydroxide until a target pH of 6.0±0.1 was reached. For serotype 14 and 18C, glacial acetic acid (0.2M final acid concentration) was added and the solution was incubated at 94°C ±2°C. Then, the solution was cooled to 21 to 25°C and the hydrolysis was stopped by adding 1 M sodium phosphate until a target pH of 6.0±0.1 was reached.

Petitioner Merck Ex-1004, 0530

Step 2: Periodate reaction

[0045] The required sodium periodate molar equivalents for pneumococcal saccharide activation was determined using total saccharide content. With thorough mixing, the oxidation reaction was allowed to proceed for between 16 to 20 hours at 21 - 25°C for all serotypes except 1, 7F, and 19F, for which the temperature was ≤ 10°C.

Step 3: Ultrafiltration

[0046] The oxidized saccharide was concentrated and diafiltered with water for injection (WFI) on a 100 kDa MWCO ultrafilter (30kDa ultrafilter for serotype 1 and 5 kDa ultrafilter for serotype 18C). Diafiltration was accomplished using 0.9% sodium chloride solution for serotype 1, 0.01 M sodium acetate buffer (pH 4.5) for serotype 7F, and 0.01 M sodium phosphate buffer (pH 6.0) for serotype 19F. The permeate was discarded and the retentate was filtered through a 0.22 μm filter

Step 4: Lyophilization

[0047] For serotypes 3, 4, 5, 9N, 9V, and 14, the concentrated saccharide was mixed with CRM_{197} carrier protein, filled into glass bottles, lyophilized and then stored at -25° ± 5°C.

[0048] For serotypes 2, 6A, 6B, 7F, 19A, 19F, and 23F, a specified amount of sucrose was added which was calculated to achieve a 5% ± 3% sucrose concentration in the conjugation reaction mixture. Serotypes 1 and 18C did not require sucrose addition. The concentrated saccharide was then filled into glass bottles, lyophilized and then stored at -25° ± 5°C.

(2) Conjugation process

[0049] Aqueous conjugation was conducted for serotypes 1, 3, 4, 5, 9N, 9V, 14, and 18C, and DMSO conjugation was conducted for serotypes 2, 6A, 6B, 7F, 19A, 19F and 23F.

Step 1: Dissolution

Aqueous Conjugation

[0050] For serotypes 3, 4, 5, 9N, 9V and 14, the lyophilized activated saccharide-CRM_{197} mixture was thawed and equilibrated at room temperature. The lyophilized activated saccharide-CRM_{197} was then reconstituted in a 0.1M sodium phosphate buffer at a typical ratio by serotype. For serotypes 1 and 18C, the lyophilized saccharide was reconstituted in a solution of CRM_{197} in 1 M dibasic sodium phosphate at a typical ratio of 0.11 L of sodium phosphate per 1 L of CRM197 solution.

Dimethyl sulfoxide (DMSO) Conjugation

[0051] The lyophilized activated saccharide serotypes 2, 6A, 6B, 7F, 19A, 19F, 23F and the lyophilized CRM_{197} carrier protein were equilibrated at room temperature and reconstituted in DMSO.

Step 2: Conjugation Reaction

Aqueous Conjugation

[0052] For serotypes 1, 3, 4, 5, 9N, 9V, 14 and 18C, the conjugation reaction was initiated by adding the sodium cyanoborohydride solution (100 mg/mL) to achieve 1.0 - 1.2 moles sodium cyanoborohydride per mole of saccharide. The reaction mixture was incubated for 44 - 96 hours at 23 °C to 37 °C. The temperature and reaction time were adjusted by serotype. The temperature was then reduced to 23° ± 2°C and sodium chloride 0.9% was added to the reactor. Sodium borohydride solution (100 mg/mL) was added to achieve 1.8 - 2.2 molar equivalents of sodium borohydride per mole saccharide. The mixture was incubated for 3 - 6 hours at 23° ± 2°C. This procedure reduced any unreacted aldehydes present on the saccharides. The mixture was diluted with sodium chloride 0.9% and the diluted conjugation mixture was filtered using a 1.2 μm pre-filter into a holding vessel.

DMSO conjugation

[0053] For serotypes 2, 6A, 6B, 7F, 19A, 19F and 23F, activated saccharide and CRM_{197} carrier protein were mixed

Petitioner Merck Ex-1004, 0531

at a ratio range of 0.8 g - 1.25 g saccharide/g CRM_{197}. The conjugation reaction was initiated by adding the sodium cyanoborohydride solution (100 mg/mL) at a ratio of 0.8 - 1.2 molar equivalents of sodium cyanoborohydride to one mole activated saccharide. WFI was added to the reaction mixture to a target of 1 % (v/v), and the mixture was incubated for 11 - 27 hours at 23° ± 2°C. Sodium borohydride solution, 100 mg/mL (typical 1.8 - 2.2 molar equivalents sodium borohydride per mole activated saccharide) and WFI (target 5% v/v) were added to the reaction and the mixture was incubated for 3 - 6 hours at 23° ± 2°C. This procedure reduced any unreacted aldehydes present on the saccharides. Then, the reaction mixture was diluted with sodium chloride 0.9%, and the diluted conjugation mixture was filtered using a 1.2 μm pre-filter into a holding vessel.

Step 3: Ultrafiltration

[0054] The diluted conjugate mixture was concentrated and diafiltered on a 100 kDa MWCO ultrafiltration filter with a minimum of 20 volumes of 0.9% sodium chloride or buffer. The permeate was discarded.

Step 4: Sterile Filtration

[0055] The retentate after the 100 kDa MWCO dialfiltration was filtered through a 0.22 μm filter. In-process controls (saccharide content, free protein, free saccharide, residual DMSO and residual cyanide; for DMSO conjugation, residual DMSO in addition thereto) were performed on the filtered product. In-process controls on filtered retentate were performed to determine whether additional concentration, diafiltration, and/or dilution were needed. As necessary, the filtered conjugate was diluted with 0.9% sodium chloride to achieve a final concentration of less than 0.55 g/L. Release tests for saccharide content, protein content and saccharide:protein ratio were performed at this stage. Finally, the conjugate was filtered (0.22 μm) and release test (appearance, free protein, free saccharide, endotoxin, molecular size determination, residual cyanide, residual DMSO, saccharide identity and CRM197 identity) was performed. The final bulk concentrated solution was stored at 2 - 8°C.

Example 3. Formulation of a Multivalent Pneumococcal Conjugate Vaccine

[0056] The required volumes of final bulk concentrates were calculated based on the batch volume and the bulk saccharide concentrations. After the required amounts of the 0.85% sodium chloride (physiological saline), polysorbate 80 and succinate buffer were added to the pre-labeled formulation vessel, bulk concentrates were added. The preparation was then thoroughly mixed and sterile filtered through a 0.22 μm membrane. The formulated bulk was mixed gently during and following the addition of bulk aluminum phosphate. The pH was checked and adjusted if necessary. The formulated bulk product was stored at 2-8°C. The product contained, in a 0.5 ml volume, 2 μg of each saccharide, except for 6B at 4 μg; approximately 34 μg CRM197 carrier protein; 0.125 mg of elemental aluminum (0.5 mg aluminum phosphate) adjuvant; 4.25 mg of sodium chloride; 295 μg of sodium succinate buffer; and 100 μg of polysorbate 80.

Example 4. Immunogenicity of the Multivalent Pneumococcal Conjugate Vaccine

[0057] The multivalent pneumococcal vaccine, i.e., the vaccine composition (SK-15) prepared in Example 3 was tested for its ability to induce an immunogenic response in rabbits. These immunogenic effects were characterized by antigen-specific ELISA for serum IgG concentrations and by opsonophagocytic assay (OPA) for antibody function. New Zealand White rabbits were immunized intramuscularly at week 0 and week 3 with the planned human clinical dose of each polysaccharide (2 μg of each PS, except for 6B at 4μg). Sera were sampled every 3 weeks post immunization.

Measurement of Serotype specific IgG concentration

[0058] Capsular polysaccharides (PnPs) for each serotype were coated on a 96-well plate at 500ng/well. An equivalent amount of serum was sampled from each subject and was pooled by group. The serum pool was serially diluted by 10 times with an antibody dilution buffer comprising Tween 20, C-PS 4μg/mL and serotype 22F capsular polysaccharide (PnPs 22F) 4μg/mL and then reacted at room temperature for 30 minutes. The plate was washed 5 times with a washing buffer and then the well plate was coated with pre-adsorbed and diluted serum 50μL and was incubated at room temperature for 18 hours. The well plate was washed in the same way and then goat anti-Rabbit IgG-alkaline phosphatase conjugates (1:50000) were added to each well, followed by incubation at room temperature for 2 hours. Plates were washed as described above and 1 mg/mL p-nitrophenylamine buffer as substrate was added to each well and then reacted at room temperature for 2 hours. The reaction was quenched by adding 50μL of 3M NaOH and absorbances at 405 nm and 690 nm were measured. As comparative examples, 7-valent vaccine (Prevnar 7, Pfizer) and 13-valent vaccine (Prevnar 13, Pfizer) were subjected to the same procedure. The results are shown in Figures 1-15.

Petitioner Merck Ex-1004, 0532

Functional Immunogenicity Test (OPA)

[0059] Antibody functions were evaluated by testing serum in OPA assay. An equivalent amount of serum was sampled from each subject, pooled by group and diluted by 10 times. S. pneumonia was cultured in a THY medium by serotype and diluted to 1000CFU/10uL. Opsonization buffer 200μL, diluted serum 10μL, and diluted S. pneumonia 10μL were mixed and reacted at room temperature for 1 hour. A mixture of pre-differentiated HL-60 cells and complements was added and reacted in a CO_2 incubater (37°C) for one hour. The temperature was reduced to stop phagocytosis and the reaction 5uL was smeared onto an agar plate pre-dried for 30 - 60 minutes. The plate was incubated in a CO_2 incubater (37°C) for 12 - 18 hours and then colonies were counted. OPA titer was expressed as a dilution rate at which 50% killings were observed. As a comparative example, 13-valent vaccine (Prevnar 13, Pfizer) was subjected to the same procedure. The results are shown in Tables 1-3.

[Table 1]

OPA Titers of 15 polysaccharide serotypes 3 weeks after primary immunization					
Serotype	Prevnar 13	SK-15	Serotype	Prevnar 13	SK-15
1	1:16	1:4	9V	1:256	1:256
2	-	1:128	9N	-	1:512
3	No dilution	No dilution	14	1:256	1:256
4	1:128	1:128	18C	1:1024	1:1024
5	1:64	1:32	19A	1:512	1:256
6A	1:512	1:256	19F	1:256	1:128
6B	1:256	1:128	23F	1:256	1:256
7F	1:1024	1:1024	-	-	-

[Table 2]

OPA Titers of 15 polysaccharide serotypes 3 weeks after second immunization					
Serotype	Prevnar13	SK-15	Serotype	Prevnar 13	SK-15
1	1:64	1:64	9V	1:512	1:512
2	-	1:512	9N	-	1:2048
3	1:2	1:4	14	1:1024	1:1024
4	1:1024	1:1024	18C	1:1024	1:512
5	1:256	1:256	19A	1:1024	1:1024
6A	1:2048	1:2048	19F	1:1024	1:512
6B	1:2048	1:2048	23F	1:2048	1:2048
7F	1:2048	1:2048	-	-	-

[Table 3]

OPA Titers of 15 polysaccharide serotypes 6 weeks after second immunization					
Serotype	Prevnar 13	SK-15	Serotype	Prevnar 13	SK-15
1	1:64	1:64	9V	1:512	1:512
2	-	1:512	9N	-	1:2048
3	1:4	1:4	14	1:1024	1:1024
4	1:1024	1:1024	18C	1:2048	1:2048

Petitioner Merck Ex-1004, 0533

(continued)

OPA Titers of 15 polysaccharide serotypes 6 weeks after second immunization					
Serotype	Prevnar 13	SK-15	Serotype	Prevnar 13	SK-15
5	1:512	1:512	19A	1:2048	1:1024
6A	1:2048	1:2048	19F	1:1024	1:512
6B	1:2048	1:1024	23F	1:2048	1:2048
7F	1:2048	1:2048	-	-	-

[0060] Serotype-specific immune response by the vaccine formulation of the present invention and that of the comparative example were evaluated via IgG ELISA and complement-mediated OPA for functional antibody. Figs. 1 to 15 show the results of IgG ELISA and Tables 1 to 3 show the results of functional immunogenicity measurements obtained by OPA, comparing the immune responses among treatment groups. These results indicate that the 15-valent pneumococcal polysaccharide conjugate vaccine would induce IgG titer and functional antibody activity equivalent to or better than Prevnar-13.

Claims

1. A multivalent immunogenic composition, comprising: 15 distinct polysaccharide-protein conjugates, together with a physiologically acceptable vehicle, wherein each of the polysaccharide-protein conjugates comprises a capsular polysaccharide from a different serotype of Streptococcus pneumoniae conjugated to a carrier protein, and the capsular polysaccharides are prepared from serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9N, 9V, 14, 18C, 19A, 19F and 23F.

2. The multivalent immunogenic composition of claim 1, wherein the carrier protein is CRM_{197}.

3. The multivalent immunogenic composition of claim 1, further comprising an adjuvant.

4. The multivalent immunogenic composition of claim 3, wherein the adjuvant is an aluminum-based adjuvant.

5. The multivalent immunogenic composition of claim 4, wherein the adjuvant is selected from the group consisting of aluminum phosphate, aluminum sulfate and aluminum hydroxide.

6. The multivalent immunogenic composition of claim 5, wherein the adjuvant is aluminum phosphate.

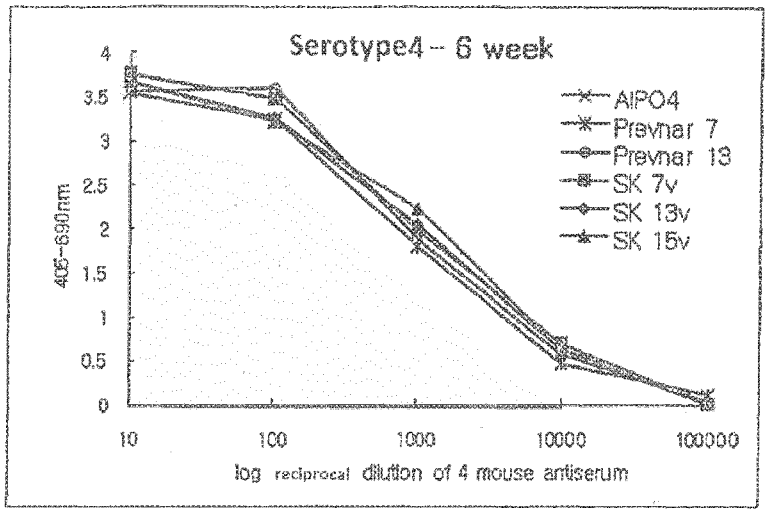
7. A pharmaceutical composition for inducing an immune response to a Streptococcus pneumoniae capsular polysaccharide conjugate, comprising an immunologically effective amount of the multivalent immunogenic composition of any one of claims 1 to 6.

8. The pharmaceutical composition of claim 7, wherein the multivalent immunogenic composition is a single 0.5 ml dose formulated to contain:

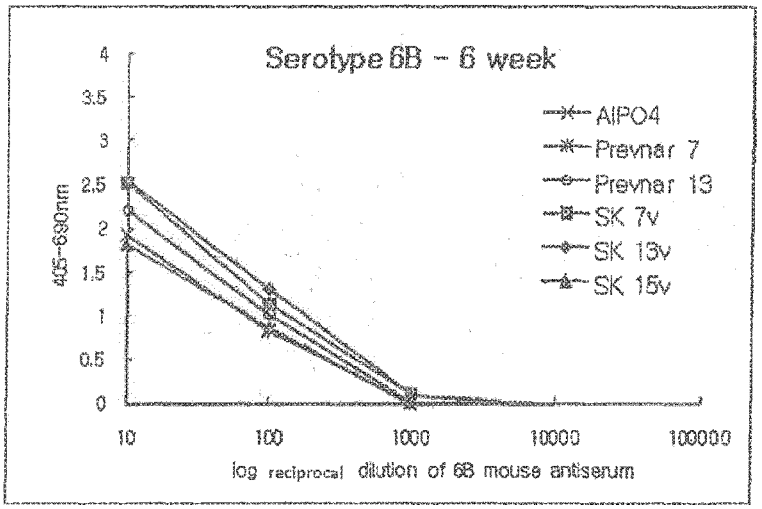
   2 μg of each saccharide, except for 6B at 4 μg;
   approximately 34 μg of the CRM_{197} carrier protein;
   0.125 mg of elemental aluminum (0.5 mg aluminum phosphate) adjuvant; and
   sodium chloride and sodium succinate buffer as excipients.

Petitioner Merck Ex-1004, 0534

[Fig. 1]

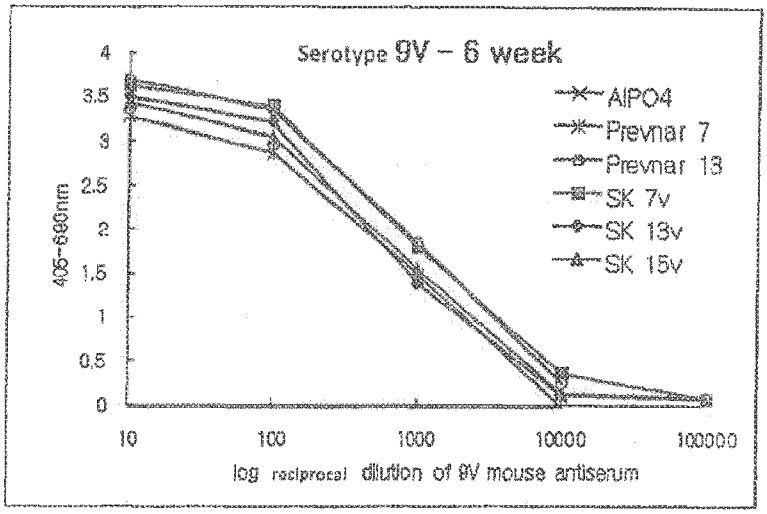


[Fig. 2]

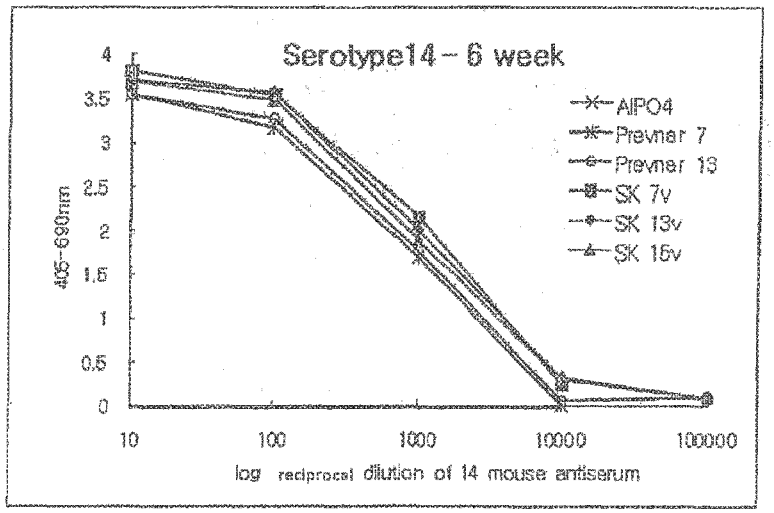


Petitioner Merck Ex-1004, 0535

[Fig. 3]

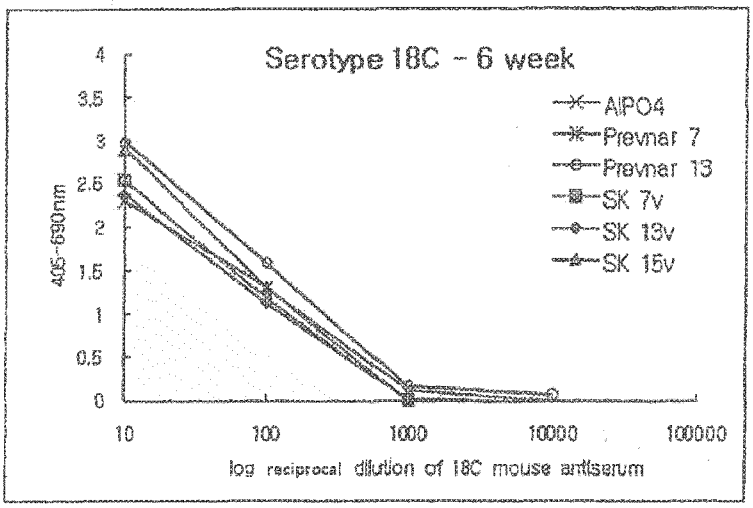


[Fig. 4]

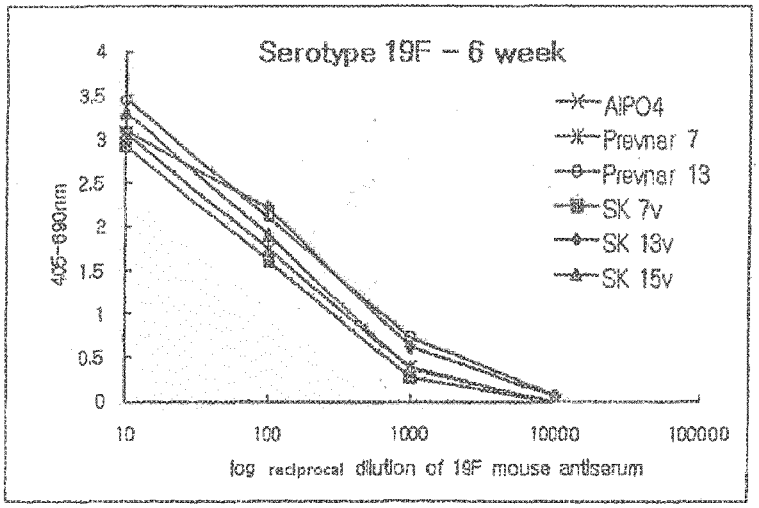


Petitioner Merck Ex-1004, 0536

[Fig. 5]

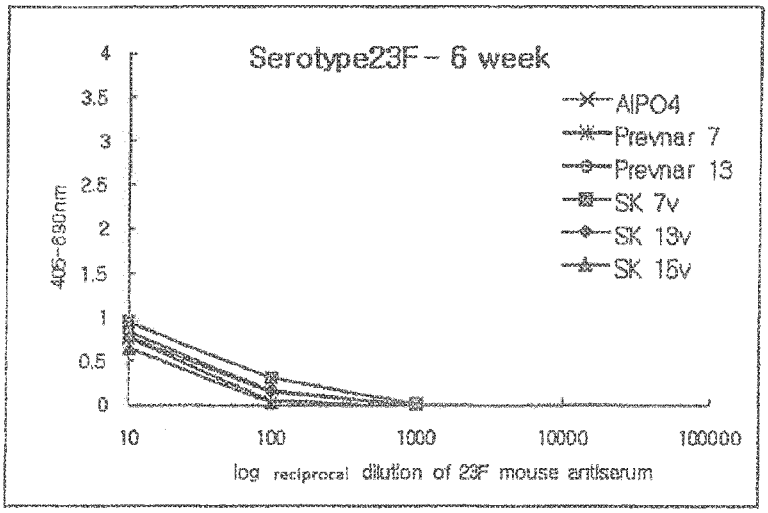


[Fig. 6]

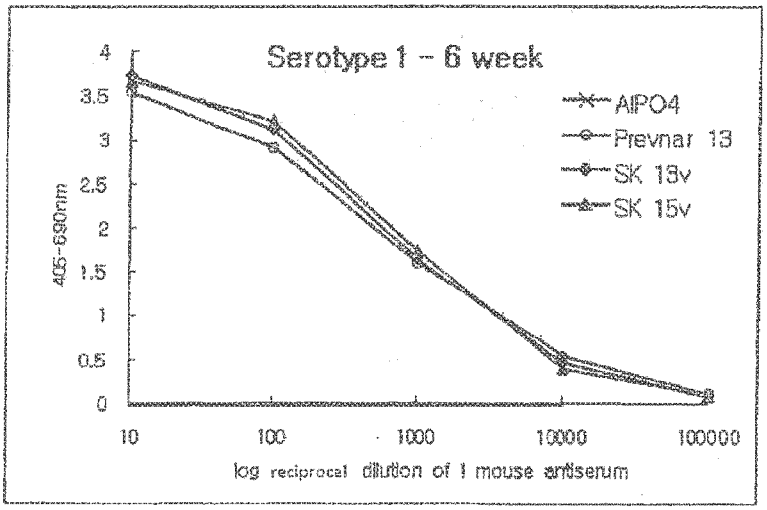


Petitioner Merck Ex-1004, 0537

[Fig. 7]

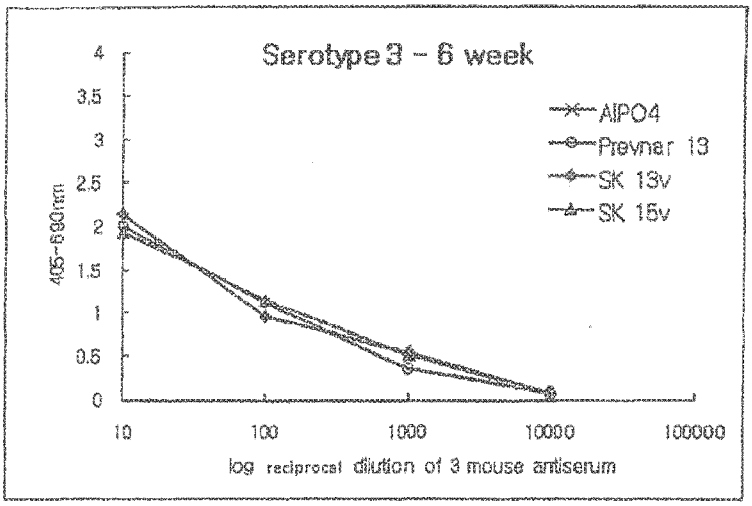


[Fig. 8]

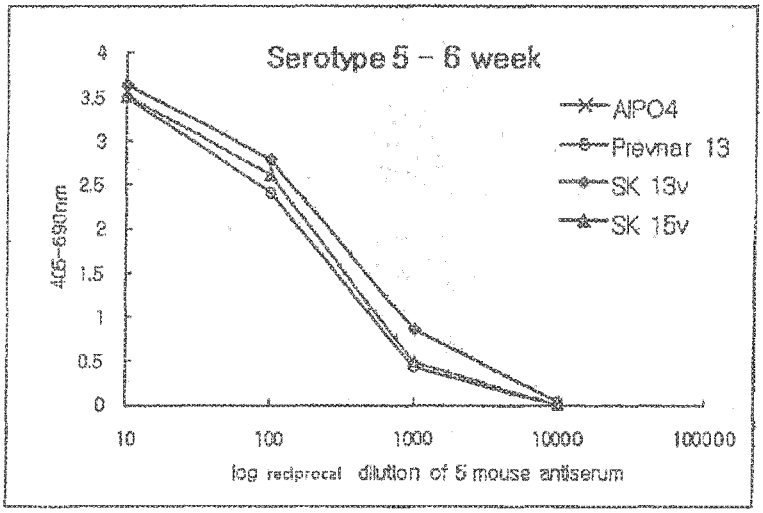


Petitioner Merck Ex-1004, 0538

[Fig. 9]

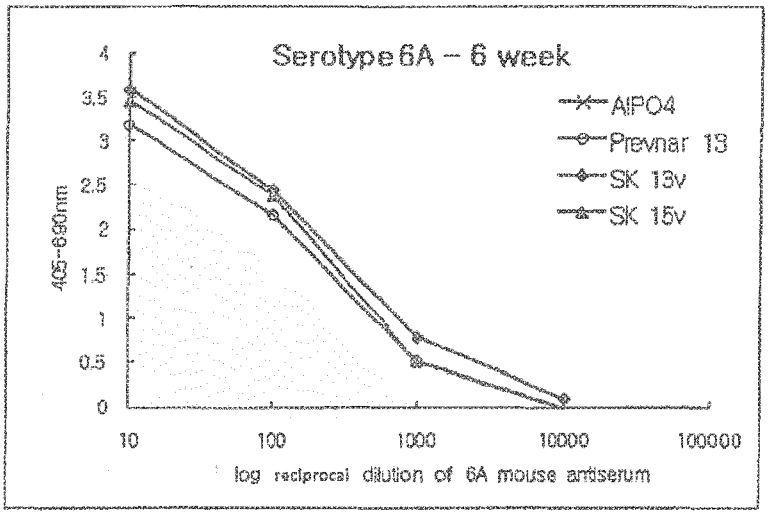


[Fig. 10]

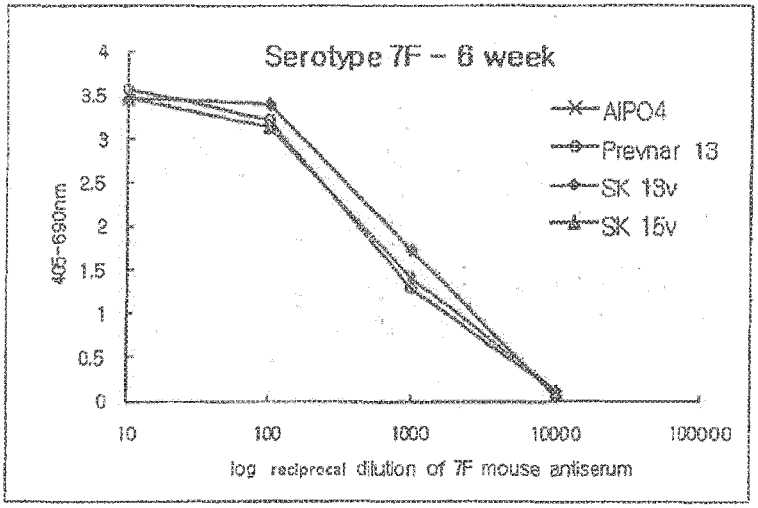


Petitioner Merck Ex-1004, 0539

[Fig. 11]

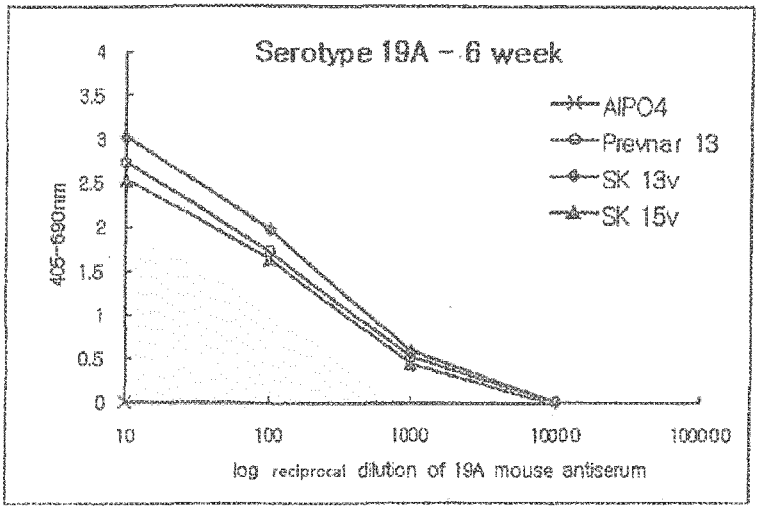


[Fig. 12]

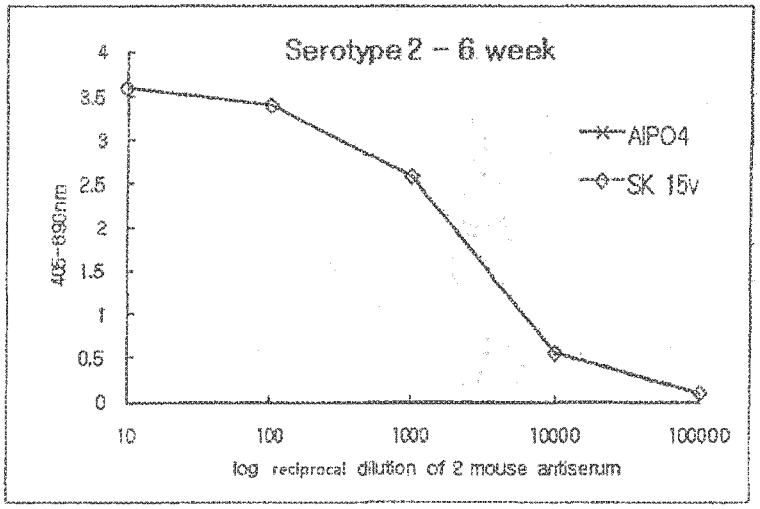


Petitioner Merck Ex-1004, 0540

[Fig. 13]

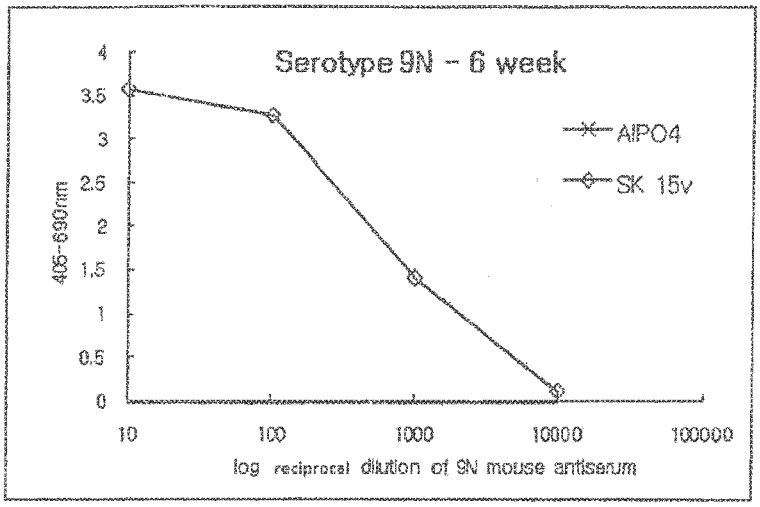


[Fig. 14]



Petitioner Merck Ex-1004, 0541

[Fig. 15]



Petitioner Merck Ex-1004, 0542

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2013/005392

A. CLASSIFICATION OF SUBJECT MATTER

A61K 47/48(2006.01)i, A61K 31/70(2006.01)i, A61K 38/00(2006.01)i, A61P 31/00(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K 47/48; C07K 15/14; A61K 39/385; A61P 31/04; C07K 14/00; A61K 39/38; A61K 39/00; A61K 39/09; A61K 31/70; A61K 38/00; A61P 31/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean Utility models and applications for Utility models: IPC as above
Japanese Utility models and applications for Utility models: IPC as above

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

eKOMPASS (KIPO internal) & Keywords: Streptococcus pneumoniae(Streptococcus pneumoniae), polysaccharide-protein conjugates, capsular polysaccharide(capsular polysaccharide), immunity, vaccine, serotype 2, serotype 9N, 15-valent, PCV-15

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CN 101590224 A (GUANGZHOU JINGDA MEDICAL SCIENCE AND TECHNOLOGY CO., LTD.) 02 December 2009 See abstract and claim 1.	1-8
A	US 2010-0316666 A1 (HAUSDORFF, W. P. et al.) 16 December 2010 See abstract, claim 17, paragraphs [0006]-[0008].	1-8
A	WO 2011-151760 A2 (WYETH LLC) 08 December 2011 See abstract, claim 1, paragraph [0004].	1-8
A	US 2003-0147922 A1 (CAPIAU, C. et al.) 07 August 2003 See abstract, claims 1-19.	1-8
A	US 5153312 A (PORRO, M.) 06 October 1992 See abstract, claims 1, 11, 16-17, and 21-22.	1-8
A	US 2004-0202668 A1 (BOUTRIAU, D. et al.) 14 October 2004 See abstract, claims 1-2, 14, 28 and 31.	1-8

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

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 SEPTEMBER 2013 (30.09.2013)	**30 SEPTEMBER 2013 (30.09.2013)**
Name and mailing address of the ISA/KR Korean Intellectual Property Office Government Complex-Daejeon, 189 Seonsa-ro, Daejeon 302-701, Republic of Korea Facsimile No. 82-42-472-7140	Authorized officer Telephone No.

Form PCT/ISA/210 (second sheet) (July 2009)

Petitioner Merck Ex-1004, 0543

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/KR2013/005392

Patent document cited in search report	Publication date	Patent family member	Publication date
CN 101590224 A	02/12/2009	NONE	
US 2010-0316666 A1	16/12/2010	AT 545431 T	15/03/2012
		AU 2006-235013 A1	19/10/2006
		AU 2006-235013 B2	03/11/2011
		AU 2007-337101 A1	03/07/2008
		AU 2007-337214 A1	03/07/2008
		AU 2007-353769 A1	27/11/2008
		AU 2007-353769 B2	10/01/2013
		CA 2604363 A1	19/10/2006
		CA 2673477 A1	27/11/2008
		CA 2673543 A1	03/07/2008
		CA 2673558 A1	03/07/2008
		CN 101180079 A	14/05/2008
		CN 101180079 B	18/07/2012
		CN 101600459 A	09/12/2009
		CN 101610785 A	23/12/2009
		CN 101610788 A	23/12/2009
		CN 102716480 A	10/10/2012
		DK 2094298 T3	26/03/2012
		EP 1868645 A1	26/12/2007
		EP 1868645 B1	07/03/2012
		EP 2094298 A2	02/09/2009
		EP 2094298 B1	15/02/2012
		EP 2094304 A1	02/09/2009
		EP 2099494 A2	16/09/2009
		EP 2417983 A1	15/02/2012
		EP 2417983 B1	26/06/2013
		EP 2425851 A1	07/03/2012
		EP 2425852 A1	07/03/2012
		EP 2425853 A1	07/03/2012
		EP 2425854 A1	07/03/2012
		EP 2425855 A1	07/03/2012
		EP 2425856 A1	07/03/2012
		ES 2379834 T3	04/05/2012
		IL 199350 A	30/05/2013
		IL 222793 D	31/12/2012
		JP 04472770 B2	02/06/2010
		JP 05173920 B2	03/04/2013
		JP 2008-535838 A	04/09/2008
		JP 2009-161567 A	23/07/2009
		JP 2010-513549 A	30/04/2010
		JP 2010-513550 A	30/04/2010
		JP 2010-513559 A	30/04/2010
		JP 2013-006881 A	10/01/2013
		KR 10-1298053 B1	20/08/2013
		KR 10-2009-0094163 A	03/09/2009
		KR 10-2009-0094164 A	03/09/2009
		KR 10-2009-0096731 A	14/09/2009

Form PCT/ISA/210 (patent family annex) (July 2009)

Petitioner Merck Ex-1004, 0544

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/KR2013/005392

Patent document cited in search report	Publication date	Patent family member	Publication date
		MX 2009006705 A	30/06/2009
		RU 2009125216 A	27/01/2011
		SI 2094298 T1	30/04/2012
		US 2006-0228380 A1	12/10/2006
		US 2007-0184071 A1	09/08/2007
		US 2007-0184072 A1	09/08/2007
		US 2007-0231340 A1	04/10/2007
		US 2009-0130137 A1	21/05/2009
		US 2009-0234108 A1	17/09/2009
		US 2011-0071279 A1	24/03/2011
		US 7709001 B2	04/05/2010
		US 7955605 B2	07/06/2011
		WO 2006-110381 A1	19/10/2006
		WO 2008-079653 A1	03/07/2008
		WO 2008-079732 A2	03/07/2008
		WO 2008-079732 A3	24/12/2008
		WO 2008-143709 A2	27/11/2008
		WO 2008-143709 A3	08/01/2009
WO 2011-151760 A2	08/12/2011	AU 2011-262346 A1	06/12/2012
		CA 2799747 A1	08/12/2011
		CN 102933229 A	13/02/2013
		EP 2575870 A2	10/04/2013
		IL 223206 D	03/02/2013
		JP 2011-256168 A	22/12/2011
		KR 10-2013-0041064 A	24/04/2013
		MX 2012013664 A	24/01/2013
		US 2013-0072881 A1	21/03/2013
		WO 2011-151760 A3	01/03/2012
US 2003-0147922 A1	07/08/2003	AR 022963 A1	04/09/2002
		AR 022964 A1	04/09/2002
		AT 346608 T	15/12/2006
		AT 387214 T	15/03/2008
		AT 459373 T	15/03/2010
		AU 2000-32919 B2	25/07/2002
		AU 2000-34307 B2	01/08/2002
		AU 2000-38136 B2	25/07/2002
		AU 2000-45525 A1	02/11/2000
		AU 2000-45525 B2	10/07/2003
		AU 2000-55233 A1	02/11/2000
		AU 2000-55233 B2	07/11/2002
		AU 3291900 A	09/10/2000
		AU 3430700 A	09/10/2000
		AU 3813600 A	09/10/2000
		AU 750762 B2	25/07/2002
		AU 750788 B2	25/07/2002
		AU 750913 B2	01/08/2002
		BR 0009154 A	26/12/2001
		BR 0009163 A	26/12/2001

Form PCT/ISA/210 (patent family annex) (July 2009)

Petitioner Merck Ex-1004, 0545

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/KR2013/005392

Patent document cited in search report	Publication date	Patent family member	Publication date
		BR 0009166 A	26/12/2001
		CA 2365296 A1	28/09/2000
		CA 2365579 A1	26/10/2000
		CA 2366152 A1	28/09/2000
		CA 2366314 A1	28/09/2000
		CA 2366314 C	10/01/2012
		CA 2370708 A1	26/10/2000
		CN 100339130 C	26/09/2007
		CN 101130071 A	27/02/2008
		CN 101130071 B	21/03/2012
		CN 1191852 C	09/03/2005
		CN 1192797 C	16/03/2005
		CN 1192798 C	16/03/2005
		CN 1351501 A	29/05/2002
		CN 1351503 A	29/05/2002
		CN 1355710 A	26/06/2002
		CN 1391481 A	15/01/2003
		CZ 20013378 A3	13/03/2002
		CZ 20013379 A3	13/03/2002
		CZ 20013380 A3	13/03/2002
		CZ 301445 B6	03/03/2010
		CZ 303653 B6	30/01/2013
		DE 60032120 D1	11/01/2007
		DE 60032120 T2	20/09/2007
		DE 60038166 D1	10/04/2008
		DE 60038166 T2	12/03/2009
		DE 60043930 D1	15/04/2010
		DK 1162999 T3	26/03/2007
		DK 1163000 T3	28/04/2008
		EP 1162998 A2	19/12/2001
		EP 1162998 B1	03/03/2010
		EP 1162999 A2	19/12/2001
		EP 1162999 B1	29/11/2006
		EP 1163000 A2	19/12/2001
		EP 1163000 B1	27/02/2008
		EP 1171158 A2	16/01/2002
		EP 1171159 A2	16/01/2002
		EP 1171159 B1	14/05/2008
		EP 1776962 A1	25/04/2007
		EP 1880735 A2	23/01/2008
		EP 1880735 A3	12/03/2008
		EP 1927366 A1	04/06/2008
		EP 2277535 A2	26/01/2011
		EP 2277535 A3	09/03/2011
		ES 2275199 T3	16/06/2007
		ES 2300255 T3	16/06/2008
		ES 2339737 T3	25/05/2010
		HK 1043728 A1	22/06/2007
		HK 1043731 A1	23/01/2009
		HU 0200367 A2	29/05/2002

Form PCT/ISA/210 (patent family annex) (July 2009)

Petitioner Merck Ex-1004, 0546

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/KR2013/005392

Patent document cited in search report	Publication date	Patent family member	Publication date
		HU 0200373 A2	29/06/2002
		HU 0200664 A2	29/06/2002
		HU 228499 B1	28/03/2013
		IL 145043 A	17/06/2007
		IL 145044 A	08/03/2007
		IL 145045 D	30/06/2002
		JP 04846906 B2	28/12/2011
		JP 2002-539273 A	19/11/2002
		JP 2002-540074 A	26/11/2002
		JP 2002-540075 A	26/11/2002
		JP 2002-542204 A	10/12/2002
		JP 2011-057713 A	24/03/2011
		KR 10-0632835 B1	16/10/2006
		KR 10-0642044 B1	10/11/2006
		KR 10-0798212 B1	24/01/2008
		KR 10-2002-0000785 A	05/01/2002
		LU 91652 A9	13/10/2010
		MX PA01009452 A	06/08/2002
		NL 300415 I1	01/12/2009
		NO 20014322 A	14/11/2001
		NO 20014323 A	14/11/2001
		NO 20014325 A	14/11/2001
		NO 330532 B1	09/05/2011
		NO 330736 B1	27/06/2011
		NZ 513840 A	27/02/2004
		NZ 513841 A	28/09/2001
		NZ 513842 A	28/05/2004
		PL 203917 B1	30/11/2009
		PL 204890 B1	26/02/2010
		PL 355178 A1	05/04/2004
		PL 355180 A1	05/04/2004
		PL 355264 A1	05/04/2004
		PT 1162999 E	28/02/2007
		PT 1163000 E	20/03/2008
		SI 1162999 T1	30/04/2007
		SI 1163000 T1	30/06/2008
		TR 200102735 T2	22/04/2002
		TR 200102736 T2	22/04/2002
		TR 200102739 T2	21/12/2001
		TW 234463 A	21/06/2005
		TW 235064 A	01/07/2005
		TW 281403 A	21/05/2007
		TW 286938 A	21/09/2007
		US 2004-0228879 A1	18/11/2004
		US 2005-0031646 A1	10/02/2005
		US 2006-0002961 A1	05/01/2006
		US 2006-0093626 A1	04/05/2006
		US 2010-0119544 A1	13/05/2010
		US 2010-0291138 A1	18/11/2010
		US 2011-0217329 A1	08/09/2011

Form PCT/ISA/210 (patent family annex) (July 2009)

Petitioner Merck Ex-1004, 0547

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/KR2013/005392

Patent document cited in search report	Publication date	Patent family member	Publication date
		WO 00-56358 A2	28/09/2000
		WO 00-56358 A3	04/01/2001
		WO 00-56359 A2	28/09/2000
		WO 00-56359 A3	01/02/2001
		WO 00-56360 A2	28/09/2000
		WO 00-56360 A3	25/01/2001
		WO 00-62801 A2	26/10/2000
		WO 00-62801 A3	11/01/2001
		WO 00-62802 A2	26/10/2000
		WO 00-62802 A3	11/01/2001
US 05153312 A	06/10/1992	CA 2052323 A1	29/03/1992
		CA 2052323 C	17/04/2001
		CN 1034054 C	19/02/1997
		CN 1060294 A	15/04/1992
		EP 0477508 A1	01/04/1992
		EP 0477508 B1	12/07/1995
		JP 03027452 B2	04/04/2000
		JP 06-340550 A	13/12/1994
		KR 10-0217317 B1	01/10/1999
		US 05306492 A	26/04/1994
US 2004-0202668 A1	14/10/2004	AR 040922 A1	27/04/2005
		AT 553775 T	15/05/2012
		AT 553776 T	15/05/2012
		AU 2001-281895 B2	28/04/2005
		AU 2001-81895 A1	08/01/2002
		BR 0208595 A	21/02/2006
		CA 2412497 A1	03/01/2002
		CA 2412497 C	02/10/2012
		CA 2442865 A1	17/10/2002
		CA 2442865 C	11/09/2012
		CA 2727715 A1	17/10/2002
		CA 2727715 C	28/05/2013
		CA 2783274 A1	03/01/2002
		CA 2810326 A1	17/10/2002
		CN 101112618 A	30/01/2008
		CN 101112618 B	08/08/2012
		CN 101112619 A	30/01/2008
		CN 101112619 B	18/05/2011
		CN 101708333 A	19/05/2010
		CN 1547485 A	17/11/2004
		CN 1547485 C	06/09/2006
		CZ 20032698 A3	15/12/2004
		DK 1390066 T3	16/07/2012
		DK 1946771 T3	03/09/2012
		DK 1946772 T3	16/07/2012
		EP 1296715 A2	02/04/2003
		EP 1296715 B1	23/11/2011
		EP 1390066 A2	25/02/2004

Form PCT/ISA/210 (patent family annex) (July 2009)

Petitioner Merck Ex-1004, 0548

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/KR2013/005392

Patent document cited in search report	Publication date	Patent family member	Publication date
		EP 1390066 B1	18/04/2012
		EP 1946769 A2	23/07/2008
		EP 1946769 A3	30/07/2008
		EP 1946769 B1	30/05/2012
		EP 1946771 A1	23/07/2008
		EP 1946771 B1	27/06/2012
		EP 1946772 A1	23/07/2008
		EP 1946772 B1	18/04/2012
		EP 2277541 A1	26/01/2011
		EP 2279748 A1	02/02/2011
		EP 2311488 A2	20/04/2011
		EP 2311488 A3	31/08/2011
		ES 2384041 T3	28/06/2012
		ES 2384065 T3	29/06/2012
		ES 2388690 T3	17/10/2012
		GB 0108364 D	23/05/2001
		HK 1062891 A1	28/09/2012
		HK 1114779 A1	28/09/2012
		HK 1114781 A1	16/11/2012
		HU 0303996 A2	01/03/2004
		HU 0303996 A3	28/03/2011
		IL 158066 D	28/03/2004
		JP 04870895 B2	08/02/2012
		JP 05095595 B2	12/12/2012
		JP 2004-501873 A	22/01/2004
		JP 2005-508849 A	07/04/2005
		JP 2009-102355 A	14/05/2009
		JP 2010-163453 A	29/07/2010
		JP 2012-051943 A	15/03/2012
		KR 10-0837917 B1	13/06/2008
		KR 10-0870280 B1	25/11/2008
		KR 10-0898845 B1	21/05/2009
		KR 10-1045412 B1	30/06/2011
		KR 10-1292708 B1	02/08/2013
		KR 10-2007-0091698 A	11/09/2007
		KR 10-2008-0048094 A	30/05/2008
		KR 10-2009-0026371 A	12/03/2009
		KR 10-2009-0120017 A	23/11/2009
		KR 10-2011-0036641 A	07/04/2011
		KR 10-2012-0005519 A	16/01/2012
		KR 10-2012-0005534 A	16/01/2012
		MX PA03008961 A	12/02/2004
		MY 129263 A	30/03/2007
		NO 20034410 A	25/11/2003
		NZ 551621 A	31/07/2008
		NZ 568317 A	29/01/2010
		NZ 581373 A	30/06/2011
		PL 211909 B1	31/07/2012
		PL 211910 B1	31/07/2012
		PL 211911 B1	31/07/2012

Form PCT/ISA/210 (patent family annex) (July 2009)

Petitioner Merck Ex-1004, 0549

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/KR2013/005392

Patent document cited in search report	Publication date	Patent family member	Publication date
		PL 211912 B1	31/07/2012
		PL 211958 B1	31/07/2012
		PL 374106 A1	19/09/2005
		PL 393582 A1	09/05/2011
		PL 393583 A1	09/05/2011
		PL 393585 A1	09/05/2011
		PL 393618 A1	09/05/2011
		PT 1390066 E	27/06/2012
		PT 1946771 E	27/08/2012
		PT 1946772 E	09/07/2012
		SI 1390066 T1	31/08/2012
		SI 1946771 T1	30/10/2012
		SI 1946772 T1	31/08/2012
		UA 76952 C2	15/07/2003
		US 2003-180316 A1	25/09/2003
		US 2008-0069835 A1	20/03/2008
		US 2011-212124 A1	01/09/2011
		US 2012-0207780 A1	16/08/2012
		WO 02-00249 A2	03/01/2002
		WO 02-00249 A3	13/06/2002
		WO 02-080965 A2	17/10/2002
		WO 02-080965 A3	04/12/2003
		ZA 200307640 A	23/05/2005

Form PCT/ISA/210 (patent family annex) (July 2009)

Petitioner Merck Ex-1004, 0550

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- US 5614382 A **[0017]**
- WO 2004083251 A **[0018]**
- US 4673574 A **[0019]**
- US 4902506 A **[0019]**
- WO 9014837 A **[0021]**
- US 4912094 A, Corixa **[0021]**
- US 5057540 A **[0021]**
- US 6113918 A **[0021]**
- US 6207646 B **[0021]**
- WO 0018434 A **[0021]**
- WO 02098368 A **[0021]**
- WO 02098369 A **[0021]**
- WO 9313302 A **[0021]**
- WO 9219265 A **[0021]**
- WO 02083855 A **[0029]**
- WO 02053761 A **[0029]**
- US 5847112 A **[0036]**

Non-patent literature cited in the description

- **HARBOE ZB ; BENFIELD TL ; VALENTINER-BRANTH P et al.** Temporal Trends in Invasive Pneumococcal Disease and Pneumococcal Serotypes over 7 Decades. *Clin Infect Dis*, 2010, vol. 50, 329-37 **[0012]**
- **SAHA SK ; AL EMRAN HM ; HOSSAIN B ; DARMSTADT GL ; SAHA S et al.** Streptococcus pneumoniae Serotype-2 Childhood Meningitis in Bangladesh: A Newly Recognized Pneumococcal Infection Threat. *PLoS ONE 2012*, 2012, vol. 7 (3), e32134 **[0013]**

Petitioner Merck Ex-1004, 0551

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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 November 2008 (27.11.2008)

PCT

(10) International Publication Number
WO 2008/143709 A2

(51) International Patent Classification:
A61K 39/00 (2006.01) *A61P 35/00* (2006.01)

(21) International Application Number:
PCT/US2007/086941

(22) International Filing Date:
10 December 2007 (10.12.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
11/644,924 22 December 2006 (22.12.2006) US

(71) Applicant *(for all designated States except US)*: **WYETH** [US/US]; 5 Giralda Farms, Madison, NJ 07940 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **HAUSDORFF, William, P.** [US/BE]; Avenue De La Chapelle, 19, B-1200 Woluwe-Saint-Lambert (BE). **SIBER, George, Rainer** [US/US]; 160 West 66th Street, New York, NY 10023 (US). **PARADISO, Peter, R.** [US/US]; 445 Freedomview Lane, Valley Forge, PA 19481 (US). **PRASAD, A., Krishna** [IN/US]; 105 Worsham Drive, Chapel Hill, NC 27516 (US).

(74) Agent: **CASH, David E.**; Alston & Bird LLP, Bank Of America Plaza, 101 South Tryon Street, Suite 4000, Charlotte, NC 28280-4000 (US).

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

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

WO 2008/143709 A2

(54) Title: MULTIVALENT PNEUMOCOCCAL POLYSACCHARIDE-PROTEIN CONJUGATE COMPOSITION

(57) Abstract: An immunogenic composition having 13 distinct polysaccharide-protein conjugates and optionally, an aluminum-based adjuvant, is described. Each conjugate contains a capsular polysaccharide prepared from a different serotype of *Streptococcus pneumoniae* (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F) conjugated to a carrier protein. The immunogenic composition, formulated as a vaccine, increases coverage against pneumococcal disease in infants and young children globally, and provides coverage for serotypes 6A and 19A that is not dependent on the limitations of serogroup cross-protection. Also described is a method for making an immunogenic conjugate comprising Streptococcus pneumoniae serotype 3 polysaccharide covalently linked to a carrier protein, the method including periodic acid oxidation of the polysaccharide in the presence of bivalent cations.

Petitioner Merck Ex-1004, 0552

MULTIVALENT PNEUMOCOCCAL POLYSACCHARIDE-PROTEIN CONJUGATE COMPOSITION

FIELD OF THE INVENTION

The present invention relates generally to the field of medicine, and specifically to microbiology, immunology, vaccines and the prevention of infection by a bacterial pathogen by immunization.

BACKGROUND OF THE INVENTION

Streptococcus pneumoniae is a leading cause of meningitis, pneumonia, and severe invasive disease in infants and young children throughout the world. The multivalent pneumococcal polysaccharide vaccines have been licensed for many years and have proved valuable in preventing pneumococcal disease in elderly adults and high-risk patients. However, infants and young children respond poorly to most pneumococcal polysaccharides. The 7-valent pneumococcal conjugate vaccine (7vPnC, Prevnar®) was the first of its kind demonstrated to be highly immunogenic and effective against invasive disease and otitis media in infants and young children. This vaccine is now approved in many countries around the world. Prevnar contains the capsular polysaccharides from serotypes 4, 6B, 9V, 14, 18C, 19F and 23F, each conjugated to a carrier protein designated CRM_{197}. Prevnar covers approximately 80-90%, 60-80%, and 40-80% of invasive pneumococcal disease (IPD) in the US, Europe, and other regions of the world, respectively [1,2]. Surveillance data gathered in the years following Prevnar's introduction has clearly demonstrated a reduction of invasive pneumococcal disease in US infants as expected (FIG. 1) [3,4].

Surveillance of IPD conducted in US infants prior to the introduction of Prevnar demonstrated that a significant portion of disease due to serogroups 6 and 19 was due to the 6A (approximately one-third) and 19A (approximately one-fourth) serotypes [5,6]. Pneumococcal invasive disease surveillance conducted in the US after licensure of Prevnar suggests that a large burden of disease is still attributable to serotypes 6A and 19A (FIG. 1) [3]. Moreover, these two serotypes account for more cases of invasive disease than serotypes 1, 3, 5, and 7F combined (8.2 vs. 3.3 cases/100,000 children 2 years and under). In addition, serotypes 6A and 19A are associated with high rates of antibiotic resistance (FIG. 2) [7,8,9]. While it is possible that serogroup cross-protection will result in a decline of serotype 6A and 19A

Petitioner Merck Ex-1004, 0553

disease as more children are immunized, there is evidence to suggest that there will be a limit to the decline, and a significant burden of disease due to these serotypes will remain (see below).

Given the relative burden and importance of invasive pneumococcal disease due to serotypes 1, 3, 5, 6A, 7F, and 19A, adding these serotypes to the Prevnar formulation would increase coverage for invasive disease to >90% in the US and Europe, and as high as 70%-80% in Asia and Latin America. This vaccine would significantly expand coverage beyond that of Prevnar, and provide coverage for 6A and 19A that is not dependent on the limitations of serogroup cross-protection.

SUMMARY OF THE INVENTION

Accordingly, the present invention provides generally a multivalent immunogenic composition comprising 13 distinct polysaccharide-protein conjugates, wherein each of the conjugates contains a capsular polysaccharide from a different serotype of *Streptococcus pneumoniae* conjugated to a carrier protein, together with a physiologically acceptable vehicle. Optionally, an adjuvant, such as an aluminum-based adjuvant, is included in the formulation. More specifically, the present invention provides a 13-valent pneumococcal conjugate (13vPnC) composition comprising the seven serotypes in the 7vPnC vaccine (4, 6B, 9V, 14, 18C, 19F and 23F) plus six additional serotypes (1, 3, 5, 6A, 7F and 19A).

The present invention also provides a multivalent immunogenic composition, wherein the capsular polysaccharides are from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F of *Streptococcus pneumoniae* and the carrier protein is CRM_{197}.

The present invention further provides a multivalent immunogenic composition, wherein the capsular polysaccharides are from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9v, 14, 18C, 19A, 19F and 23F of *Streptococcus pneumoniae*, the carrier protein is CRM_{197}, and the adjuvant is an aluminum-based adjuvant, such as aluminum phosphate, aluminum sulfate and aluminum hydroxide. In a particular embodiment of the invention, the adjuvant is aluminum phosphate.

The present invention also provides a multivalent immunogenic composition, comprising polysaccharide-protein conjugates together with a physiologically acceptable vehicle, wherein each of the conjugates comprises a capsular

Petitioner Merck Ex-1004, 0554

polysaccharide from a different serotype of *Streptococcus pneumoniae* conjugated to a carrier protein, and the capsular polysaccharides are prepared from serotype 3 and at least one additional serotype.

In one embodiment of this multivalent immunogenic composition, the additional serotype is selected from the group consisting of serotypes 1, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. In another embodiment, the carrier protein is CRM_{197}. In yet another embodiment, the composition comprises an adjuvant, such as an aluminum-based adjuvant selected from aluminum phosphate, aluminum sulfate and aluminum hydroxide. In a particular embodiment, the adjuvant is aluminum phosphate.

The present invention also provides a multivalent immunogenic composition, comprising polysaccharide-protein conjugates together with a physiologically acceptable vehicle, wherein each of the conjugates comprises a capsular polysaccharide from a different serotype of *Streptococcus pneumoniae* conjugated to a carrier protein, and the capsular polysaccharides are prepared from serotypes 4, 6B, 9V, 14, 18C, 19F, 23F and at least one additional serotype.

In one embodiment of this multivalent immunogenic composition, the additional serotype is selected from the group consisting of serotypes 1, 3, 5, 6A, 7F, and 19A. In another embodiment, the carrier protein is CRM_{197}. In yet another embodiment, the composition comprises an adjuvant, such as an aluminum-based adjuvant selected from aluminum phosphate, aluminum sulfate and aluminum hydroxide. In a particular embodiment, the adjuvant is aluminum phosphate.

The present invention also provides a method of inducing an immune response to a *Streptococcus pneumoniae* capsular polysaccharide conjugate, comprising administering to a human an immunologically effective amount of any of the immunogenic compositions just described.

The present invention further provides that any of the immunogenic compositions administered is a single 0.5 mL dose formulated to contain: 2 µg of each saccharide, except for 6B at 4 µg; approximately 29 µg CRM_{197} carrier protein; 0.125 mg of elemental aluminum (0.5 mg aluminum phosphate) adjuvant; and sodium chloride and sodium succinate buffer as excipients.

Methods for making an immunogenic conjugate comprising *Streptococcus pneumoniae* serotype 3 (Pn 3) polysaccharide covalently linked to a carrier protein are also provided. In one embodiment, the method comprises (i) reacting purified Pn 3 polysaccharide with a mild acid resulting in a hydrolyzed Pn 3 polysaccharide; (ii) reacting the hydrolyzed Pn 3 polysaccharide with an oxidizing agent in the presence

Petitioner Merck Ex-1004, 0555

of bivalent cations resulting in an activated Pn 3 polysaccharide; (iii) compounding the activated Pn 3 polysaccharide with a carrier protein; (iv) reacting the compounded, activated Pn 3 polysaccharide and carrier protein with a reducing agent resulting in a Pn 3 polysaccharide:carrier protein conjugate; and (v) capping unreacted aldehydes in the Pn 3 polysaccharide:carrier protein conjugate resulting in an immunogenic conjugate comprising *Streptococcus pneumoniae* Pn 3 polysaccharide covalently linked to a carrier protein.

In a further embodiment, the method comprises (i) reacting purified Pn 3 polysaccharide with acetic acid resulting in a hydrolyzed Pn 3 polysaccharide; (ii) reacting the hydrolyzed Pn 3 polysaccharide with periodic acid in the presence of $MgCl_2$ resulting in an activated Pn 3 polysaccharide; (iii) purifying the activated Pn 3 polysaccharide; (iv) compounding the activated Pn 3 polysaccharide with a carrier protein; (v) co-lyophilizing the compounded, activated Pn 3 polysaccharide and carrier protein; (vi) reacting the co-lyophilized, activated Pn 3 polysaccharide and carrier protein with sodium cyanoborohydride resulting in a Pn 3 polysaccharide:carrier protein conjugate; and (vii) capping unreacted aldehydes in the Pn 3 polysaccharide:carrier protein conjugate with sodium borohydride resulting in an immunogenic conjugate comprising *Streptococcus pneumoniae* Pn 3 polysaccharide covalently linked to a carrier protein.

A method for making an activated *Streptococcus pneumoniae* Pn 3 polysaccharide is also provided. The method comprises (i) reacting purified Pn 3 polysaccharide with a mild acid resulting in a hydrolyzed Pn 3 polysaccharide; and (ii) reacting the hydrolyzed Pn 3 polysaccharide with an oxidizing agent in the presence of bivalent cations resulting in an activated Pn 3 polysaccharide.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts the changes in IPD rates by serotype in US children <2 years of age from baseline (1998/1999) to 2001.

FIG. 2 depicts the distribution of pneumococcal isolates with resistance to penicillin (PCN) in children <5 years of age (1998).

FIG. 3 depicts the reverse cumulative distribution curves (RCDC) of OPA post-third dose results from the D118-P16 Prevnar trial.

Petitioner Merck Ex-1004, 0556

DETAILED DESCRIPTION OF THE INVENTION

Inclusion of Prevnar Serotypes 4, 6B, 9V, 14, 18C, 19F, 23F

Data from IPD surveillance between 1995-1998 estimated that the seven serotypes in Prevnar were responsible for around 82% of IPD in children <2 years of age [5]. In Northern California, the site of the efficacy trial, the Prevnar serotypes accounted for 90% of all cases of IPD in infants and young children [10]. Since introduction of the Prevnar vaccine in 2000, there has been a significant decrease in the overall IPD rates due to a decrease in disease due to the vaccine serotypes [3,4]. Therefore, there is no justification at this time to remove any of the Prevnar serotypes from the next generation of pneumococcal conjugate vaccines but rather to add serotypes to obtain wider coverage.

Inclusion of Serotypes 1, 3, 5 and 7F

In the US, the rate of IPD caused by serotype 1 in children under the age of 5 years is <2%, about the same as for each of types 3 and 7F [1,6]. Serotypes 1 and 5 account for higher rates of IPD in US populations at high risk for invasive pneumococcal disease. Specifically, serotype 1 causes 3.5% of IPD in Alaskan native children <2 years of age, and 18% in children 2-4 years of age [11]. Both serotype 1 and serotype 5 significantly cause disease in other parts of the world and in indigenous populations in developed countries [12,13,14].

Serotype 1 may also be associated with more severe disease as compared with other pneumococcal serotypes [15]. This observation is based on the difference in rates of case identification between the US and Europe, and the associated difference in medical practice. Overall, the incidence of IPD is lower in Europe than in the US. However, the percent of IPD caused by serotype 1 in Europe is disproportionately higher than in the US (6-7%, vs. 1-2%, respectively). In Europe, blood cultures are obtained predominantly from hospitalized children. In the US, it is routine medical practice to obtain blood cultures in an outpatient setting from children presenting with fever $\geq$39°C and elevated white blood cell counts. Given the difference in medical practice, it is postulated that the lower percent of disease caused by serotype 1 in the US may be diluted by higher rates of other serotypes causing milder disease, while the higher percent in Europe reflects more serious disease. In addition, seroepidemiology studies of children with complicated pneumonia demonstrate that serotype 1 is disproportionately represented [16,17,18].

Petitioner Merck Ex-1004, 0557

This suggests that inclusion of serotype 1 may reduce the amount of severe pneumococcal disease, as well as, contribute to a total reduction in invasive pneumococcal disease.

The addition of serotypes 3 and 7F will increase coverage against IPD in most areas of the world by approximately 3%-7%, and in Asia by around 9%. Thus, an 11-valent vaccine would cover 50% in Asia and around 80% of IPD in all other regions [1,2]. These serotypes are also important with respect to otitis media coverage [19]. In a multinational study of pneumococcal serotypes causing otitis media, Hausdorff et al. found serotype 3 to be the 8th most common middle ear fluid isolate overall [20]. Serotype 3 accounted for up to 8.7% of pneumococcal serotypes associated with otitis media. Thus, the importance of types 3 and 7F in otitis media, as well as in IPD, warrants their inclusion in a pneumococcal conjugate vaccine.

However, attempts to produce a multivalent pneumococcal conjugate vaccine that exhibits significant immunogenicity with respect to serotype 3 polysaccharides have been unsuccessful. For example, in a study of the immunogenicity and safety of an 11-valent pneumococcal protein D conjugate vaccine (11-Pn-PD), no priming effect was observed for serotype 3 in infants who had received three doses of the vaccine followed by a booster dose of either the same vaccine or a pneumococcal polysaccharide vaccine (Nurkka et al. (2004) *Ped. Inf. Dis. J.*, 23:1008-1014). In another study, opsonophagocytic assay (OPA) results from infants who had received doses of 11-Pn-PD failed to show antibody responses for serotype 3 at levels comparable to other tested serotypes (Gatchalian et al., 17th Annual Meeting of the Eur. Soc. Paed. Inf. Dis. (ESPID), Poster No. 4, P1A Poster Session 1, Istanbul Turkey, Mar. 27, 2001). In yet another study, which assessed the efficacy of an 11-Pn-PD in the prevention of acute otitis media, the vaccine did not provide protection against episodes caused by serotype 3 (Prymula et al. (2006) *Lancet*, 367:740-748). Accordingly, a pneumococcal conjugate vaccine comprising capsular polysaccharides from serotype 3 and capable of eliciting an immunogenic response to serotype 3 polysaccharides provides a significant improvement over the existing state of the art.

Inclusion of Serotypes 6A and 19A

a. Epidemiology of Serotypes 6A and 19A

Surveillance data in the literature suggest that serotypes 6A and 19A account for more invasive pneumococcal disease in US children <2 years of age than serotypes 1, 3, 5, and 7F combined (FIG. 1) [1,5]. In addition, these serotypes are

Petitioner Merck Ex-1004, 0558

commonly associated with antibiotic resistance (FIG. 2) and play an important role in otitis media [6,19,20]. The ability of the current Prevnar vaccine to protect against disease due to 6A and 19A is not clear. The rationale for inclusion of 6A and 19A components in a 13vPnC vaccine is discussed below.

b. Responses to 6A and 19A Induced by 6B and 19F Polysaccharides

The licensed unconjugated pneumococcal polysaccharide vaccines (for use in persons at least two years of age) have contained 6A or 6B capsular polysaccharide but not both [21]. Immunogenicity data generated at the time of formulation of the 23-valent pneumococcal polysaccharide vaccine demonstrated that a 6B monovalent vaccine induced antibody to both the 6A and 6B capsules. The data from several trials assessing IgG and opsonophagocytic assay (OPA) responses in a variety of populations with free polysaccharide and with pneumococcal conjugate vaccines suggested that IgG responses to 6A are induced by 6B antigens, but the responses are generally lower, and the OPA activity with 6A organisms is different than with 6B organisms [22,23,24,25]. In addition, subjects responding with high 6B antibody may have little or no activity against 6A.

In contrast to the chemical composition of the 6A and 6B capsular polysaccharides where there exists a high degree of similarity, the 19A and 19F capsules are quite different due to the presence of two additional side chains in the 19A polysaccharide. Not surprisingly, immune responses measured in human volunteers immunized with 19F polysaccharide vaccine showed that responses to 19F were induced in 80% of subjects, but only 20% of subjects had a response to 19A [26]. Low levels of cross-reactive IgG and OPA responses to serotype 19A after immunization with 19F polysaccharide have also been documented in trials with conjugate vaccines as well [24,26].

Internal data on cross-reactive OPA responses to 6A and 19A have been generated from the 7vPnC bridging trial (D118-P16) conducted in US infants (FIG. 3). These studies are consistent with the findings of others, and demonstrate induction of cross-reactive functional antibody to 6A polysaccharide after immunization with 6B polysaccharide, although at a lower level, and very little functional antibody to 19A after immunization with 19F.

Petitioner Merck Ex-1004, 0559

Impact of 6B and 19F Immunization on 6A and 19A in Animal Models

Animal models have been used to evaluate the potential for cross-protection with polysaccharide immunization. In an otitis media model developed by Giebink et al., chinchillas were immunized with a tetravalent polysaccharide outer membrane protein (OMP) conjugate vaccine (containing 6B, 14, 19F, 23F saccharides) or placebo [27]. In this trial there appeared to be some cross-protection for 6A; however this did not reach statistical significance and the level of protection was lower than with 6B against otitis media. In this same model there was 100% protection against 19F otitis media, but only 17% protection against 19A otitis media.

Saeland et al. used sera from infants immunized with an 8-valent pneumococcal tetanus conjugate vaccine (containing 6B and 19F) to passively immunize mice prior to an intranasal challenge with 6A organisms, in a lung infection model [28]. Of the 59 serum samples, 53% protected mice against bacteremia with 6B and 37% protected against 6A. Mice passively immunized with sera from infants immunized with four doses of an 11-valent pneumococcal conjugate vaccine (containing 19F conjugated to tetanus toxoid) were given an intranasal challenge with 19A organisms in the same model [29]. Of 100 mice passively immunized and then challenged, 60 mice had no 19A organisms detected in lung tissue, whereas organisms were identified in all mice given saline placebo. However, passive immunization did not protect against challenge with 19F organisms in this model; therefore, the relevance of the model for serogroup 19 is questionable. In general these models provide evidence of some biological impact of 6B immunization on 6A organisms although the effect on the heterologous serotype was not as great as that observed with the homologous serotype. The impact of 19F immunization on 19A organisms is not well understood from these models.

Impact of 6B and 19F Polysaccharide Conjugate Immunization on 6A and 19A Disease in Efficacy/Effectiveness Trials

The number of cases of disease due to the 6B, 6A, 19F and 19A serotypes in 7vPnC and 9vPnC (7vPnC plus serotypes 1 and 5) efficacy trials is noted in Table 1 [30,10,31]. The numbers of invasive disease cases are too small to allow any conclusions to be drawn for serotypes 6A and 19A. However, the Finnish otitis media trial generated a large number of pneumococcal isolates [32]. In the per protocol analysis 7vPnC was 84% (95% CI 62%, 93%) efficacious against otitis

Petitioner Merck Ex-1004, 0560

media due to serotype 6B and 57% (95% CI 24%, 76%) efficacious against otitis media due to serotype 6A (Table 1). In contrast, serotype-specific efficacy with the 7vPnC was not demonstrated for otitis media due to either 19F or 19A.

Table 1. Cases of Pneumococcal Disease Due to Serotypes 6B, 6A, 19F, and 19A in Efficacy Trials with the 7vPnC and 9vPnC Vaccines

	6B		6A		19F		19A	
	PnC	Contr.	PnC	Contr.	PnC	Contr.	PnC	Contr.
Kaiser Efficacy Trial – 7vPnC (ITT)	1	7	0	1	2*	13	0	1
Navajo Efficacy Trial – 7vPnC (ITT)	0	5	1	0	1	1	1	0
South African Efficacy Trial – 9vPnC HIV (-) (ITT)	1	2	1	0	0	1	3	1
South African Efficacy Trial – 9vPnC HIV (+) (ITT)	1	7	3	10	2	3	2	3
Finnish Otitis Media Trial – 7vPnC (PP)	9*	56	19*	45	43	58	17	26

*Statistically significant efficacy demonstrated
From references 30, 10 and 33, and personal communications
Contr = control
ITT = intention to treat analysis
PP = per protocol analysis

Post-marketing IPD surveillance data is also available from a case-control trial conducted by the Centers for Disease Control to evaluate the effectiveness of Prevnar [33]. Cases of pneumococcal invasive disease occurring in children 3 to 23 months of age were identified in the surveillance laboratories and matched with three control cases by age and zip code. After obtaining consent, medical and immunization history (subjects were considered immunized if they had received at least one dose of Prevnar) was obtained from parents and medical providers for cases and controls. The preliminary results were presented at the 2003 ICAAC meeting and a summary of the findings for 6B, 19F, 19A and 6A disease is presented in Table 2. These data indicate that Prevnar is able to prevent disease due to 6A, although at a level that may be somewhat lower than serotype 6B disease. These data also indicate that the cross-protection for invasive disease due to 19A is limited.

Petitioner Merck Ex-1004, 0561

Table 2. Preliminary results of a Case Control Trial Performed by the CDC (presented at ICAAC, 2003)

Serotype	Informative Sets, n	VE* (95% CI)
Vaccine Type, All	115	94 (87, 97)
Vaccine Related, All	36	70 (38, 86)
Non-Vaccine Type, All	43	-4 (-106, 48)
6B	27	94 (72, 99)
19F	19	73 (16, 92)
6A	15	87 (53, 97)
19A	16	40 (-87, 80)

*Vaccine effectiveness comparing vaccinated (≥1 dose) vs. unvaccinated, and adjusted for underlying conditions
Reference 40 and personal/confidential communication

A published analysis [3] of the use of Prevnar also indicated that serotypes 6B and 19F conferred a moderate reduction in IPD caused by serotypes 6A and 19A among children under two years of age (Table 1 in [3]). Disease rates among unimmunized adults caused by serotypes 6A, 9A, 9L, 9N, 18A, 18B, 18F, 19A, 19B, 19C, 23A and 23B ("all vaccine-related serotypes") were somewhat reduced (Table 2 in [3]). These data establish that herd immunity from the use of Prevnar in children under two years of age was modest for serotypes 6A and 19A, and provide a basis for the inclusion of serotypes 6A and 19A in the 13vPnC vaccine of this invention.

Conclusion for addition of 6A and 19A

The post-marketing surveillance data and the case-control study results noted in FIG. 1 and Table 2 with the 7vPnC vaccine suggest that, consistent with the other information on immune responses and performance in the animals models described above, there may be some cross-protection against 6A disease, but to a lesser extent than to 6B disease. Furthermore, it appears the protection against 19A is limited. Therefore, a 13vPnC vaccine containing serotypes 6A and 19A provides coverage that is not dependent on the limitations of serogroup cross-protection by serotypes 6B and 19F.

Petitioner Merck Ex-1004, 0562

Accordingly, the present invention provides a multivalent immunogenic composition comprising 13 distinct polysaccharide-protein conjugates, wherein each of the conjugates contains a different capsular polysaccharide conjugated to a carrier protein, and wherein the capsular polysaccharides are prepared from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F of *Streptococcus pneumoniae*, together with a physiologically acceptable vehicle. One such carrier protein is the diphtheria toxoid designated CRM_{197}. The immunogenic composition may further comprise an adjuvant, such as an aluminum-based adjuvant, such as aluminum phosphate, aluminum sulfate and aluminum hydroxide.

Capsular polysaccharides are prepared by standard techniques known to those skilled in the art. In the present invention, capsular polysaccharides are prepared from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F of *Streptococcus pneumoniae*. These pneumococcal conjugates are prepared by separate processes and formulated into a single dosage formulation. For example, in one embodiment, each pneumococcal polysaccharide serotype is grown in a soy-based medium. The individual polysaccharides are then purified through centrifugation, precipitation, ultra-filtration, and column chromatography. The purified polysaccharides are chemically activated to make the saccharides capable of reacting with the carrier protein.

Once activated, each capsular polysaccharide is separately conjugated to a carrier protein to form a glycoconjugate. In one embodiment, each capsular polysaccharide is conjugated to the same carrier protein. In this embodiment, the conjugation is effected by reductive amination.

The chemical activation of the polysaccharides and subsequent conjugation to the carrier protein are achieved by conventional means. See, for example, U.S. Pat. Nos. 4,673,574 and 4,902,506 [34,35].

Carrier proteins are preferably proteins that are non-toxic and non-reactogenic and obtainable in sufficient amount and purity. Carrier proteins should be amenable to standard conjugation procedures. In a particular embodiment of the present invention, CRM_{197} is used as the carrier protein.

CRM_{197} (Wyeth, Sanford, NC) is a non-toxic variant (i.e., toxoid) of diphtheria toxin isolated from cultures of *Corynebacterium diphtheria* strain C7 (β197) grown in casamino acids and yeast extract-based medium. CRM_{197} is purified through ultra-filtration, ammonium sulfate precipitation, and ion-exchange chromatography.

Petitioner Merck Ex-1004, 0563

Alternatively, CRM_{197} is prepared recombinantly in accordance with U.S. Patent No. 5,614,382, which is hereby incorporated by reference. Other diphtheria toxoids are also suitable for use as carrier proteins.

Other suitable carrier proteins include inactivated bacterial toxins such as tetanus toxoid, pertussis toxoid, cholera toxoid (e.g., as described in International Patent Application WO2004/083251 [38]), *E. coli* LT, *E. coli* ST, and exotoxin A from *Pseudomonas aeruginosa*. Bacterial outer membrane proteins such as outer membrane complex c (OMPC), porins, transferrin binding proteins, pneumolysin, pneumococcal surface protein A (PspA), pneumococcal adhesin protein (PsaA), C5a peptidase from Group A or Group B streptococcus, or *Haemophilus influenzae* protein D, can also be used. Other proteins, such as ovalbumin, keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA) or purified protein derivative of tuberculin (PPD) can also be used as carrier proteins.

After conjugation of the capsular polysaccharide to the carrier protein, the polysaccharide-protein conjugates are purified (enriched with respect to the amount of polysaccharide-protein conjugate) by a variety of techniques. These techniques include concentration/diafiltration operations, precipitation/elution, column chromatography, and depth filtration. See examples below.

After the individual glycoconjugates are purified, they are compounded to formulate the immunogenic composition of the present invention, which can be used as a vaccine. Formulation of the immunogenic composition of the present invention can be accomplished using art-recognized methods. For instance, the 13 individual pneumococcal conjugates can be formulated with a physiologically acceptable vehicle to prepare the composition. Examples of such vehicles include, but are not limited to, water, buffered saline, polyols (e.g., glycerol, propylene glycol, liquid polyethylene glycol) and dextrose solutions.

In certain embodiments, the immunogenic composition will comprise one or more adjuvants. As defined herein, an "adjuvant" is a substance that serves to enhance the immunogenicity of an immunogenic composition of this invention. Thus, adjuvants are often given to boost the immune response and are well known to the skilled artisan. Suitable adjuvants to enhance effectiveness of the composition include, but are not limited to:

(1) aluminum salts (alum), such as aluminum hydroxide, aluminum phosphate, aluminum sulfate, etc.;

Petitioner Merck Ex-1004, 0564

(2) oil-in-water emulsion formulations (with or without other specific immunostimulating agents such as muramyl peptides (defined below) or bacterial cell wall components), such as, for example,

(a) MF59 (PCT Publ. No. WO 90/14837), containing 5% Squalene, 0.5% Tween 80, and 0.5% Span 85 (optionally containing various amounts of MTP-PE (see below, although not required)) formulated into submicron particles using a microfluidizer such as Model 110Y microfluidizer (Microfluidics, Newton, MA),

(b) SAF, containing 10% Squalene, 0.4% Tween 80, 5% pluronic-blocked polymer L121, and thr-MDP (see below) either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion, and

(c) Ribi™ adjuvant system (RAS), (Corixa, Hamilton, MT) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of 3-O-deaylated monophosphorylipid A (MPL™) described in U.S. Patent No. 4,912,094 (Corixa), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (Detox™);

(3) saponin adjuvants, such as Quil A or STIMULON™ QS-21 (Antigenics, Framingham, MA) (U.S. Patent No. 5,057,540) may be used or particles generated therefrom such as ISCOMs (immunostimulating complexes);

(4) bacterial lipopolysaccharides, synthetic lipid A analogs such as aminoalkyl glucosamine phosphate compounds (AGP), or derivatives or analogs thereof, which are available from Corixa, and which are described in U.S. Patent No. 6,113,918; one such AGP is 2-[(R)-3-Tetradecanoyloxytetradecanoylamino]ethyl 2-Deoxy-4-O-phosphono-3-O-[(R)-3-tetradecanoyloxytetradecanoyl]-2-[(R)-3-tetradecanoyloxytetradecanoylamino]-b-D-glucopyranoside, which is also know as 529 (formerly known as RC529), which is formulated as an aqueous form or as a stable emulsion, synthetic polynucleotides such as oligonucleotides containing CpG motif(s) (U.S. Patent No. 6,207,646);

(5) cytokines, such as interleukins (e.g., IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, IL-15, IL-18, etc.), interferons (e.g., gamma interferon), granulocyte macrophage colony stimulating factor (GM-CSF), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF), costimulatory molecules B7-1 and B7-2, etc.;

(6) detoxified mutants of a bacterial ADP-ribosylating toxin such as a cholera toxin (CT) either in a wild-type or mutant form, for example, where the glutamic acid at amino acid position 29 is replaced by another amino acid, preferably a histidine, in accordance with published international patent application number WO 00/18434

Petitioner Merck Ex-1004, 0565

(see also WO 02/098368 and WO 02/098369), a pertussis toxin (PT), or an *E. coli* heat-labile toxin (LT), particularly LT-K63, LT-R72, CT-S109, PT-K9/G129 (see, e.g., WO 93/13302 and WO 92/19265); and

(7) other substances that act as immunostimulating agents to enhance the effectiveness of the composition.

Muramyl peptides include, but are not limited to, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanine-2-(1'-2' dipalmitoyl-*sn*-glycero-3-hydroxyphosphoryloxy)-ethylamine (MTP-PE), etc.

The vaccine formulations of the present invention can be used to protect or treat a human susceptible to pneumococcal infection, by means of administering the vaccine via a systemic or mucosal route. These administrations can include injection via the intramuscular, intraperitoneal, intradermal or subcutaneous routes; or via mucosal administration to the oral/alimentary, respiratory or genitourinary tracts. In one embodiment, intranasal administration is used for the treatment of pneumonia or otitis media (as nasopharyngeal carriage of pneumococci can be more effectively prevented, thus attenuating infection at its earliest stage).

The amount of conjugate in each vaccine dose is selected as an amount that induces an immunoprotective response without significant, adverse effects. Such amount can vary depending upon the pneumococcal serotype. Generally, each dose will comprise 0.1 to 100 µg of polysaccharide, particularly 0.1 to 10 µg, and more particularly 1 to 5 µg.

Optimal amounts of components for a particular vaccine can be ascertained by standard studies involving observation of appropriate immune responses in subjects. Following an initial vaccination, subjects can receive one or several booster immunizations adequately spaced.

In a particular embodiment of the present invention, the 13vPnC vaccine is a sterile liquid formulation of pneumococcal capsular polysaccharides of serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F individually conjugated to CRM_{197}. Each 0.5 mL dose is formulated to contain: 2 µg of each saccharide, except for 6B at 4 µg; approximately 29 µg CRM_{197} carrier protein; 0.125 mg of elemental aluminum (0.5 mg aluminum phosphate) adjuvant; and sodium chloride and sodium succinate buffer as excipients. The liquid is filled into single dose syringes without a preservative. After shaking, the vaccine is a homogeneous, white suspension ready for intramuscular administration.

Petitioner Merck Ex-1004, 0566

The choice of dose level for the 13vPnC vaccine is similar to the marketed 7vPnC vaccine (Prevnar). The 2 µg saccharide dose level was selected for all serotypes, except for 6B, which is at 4 µg per dose. The 7vPnC vaccine has shown desirable safety, immunogenicity, and efficacy against IPD in the 2 µg saccharide dose level for serotypes 4, 9V, 14, 18C, 19F and 23F, and at the 4 µg dose for 6B.

The immunization schedule can follow that designated for the 7vPnC vaccine. For example, the routine schedule for infants and toddlers against invasive disease caused by *S. pneumoniae* due to the serotypes included in the 13vPnC vaccine is 2, 4, 6 and 12-15 months of age. The compositions of this invention are also suitable for use with older children, adolescents and adults.

The compositions of this invention may further include one or more additional antigens for use against otitis media caused by infection with other bacteria. Such bacteria include nontypable *Haemophilus influenza*, *Moraxella catarrhalis* (formerly known as *Branhamella catarrhalis*) and *Alloiococcus otitidis*.

Examples of nontypable *Haemophilus influenzae* antigens suitable for inclusion include the P4 protein, also known as protein "e" (U.S. Patent No. 5,601,831; International Patent Application WO03/078453), the P6 protein, also known as the PAL or the PBOMP-1 protein (U.S. Patent No. 5,110,908; International Patent Application WO0100790), the P5 protein (U.S. Reissue Patent No. 37,741), the *Haemophilus* adhesion and penetration protein (U.S. Patent Nos. 6,245,337 and 6,676,948), the LKP tip adhesin protein (U.S. Patent No. 5,643,725) and the NucA protein (U.S. Patent No. 6,221,365).

Examples of *Moraxella catarrhalis* antigens suitable for inclusion include the UspA2 protein (U.S. Patent Nos. 5,552,146, 6,310,190), the CD protein (U.S. Patent No. 5,725,862), the E protein (U.S. Patent No. 5,948,412) and the 74 kilodalton outer membrane protein (U.S. Patent No. 6,899,885).

Examples of *Alloiococcus otitidis* antigens suitable for inclusion include those identified in International Patent Application WO03/048304.

The compositions of this invention may also include one or more proteins from *Streptococcus pneumoniae*. Examples of *Streptococcus pneumoniae* proteins suitable for inclusion include those identified in International Patent Application WO02/083855, as well as that described in International Patent Application WO02/053761.

Petitioner Merck Ex-1004, 0567

The compositions of this invention may further include one or more proteins from *Neisseria meningitidis* type B. Examples of *Neisseria meningitidis* type B proteins suitable for inclusion include those identified in International Patent Applications WO03/063766, WO2004/094596, WO01/85772, WO02/16612 and WO01/87939.

Activation of S. pneumoniae Serotype 3 Polysaccharide for Conjugation

Partial oxidation of carbohydrates in polysaccharides has been effectively utilized to generate aldehyde groups which are then coupled to the lysine residues of carrier proteins to generate immunogenic conjugates (i.e., compositions, such as vaccines, useful for stimulating or eliciting a specific immune response in a vertebrate). For many applications, the general purpose oxidizing agent sodium periodate provides effective oxidation of carbohydrates in polysaccharides prior to conjugation with carrier proteins. However, direct oxidation of Pn 3 polysaccharide with sodium periodate can result in degraded polysaccharide with few remaining aldehydes present. An alternate oxidation procedure incorporates periodic acid in the presence of bivalent cations (e.g., Mg^{2+} or Ca^{2+}) and provides effective oxidation of Pn 3 polysaccharide (Table 3).

Table 3: Oxidation Data of Pn 3 using Periodic Acid/$MgCl_2$

Sample ID	Periodic Acid amount (meq)**	Oxidation (hours)	Temp (°C)	DO#***	Kd # (CL4B)
PP3* Native	0	--	4	0.0	0.048
PP3 Oxidized	0.2	16	25	5.8	0.24
PP3 Oxidized	0.2	72	4	9.0	0.1
PP3 Oxidized	1	16	4	13.2	0.24
PP3 Oxidized	1	16	25	4.1	0.24
PP3 Oxidized	1	72	4	7.9	0.29

*PP3: native Pneumo Polysaccharide type 3
**Meq: mole equivalent
***DO: degree of oxidation

In order to reduce viscosity and associated filtration issues, the Pn 3 polysaccharide is first subjected to hydrolysis/depolymerization using a mild acid, such as acetic acid, citric acid or carbonic acid. Partial oxidation to produce "activated" Pn 3 polysaccharide is carried out following hydrolysis/depolymerization using controlled amounts of an oxidizing agent. Any selective oxidizing agent which oxidizes a terminal hydroxyl group to an aldehyde, including specific sugar oxidases, can be used (e.g., sodium or potassium periodate, or periodic acid). As discussed

Petitioner Merck Ex-1004, 0568

above, a preferred oxidizing agent is periodic acid in the presence of bivalent cations. The activated Pn 3 polysaccharide is then optionally purified (e.g., by diafiltration versus buffered saline).

Activated Pn 3 polysaccharide can be compounded with a carrier protein. Carrier proteins are chosen to increase the immunogenicity of the bound Pn 3 polysaccharide and/or to elicit antibodies against the carrier protein which are diagnostically, analytically and/or therapeutically beneficial. Covalent linking of an antigenic molecule (e.g., a polysaccharide) to a carrier confers enhanced immunogenicity and T-cell dependence (Pozsgay et al. (1999) *PNAS*, 96:5194-97; Lee et al. (1976) *J. Immunol.*, 116:1711-18; Dintzis et al. (1976) *PNAS*, 73:3671-75). As described herein, useful carrier proteins include inactivated bacterial toxins such as tetanus toxoid, pertussis toxoid, cholera toxoid (e.g., as described in International Patent Application WO2004/083251), *E. coli* LT, *E. coli* ST, and exotoxin A from *Pseudomonas aeruginosa*. Bacterial outer membrane proteins such as outer membrane complex c, porins, transferrin binding proteins, pneumolysin, pneumococcal surface protein A, pneumococcal adhesin protein, C5a peptidase from Group A or Group B streptococcus, or *Haemophilus influenzae* protein D, can also be used. Other proteins, such as ovalbumin, keyhole limpet hemocyanin, bovine serum albumin, or purified protein derivative of tuberculin can also be used as carrier proteins. A preferred carrier protein is the mutated diphtheria toxin CRM_{197}. Following compounding with the carrier protein, the activated Pn 3 polysaccharide/carrier protein mixture is optionally frozen (e.g., shell frozen) and co-lyophilized.

Conjugation is carried out by reacting the activated Pn 3 polysaccharide/carrier protein mixture with a reducing agent, such as sodium cyanoborohydride. Unreacted aldehydes are then capped via sodium borohydride reduction. The conjugate is purified (e.g., by diafiltration versus phosphate buffer followed by buffered saline), giving a final batch concentrate of the glycoconjugate in buffered saline.

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples. These examples are described solely for the purpose of illustration and are not intended to limit the scope of the invention.

Petitioner Merck Ex-1004, 0569

EXAMPLES

Example 1

Preparation of *S. Pneumoniae* Capsular Polysaccharide Serotype 1

Preparation of Master and Working Cell Banks

S. pneumoniae serotype 1 was obtained from the American Type Culture Collection, ATCC, strain 6301. Several generations of seed stocks were created in order to expand the strain and remove components of animal origin (generations F1, F2, and F3). Two additional generations of seed stocks were produced. The first additional generation was made from an F3 vial, and the subsequent generation was made from a vial of the first additional generation. Seed vials were stored frozen (<-70°C) with synthetic glycerol as a cryopreservative. In addition to frozen vials, lyophilized vials were prepared for the F4 generation. For cell bank preparation, all cultures were grown in a soy-based medium. Prior to freezing, cells were concentrated by centrifugation, spent medium was removed, and cell pellets were re-suspended in fresh medium containing a cryopreservative, such as synthetic glycerol.

Fermentation and Harvesting

Cultures from the working cell bank were used to inoculate seed bottles containing a soy-based medium. The bottles were incubated at 36°C ± 2° C without agitation until growth requirements were met. A seed bottle was used to inoculate a seed fermentor containing soy-based medium. A pH of about 7.0 was maintained with sterile sodium carbonate solution. After the target optical density was reached, the seed fermentor was used to inoculate the production fermentor containing soy-based medium. The pH was maintained with sterile sodium carbonate solution. The fermentation was terminated after cessation of growth or when the working volume of the fermentor was reached. An appropriate amount of sterile 12% deoxycholate sodium was added to the culture to lyse the bacterial cells and release cell-associated polysaccharide. After lysing, the fermentor contents were cooled. The pH of the lysed culture broth was adjusted to approximately pH 6.6 with acetic acid. The lysate was clarified by continuous flow centrifugation followed by depth filtration and 0.45 µm microfiltration.

Petitioner Merck Ex-1004, 0570

In an alternate process, the fermentation pH of about 7.0 was maintained with 3N NaOH. After the target optical density was reached, the seed fermentor was used to inoculate the production fermentor containing soy-based medium. The pH was maintained with 3N NaOH. The fermentation was terminated after cessation of growth or when the working volume of the fermentor was reached. An appropriate amount of sterile 12% deoxycholate sodium was added to the culture to obtain a 0.12% concentration in the broth, to lyse the bacterial cells and release cell-associated polysaccharide. After lysing, the fermentor contents were held, with agitation, for a time interval between 8 and 24 hours at a temperature between 7°C and 13°C, to assure that complete cellular lysis and polysaccharide release had occurred. Agitation during this hold period prevented lysate sediment from settling on the fermentor walls and pH probe, thereby allowing the pH probe integrity to be maintained. Next, the pH of the lysed culture broth was adjusted to approximately pH 5.0 with 50% acetic acid. After a hold time without agitation, for a time interval between 12 and 24 hours at a temperature between 15°C and 25°C, a significant portion of the previously soluble proteins dropped out of solution as a solid precipitate with little loss or degradation of the polysaccharide, which remained in solution. The solution with the precipitate was then clarified by continuous flow centrifugation followed by depth filtration and 0.45 µm microfiltration.

Purification

The purification of the pneumococcal polysaccharide consisted of several concentration/diafiltration operations, precipitation/elution, column chromatography, and depth filtration steps. All procedures were performed at room temperature unless otherwise specified.

Clarified broth from the fermentor cultures of *S. pneumoniae* serotype 1 were concentrated and diafiltered using a 100 kDa MWCO (kilodalton molecular weight cutoff) filter. Diafiltration was accomplished using sodium phosphate buffer at neutral pH. Diafiltration removed the low molecular weight medium components from the higher molecular weight biopolymers such as nucleic acid, protein and polysaccharide.

The polysaccharide was precipitated from the concentrated and diafiltered solution by adding hexadecyltrimethyl ammonium bromide (HB) from a stock solution to give a final concentration of 1% HB (w/v). The polysaccharide/HB precipitate was captured on a depth filter and the filtrate was discarded. The polysaccharide

Petitioner Merck Ex-1004, 0571

precipitate was resolubilized and eluted by recirculating a sodium chloride solution through the precipitate-containing depth filter. The filters were then rinsed with additional sodium chloride solution.

Sodium iodide (NaI) was added to the polysaccharide solution from a stock NaI solution to achieve a final concentration of 0.5% to precipitate HB. The precipitate was removed by depth filtration. The filtrate contains the target polysaccharide. The precipitation vessel and the filter were rinsed with a NaCl/NaI solution and the rinse was combined with the partially purified polysaccharide solution. The filter was discarded. The polysaccharide was then filtered through a 0.2 µm filter.

The polysaccharide solution was concentrated on a 30 kDa MWCO ultrafilter and diafiltered with a sodium chloride solution.

The partially purified polysaccharide solution was further purified by filtration through a depth filter impregnated with activated carbon. After filtration, the carbon filter was rinsed with a sodium chloride solution. The rinse is combined with the polysaccharide solution, which is then filtered through a 0.2 µm filter.

The polysaccharide solution was concentrated on a 30 kDa MWCO ultrafilter and adjusted with a 1M sodium phosphate buffer to achieve a final concentration of 0.025 M sodium phosphate. The pH was checked and adjusted to 7.0 ± 0.2.

The ceramic hydroxyapatite (HA) column was equilibrated with sodium phosphate buffer containing sodium chloride to obtain the appropriate conductivity (<15 µS). The polysaccharide solution was then loaded onto the column. Under these conditions, impurities bound to the resin and the polysaccharide was recovered in the flow-through from the column. The polysaccharide solution was filtered through 0.2µm inline filters located before and after the column.

The polysaccharide solution was concentrated using a 30 kDa MWCO filter. The concentrate was then diafiltered with Water for Injection (WFI).

The diafiltered polysaccharide solution was filtered through a 0.2 µm membrane filter into polypropylene bottles. Samples were removed for release testing and the purified polysaccharide was stored frozen at –25° ± 5°C.

Characterization

The 1H-NMR data was consistent with the chemical structure by the assignment of signals assigned to the protons of the polysaccharide molecule. The 1H-NMR spectrum showed a series of well-resolved signals (protons from the methyl group) for the quantitation of the O-acetyl functional group in the polysaccharide.

Petitioner Merck Ex-1004, 0572

The identity of the monovalent polysaccharide was confirmed by countercurrent immunoelectrophoresis using specific antisera.

High performance gel filtration chromatography coupled with refractive index and multiangle laser light scattering (MALLS) detectors was used in conjunction with the sample concentration to calculate the molecular weight.

Size exclusion chromatography media (CL-4B) was used to profile the relative molecular size distribution of the polysaccharide.

Example 2

Preparation of Serotype 1 Pneumococcal Saccharide – CRM_{197} Conjugate

Activation and Conjugation

Containers of purified polysaccharide were thawed and combined in a reaction vessel. To the vessel, 0.2 M sodium carbonate, pH 9.0 was added for partial deacetylation (hydrolysis) for 3 hours at 50°C. The reaction was cooled to 20°C and neutralization was performed by 0.2 M acetic acid. Oxidation in the presence of sodium periodate was performed by incubation at 2-8°C, and the mixture was stirred for 15-21 hours.

The activation reaction mixture was concentrated and diafiltered 10x with 0.9% NaCl using a 30K MWCO membrane. The retentate was 0.2 µm filtered. The activated saccharide was filled into 100 mL glass lyophilization bottles and shell-frozen at -75°C and lyophilized.

"Shell-freezing" is a method for preparing samples for lyophilization (freeze-drying). Flasks are automatically rotated by motor driven rollers in a refrigerated bath containing alcohol or any other appropriate fluid. A thin coating of product is evenly frozen around the inside "shell" of a flask, permitting a greater volume of material to be safely processed during each freeze-drying run. These automatic, refrigerated units provide a simple and efficient means of pre-freezing many flasks at a time, producing the desired coatings inside, and providing sufficient surface area for efficient freeze-drying.

Bottles of lyophilized material were brought to room temperature and resuspended in CRM_{197} solution at a saccharide/protein ratio of 2:1. To the saccharide/protein mixture 1M sodium phosphate buffer was added to a final 0.2M ionic strength and a pH of 7.5, then sodium cyanoborohydride was added. The reaction was incubated at 23°C for 18 hours, followed by a second incubation at 37°C for 72 hours. Following the cyanoborohydride incubations, the reaction mixture

Petitioner Merck Ex-1004, 0573

was diluted with cold saline followed by the addition of 1M sodium carbonate to adjust the reaction mixture to pH 9.0. Unreacted aldehydes were quenched by addition of sodium borohydride by incubation at 23°C for 3-6 hours.

The reaction mixture was diluted 2-fold with saline and transferred through a 0.45 – 5 µm prefilter into a retentate vessel. The reaction mixture is diafiltered 30x with 0.15 M phosphate buffer, pH 6, and 20x with saline. The retentate was filtered through a 0.2 µm filter.

The conjugate solution was diluted to a target of 0.5 mg/mL in 0.9% saline, and then sterile filtered into final bulk concentrate (FBC) containers in a Class 100 hood. The conjugate was stored at 2 – 8°C.

Characterization

Size exclusion chromatography media (CL-4B) was used to profile the relative molecular size distribution of the conjugate.

The identity of the conjugate was confirmed by the slot-blot assay using specific antisera.

The saccharide and protein concentrations were determined by the uronic acid and Lowry assays, respectively. The ratio of saccharide to protein in the covalently bonded conjugate complex was obtained by the calculation:

$$\text{Ratio} = \frac{\text{µg/mL saccharide}}{\text{µg/mL protein}}$$

O-acetyl content was measured by the Hestrin method (Hestrin et. al., J. Biol. Chem. 1949, 180, p. 249). The ratio of O-acetyl concentration to total saccharide concentration gave µmoles of O-acetyl per mg of saccharide.

Example 3

Preparation of *S. Pneumoniae* Capsular Polysaccharide Serotype 3

Preparation of Master and Working Cell Banks

S. pneumoniae serotype 3 was obtained from Dr. Robert Austrian, University of Pennsylvania, Philadelphia, Pennsylvania. For preparation of the cell bank system, see Example 1.

Petitioner Merck Ex-1004, 0574

Fermentation and Harvesting

Cultures from the working cell bank were used to inoculate seed bottles containing soy-based medium. The bottles were incubated at 36°C ± 2° C without agitation until growth requirements were met. A seed bottle was used to inoculate a seed fermentor containing soy-based medium. A pH of about 7.0 was maintained with sterile sodium carbonate solution. After the target optical density was reached, the seed fermentor was used to inoculate an intermediate seed fermentor. After the target optical density was reached, the intermediate seed fermentor was used to inoculate the production fermentor. The pH was maintained with sterile sodium carbonate solution. The fermentation was terminated after the working volume of the fermentor was reached. An appropriate amount of sterile 12% sodium deoxycholate was added to the culture to lyse the bacterial cells and release cell-associated polysaccharide. After lysing, the fermentor contents were cooled. The pH of the lysed culture broth was adjusted to approximately pH 6.6 with acetic acid. The lysate was clarified by continuous flow centrifugation followed by depth filtration and 0.45 µm microfiltration.

Purification

The purification of the pneumococcal polysaccharide consisted of several concentration/diafiltration operations, precipitation/elution, column chromatography, and depth filtration steps. All procedures were performed at room temperature unless otherwise specified.

Clarified broth from the fermentor cultures of *S. pneumoniae* serotype 3 were concentrated and diafiltered using a 100 kDa MWCO filter. Diafiltration was accomplished using sodium phosphate buffer at neutral pH. Diafiltration removed the low molecular weight medium components from the higher molecular weight biopolymers such as nucleic acid, protein and polysaccharide.

Prior to the addition of hexadecyltrimethyl ammonium bromide (HB), a calculated volume of a NaCl stock solution was added to the concentrated and diafiltered polysaccharide solution to give a final concentration of 0.25 M NaCl. The polysaccharide was then precipitated by adding HB from a stock solution to give a final concentration of 1% HB (w/v). The polysaccharide/HB precipitate was captured on a depth filter and the filtrate was discarded. The polysaccharide precipitate was resolubilized and eluted by recirculating a sodium chloride solution through the precipitate-containing depth filter. The filters were then rinsed with additional sodium chloride solution.

Petitioner Merck Ex-1004, 0575

Sodium iodide (NaI) was added to the polysaccharide solution from a stock NaI solution to achieve a final concentration of 0.5% to precipitate HB. The precipitate was removed by depth filtration. The filtrate contained the target polysaccharide. The precipitation vessel and the filter were rinsed with a NaCl/NaI solution and the rinse was combined with the partially purified polysaccharide solution. The filter was discarded. The polysaccharide was then filtered through a 0.2 µm filter.

The polysaccharide solution was concentrated on a 30 kDa MWCO ultrafilter and diafiltered with a sodium chloride solution.

The partially purified polysaccharide solution was further purified by filtration through a depth filter impregnated with activated carbon. After filtration, the carbon filter was rinsed with a sodium chloride solution. The rinse was combined with the polysaccharide solution, which was then filtered through a 0.2 µm filter.

The polysaccharide solution was concentrated on a 30 kDa MWCO ultrafilter and adjusted with a 1M sodium phosphate buffer to achieve a final concentration of 0.025M sodium phosphate. The pH was checked and adjusted to 7.0 ± 0.2.

The ceramic hydroxyapatite (HA) column was equilibrated with sodium phosphate buffer containing sodium chloride to obtain the appropriate conductivity (15 µS). The polysaccharide solution was then loaded onto the column. Under these conditions, impurities bound to the resin and the polysaccharide was recovered in the flow-through from the column. The polysaccharide was flushed through the column with buffer and was filtered through a 0.2µm filter.

The polysaccharide solution was concentrated using a 30 kDa MWCO filter. The concentrate was then diafiltered with WFI.

The diafiltered polysaccharide solution was filtered through a 0.2 µm membrane filter into stainless steel containers. Samples were removed for release testing and the purified polysaccharide was stored frozen at –25° ± 5°C.

Characterization

The 1H-NMR data was consistent with the chemical structure by the assignment of signals assigned to the protons of the polysaccharide molecule.

The identity of the monovalent polysaccharide was confirmed by countercurrent immunoelectrophoresis using specific antisera.

High performance gel filtration chromatography, coupled with refractive index and multiangle laser light scattering (MALLS) detectors, was used in conjunction with the sample concentration to calculate the molecular weight.

Petitioner Merck Ex-1004, 0576

Size exclusion chromatography media (CL-4B) was used to profile the relative molecular size distribution of the polysaccharide.

Example 4

Preparation of Serotype 3 Pneumococcal Saccharide – CRM_{197} Conjugate

Activation and Conjugation

Containers of purified serotype 3 saccharide were thawed and combined in a reaction vessel. To the vessel, WFI and 2M acetic acid were added to a final concentration of 0.2M and 2mg/mL saccharide. The temperature of the solution was raised to 85°C for one hour to hydrolyze the polysaccharide. The reaction was cooled to ≤25°C and 1M magnesium chloride was added to a final concentration of 0.1M. Oxidation in the presence of sodium periodate was performed by incubation for 16-24 hours at 23°C.

The activation reaction mixture was concentrated and diafiltered 10x with WFI using a 100K MWCO membrane. The retentate was filtered through a 0.2-µm filter.

For compounding, 0.2M sodium phosphate, pH 7.0, was added to the activated saccharide to a final concentration of 10mM and a pH of 6.0-6.5. CRM_{197} carrier protein was mixed with the saccharide solution to a ratio of 2g of saccharide per 1g of CRM_{197}. The combined saccharide/protein solution was filled into 100 mL glass lyophilization bottles with a 50mL target fill, shell-frozen at -75°C, and lyophilized.

Bottles of co-lyophilized saccharide/protein material were brought to room temperature and resuspended in 0.1M sodium phosphate buffer, pH 7.0, to a final saccharide concentration of 20 mg/mL. The pH was adjusted to 6.5 and then a 0.5 molar equivalent of sodium cyanoborohydride was added. The reaction was incubated at 37°C for 48 hours. Following the cyanoborohydride incubation, the reaction mixture was diluted with cold 5mM succinate/0.9% saline buffer. Unreacted aldehydes were quenched by the addition of sodium borohydride and incubation at 23°C for 3-6 hours. The reaction mixture was transferred through a 0.45-5 µm prefilter into a retentate vessel.

The reaction mixture was diafiltered 30x with 0.1M phosphate buffer (pH 9), 20x with 0.15M phosphate butter (pH 6), and 20x with 5mM succinate/0.9% saline. The retentate was filtered through a 0.2-µm filter.

Petitioner Merck Ex-1004, 0577

The conjugate solution was diluted to a saccharide target of 0.5 mg/mL, and then sterile filtered into FBC containers in a Class 100 hood. The conjugate was stored at 2 – 8°C.

Characterization

Size exclusion chromatography media (CL-4B) was used to profile the relative molecular size distribution of the conjugate.

The identity of the conjugate was confirmed by the slot-blot assay using specific antisera.

The saccharide and protein concentrations were determined by the Anthrone and Lowry assays, respectively. The ratio of saccharide to protein in the covalently bonded conjugate complex was obtained by the calculation:

$$\text{Ratio} = \frac{\mu g/mL \text{ saccharide}}{\mu g/mL \text{ protein}}$$

Example 5

Preparation of *S. Pneumoniae* Capsular Polysaccharide Serotype 5

S. pneumoniae serotype 5 was obtained from Dr. Gerald Schiffman of the State University of New York, Brooklyn, New York. For preparation of the cell bank system, see Example 1. For fermentation, harvesting, purification and characterization of the polysaccharide, see Example 1.

Alternate Fermentation Process

Cultures from the working cell bank were used to inoculate seed bottles containing a soy-based medium and a 10mM sterile $NaHCO_3$ solution. The bottles were incubated at 36°C ± 2° C without agitation until growth requirements were met. A seed bottle was used to inoculate a seed fermentor containing soy-based medium and a 10mM sterile $NaHCO_3$ solution. A pH of about 7.0 was maintained with 3N NaOH. After the target optical density was reached, the seed fermentor was used to inoculate the production fermentor containing soy-based medium with a 10mM $NaHCO_3$ concentration. The pH was maintained with 3N NaOH. The fermentation was terminated after cessation of growth or when the working volume of the fermentor was reached. An appropriate amount of sterile 12% sodium deoxycholate was added to the culture to obtain a 0.12% concentration in the broth. to lyse the

Petitioner Merck Ex-1004, 0578

bacterial cells and release cell-associated polysaccharide. After lysing, the fermentor contents were held, with agitation, for a time interval between 8 and 24 hours at a temperature between 7°C and 13°C to assure that complete cellular lysis and polysaccharide release had occurred. Agitation during this hold period prevented lysate sediment from settling on the fermentor walls and pH probe, thereby allowing the pH probe integrity to be maintained. Next, the pH of the lysed culture broth was adjusted to approximately pH 4.5 with 50% acetic acid. After a hold time without agitation, for a time interval between 12 and 24 hours at a temperature between 15°C and 25°C, a significant portion of the previously soluble proteins dropped out of solution as a solid precipitate with little loss or degradation of the polysaccharide, which remained in solution. The solution with the precipitate was then clarified by continuous flow centrifugation followed by depth filtration and 0.45 µm microfiltration.

Example 6

Preparation of Serotype 5 Pneumococcal Saccharide – CRM_{197} Conjugate

Activation and Conjugation

Containers of serotype 5 saccharide were thawed and combined in a reaction vessel. To the vessel, 0.1M sodium acetate, pH 4.7, was added followed by oxidation in the presence of sodium periodate by incubation for 16-22 hours at 23°C.

The activation reaction mixture was concentrated and diafiltered 10x with WFI using a 100K MWCO membrane. The retentate was filtered through a 0.2 µm filter.

The serotype 5 activated saccharide was combined with CRM_{197} at a ratio of 0.8:1. The combined saccharide/protein solution was filled into 100 mL glass lyophilization bottles (50 mL target fill), shell-frozen at -75°C, and co-lyophilized.

Bottles of co-lyophilized material were brought to room temperature and resuspended in 0.1M sodium phosphate, pH 7.5, and sodium cyanoborohydride was added. The reaction was incubated at 30°C for 72 hours, followed by a second addition of cyanoborohydride and incubated at 30°C for 20-28 hours.

Following the cyanoborohydride incubations, the reaction mixture was diluted 2-fold with saline and transferred through a 0.45-5 µm prefilter into a retentate vessel. The reaction mixture was diafiltered 30x with 0.01M phosphate buffer, pH 8, 20x with 0.15M phosphate buffer, pH 6, and 20x with saline. The retentate was filtered through a 0.2 µm filter.

Petitioner Merck Ex-1004, 0579

The conjugate solution was diluted to a saccharide target of 0.5 mg/mL, and then sterile filtered into FBC containers in a Class 100 hood. The conjugate was stored at 2 – 8°C.

For the characterization of the conjugate, see Example 2.

5

Example 7

Preparation of *S. Pneumoniae* Capsular Polysaccharide Serotype 6A

S. pneumoniae serotype 6A was obtained from Dr. Gerald Schiffman of the State University of New York, Brooklyn, New York. For preparation of the cell bank
10 system, see Example 1. For fermentation, harvesting and purification of the polysaccharide, see Example 1, except that during purification, the 30 kDa MWCO concentration step, prior to the chromatography step, is omitted.

Example 8

15 **Preparation of Serotype 6A Pneumococcal Saccharide – CRM_{197} Conjugate**

Activation and Conjugation

Serotype 6A polysaccharide is a high molecular weight polymer that had to be reduced in size prior to oxidation. Containers of serotype 6A saccharide were
20 thawed and combined in a reaction vessel. To the vessel, 2 M acetic acid was added to a final concentration of 0.1 M for hydrolysis for 1.5 hours at 60°C. The reaction was cooled to 23°C and neutralization was performed by adjusting the reaction mixture with 1 M NaOH to pH 6. Oxidation in the presence of sodium periodate was performed by incubation at 23°C for 14-22 hours.

25 The activation reaction mixture was concentrated and diafiltered 10x with WFI using a 100K MWCO membrane. The retentate was filtered through a 0.2 µm filter.

Serotype 6A was compounded with sucrose and filled into 100 mL glass lyophilization bottles (50mL target fill) and shell-frozen at -75°C and lyophilized.

Bottles of lyophilized material were brought to room temperature and
30 resuspended in dimethylsulfoxide (DMSO) at a saccharide/protein ratio of 1:1. After addition of sodium cyanoborohydride, the reaction mixture was incubated at 23°C for 18 hours. Following the cyanoborohydride incubation, the reaction mixture was diluted with cold saline. Unreacted aldehydes were quenched by addition of sodium borohydride by incubation at 23°C for 3-20 hours.

Petitioner Merck Ex-1004, 0580

The diluted reaction mixture was transferred through a 5 µm prefilter into a retentate vessel. The reaction mixture was diafiltered 10x with 0.9% NaCl and 30x with succinate-buffered NaCl. The retentate was filtered through a 0.2 µm filter.

The conjugate solution was diluted to a saccharide target of 0.5 mg/mL, and then sterile filtered into FBC containers in a Class 100 hood. The conjugate was stored at 2 - 8°C.

For the characterization of the conjugate, see Example 2.

Example 9

Preparation of *S. Pneumoniae* Capsular Polysaccharide Serotype 7F

S. pneumoniae serotype 7F was obtained from Dr. Gerald Schiffman of the State University of New York, Brooklyn, New York. For preparation of the cell bank system, and for fermentation and harvesting of the polysaccharide, see Example 3. For an alternate fermentation and harvesting process, see the alternate process described in Example 1.

Purification

The purification of the pneumococcal polysaccharide consisted of several concentration/diafiltration operations, precipitation/elution, column chromatography, and depth filtration steps. All procedures were performed at room temperature unless otherwise specified.

Clarified broth from fermentor cultures of *S. pneumoniae* serotype 7F were concentrated and diafiltered using a 100 kDa MWCO filter. Diafiltration was accomplished using sodium phosphate buffer at neutral pH. Diafiltration removed the low molecular weight medium components from the higher molecular weight biopolymers such as nucleic acid, protein and polysaccharide.

Serotype 7F does not form a precipitate with HB. Instead, impurities were precipitated from the concentrated and diafiltered solution by adding the HB from a stock solution to a final concentration of 1% HB. The precipitate was captured on a depth filter and the filter was discarded. The polysaccharide was contained in the filtrate.

Sodium iodide (NaI) was added to the polysaccharide solution from a stock NaI solution to achieve a final concentration of 0.5% to precipitate HB. The precipitate was removed by depth filtration. The filtrate contained the target polysaccharide. The precipitation vessel and the filter were rinsed with a NaCl/NaI

Petitioner Merck Ex-1004, 0581

solution and the rinses were combined with the partially purified polysaccharide solution. The filter was discarded. The polysaccharide was then filtered through a 0.2µm filter.

The polysaccharide solution was concentrated on a 30 kDa MWCO ultrafilter and diafiltered with a sodium chloride solution.

The partially purified polysaccharide solution was further purified by filtration through a depth filter impregnated with activated carbon. After filtration, the carbon filter was rinsed with a sodium chloride solution. The rinse was combined with the polysaccharide solution, which was then filtered through a 0.2 µm filter.

The polysaccharide solution was concentrated on a 30 kDa MWCO ultrafilter and adjusted with a 1M sodium phosphate buffer to achieve a final concentration of 0.025M sodium phosphate. The pH was checked and adjusted to 7.0 ± 0.2.

The ceramic hydroxyapatite (HA) column was equilibrated with sodium phosphate buffer containing sodium chloride to obtain the appropriate conductivity (15 µS). The polysaccharide solution was then loaded onto the column. Under these conditions, impurities bound to the resin and the polysaccharide was recovered in the flow-through from the column. The polysaccharide was flushed through the column with buffer and was filtered through a 0.2 µm filter.

The polysaccharide solution was concentrated using a 30 kDa MWCO filter. The concentrate was then diafiltered with WFI.

The diafiltered polysaccharide solution was filtered through a 0.2 µm membrane filter into stainless steel containers. Samples were removed for release testing and the purified polysaccharide was stored at 2° - 8°C.

For characterization of the polysaccharide, see Example 3.

Example 10

Preparation of Serotype 7F Pneumococcal Saccharide – CRM_{197} Conjugate

Activation and Conjugation

Oxidation in the presence of sodium periodate was performed by incubation for 16-24 hrs at 23°C.

The activation reaction mixture was concentrated and diafiltered 10x with 10mM NaOAc, pH 4.5, using a 100K MWCO membrane. The retentate was filtered through a 0.2 µm filter.

Serotype 7F was filled into 100 mL glass lyophilization bottles (50 mL target fill) and shell-frozen at -75°C and lyophilized.

Petitioner Merck Ex-1004, 0582

Bottles of lyophilized serotype 7F and CRM_{197} were brought to room temperature and resuspended in DMSO at a saccharide/protein ratio of 1.5:1. After the addition of sodium cyanoborohydride, the reaction was incubated at 23°C for 8-10 hours. Unreacted aldehydes were quenched by the addition of sodium borohydride by incubation at 23°C for 16 hours.

The reaction mixture was diluted 10-fold with cold saline and transferred through a 5 µm prefilter into a retentate vessel. The reaction mixture was diafiltered 10x with 0.9% saline and 30x with succinate-buffered saline. The retentate was filtered through a 0.2 µm filter.

The conjugate solution was diluted to a saccharide target of 0.5 mg/mL 0.9% saline, and then sterile filtered into FBC containers in a Class 100 hood. The conjugate was stored at 2 - 8°C.

For characterization of the conjugate, see Example 4.

Example 11

Preparation of *S. Pneumoniae* Capsular Polysaccharide Serotype 19A

S. pneumoniae serotype 19A was obtained from Dr. Gerald Schiffman of the State University of New York, Brooklyn, New York. For preparation of the cell bank system, see Example 1. For fermentation, harvesting and purification of the polysaccharide, see Example 7. For characterization, see Example 3.

Example 12

Preparation of Serotype 19A Pneumococcal Saccharide – CRM_{197} Conjugate

Activation and Conjugation

Containers of serotype 19A saccharide were thawed and combined in a reaction vessel. Sodium acetate was added to 10 mM (pH 5.0) and oxidation was carried out in the presence of sodium periodate by incubation for 16-24 hrs at 23°C.

The activation reaction mixture was concentrated and diafiltered 10x with 10mM acetate, pH 5.0, using a 100K MWCO membrane. The retentate was filtered through a 0.2 µm filter.

The activated saccharide was compounded with sucrose followed by the addition of CRM_{197}. The serotype 19A activated saccharide and CRM_{197} mixture (0.8:1 ratio) was filled into 100 mL glass lyophilization bottles (50 mL target fill) and shell-frozen at -75°C and lyophilized.

Petitioner Merck Ex-1004, 0583

Bottles of lyophilized material were brought to room temperature and resuspended in DMSO. To the saccharide/protein mixture, sodium cyanoborohydride (100 mg/ml) was added. The reaction was incubated at 23°C for 15 hours. Following the cyanoborohydride incubation, unreacted aldehydes were quenched by the addition of sodium borohydride by incubation at 23°C for 3-20 hours.

The reaction mixture was diluted 10-fold with cold saline and transferred through a 5 µm prefilter into a retentate vessel. The reaction mixture was diafiltered 10x with 0.9% NaCl, 0.45-µm filtered, and 30x with diafiltration using 5mM succinate/ 0.9% NaCl buffer, pH 6. The retentate was filtered through a 0.2 µm filter.

The conjugate solution was diluted to a target of 0.5 mg/mL using 5mM succinate/0.9% saline, and then sterile filtered into FBC containers in a Class 100 hood. The conjugate was stored at 2 - 8°C.

For characterization of the conjugate, see Example 4.

Example 13

Preparation of *S. Pneumoniae* Capsular Polysaccharide Serotypes 4, 6B, 9V, 14, 18C, 19F and 23F

Preparation of the *S. pneumoniae* Seed Culture

S. pneumoniae serotypes 4, 6B, 9V, 18C, 19F and 23F were obtained from Dr. Gerald Schiffman, State University of New York, Brooklyn, New York. *S. pneumoniae* serotype 14 was obtained from the ATCC, strain 6314.

Separately, one vial of each of the desired serotypes of *Streptococcus pneumoniae* was used to start a fermentation batch. Two bottles containing a soy-based medium and phenol red were adjusted to a pH range of 7.4 ± 0.2 using sodium carbonate, and the required volume of 50% dextrose/1% magnesium sulfate solution was then added to the bottles. The two bottles were inoculated with different amounts of seed. The bottles were incubated at 36° ± 2°C until the medium turned yellow. Following incubation, samples were removed from each bottle and tested for optical density (OD) (0.3 to 0.9) and pH (4.6 to 5.5). One of the two bottles was selected for inoculation of the seed fermentor.

Soy-based medium was transferred to the seed fermentor and sterilized. Then a volume of 50% dextrose/1% magnesium sulfate solution was added to the fermentor. The pH and agitation of the seed fermentor were monitored and controlled (pH 6.7 to 7.4). The temperature was maintained at 36° ± 2°C. The seed

Petitioner Merck Ex-1004, 0584

inoculum (bottle) was aseptically connected to the seed fermentor and the inoculum was transferred. The fermentor was maintained in pH control and samples were periodically removed and tested for OD and pH. When the desired OD of 0.5 at 600 nm was reached, the intermediate fermentor was inoculated with the fermentation broth from the seed fermentor.

Soy-based medium was transferred to the intermediate fermentor and sterilized. Then a volume of 50% dextrose/1% magnesium sulfate solution was added to the fermentor. The pH and agitation of the intermediate fermentor were monitored and controlled (pH 6.7 to 7.4). The temperature was maintained at 36° ± 2°C. The contents of the seed fermentor were transferred to the intermediate fermentor. The fermentor was maintained in pH control and samples were periodically removed and tested for OD and pH. When the desired OD of 0.5 at 600 nm was reached, the production fermentor was inoculated with the fermentation broth from the intermediate fermentor.

Soy-based medium was transferred to the production fermentor and sterilized. Then a volume of 50% dextrose/1% magnesium sulfate solution was added to the fermentor. The pH and agitation of the production fermentor were monitored and controlled (pH 6.7 to 7.4). The temperature was maintained at 36° ± 2°C. The fermentor was maintained in pH control and samples were periodically removed and tested for OD and pH, until the fermentation was complete.

Deoxycholate sodium was added to the fermentor to a final concentration of approximately 0.12% w/v. The culture was mixed for a minimum of thirty minutes and the temperature set point was reduced to 10°C. The culture was incubated overnight and following confirmation of inactivation, the pH of the culture was adjusted to between 6.4 and 6.8, as necessary, with 50% acetic acid. The temperature of the fermentor was increased to 20° ± 5°C and the contents were transferred to the clarification hold tank.

The contents of the clarification hold tank (including the cellular debris) were processed through a centrifuge at a flow rate between 25 and 600 liters per hour (except Serotype 4, wherein the cell debris was discarded and the flow rate tightened to between 25 and 250 liters per hour). Samples of the supernatant were removed and tested for OD. The desired OD during the centrifugation was ≤ 0.15.

Initially, the supernatant was recirculated through a depth filter assembly until an OD of 0.05 ± 0.03 was achieved. Then the supernatant was passed through the depth filter assembly and through a 0.45 μm membrane filter to the filtrate hold tank.

Petitioner Merck Ex-1004, 0585

Subsequently, the product was transferred through closed pipes to the purification area for processing.

All of the above operations (centrifugation, filtration and transfer) were performed between 10°C to 30°C.

For an alternate fermentation and harvesting process for serotypes 4 and 6B, see the alternate process described in Example 1.

Purification

The purification of each pneumococcal polysaccharide consisted of several concentration/diafiltration operations, precipitation/elution, column chromatography, and depth filtration steps. All procedures were performed at room temperature unless otherwise specified.

Clarified broth from the fermentor cultures of the desired *S. pneumoniae* serotype was concentrated and diafiltered using a 100 kDa MWCO filter. Diafiltration was accomplished using sodium phosphate buffer at pH < 9.0. Diafiltration removed the low molecular weight medium components from the higher molecular weight biopolymers such as nucleic acid, protein and polysaccharide.

The polysaccharide was precipitated from the concentrated and diafiltered solution by adding HB from a stock solution to give a final concentration of 1% HB (w/v) (except Serotype 23F, which had a final concentration of 2.5%). The polysaccharide/HB precipitate was captured on a depth filter and the filtrate was discarded. (Note: Serotype 14 does not precipitate; therefore the filtrate was retained.) The polysaccharide precipitate was resolubilized and eluted by recirculating a sodium chloride solution through the precipitate-containing depth filter. The filters were then rinsed with additional sodium chloride solution.

Sodium iodide (NaI) was added to the polysaccharide solution from a stock NaI solution to achieve a final concentration of 0.5% to precipitate HB (except for Serotype 6B, which had a final concentration of 0.25%). The precipitate was removed by depth filtration. The filtrate contained the target polysaccharide. The filter was discarded. The polysaccharide was then filtered through a 0.2µm filter.

The polysaccharide solution was concentrated on a 30 kDa MWCO ultrafilter and diafiltered with a sodium chloride solution.

The partially purified polysaccharide solution was further purified by filtration through a depth filter impregnated with activated carbon. After filtration, the carbon filter was rinsed with a sodium chloride solution. The rinse was combined with the polysaccharide solution, which was then filtered through a 0.2µm filter.

Petitioner Merck Ex-1004, 0586

The polysaccharide solution was concentrated on a 30 kDa MWCO ultrafilter and the filter was rinsed with a sodium chloride solution. The pH was checked and adjusted to 7.0 ± 0.3.

The ceramic hydroxyapatite (HA) column was equilibrated with sodium phosphate buffer containing sodium chloride until the pH is 7.0 ± 0.3 and the conductivity was 26 ± 4μS. The polysaccharide solution was then loaded onto the column. Under these conditions, impurities bound to the resin and the polysaccharide was recovered in the flow through from the column. The polysaccharide solution was filtered through a 0.2μm filter.

The polysaccharide solution was concentrated using a 30 kDa MWCO filter. The concentrate was then diafiltered with WFI until the conductivity was < 15μS.

The diafiltered polysaccharide solution was filtered through a 0.2μm membrane filter into bulk containers and stored at 2-8°C.

Example 14

Preparation of Pneumococcal Saccharide – CRM_{197} Conjugates For Serotypes 4, 6B, 9V, 14, 18C, 19F and 23F

Activation Process

The different serotype saccharides follow different pathways for activation (hydrolysis or no hydrolysis prior to activation) and conjugation (aqueous or DMSO reactions) as described in this example.

Polysaccharide was transferred from the bulk containers to the reactor vessel. The polysaccharide was then diluted in WFI and sodium phosphate to a final concentration range of 1.6 - 2.4 mg/mL.

Step 1.

For serotypes 6B, 9V, 14, 19F and 23F, pH was adjusted to pH 6.0 ± 0.3.

For serotype 4, hydrochloric acid (0.01 M final acid concentration) was added and the solution was incubated for 25 - 35 minutes at 45° ± 2°C. Hydrolysis was stopped by cooling to 21 - 25°C and adding 1M sodium phosphate to a target of pH 6.7 ± 0.2. An in-process test was done to confirm an appropriate level of depyruvylation.

Petitioner Merck Ex-1004, 0587

For serotype 18C, glacial acetic acid (0.2 M final acid concentration) was added and the solution was incubated for 205 - 215 minutes at 94° ± 2°C. Temperature was then decreased to 21 - 25°C and 1 - 2 M sodium phosphate was added to a target of pH 6.8 ± 0.2.

Step 2: Periodate Reaction

The required sodium periodate molar equivalents for pneumococcal saccharide activation was determined using total saccharide content (except for serotype 4). For serotype 4, a ratio of 0.8-1.2 moles of sodium periodate per mole of saccharide was used. With thorough mixing, the oxidation reaction was allowed to proceed between 16 to 20 hours at 21 - 25°C for all serotypes except 19F for which the temperature was ≤ 15°C.

Step 3: Ultrafiltration

The oxidized saccharide was concentrated and diafiltered with WFI (0.01 M sodium phosphate buffer pH 6.0 for serotype 19F) on a 100 kDa MWCO ultrafilter (5 kDa ultrafilter for 18C). The permeate was discarded and the retentate was filtered through a 0.22 μm filter.

Step 4: Lyophilization

For serotypes 4, 9V, and 14 the concentrated saccharide was mixed with CRM_{197} carrier protein, filled into glass bottles, shell-frozen and stored at ≤ -65°C. The frozen concentrated saccharide-CRM_{197} was lyophilized and then stored at –25° ± 5°C.

For serotypes 6B, 19F, and 23F a specified amount of sucrose was added which was calculated to achieve a 5% ± 3% sucrose concentration in the conjugation reaction mixture. Serotype 18C did not require sucrose addition. The concentrated saccharide was then filled into glass bottles, shell-frozen and stored at ≤ -65°C. The frozen concentrated saccharide was lyophilized and then stored at -25° ± 5°C.

Conjugation Process

Two conjugation processes were used: aqueous conjugation for serotypes 4, 9V, 14 and 18C, and DMSO conjugation for serotypes 6B, 19F and 23F.

Petitioner Merck Ex-1004, 0588

Aqueous Conjugation

Step 1: Dissolution

For serotypes 4, 9V and 14, the lyophilized activated saccharide-CRM_{197} mixture was thawed and equilibrated at room temperature. The lyophilized activated saccharide-CRM_{197} was then reconstituted in 0.1M sodium phosphate buffer at a typical ratio of:

- 1L of buffer per 16 - 24 g of saccharide for serotype 4 and 9V
- 1L of buffer per 6 - 10 g of saccharide for serotype 14

The reaction mixture was incubated at 37° ± 2°C until total dissolution for the serotype 9V and at 23° ± 2°C for serotypes 4 and 14.

For serotype 18C, the lyophilized saccharide was reconstituted in a solution of CRM_{197} in 1M dibasic sodium phosphate at a typical ratio of 0.11 L of sodium phosphate per 1 L of CRM_{197} solution. The reaction mixture (8-12 g/L saccharide concentration) was incubated at 23° ± 2°C until total dissolution.

The pH was tested as an in-process control at this stage.

Step 2: Conjugation Reaction

For serotypes 4 and 9V, the conjugation reaction was initiated by adding the sodium cyanoborohydride solution (100 mg/mL) to achieve 1.0 - 1.4 moles sodium cyanoborohydride per mole of saccharide. The reaction mixture was incubated for 44 - 52 hours at 37° ± 2°C. The temperature was then reduced to 23° ± 2°C and sodium chloride 0.9% was added to the reactor. Sodium borohydride solution (100 mg/mL) was added to achieve 1.8 - 2.2 molar equivalents of sodium borohydride per mole saccharide. The mixture was incubated for 3 - 6 hours at 23° ± 2°C. The mixture was diluted with sodium chloride 0.9% and the reactor was rinsed. The diluted conjugation mixture was filtered using a 1.2 μm pre-filter into a holding vessel.

For serotypes 14 and 18C, the conjugation reaction was initiated by adding the cyanoborohydride solution (100 mg/mL) to achieve 1.0 - 1.4 moles of sodium cyanoborohydride per mole of saccharide. The reaction mixture was incubated for 12 - 24 hours at 23° ± 2°C. The temperature was increased to 37° ± 2°C and the reaction was incubated for 72 - 96 hours. The temperature was then reduced to 23° ± 2°C and 0.9% sodium chloride was added to the reactor. Sodium borohydride solution (100mg/mL) was added to achieve 1.8 - 2.2 molar equivalents of sodium borohydride per mole of saccharide. The mixture was incubated for 3 - 6 hours at

Petitioner Merck Ex-1004, 0589

23° ± 2°C. The mixture was diluted with 0.9% sodium chloride and the reactor was rinsed. The diluted conjugation mixture was then filtered using a 1.2 μm pre-filter into a holding vessel.

Step 3: Ultrafiltration 100 kDa

The diluted conjugation mixture was concentrated and diafiltrated on a 100 kDa MWCO ultrafilter with either a minimum of 15 volumes (serotype 4) or 40 volumes (serotypes 9V, 14, and 18C) of 0.9% sodium chloride.

The permeate was discarded.

For serotype 4, the retentate was filtered through a 0.45μm filter.

An in-process control (saccharide content) was performed at this step.

Step 4: HA Column Purification

This step was only performed for the serotype 4 conjugate.

The HA column was first neutralized using 0.5M sodium phosphate buffer (pH 7.0 ± 0.3) and then equilibrated with 0.9% sodium chloride. The filtered retentate (serotype 4) was loaded onto the column at a flow rate of 1.0 L/min. The column was washed with 0.9% sodium chloride at a flow rate of ≤ 2.0 L/min. The product was then eluted with 0.5M sodium phosphate buffer at a flow rate of ≤ 2.0 L/min.

The HA fraction was then concentrated and diafiltered on a 100 kDa MWCO membrane with a minimum of 20 volumes of 0.9% sodium chloride. The permeate was discarded.

Step 5: Sterile Filtration

The retentate after the 100 kDa MWCO diafiltration was filtered through a 0.22μm filter. In-process controls (saccharide content, free protein, free saccharide and cyanide) were performed on the filtered product. In-process controls on filtered retentate were performed to determine whether additional concentration, diafiltration, and/or dilution were needed to meet FBC targets. These and additional tests were repeated in FBC samples.

As necessary, the filtered conjugate was diluted with 0.9% sodium chloride in order to achieve a final concentration of less than 0.55 g/L. Release tests for saccharide content, protein content and saccharide:protein ratio were performed at this stage.

Petitioner Merck Ex-1004, 0590

Finally, the conjugate was filtered (0.22 μm) and filled into 10 L stainless steel canisters at a typical quantity of 2.64 g/canister. At this stage, yield, saccharide content, protein content, pH, saccharide:protein ratio and lysine content were performed as in-process controls. Release testing (appearance, free protein, free saccharide, endotoxin, molecular size determination, residual cyanide, saccharide identity, CRM_{197} identity) was performed at this stage.

DMSO Conjugation

Step I: Dissolution

The lyophilized activated saccharide serotypes 6B, 19F, 23F and the lyophilized CRM_{197} carrier protein were equilibrated at room temperature and reconstituted in DMSO. The dissolution concentration typically ranged from 2-3 grams of saccharide (2-2.5 g protein) per liter of DMSO.

Step II: Conjugation Reaction

The activated saccharide and CRM_{197} carrier protein were mixed for 60 - 75 minutes at 23° ± 2°C at a ratio range of 0.6 g – 1.0 g saccharide/g CRM_{197} for serotypes 6B and 19F or 1.2 to 1.8 g saccharide/g CRM_{197} for serotype 23F.

The conjugation reaction was initiated by adding the sodium cyanoborohydride solution (100mg/mL) at a ratio of 0.8 - 1.2 molar equivalents of sodium cyanoborohydride to one mole activated saccharide. WFI was added to the reaction mixture to a target of 1% (v/v) and the mixture was incubated for over 40 hours at 23° ± 2°C.

Sodium borohydride solution, 100 mg/mL (typical 1.8 - 2.2 molar equivalents sodium borohydride per mole activated saccharide) and WFI (target 5% v/v) were added to the reaction and the mixture was incubated for 3 - 6 hours at 23° ± 2°C. This procedure reduced any unreacted aldehydes present on the saccharides. Then the reaction mixture was transferred to a dilution tank containing 0.9% sodium chloride at < 15°C.

Step III: 100 kDa Ultrafiltration

The diluted conjugate mixture was filtered through a 1.2 μm filter and concentrated and diafiltered on a 100 kDa MWCO membrane with a minimum of 15 volumes of 0.9% sodium chloride (0.01M sodium phosphate/0.05M NaCl buffer was

Petitioner Merck Ex-1004, 0591

used for serotype 23F). The permeate was discarded. The retentate was filtered through a 0.45 μm filter. An in-process saccharide content sample was taken at this stage.

Step IV: DEAE Column Purification

This step was only performed for serotype 23F.

The DEAE column was equilibrated with 0.01M sodium phosphate/0.05M sodium chloride buffer. The filtered retentate (serotype 23F) was loaded onto the column and washed with 0.01M sodium phosphate/0.05M sodium chloride buffer. The column was then washed with 0.01M sodium phosphate/0.9% NaCl buffer. The product was then eluted with 0.01M sodium phosphate/0.5M sodium chloride buffer.

Step V: 100 kDa Ultrafiltration

The retentate from 6B and 19F was concentrated and diafiltered with at least 30 volumes of 0.9% sodium chloride. The permeate was discarded.

The eluate from serotype 23F was concentrated and diafiltered with a minimum of 20 volumes of 0.9% sodium chloride. The permeate was discarded.

Step VI: Sterile Filtration

The retentate after the 100 kDa MWCO diafiltration was filtered through 0.22 μm filter. In-process controls (saccharide content, free protein, free saccharide, residual DMSO and residual cyanide) were performed on the filtered product. In-process controls on filtered retentate were performed to determine whether additional concentration, diafiltration, and/or dilution were needed to meet FBC targets. These and additional tests were repeated in FBC samples.

As necessary, the filtered conjugate was diluted with 0.9% sodium chloride to achieve a final concentration of less than 0.55 g/L. Release tests for saccharide content, protein content and saccharide:protein ratio were performed at this stage.

Finally, the conjugate was filtered (0.22 μm) and filled into 10 L stainless steel canisters at a quantity of 2.64 g/canister. At this stage, yield, saccharide content, protein content, pH, saccharide:protein ratio and lysine content were performed as in-process controls. Release testing (appearance, free protein, free saccharide, endotoxin, molecular size determination, residual cyanide, residual DMSO, saccharide identity and CRM_{197} identity) was performed at this stage.

Petitioner Merck Ex-1004, 0592

Example 15

Formulation of a Multivalent Pneumococcal Conjugate Vaccine

The final bulk concentrates of the 13 conjugates contain 0.85% sodium chloride. Type 3, 6A, 7F and 19A bulk concentrates also contain 5 mM sodium succinate buffer at pH 5.8. The required volumes of bulk concentrates were calculated based on the batch volume and the bulk saccharide concentrations. After 80% of the 0.85% sodium chloride (physiological saline) and the required amount of succinate buffer were added to the pre-labeled formulation vessel, bulk concentrates were added. The preparation was then sterile filtered through a 0.22 µm membrane into a second container by using a Millipore Durapore membrane filter unit. The first container was washed with the remaining 20% of 0.85% sodium chloride and the solution was passed through the same filter and collected into the second container. The formulated bulk was mixed gently during and following the addition of bulk aluminum phosphate. The pH was checked and adjusted if necessary. The formulated bulk product was stored at 2-8°C.

The formulated bulk product was filled into Type 1 borosilicate glass syringes obtained from Becton Dickinson. The vaccine was monitored at regular intervals for turbidity to ensure the uniformity of the filling operation. The filled vaccine (Final Product) was stored at 2-8°C.

Example 16

Immunogenicity of the 13-Valent Conjugate Vaccine

To date, the preclinical studies performed on the 13vPnC vaccine have been in rabbits. Studies #HT01-0021 and #HT01-0036 were designed to independently examine the effect of chemical conjugation of capsular polysaccharides (PSs) from *S. pneumoniae* to CRM_{197} and the effect of aluminum phosphate ($AlPO_4$) adjuvant on the immune response to the 13vPnC vaccine in rabbits. These effects were characterized by antigen-specific ELISA for serum IgG concentrations and for antibody function by opsonophagocytic assay (OPA).

Study #HT01-0021

Study #HT01-0021 examined the ability of the 13vPnC vaccine with $AlPO_4$ adjuvant to elicit vaccine serotype-specific immune responses. The pneumococcal serotypes represented in the 13vPnC vaccine include types 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F. Secondary objectives included an evaluation of the kinetics and duration of the antibody response. New Zealand White rabbits were

Petitioner Merck Ex-1004, 0593

immunized intramuscularly at week 0 and week 2 with the planned human clinical dose of each polysaccharide (2 µg of each PS, except 4 µg of 6B) formulated with or without $AlPO_4$ (100 µg /dose). Sera were collected at various time points. Serotype specific IgG was measured by ELISA and functional activity was assessed by OPA.

Table 4 shows the geometric mean titer (GMT) achieved in pooled serum samples, following two doses of the 13vPnC vaccine. A ratio of the IgG GMTs was used to compare responses from week 4 to week 0. These data demonstrate that the inclusion of $AlPO_4$ in the 13vPnC formulation elicited higher levels of IgG antibody in comparison to the same vaccine without adjuvant. Although the antibody responses were greater when $AlPO_4$ was included in the formulation, these increases were not statistically significant.

Functional antibody responses were also assessed in rabbits following immunization with the two 13vPnC formulations (Table 5). When comparing vaccine formulations with or without adjuvant, higher OPA GMTs were observed in the 13vPnC + $AlPO_4$ vaccine treatment group. OPA titers were detected in week 4 serum pools to all vaccine serotypes in both groups. For the majority of the serotypes, OPA titers measured at week 4 were at least 4-fold higher than those at week 0 (baseline).

The kinetic responses to each of the 13vPnC vaccine serotypes were evaluated from serum pools of both treatment groups. IgG titers to each serotype were measured from blood draws at week 0 and weeks 1, 2, 3, 4, 8, 12, 26, and 39 and then compared. With the exception of serotype 1, antibody responses in animals receiving adjuvanted vaccine were superior to those that received non-adjuvanted vaccine and peaked at week 2 of the immunization schedule (data not shown).

Overall, the data indicate that the 13vPnC vaccine formulated with aluminum phosphate is immunogenic in rabbits, eliciting substantial antibody responses to the pneumococcal capsular polysaccharides contained in the vaccine and these responses are associated with functional activity. The responses observed to the seven core serotypes following immunization with 13vPnC + $AlPO_4$ are consistent with historical responses of rabbits to the heptavalent formulation.

Petitioner Merck Ex-1004, 0594

Table 4. Rabbit IgG Immune Responses (GMTs) Following Immunization with Two Doses of 13-valent Pneumococcal Glycoconjugate

	Diluent with $ALPO_4$[a]			13vPnC[a]			13vPnC + $ALPO_4$[a]		
Serotype	Week 0	Week 4	Ratio Wk4:Wk0	Week 0	Week 4 (95% CI)	Ratio Wk4:Wk0	Week 0	Week 4 (95% CI)	Ratio Wk4:Wk0
1	<100	<100	1.0	50	5,926 (2,758-12,733)	119	50	11,091 (5,327-23,093)	222
3	<100	<100	1.0	50	6,647 (2,773-15,932)	133	58	16,443 (7,096-38,106)	284
4	<100	<100	1.0	50	13,554 (8,031-22,875)	271	50	29,183 (15,342-55,508)	584
5	134	<100	0.4	50	5,859 (2,450-14,009)	117	50	16,714 (6,959-40,140)	334
6A	141	<100	0.4	74	22,415 (11,987-41,914)	303	83	63,734 (21,141-192,146)	768
6B	<100	<100	1.0	57	8,108 (3,564-18,444)	142	54	23,505 (11,286-48,955)	435
7F	3,859	579	0.2	171	43,591 (26,931-70,557)	444	143	84,888 (46,445-155,151)	496
9V	289	995	3.4	205	15,780 (7,193-34,616)	125	208	43,331[b] (23,256-71,510)	217
14	437	177	0.4	61	6,906 (3,416-13,962)	113	70	16,076 (9,649-26,785)	322
18C	<100	<100	1.0	50	21,283 (15,770-28,725)	426	50	35,040 (24,708-49,692)	701
19A	<100	<100	1.0	121	113,599 (54,518-236,707)	939	144	280,976 (119,587-660,167)	1,951
19F	<100	<100	1.0	50	14,365 (7,346-28,090)	287	50	24,912 (9,243-67,141)	498
23F	<100	<100	1.0	50	5,323 (1,894-14,962)	106	50	15,041 (4,711-48,018)	301

a: GMTs of pooled sera consisted of equal volumes of serum from each individual rabbit within a group
b: Statistically different (p=0.022) from treatment group without $ALPO_4$

Petitioner Merck Ex-1004, 0595

Table 5. ***S. pneumoniae*** **OPA GMTs for NZW Rabbit Serum Pools Following Immunization with Two Doses of 13-valent Pneumococcal Glycoconjugate**

	13vPnC[a]			13vPnC + $ALPO_4$[a]		
Serotype	Week 0	Week 4	Ratio Wk4:Wk0	Week 0	Week 4	Ratio Wk4:Wk0
1	<8	64	16	<8	64	16
3	<8	8	2	<8	16	4
4	<8	16	4	<8	32	8
5	<8	128	32	<8	512	128
6A	8	128	16	8	512	64
6B	<8	256	64	8	1,024	128
7F	8	64	8	8	128	16
9V	8	64	8	8	128	16
14	16	32	2	16	32	2
18C	8	256	32	<8	256	64
19A	<8	256	64	<8	1,024	256
19F	<8	128	32	<8	512	128
23F	8	64	8	<8	256	64

A: Pools consisted of equal volumes of serum from individual rabbits within a treatment group (n=12)

Study #HT01-0036

Study #HT01-0036 compared rabbit immune responses to the polysaccharides (PSs) contained in the vaccine, after immunization with the 13vPnC vaccine with or without conjugation to the CRM_{197} protein. New Zealand White rabbits were immunized intramuscularly at week 0 and week 2 with a dose of 2.2 μg of each PS (except 4.4 μg of 6B). Animals received one of three vaccine preparations: (a) 13vPnC (PS directly conjugated to CRM_{197}), (b) 13vPnPS, (free PS) or (c) 13vPnPS + CRM_{197} (free PS mixed with CRM_{197}). All vaccine preparations contained $AlPO_4$ as the adjuvant at 125 μg/dose.

Serotype specific immune responses for all vaccine preparations were evaluated in an IgG ELISA and complement-mediated OPA measuring functional antibody. The immune responses were compared between the treatment groups.

Table 6 presents GMT data obtained from week 4 bleeds analyzed in antigen specific IgG ELISAs. Additional analyses show the ratio of GMT values at week 4 to week 0. The data indicate that the conjugate vaccine preparation elicited greater serum IgG titers than free PS or free PS + CRM_{197} vaccine. With the exception of *S. pneumoniae* type 14, the 13vPnC vaccine was able to induce functional antibodies to the representative strains of *S. pneumoniae* in an OPA (Table 7). After two

Petitioner Merck Ex-1004, 0596

immunizations with either the 13vPnPS or 13vPnPS + CRM_{197} vaccine, neither could induce OPA titers ≥ 8-fold at week 4 relative to week 0 for 10 out of the 13 serotypes measured (Table 7).

In conclusion, these results indicate that conjugation of the 13-valent pneumococcal vaccine polysaccharides produces higher serum IgG titers and overall greater functional antibody activity than seen with free polysaccharide alone or mixed with unconjugated CRM_{197}.

Petitioner Merck Ex-1004, 0597

Table 6. Rabbit IgG Responses (GMTs) to PnPS by ELISA Following Immunization with Two Doses of 13-valent Pneumococcal Glycoconjugate

	13vPnPS (free PS)			13vPnPS = CRM 197 (PS mixed with CRM197)			13vPnC		
Serotype	Week 0	Week 4 (95% CI)	Ratio Wk4:Wk0	Week 0	Week 4 (95% CI)	Ratio Wk4:Wk0	Week 0	Week 4 (95% CI)	Ratio Wk4:Wk0
1	378	2,290 (843-5,790)	5.8	395	1,959 (809-4,739)	5.0	472	35,970 (29,130-44,417)	76.2
3	57	240 (64-908)	4.2	89	163 (74-358)	1.8	50	10,414 (10,414-16,676)	208.3
4	50	379 (150-959)	7.6	50	607 (313-1,178)	12.1	50	12,890 (9,117-18,224)	257.8
5	343	226 (113-450)	4.5	50	321 (147-701)	6.4	50	35,264 (24,467-50,824)	705.3
6A	154	466 (316-688)	3.0	98	210 (95-464)	2.1	163	234,245 (167,152-328,283)	1,437.1
6B	63	727 (384-1,375)	11.6	62	745 (384-1,440)	12.0	131	33,599 (22,934-49,222)	256.5
7F	50	61 (39-95)	1.2	50	72 (47-111)	1.4	50	35,702 (24,350-52,347)	714.0
9V	50	104 (48-195)	2.1	55	169 (74-390)	3.0	50	50,033 (34,765-72,007)	1,000.7
14	66	298 (117-757)	4.5	50	195 (71-535)	3.9	50	20,121 (12,087-32,138)	402.4
18C	89	1,555 (655-3,688)	17.5	66	761 (300-1,935)	11.5	101	71,451 (32,745-124,641)	707.4
19A	50	89 (44-179)	1.8	50	80 (39-163)	1.6	50	23,485 (12,857-42,723)	469.7
19F	61	1,362 (559-3,317)	22.3	61	991 (370-2,654)	16.3	67	19,358 (12,553-33,173)	288.9
23F	73	1,085 (487-2,420)	14.9	121	638 (311-1,311)	5.3	68	45,972 (25,134-84,089)	676.1

Petitioner Merck Ex-1004, 0598

Table 7. *S. pneumoniae* OPA Titers for Rabbit Serum Pools Following Immunization with Two Doses of 13-valent Pneumococcal Vaccines

	OPA Titers						
	No Treatment	13vPnPS (free PS)		13vPnPS + CRM_{197} (free PS mixed with CRM_{197})		13vPnC	
Serotype	Week 0[a]	Week 4	Ratio Wk4:Wk0	Week 4	Ratio Wk4:Wk0	Week 4	Ratio Wk4:Wk0
1	4	16	4	16	4	8	32
3	4	4	1	4	1	4	8
4	4	4	1	4	1	4	64
5	4	32	8	16	4	16	64
6A	8	64	8	32	4	32	664
6B	8	64	8	32	4	32	32
7F	16	32	2	16	1	16	16
9V	16	16	1	32	2	32	8
14	16	16	1	16	1	16	2
18C	4	16	4	16	4	8	64
19A	8	8	1	8	1	16	64
19F	4	4	1	4	1	8	64
23F	16	32	2	16	1	32	32

a. Used as week 0 values for all groups

Example 17

Alternate Procedure for Serotype 3 Pneumococcal Saccharide – CRM_{197} Conjugation

Containers of purified polysaccharide were thawed and combined in a reaction vessel and heated to a temperature of 85°C. When the temperature of the mixture reached approximately 35°C during temperature ramp-up, 2 M acetic acid was added to achieve a 0.1 M acetic acid concentration. When the temperature of the mixture reached 85°C, it was maintained at this temperature for 1 hour to achieve hydrolysis/depolymerization. The hydrolyzed/depolymerized polysaccharide was cooled to 23°C and 1 M $MgCl_2$ was added to achieve a 0.1 M $MgCl_2$ concentration. Oxidation was carried out using 1-1.25 mole equivalent of periodic acid, added as a 50 mg/mL solution in WFI. The mixture was incubated at 23°C for 16-24 hours.

The reaction mixture was purified by diafiltration against 10x volumes of WFI using a 100K MWCO polyethersulfone (PES) membrane. The purified, activated polysaccharide was adjusted to a pH of 6.0 using 0.2 M phosphate buffer (pH 7.0), compounded with CRM_{197} at a polysaccharide/protein ratio of 2:1, shell-frozen at -75°C, and co-lyophilized for 71 hours. Following co-lyophilization, the polysaccharide/CRM_{197} mixture was stored at -25° ± 5°C until conjugation. For conjugation, the co-lyophilized polysaccharide/CRM_{197} mixture was dissolved in 0.1 M phosphate buffer at a concentration of 20 mg/mL polysaccharide and the pH was adjusted to 6.2 with a combination of 0.2 M phosphate buffer, pH 4.6, and 1 M

Petitioner Merck Ex-1004, 0599

NaOH, followed by the addition of 0.5 mole equivalent of sodium cyanoborohydride as a 100 mg/mL solution in 0.15 M phosphate buffer, pH 7.5. The reaction mixture was stirred and incubated at 37°C for 44 hours. Following the cyanoborohydride incubation, the reaction mixture was diluted 1x with cold (2-8°C) 0.9% saline followed by the addition of 1 mole equivalent of sodium borohydride as a 100 mg/mL solution in WFI to cap unreacted aldehydes at 23°C for 3-6 hours.

The reaction mixture was transferred through a 0.45-5 µm prefilter into a retentate vessel and then purified by diafiltration using a 100K MWCO regenerated cellulose membrane. The reaction mixture was then diafiltered 30x against 0.1 M phosphate buffer, pH 9.0, and 20x against 0.15 M phosphate buffer, pH 6.0. A final 20x diafiltration was performed against 5 mM succinate/0.9% saline, pH 6.0. The diafiltered conjugate was filtered through a 0.2 µm filter and assayed for saccharide content. Based on saccharide analysis, the conjugate solution was diluted with 5 mM succinate/0.9% saline, pH 6.0, to a target of 0.5 mg/mL and again sterile filtered (0.2 µm) to give the final batch concentrate conjugate. The conjugate was stored at 2-8°C.

For characterization of the conjugate, see Example 4.

It should be understood that the foregoing discussion and examples merely present a detailed description of certain embodiments. It therefore should be apparent to those of ordinary skill in the art that various modifications and equivalents can be made without departing from the spirit and scope of the invention.

All journal articles, other references, patents and patent applications that are identified in this patent application are incorporated by reference in their entirety.

Petitioner Merck Ex-1004, 0600

REFERENCES

1. Hausdorff WP, Bryant J, Paradiso PR, Siber GR. Which pneumococcal serogroups cause the most invasive disease: implications for conjugate vaccine formulation and use, part I. *Clin Infect Dis* 2000; 30:100-21.

2. Hausdorff WP, Bryant J, Kloek C, Paradiso PR, Siber GR. The contribution of specific pneumococcal serogroups to different disease manifestations: implications for conjugate vaccine formulation and use, part I. *Clin Infect Dis* 2000; 30:122-40.

3. Whitney CG, Farley MM, Hadler J, et al. Decline in invasive pneumococcal disease after the introduction of protein-polysaccharide conjugate vaccine. *New Engl J Med* 2003; 348(18):1737-46.

4. Black S, Shinefield H, Hansen J, et al. Postlicensure evaluation of the effectiveness of seven valent pneumococcal conjugate vaccine. *Pediatr Infect Dis J* 2001; 20;1105-7.

5. Robinson KA, Baughman W, Rothrock G, et al. Epidemiology of invasive *Streptococcus pneumoniae* infections in the United States, 1995-1998: Opportunities for prevention in the conjugate vaccine era. *JAMA* 2001; 285:1729-35.

6. Butler J, Breiman R, Lipman H, et al. Serotype distribution of *Streptococcus pneumoniae* infections among preschool children in the United States, 1978-1994. *J Infect Dis* 1995; 171:885-9.

7. Whitney CG, Farley MM, Hadler J, et al. Increasing prevalence of multidrug-resistant *Streptococcus pneumoniae* in the United States. *N Engl J Med* 2000; 343:1917-24.

8. Hofmann J, Cetron MS, Farley MM, et al. The prevalence of drug-resistant *Streptococcus pneumoniae* in Atlanta. *N Engl J Med* 1995; 333:481-6.

9. Joloba ML, Windau A, Bajaksouzian S, Appelbaum PC Hausdorff WP, Jacobs MR. Pneumococcal conjugate vaccine serotypes of *Streptococcus pneumoniae* isolates and the antimicrobial susceptibility of such isolates in children with otitis media. *Clin Infect Dis* 2001; 33:1489-94.

Petitioner Merck Ex-1004, 0601

10. Black S, Shinefield H, Fireman B, et al. Efficacy, safety, and immunogenicity of heptavalent pneumococcal conjugate vaccine in children. *Pediatr Infect Dis J* 2000; 19:187-95.

11. Rudolph KM, Parkinson AJ, Reasonover AL, Bulkow LR, Parks DJ, Butler JC. Serotype distribution and antimicrobial resistance patternrs of invasive isolates of *Streptococcus pneumoniae*: Alaska, 1991-1998. *J Infect Dis* 2000; 182:490-6.

12. Sniadack DH, Schwartz B, Lipman H, et al. Potential interventions for the prevention of childhood pneumonia: geographic and temporal differences in serotype and serogroup distribution of sterile site pneumococcal isolates from children: implications for vaccine strategies. *Pediatr Infect Dis J* 1995; 14:503-10.

13. Fagan RL, Hanna JN, Messer RD, Brookes DL, Murphy DM. The epidemiology of invasive pneumococcal disease in children in Far North Queensland. *J. Paediatr Child Health* 2001; 37:571-5.

14. Kertesz DA, Di Fabio JL, de Cunto Brandileone MC, et al. Invasive *Streptococcus pneumoniae* infection in Latin American children: results of the Pan American Health Organization Surveillance Study. *Clin Infect Dis* 1998; 26:1355-61.

15. Hausdorff W, Siber G, Paradiso P. Geographical differences in invasive pneumococcal disease rates and serotype frequency in young children. *Lancet* 2001; 357:950-52.

16. Buckingham SC, King MD, Miller ML. Incidence and etiologies of complicated parapneumonic effusions in children, 1996 to 2001. *Pediatr Infect Dis J* 2003; 22:499-504.

17. Byington C, Spencer L, Johnson T, et al. An epidemiological investigation of a sustained high rate of pediatric parapneumonic empyema: risk factors and microbiological associations. *Clin Infect Dis* 2002; 34:434-40.

18. Tan T, Mason E, Wald E, et al. Clinical characteristics with complicated pneumonia caused by *Streptococcus pneumoniae*. *Pediatrics* 2002; 110:1-6.

Petitioner Merck Ex-1004, 0602

19. Block SL, Hedrick J, Harrison CJ, et al. Pneumococcal serotypes from acute otitis media in rural Kentucky. *Pediatr Infect Dis J* 2002; 21:859-65.

20. Hausdorff WP, Yothers G, Dagan R, et al. Multinational study of pneumococcal serotypes causing acute otitis media in children. *Pediatr Infect Dis J* 2002; 21:1008-16.

21. Robbins JB, Austrian R, Lee CJ, et al. Considerations for formulating the second-generation pneumococcal capsular polysaccharide vaccine with emphasis on the cross-reactive types within groups. *J Infect Dis* 1983; 148:1136-59.

22. Nahm MH, Olander JV, Magyarlaki M. Identification of cross-reactive antibodies with low opsonophagocytic activity for *Streptococcus pneumoniae*. *J Infect Dis* 1997; 176:698-703.

23. Yu X, Gray B, Chang S, Ward JI, Edwards KM, Nahm MH. Immunity to cross-reactive serotypes induced by pneumococcal conjugate vaccines in infants. *J Infect Dis* 1999; 180:1569-76.

24. Vakevainen M, Eklund C, Eskola J, Kayhty H. Cross-reactivity of antibodies to type 6B and 6A polysaccharides of *Streptococcus pneumoniae*, evoked by pneumococcal conjugate vaccines, in infants. *J Infect Dis* 2001; 184:789-93.

25. Ekstrom N, Kilpi T, Lahdenkari M, Lehtonen H, Ahman H, Kayhty, H. Immune response to cross-reacting pneumococcal serotypes 6A/6B and 19A/19F in the FinOM vaccine trial, Third World of Congress of Pediatric Infectious Diseases, Santiago, Chile, November 19-23, 2003.

26. Penn RL, Lewin EB, Douglas RG, Jr., Schiffman G, Lee CJ, Robbins JB. Antibody responses in adult volunteers to pneumococcal polysaccharide types 19F and 19A administered singly and in combination. *Infect Immun* 1982; 36:1261-2.

27. Giebink GS, Meier JD, Quartey MK, Liebeler CL, Le CT. Immunogenicity and efficacy of *Streptococcus pneumoniae* polysaccharide-protein conjugate vaccines against homologous and heterologous serotypes in the chinchilla otitis media model. *J Infect Dis* 1996; 173:119-27.

Petitioner Merck Ex-1004, 0603

28. Saeland E, Jakobsen H, Ingolfsdottir G, Sigurdardottir ST, Jonsdottir I. Serum samples from infants vaccinated with a pneumococcal conjugate vaccine, PncT, protect mice against invasive infection caused by *Streptococcus pneumoniae* serotypes 6A and 6B. *J Infect Dis* 2001; 183:253-60.

29. Jakobsen H, Sigurdsson VD, Sigurdardottir S, Schulz D, Jonsdottir I. Pneumococcal serotype 19F conjugate vaccine induces cross-protective immunity in serotype 19A in a murine pneumococcal pneumonia model. *Infect Immun* 2003; 71:2956-9.

30. Klugman KP, Madhi SA, Huebner RE, Kohberger R, Mbelle N, Pierce N. A trial of a 9-valent pneumococcal conjugate vaccine in children with and those without HIV infection. *N Engl J Med* 2003; 349:1341-8.

31. O'Brien KL, Moulton LH, Reid R, et al. Efficacy and safety of seven-valent conjugate pneumococcal vaccine in American Indian children: group randomised trial. *Lancet* 2003; 362:355-61.

32. Eskola J, Kilpi T, Palmu A, et al. Efficacy of a pneumococcal conjugate vaccine against acute otitis media. *N Engl J Med* 2001: 344:403-9.

33. Pilishvili T, Farley M, Vazquez M, Reingold A, Nyquist A, et al. Effectiveness of heptavalent pneumococcal conjugate vaccine in children. Abst G-1079, ICAAC, Chicago, IL, 2003.

34. U.S. Patent No. 4,673,574.

35. U.S. Patent No. 4,902,506.

Petitioner Merck Ex-1004, 0604

What is claimed is:

1. A method for making an immunogenic conjugate comprising *Streptococcus pneumoniae* serotype 3 polysaccharide covalently linked to a carrier protein, the method comprising:
(a) reacting purified serotype 3 polysaccharide with a mild acid resulting in a hydrolyzed serotype 3 polysaccharide;
(b) reacting the hydrolyzed serotype 3 polysaccharide with an oxidizing agent in the presence of bivalent cations resulting in an activated serotype 3 polysaccharide;
(c) compounding the activated serotype 3 polysaccharide with a carrier protein;
(d) reacting the compounded, activated serotype 3 polysaccharide and carrier protein with a reducing agent resulting in a serotype 3 polysaccharide:carrier protein conjugate; and
(e) capping unreacted aldehydes in the serotype 3 polysaccharide:carrier protein conjugate resulting in an immunogenic conjugate comprising *Streptococcus pneumoniae* serotype 3 polysaccharide covalently linked to a carrier protein.

2. The method of claim 1, further comprising purifying the activated serotype 3 polysaccharide prior to compounding with the carrier protein.

3. The method of claim 1, further comprising co-lyophilizing the compounded, activated serotype 3 polysaccharide and carrier protein prior to reacting with the reducing agent.

4. The method of claim 1, further comprising purifying the immunogenic conjugate.

5. The method of claim 1, wherein the mild acid is acetic acid.

6. The method of claim 1, wherein the oxidizing agent is periodic acid and the bivalent cations are Mg^{2+}.

Petitioner Merck Ex-1004, 0605

7. The method of claim 1, wherein the carrier protein is CRM_{197}.

8. The method of claim 1, wherein the reducing agent is sodium cyanoborohydride.

9. The method of claim 1, wherein capping unreacted aldehydes comprises reacting the serotype 3 polysaccharide:carrier protein conjugate with sodium borohydride.

10. A method for making an immunogenic conjugate comprising *Streptococcus pneumoniae* serotype 3 polysaccharide covalently linked to a carrier protein, the method comprising:

(a) reacting purified serotype 3 polysaccharide with acetic acid resulting in a hydrolyzed serotype 3 polysaccharide;

(b) reacting the hydrolyzed serotype 3 polysaccharide with periodic acid in the presence of $MgCl_2$ resulting in an activated serotype 3 polysaccharide;

(c) purifying the activated serotype 3 polysaccharide;

(d) compounding the activated serotype 3 polysaccharide with a carrier protein;

(e) co-lyophilizing the compounded, activated serotype 3 polysaccharide and carrier protein;

(f) reacting the co-lyophilized, activated serotype 3 polysaccharide and carrier protein with sodium cyanoborohydride resulting in a serotype 3 polysaccharide:carrier protein conjugate; and

(g) capping unreacted aldehydes in the serotype 3 polysaccharide:carrier protein conjugate with sodium borohydride resulting in an immunogenic conjugate comprising *Streptococcus pneumoniae* serotype 3 polysaccharide covalently linked to a carrier protein.

11. The method of claim 10, further comprising purifying the immunogenic conjugate.

12. The method of claim 10, wherein the carrier protein is CRM_{197}.

Petitioner Merck Ex-1004, 0606

13. A method for making an activated *Streptococcus pneumoniae* serotype 3 polysaccharide, the method comprising:

(a) reacting purified serotype 3 polysaccharide with a mild acid resulting in a hydrolyzed serotype 3 polysaccharide; and

(b) reacting the hydrolyzed serotype 3 polysaccharide with an oxidizing agent in the presence of bivalent cations resulting in an activated serotype 3 polysaccharide.

14. The method of claim 13, further comprising purifying the activated serotype 3 polysaccharide following oxidation.

15. The method of claim 13, wherein the mild acid is acetic acid.

16. The method of claim 13, wherein the oxidizing agent is periodic acid and the bivalent cations are Mg^{2+}.

Petitioner Merck Ex-1004, 0607

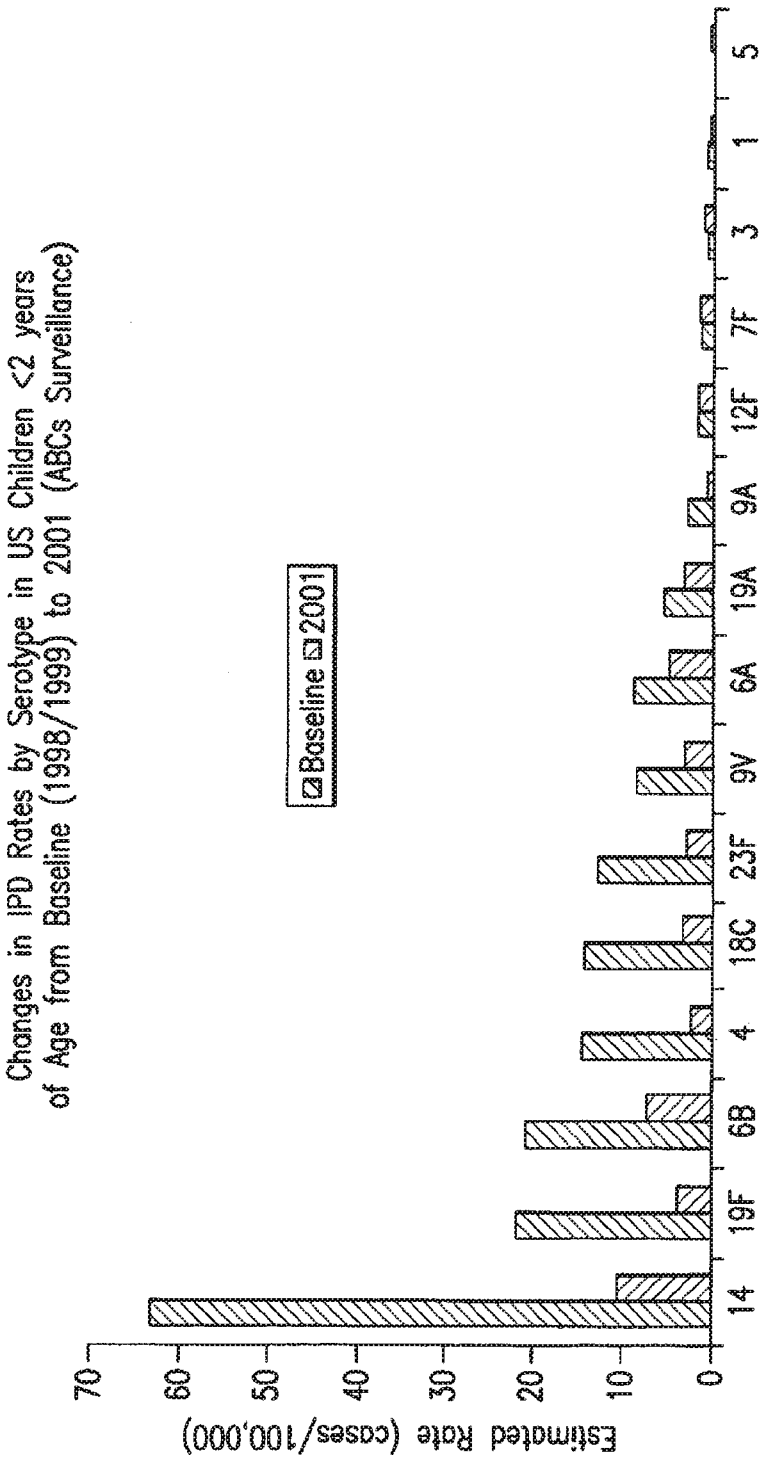


FIG. 1

Petitioner Merck Ex-1004, 0608

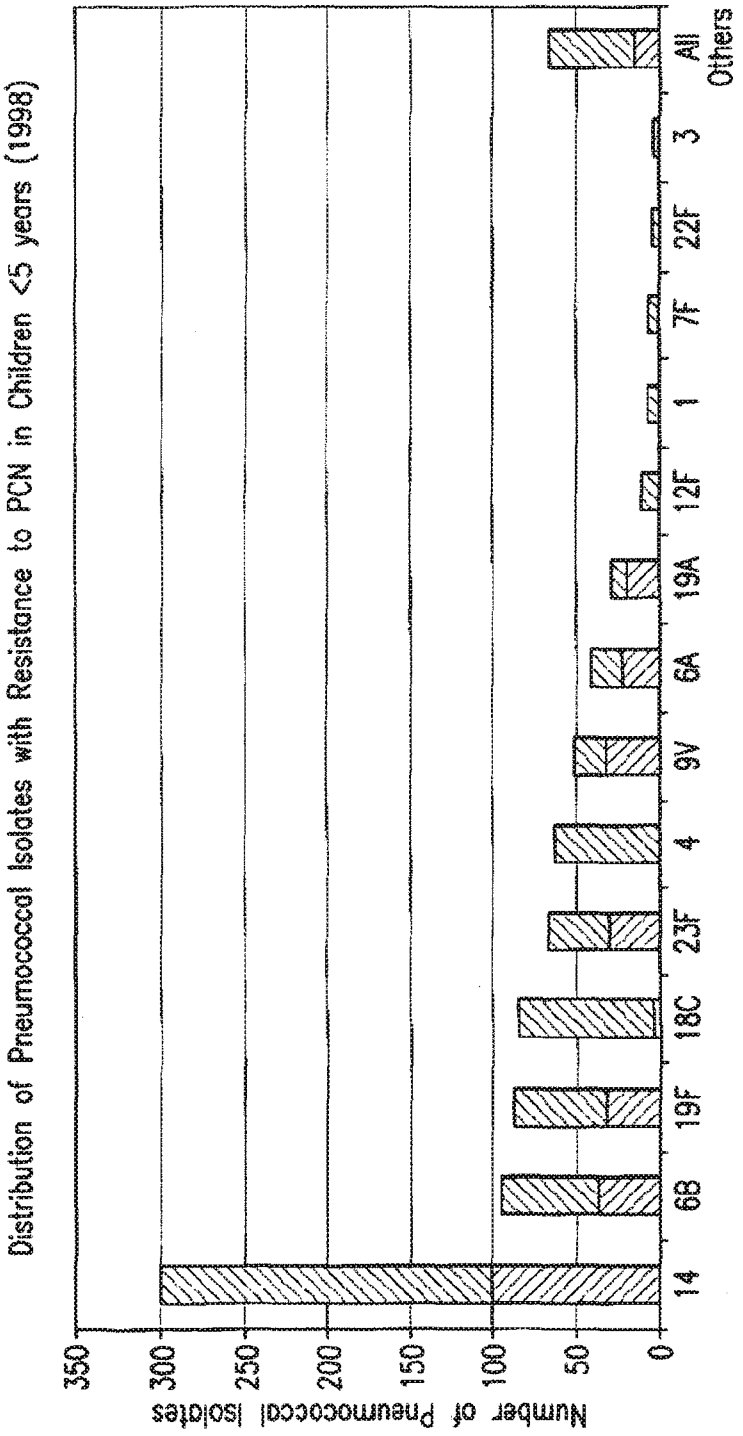


FIG. 2

Petitioner Merck Ex-1004, 0609

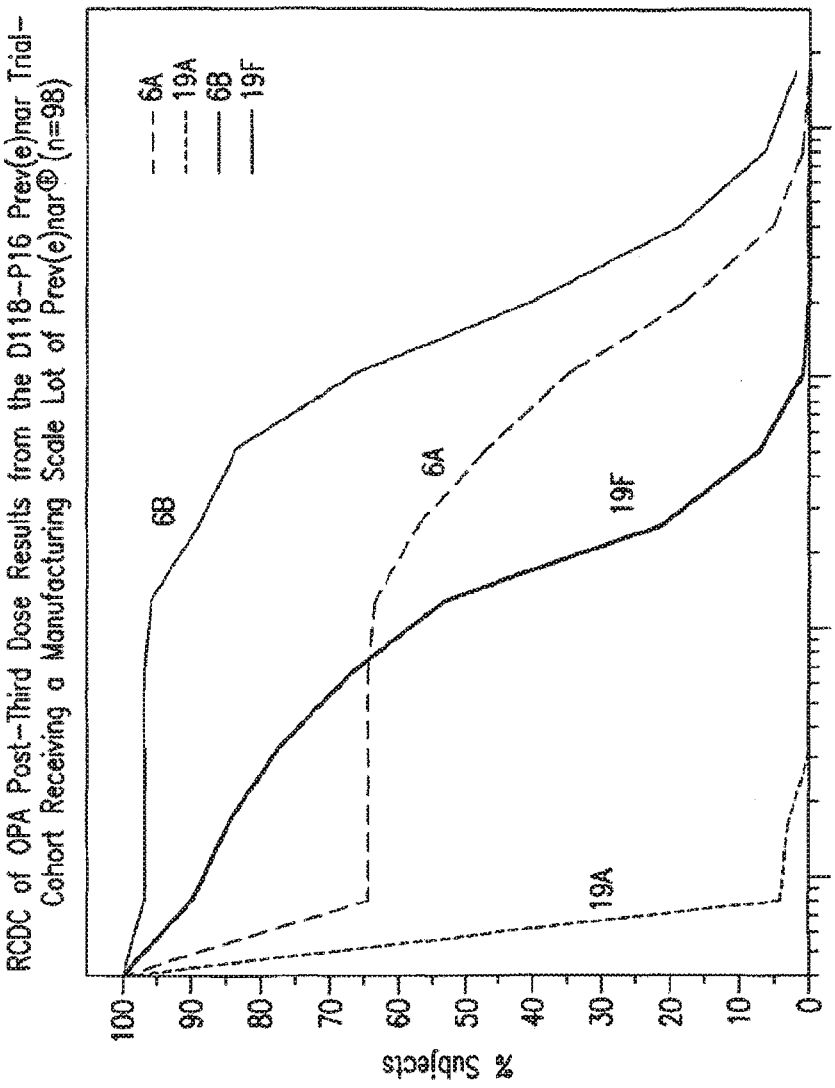


FIG.3

Petitioner Merck Ex-1004, 0610

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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
18 August 2011 (18.08.2011)

PCT

(10) International Publication Number
WO 2011/100151 A1

(51) International Patent Classification:
A61K 39/00 (2006.01)

(21) International Application Number:
PCT/US2011/023526

(22) International Filing Date:
3 February 2011 (03.02.2011)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/302,726 9 February 2010 (09.02.2010) US

(71) Applicant *(for all designated States except US)*: **MERCK SHARP & DOHME CORP.** [US/US]; 126 East Lincoln Avenue, Rahway, New Jersey 07065-0907 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **CAULFIELD, Michael, J.** [US/US]; 711 Prospect Avenue, Ft. Washington, Pennsylvania 19034 (US). **AHL, Patrick, L.** [US/US]; 770 Sumneytown Pike, West Point, Pennsylvania 19486 (US). **BLUE, Jeffrey, T.** [US/US]; 770 Sumneytown Pike, West Point, Pennsylvania 19486 (US). **CANNON, Jayme, L.** [US/US]; 770 Sumneytown Pike, West Point, Pennsylvania 19486 (US).

(74) Common Representative: **MERCK SHARP & DOHME CORP.**; 126 East Lincoln Avenue, Rahway, New Jersey 07065-0907 (US).

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

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

[Continued on next page]

(54) Title: 15-VALENT PNEUMOCOCCAL POLYSACCHARIDE-PROTEIN CONJUGATE VACCINE COMPOSITION

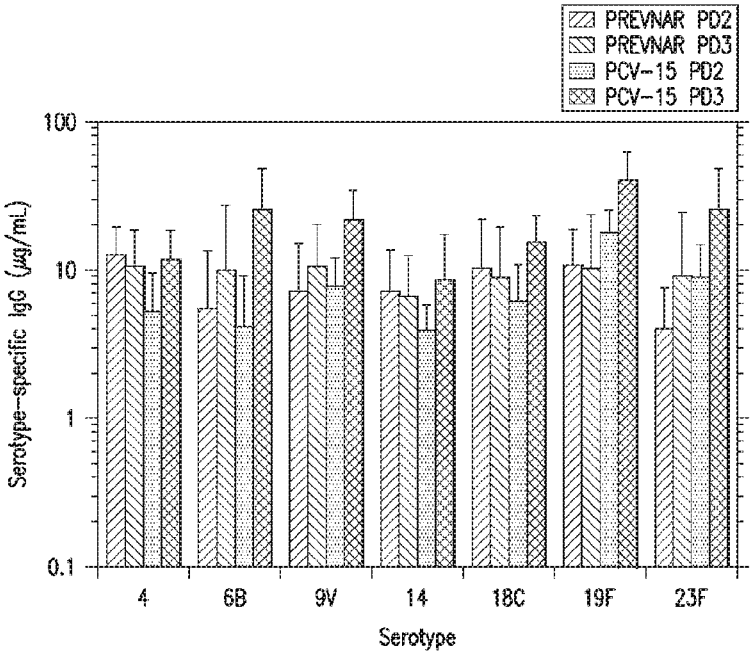


FIG.1

(57) Abstract: The present invention provides a multivalent immunogenic composition having 15 distinct polysaccharide-protein conjugates. Each conjugate consists of a capsular polysaccharide prepared from a different serotype of *Streptococcus pneumoniae* (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F or 33F) conjugated to a carrier protein, preferably CRM197. The immunogenic composition, preferably formulated as a vaccine on an aluminum-based adjuvant, provides broad coverage against pneumococcal disease, particularly in infants and young children.

WO 2011/100151 A1

Petitioner Merck Ex-1004, 0611

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

Declarations under Rule 4.17:

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

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

— *with international search report (Art. 21(3))*

TITLE OF THE INVENTION

15-VALENT PNEUMOCOCCAL POLYSACCHARIDE-PROTEIN CONJUGATE VACCINE COMPOSITION

CROSS-REFERENCE TO RELATED APPLICATIONS

None

FIELD OF INVENTION

The present invention provides a multivalent immunogenic composition having 15 distinct polysaccharide-protein conjugates. Each conjugate consists of a capsular polysaccharide prepared from a different serotype of *Streptococcus pneumoniae* (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F or 33F) conjugated to a carrier protein, preferably CRM_{197}. The immunogenic composition, preferably formulated as a vaccine on an aluminum-based adjuvant, provides broad coverage against pneumococcal disease, particularly in infants and young children.

BACKGROUND OF THE INVENTION

Streptococcus pneumoniae is a significant cause of serious disease world-wide. In 1997, the Centers for Disease Control and Prevention (CDC) estimated there were 3,000 cases of pneumococcal meningitis, 50,000 cases of pneumococcal bacteremia, 7,000,000 cases of pneumococcal otitis media and 500,000 cases of pneumococcal pneumonia annually in the United States. See Centers for Disease Control and Prevention, MMWR Morb Mortal Wkly Rep 1997, 46(RR-8):1-13. Furthermore, the complications of these diseases can be significant with some studies reporting up to 8% mortality and 25% neurologic sequelae with pneumococcal meningitis. See Arditi *et al.*, 1998, Pediatrics 102:1087-97.

The multivalent pneumococcal polysaccharide vaccines that have been licensed for many years have proved valuable in preventing pneumococcal disease in adults, particularly, the elderly and those at high-risk. However, infants and young children respond poorly to unconjugated pneumococcal polysaccharides. The pneumococcal conjugate vaccine, Prevnar®, containing the 7 most frequently isolated serotypes (4, 6B, 9V, 14, 18C, 19F and 23F) causing invasive pneumococcal disease in young children and infants at the time, was first licensed in the United States in February 2000. Following universal use of Prevnar® in the United States, there has been a significant reduction in invasive pneumococcal disease in children due to the

Petitioner Merck Ex-1004, 0613

serotypes present in Prevnar®. See Centers for Disease Control and Prevention, MMWR Morb Mortal Wkly Rep 2005, 54(36):893-7. However, there are limitations in serotype coverage with Prevnar® in certain regions of the world and some evidence of certain emerging serotypes in the United States (for example, 19A and others). See O'Brien *et al.*, 2004, Am J Epidemiol 159:634-44; Whitney *et al.*, 2003, N Engl J Med 348:1737-46; Kyaw *et al.*, 2006, N Engl J Med 354:1455-63; Hicks *et al.*, 2007, J Infect Dis 196:1346-54; Traore *et al.*, 2009, Clin Infect Dis 48:S181-S189.

U.S. Patent Application Publication No. US 2006/0228380 A1 describes a 13-valent pneumococcal polysaccharide-protein conjugate vaccine including serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F. Chinese Patent Application Publication No. CN 101590224 A describes a 14-valent pneumococcal polysaccharide-protein conjugate vaccine including serotypes 1, 2, 4, 5, 6A, 6B, 7F, 9N, 9V, 14, 18C, 19A, 19F and 23F.

Other PCVs have covered 7, 10, 11, or 13 of the serotypes contained in PCV-15, but immune interference has been observed for some serotypes (e.g. lower protection for serotype 3 in GSK's PCV-11) and lower response rates to serotype 6B in Pfizer's PCV-13. See Prymula *et al.*, 2006, Lancet 367:740-48 and Kieninger *et al.*, Safety and Immunologic Non-inferiority of 13-valent Pneumococcal Conjugate Vaccine Compared to 7-valent Pneumococcal Conjugate Vaccine Given as a 4-Dose Series in Healthy Infants and Toddlers, presented at the 48th Annual ICAAC/ISDA 46th Annual Meeting, Washington DC, October 25-28, 2008.

SUMMARY OF THE INVENTION

The present invention provides an immunogenic composition comprising (1) a multivalent polysaccharide-protein conjugate mixture consisting of capsular polysaccharides from 15 different serotypes of *S. pneumoniae* conjugated to a carrier protein, and (2) a pharmaceutically acceptable carrier. More specifically, the present invention provides a 15-valent pneumococcal conjugate vaccine (PCV-15) composition comprising a multivalent polysaccharide-protein conjugate mixture consisting of capsular polysasccharides from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F, and 33F of *S. pneumoniae* conjugated to a carrier protein; and a pharmaceutically acceptable carrier. In one specific embodiment, the immunogenic composition contains capsular polysaccharides from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F and the carrier protein is CRM_{197}.

In certain embodiments, the composition further comprises an adjuvant. In certain embodiments, the adjuvant is an aluminum-based adjuvant, such as aluminum phosphate,

Petitioner Merck Ex-1004, 0614

aluminum sulfate or aluminum hydroxide. In a particular embodiment of the invention, the adjuvant is aluminum phosphate.

The present invention also provides a method of inducing an immune response to a *S. pneumoniae* capsular polysaccharide, comprising administering to a human an immunologically effective amount of the above immunogenic composition.

The present invention further provides an immunogenic composition administered as a single 0.5 mL dose formulated to contain: 2 μg of each polysaccharide, except for 6B at 4 μg; about 32 μg CRM_{197} carrier protein; 0.125 mg of elemental aluminum (0.5 mg aluminum phosphate) adjuvant; 150 mM sodium chloride and 20 mM L-histidine buffer.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Comparison of GMCs for PCV-15 relative to Prevnar® in Infant Rhesus Monkeys (Prevnar serotypes, PD-2 and PD-3). Error bars denote 2 standard errors.

Figure 2: Serotype-specific GMCs to non- Prevnar® serotypes in infant rhesus monkeys immunized with PCV-15. Error bars denote 2 standard errors.

Figure 3: NZWR-1: Comparison of geometric mean titers in rabbits immunized with PCV-15 without (0xA) or with APA (B1) (Post-dose 2). Error bars denote 95% CI on the geometric mean fold-difference (PCV-15 without APA / PCV-15 with APA).

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a multivalent immunogenic composition comprising, consisting essentially of, or alternatively, consisting of 15 distinct polysaccharide-protein conjugates, wherein each of the conjugates contains a different capsular polysaccharide conjugated to a carrier protein, and wherein the capsular polysaccharides are prepared from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F of *S. pneumoniae*, together with a pharmaceutically acceptable carrier. In certain embodiments, the carrier protein is CRM_{197}. The immunogenic composition may further comprise an adjuvant, such as an aluminum-based adjuvant, such as aluminum phosphate, aluminum sulfate and aluminum hydroxide. The present invention also provides a method of inducing an immune response to a *S. pneumoniae* capsular polysaccharide conjugate, comprising administering to a human an immunologically effective amount of the above multivalent immunogenic composition.

As illustrated in the Examples, *infra.*, preclinical studies in infant rhesus monkeys demonstrated robust antibody responses to all 15 serotypes in PCV-15 which are comparable to the responses for the 7 common serotypes in Prevnar®. Applicants' finding that a 15 valent

Petitioner Merck Ex-1004, 0615

pneumococcal conjugate vaccine including the addition of new polysaccharide-protein conjugates containing serotypes 22F and 33F provides robust antibody responses demonstrates the feasibility of expanding coverage of pneumococcal serotypes not covered by existing pneumococcal vaccines.

The term "comprises" when used with the immunogenic composition of the invention refers to the inclusion of any other components (subject to limitations of "consisting of" language for the antigen mixture), such as adjuvants and excipients. The term "consisting of" when used with the multivalent polysaccharide-protein conjugate mixture refers to a mixture having those 15 particular *S. pneumoniae* polysaccharide protein conjugates and no other *S. pneumoniae* polysaccharide protein conjugates from a different serotype.

Streptococcus pneumoniae capsular polysaccharide – protein conjugates

Capsular polysaccharides from *Steptococcus pneumoniae* can be prepared by standard techniques known to those skilled in the art. For example, polysaccharides can be isolated from bacteria and may be sized to some degree by known methods (see, e.g., European Patent Nos. EP497524 and EP497525) and preferably by microfluidisation. Polysaccharides can be sized in order to reduce viscosity in polysaccharide samples and/or to improve filterability for conjugated products. In the present invention, capsular polysaccharides are prepared from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F of *S. pneumoniae*.

In one embodiment, each pneumococcal polysaccharide serotype is grown in a soy-based medium. The individual polysaccharides are then purified through standard steps including centrifugation, precipitation, and ultra-filtration. See, e.g., U.S. Patent Application Publication No. 2008/0286838 and U.S. Pat. No. 5,847,112.

Carrier proteins are preferably proteins that are non-toxic and non-reactogenic and obtainable in sufficient amount and purity. A carrier protein can be conjugated or joined with a *S. pneumoniae* polysaccharide to enhance immunogenicity of the polysaccharide. Carrier proteins should be amenable to standard conjugation procedures. In a particular embodiment of the present invention, CRM_{197} is used as the carrier protein. In one embodiment, each capsular polysaccharide is conjugated to the same carrier protein (each capsular polysaccharide molecule being conjugated to a single carrier protein). In another embodiment, the capsular polysaccharides are conjugated to two or more carrier proteins (each capsular polysaccharide molecule being conjugated to a single carrier protein). In such an embodiment, each capsular polysaccharide of the same serotype is typically conjugated to the same carrier protein.

Petitioner Merck Ex-1004, 0616

CRM_{197} is a non-toxic variant (i.e., toxoid) of diphtheria toxin. In one embodiment, it is isolated from cultures of *Corynebacterium diphtheria* strain C7 (β197) grown in casamino acids and yeast extract-based medium. In another embodiment, CRM_{197} is prepared recombinantly in accordance with the methods described in U.S. Pat. No. 5,614,382. Typically, CRM_{197} is purified through a combination of ultra-filtration, ammonium sulfate precipitation, and ion-exchange chromatography. In some embodiments, CRM_{197} is prepared in *Pseudomonas fluorescens* using Pfenex Expression Technology™ (Pfenex Inc., San Diego, CA).

Other suitable carrier proteins include additional inactivated bacterial toxins such as DT (Diphtheria toxoid), TT (tetanus toxid) or fragment C of TT, pertussis toxoid, cholera toxoid (e.g., as described in International Patent Application Publication No. WO 2004/083251), *E. coli* LT, *E. coli* ST, and exotoxin A from *Pseudomonas aeruginosa*. Bacterial outer membrane proteins such as outer membrane complex c (OMPC), porins, transferrin binding proteins, pneumococcal surface protein A (PspA; See International Application Patent Publication No. WO 02/091998), pneumococcal adhesin protein (PsaA), C5a peptidase from Group A or Group B streptococcus, or *Haemophilus influenzae* protein D, pneumococcal pneumolysin (Kuo *et al.*, 1995, Infect Immun 63; 2706-13) including ply detoxified in some fashion for example dPLY-GMBS (See International Patent Application Publication No. WO 04/081515) or dPLY-formol, PhtX, including PhtA, PhtB, PhtD, PhtE and fusions of Pht proteins for example PhtDE fusions, PhtBE fusions (See International Patent Application Publication Nos. WO 01/98334 and WO 03/54007), can also be used. Other proteins, such as ovalbumin, keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA) or purified protein derivative of tuberculin (PPD), PorB (from *N. meningitidis*), PD (*Haemophilus influenzae* protein D; see, e.g., European Patent No. EP 0 594 610 B), or immunologically functional equivalents thereof, synthetic peptides (See European Patent Nos. EP0378881 and EP0427347), heat shock proteins (See International Patent Application Publication Nos. WO 93/17712 and WO 94/03208), pertussis proteins (See International Patent Application Publication No. WO 98/58668 and European Patent No. EP0471177), cytokines, lymphokines, growth factors or hormones (See International Patent Application Publication No. WO 91/01146), artificial proteins comprising multiple human CD4+ T cell epitopes from various pathogen derived antigens (See Falugi *et al.*, 2001, Eur J Immunol 31:3816-3824) such as N19 protein (See Baraldoi *et al.*, 2004, Infect Immun 72:4884-7), iron uptake proteins (See International Patent Application Publication No. WO 01/72337), toxin A or B of *C. difficile* (See International Patent Publication No. WO

Petitioner Merck Ex-1004, 0617

00/61761), and flagellin (See Ben-Yedidia et al., 1998, Immunol Lett 64:9) can also be used as carrier proteins.

Other DT mutants can be used, such as CRM176, CRM228, CRM 45 (Uchida *et al.*, 1973, J Biol Chem 218:3838-3844); 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 U.S. Pat. No. 4,709,017 or U.S. Pat. No. 4,950,740; mutation of at least one or more residues Lys 516, Lys 526, Phe 530 and/or Lys 534 and other mutations disclosed in U.S. Pat. No. 5,917,017 or U.S. Pat. No. 6,455,673; or fragment disclosed in U.S. Pat. No. 5,843,711.

The purified polysaccharides are chemically activated to make the saccharides capable of reacting with the carrier protein. Once activated, each capsular polysaccharide is separately conjugated to a carrier protein to form a glycoconjugate. The polysaccharide conjugates may be prepared by known coupling techniques.

In one embodiment, the chemical activation of the polysaccharides and subsequent conjugation to the carrier protein are achieved by means described in U.S. Pat. Nos. 4,365,170, 4,673,574 and 4,902,506. Briefly, that chemistry entails the activation of pneumococcal polysaccharide by reaction with any oxidizing agent which oxidizes a terminal hydroxyl group to an aldehyde, such as periodate (including sodium periodate, potassium periodate, or periodic acid). The reaction leads to a random oxidative cleavage of vicinal hydroxyl groups of the carbohydrates with the formation of reactive aldehyde groups.

Coupling to the protein carrier (e.g., CRM_{197}) can be by reductive amination via direct amination to the lysyl groups of the protein. For example, conjugation is carried out by reacting a mixture of the activated polysaccharide and carrier protein with a reducing agent such as sodium cyanoborohydride. Unreacted aldehydes are then capped with the addition of a strong reducing agent, such as sodium borohydride.

In another embodiment, 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 haloacetylated carrier protein (for example using iodoacetimide [e.g. ethyl

Petitioner Merck Ex-1004, 0618

iodoacetimide HCl] or N-succinimidyl bromoacetate or SIAB, or SIA, or SBAP). Preferably, the cyanate ester (optionally made by CDAP chemistry) is coupled with hexane diamine or adipic acid dihydrazide (ADH) and the amino-derivatised saccharide is conjugated to the carrier protein using carbodiimide (e.g. EDAC or EDC) chemistry via a carboxyl group on the protein carrier. Such conjugates are described in International Patent Application Publication Nos. WO 93/15760, WO 95/08348 and WO 96/29094; and Chu *et al.*, 1983, Infect. Immunity 40:245-256.

Other suitable techniques use carbodiimides, hydrazides, active esters, norborane, p-nitrobenzoic acid, N-hydroxysuccinimide, S--NHS, EDC, TSTU. Many are described in International Patent Application Publication No. 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 (See Bethell *et al.*, 1979, J. Biol. Chem. 254:2572-4; Hearn *et al.*, 1981, J. Chromatogr. 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.

In one embodiment, prior to formulation, each pneumococcal capsular polysaccharide antigen is individually purified from *S. pneumoniae*, activated to form reactive aldehydes, and then covalently conjugated using reductive amination to the carrier protein CRM_{197}.

After conjugation of the capsular polysaccharide to the carrier protein, the polysaccharide-protein conjugates are purified (enriched with respect to the amount of polysaccharide-protein conjugate) by one or more of a variety of techniques. Examples of these techniques are well known to the skilled artisan and include concentration/diafiltration operations, ultrafiltration, precipitation/elution, column chromatography, and depth filtration. See, e.g., U.S. Pat. No. 6,146,902.

Pharmaceutical/Vaccine Compositions

The present invention further provides compositions, including pharmaceutical, immunogenic and vaccine compositions, comprising, consisting essentially of, or alternatively, consisting of 15 distinct polysaccharide-protein conjugates, wherein each of the conjugates contains a different capsular polysaccharide conjugated to a carrier protein, and wherein the capsular polysaccharides are prepared from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F of *S. pneumoniae*, together with a pharmaceutically acceptable carrier and

Petitioner Merck Ex-1004, 0619

an adjuvant. These pneumococcal conjugates are prepared by separate processes and bulk formulated into a single dosage formulation.

As defined herein, an "adjuvant" is a substance that serves to enhance the immunogenicity of an immunogenic composition of the invention. An immune adjuvant may enhance an immune response to an antigen that is weakly immunogenic when administered alone, e.g., inducing no or weak antibody titers or cell-mediated immune response, increase antibody titers to the antigen, and/or lowers the dose of the antigen effective to achieve an immune response in the individual. Thus, adjuvants are often given to boost the immune response and are well known to the skilled artisan. Suitable adjuvants to enhance effectiveness of the composition include, but are not limited to:

(1) aluminum salts (alum), such as aluminum hydroxide, aluminum phosphate, aluminum sulfate, etc.;

(2) oil-in-water emulsion formulations (with or without other specific immunostimulating agents such as muramyl peptides (defined below) or bacterial cell wall components), such as, for example, (a) MF59 (International Patent Application Publication No. WO 90/14837), containing 5% Squalene, 0.5% Tween 80, and 0.5% Span 85 (optionally containing various amounts of MTP-PE) formulated into submicron particles using a microfluidizer such as Model 110Y microfluidizer (Microfluidics, Newton, MA), (b) SAF, containing 10% Squalene, 0.4% Tween 80, 5% pluronic-blocked polymer L121, and thr-MDP either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion, (c) Ribi™ adjuvant system (RAS), (Corixa, Hamilton, MT) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of 3-O-deaylated monophosphorylipid A (MPL™) described in U.S. Pat. No. 4,912,094, trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL+CWS (Detox™); and (d) a Montanide ISA;

(3) saponin adjuvants, such as Quil A or STIMULON™ QS-21 (Antigenics, Framingham, MA) (see, e.g., U.S. Pat. No. 5,057,540) may be used or particles generated therefrom such as ISCOM (immunostimulating complexes formed by the combination of cholesterol, saponin, phospholipid, and amphipathic proteins) and Iscomatrix® (having essentially the same structure as an ISCOM but without the protein);

(4) bacterial lipopolysaccharides, synthetic lipid A analogs such as aminoalkyl glucosamine phosphate compounds (AGP), or derivatives or analogs thereof, which are available from Corixa, and which are described in U.S. Pat. No. 6,113,918; one such AGP is 2-[(R)-3-

Petitioner Merck Ex-1004, 0620

tetradecanoyloxytetradecanoylamino]ethyl 2-Deoxy-4-O-phosphono-3-O-[(R)-3-tetradecanoyloxytetradecanoyl]-2-[(R)-3-- tetradecanoyloxytetradecanoylamino]-b-D-glucopyranoside, which is also known as 529 (formerly known as RC529), which is formulated as an aqueous form or as a stable emulsion

(5) synthetic polynucleotides such as oligonucleotides containing CpG motif(s) (U.S. Pat. No. 6,207,646); and

(6) cytokines, such as interleukins (e.g., IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, IL-15, IL-18, etc.), interferons (e.g., gamma interferon), granulocyte macrophage colony stimulating factor (GM-CSF), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF), costimulatory molecules B7-1 and B7-2, etc; and

(7) complement, such as a trimer of complement component C3d.

In another embodiment, the adjuvant is a mixture of 2, 3, or more of the above adjuvants, e.g.,. SBAS2 (an oil-in-water emulsion also containing 3-deacylated monophosphoryl lipid A and QS21).

Muramyl peptides include, but are not limited to, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanine-2-(1'-2' dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (MTP-PE), etc.

In certain embodiments, the adjuvant is an aluminum salt. The aluminum salt adjuvant may be an alum-precipitated vaccine or an alum-adsorbed vaccine. Aluminum-salt adjuvants are well known in the art and are described, for example, in Harlow, E. and D. Lane (1988; Antibodies: A Laboratory Manual Cold Spring Harbor Laboratory) and Nicklas, W. (1992; Aluminum salts. Research in Immunology 143:489-493). The aluminum salt includes, but is not limited to, hydrated alumina, alumina hydrate, alumina trihydrate (ATH), aluminum hydrate, aluminum trihydrate, alhydrogel, Superfos, Amphogel, aluminum (III) hydroxide, aluminum hydroxyphosphate sulfate (Aluminum Phosphate Adjuvant (APA)), amorphous alumina, trihydrated alumina, or trihydroxyaluminum.

APA is an aqueous suspension of aluminum hydroxyphosphate. APA is manufactured by blending aluminum chloride and sodium phosphate in a 1:1 volumetric ratio to precipitate aluminum hydroxyphosphate. After the blending process, the material is size-reduced with a high-shear mixer to achieve a target aggregate particle size in the range of 2-8 μm. The product is then diafiltered against physiological saline and steam sterilized.

In certain embodiments, a commercially available $Al(OH)_3$ (e.g. Alhydrogel or Superfos of Denmark/Accurate Chemical and Scientific Co., Westbury, NY) is used to adsorb

Petitioner Merck Ex-1004, 0621

proteins in a ratio of 50 - 200 g protein/mg aluminum hydroxide. Adsorption of protein is dependent, in another embodiment, on the pI (Isoelectric pH) of the protein and the pH of the medium. A protein with a lower pI adsorbs to the positively charged aluminum ion more strongly than a protein with a higher pI. Aluminum salts may establish a depot of Ag that is released slowly over a period of 2-3 weeks, be involved in nonspecific activation of macrophages and complement activation, and/or stimulate innate immune mechanism (possibly through stimulation of uric acid). See, e.g., Lambrecht *et al.*, 2009, Curr Opin Immunol 21:23.

Monovalent bulk aqueous conjugates are typically blended together and diluted to target 8 μg/mL for all serotypes except 6B, which will be diluted to target 16 μg/mL. Once diluted, the batch will be filter sterilized, and an equal volume of aluminum phosphate adjuvant added aseptically to target a final aluminum concentration of 250 μg/mL. The adjuvanted, formulated batch will be filled into single-use, 0.5 mL/dose vials.

In certain embodiments, the adjuvant is a CpG-containing nucleotide sequence, for example, a CpG-containing oligonucleotide, in particular, a CpG-containing oligodeoxynucleotide (CpG ODN). In another embodiment, the adjuvant is ODN 1826, which may be acquired from Coley Pharmaceutical Group.

"CpG-containing nucleotide," "CpG-containing oligonucleotide," "CpG oligonucleotide," and similar terms refer to a nucleotide molecule of 6-50 nucleotides in length that contains an unmethylated CpG moiety. See, e.g., Wang *et al.*, 2003, Vaccine 21:4297. In another embodiment, any other art-accepted definition of the terms is intended. CpG-containing oligonucleotides include modified oligonucleotides using any synthetic internucleoside linkages, modified base and/or modified sugar.

Methods for use of CpG oligonucleotides are well known in the art and are described, for example, in Sur *et al.*, 1999, J Immunol. 162:6284-93; Verthelyi, 2006, Methods Mol Med. 127:139-58; and Yasuda *et al.*, 2006, Crit Rev Ther Drug Carrier Syst. 23:89-110.

Administration/Dosage

The compositions and formulations of the present invention can be used to protect or treat a human susceptible to pneumococcal infection, by means of administering the vaccine via a systemic or mucosal route. In one embodiment, the present invention provides a method of inducing an immune response to a *S. pneumoniae* capsular polysaccharide conjugate, comprising administering to a human an immunologically effective amount of an immunogenic composition of the present invention. In another embodiment, the present invention provides a method of

Petitioner Merck Ex-1004, 0622

vaccinating a human against a pneumococcal infection, comprising the step of administering to the human an immunogically effective amount of a immunogenic composition of the present invention.

"Effective amount" of a composition of the invention refers to a dose required to elicit antibodies that significantly reduce the likelihood or severity of infectivitiy of *S. pneumoniae* during a subsequent challenge.

The methods of the invention can be used for the prevention and/or reduction of primary clinical syndromes caused by *S. pneumoniae* including both invasive infections (meningitis, pneumonia, and bacteremia), and noninvasive infections (acute otitis media, and sinusitis).

Administration of the compositions of the invention can include one or more of: injection via the intramuscular, intraperitoneal, intradermal or subcutaneous routes; or via mucosal administration to the oral/alimentary, respiratory or genitourinary tracts. In one embodiment, intranasal administration is used for the treatment of pneumonia or otitis media (as nasopharyngeal carriage of pneumococci can be more effectively prevented, thus attenuating infection at its earliest stage).

The amount of conjugate in each vaccine dose is selected as an amount that induces an immunoprotective response without significant, adverse effects. Such amount can vary depending upon the pneumococcal serotype. Generally, each dose will comprise 0.1 to 100 µg of each polysaccharide, particularly 0.1 to 10 µg, and more particularly 1 to 5 µg. For example, each dose can comprise 100, 150, 200, 250, 300, 400, 500, or 750 ng or 1, 1.5, 2, 3, 4, 5, 6, 7, 7.5, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 20, 22, 25, 30, 40, 50, 60, 70, 80, 90, or 100 µg.

Optimal amounts of components for a particular vaccine can be ascertained by standard studies involving observation of appropriate immune responses in subjects. For example, in another embodiment, the dosage for human vaccination is determined by extrapolation from animal studies to human data. In another embodiment, the dosage is determined empirically.

In one embodiment, the dose of the aluminum salt is 10, 15, 20, 25, 30, 50, 70, 100, 125, 150, 200, 300, 500, or 700 µg, or 1, 1.2, 1.5, 2, 3, 5 mg or more. In yet another embodiment, the dose of alum salt described above is per µg of recombinant protein.

In a particular embodiment of the present invention, the PCV-15 vaccine is a sterile liquid formulation of pneumococcal capsular polysaccharides of serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F individually conjugated to CRM_{197}. Each 0.5

Petitioner Merck Ex-1004, 0623

mL dose is formulated to contain: 2 μg of each saccharide, except for 6B at 4 μg; about 32 μg CRM_{197} carrier protein (e.g., 32 μg ± 5 μg, ± 3 μg, ± 2 μg, or ± 1 μg); 0.125 mg of elemental aluminum (0.5 mg aluminum phosphate) adjuvant; and sodium chloride and L-histidine buffer. The sodium chloride concentration is about 150 mM (e.g., 150 mM ± 25 mM, ± 20 mM, ± 15 mM, ± 10 mM, or ± 5 mM) and about 20 mM (e..g, 20 mM ± 5 mM, ± 2.5 mM, ± 2 mM, ± 1 mM, or ± 0.5 mM) L-histidine buffer.

According to any of the methods of the present invention and in one embodiment, the subject is human. In certain embodiments, the human patient is an infant (less than 1 year of age), toddler (approximately 12 to 24 months), or young child (approximately 2 to 5 years). In other embodiments, the human patient is an elderly patient (> 65 years). The compositions of this invention are also suitable for use with older children, adolescents and adults (e.g., aged 18 to 45 years or 18 to 65 years).

In one embodiment of the methods of the present invention, a composition of the present invention is administered as a single inoculation. In another embodiment, the vaccine is administered twice, three times or four times or more, adequately spaced apart. For example, the composition may be administered at 1, 2, 3, 4, 5, or 6 month intervals or any combination thereof. The immunization schedule can follow that designated for pneumococcal vaccines. For example, the routine schedule for infants and toddlers against invasive disease caused by *S. pneumoniae* is 2, 4, 6 and 12-15 months of age. Thus, in a preferred embodiment, the composition is administered as a 4-dose series at 2, 4, 6, and 12-15 months of age.

The compositions of this invention may also include one or more proteins from *S. pneumoniae*. Examples of *S. pneumoniae* proteins suitable for inclusion include those identified in International Patent Application Publication Nos. WO 02/083855 and WO 02/053761.

Formulations

The compositions of the invention can be administered to a subject by one or more method known to a person skilled in the art, such as parenterally, transmucosally, transdermally, intramuscularly, intravenously, intra-dermally, intra-nasally, subcutaneously, intra-peritonealy, and formulated accordingly.

In one embodiment, compositions of the present invention are administered via epidermal injection, intramuscular injection, intravenous, intra-arterial, subcutaneous injection, or intra-respiratory mucosal injection of a liquid preparation. Liquid formulations for injection include solutions and the like.

Petitioner Merck Ex-1004, 0624

The composition of the invention can be formulated as single dose vials, multi-dose vials or as pre-filled syringes.

In another embodiment, compositions of the present invention are administered orally, and are thus formulated in a form suitable for oral administration, i.e., as a solid or a liquid preparation. Solid oral formulations include tablets, capsules, pills, granules, pellets and the like. Liquid oral formulations include solutions, suspensions, dispersions, emulsions, oils and the like.

Pharmaceutically acceptable carriers for liquid formulations are aqueous or non-aqueous solutions, suspensions, emulsions or oils. Examples of nonaqueous solvents are propylene glycol, polyethylene glycol, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Examples of oils are those of animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, olive oil, sunflower oil, fish-liver oil, another marine oil, or a lipid from milk or eggs.

The pharmaceutical composition may be isotonic, hypotonic or hypertonic. However it is often preferred that a pharmaceutical composition for infusion or injection is essentially isotonic, when it is administrated. Hence, for storage the pharmaceutical composition may preferably be isotonic or hypertonic. If the pharmaceutical composition is hypertonic for storage, it may be diluted to become an isotonic solution prior to administration.

The isotonic agent may be an ionic isotonic agent such as a salt or a non-ionic isotonic agent such as a carbohydrate. Examples of ionic isotonic agents include but are not limited to NaCl, $CaCl_2$, KCl and $MgCl_2$. Examples of non-ionic isotonic agents include but are not limited to mannitol, sorbitol and glycerol.

It is also preferred that at least one pharmaceutically acceptable additive is a buffer. For some purposes, for example, when the pharmaceutical composition is meant for infusion or injection, it is often desirable that the composition comprises a buffer, which is capable of buffering a solution to a pH in the range of 4 to 10, such as 5 to 9, for example 6 to 8.

The buffer may for example be selected from the group consisting of TRIS, acetate, glutamate, lactate, maleate, tartrate, phosphate, citrate, carbonate, glycinate, histidine, glycine, succinate and triethanolamine buffer.

The buffer may furthermore for example be selected from USP compatible buffers for parenteral use, in particular, when the pharmaceutical formulation is for parenteral use. For example the buffer may be selected from the group consisting of monobasic acids such as acetic,

Petitioner Merck Ex-1004, 0625

benzoic, gluconic, glyceric and lactic; dibasic acids such as aconitic, adipic, ascorbic, carbonic, glutamic, malic, succinic and tartaric, polybasic acids such as citric and phosphoric; and bases such as ammonia, diethanolamine, glycine, triethanolamine, and TRIS.

Parenteral vehicles (for subcutaneous, intravenous, intraarterial, or intramuscular injection) include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's and fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers such as those based on Ringer's dextrose, and the like. Examples are sterile liquids such as water and oils, with or without the addition of a surfactant and other pharmaceutically acceptable adjuvants. In general, water, saline, aqueous dextrose and related sugar solutions, glycols such as propylene glycols or polyethylene glycol, and Polysorbate-80 are preferred liquid carriers, particularly for injectable solutions. Examples of oils are those of animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, olive oil, sunflower oil, fish-liver oil, another marine oil, or a lipid from milk or eggs.

The formulations of the invention may also contain a surfactant. Preferred surfactants include, but are not limited to: the polyoxyethylene sorbitan esters surfactants (commonly referred to as the Tweens), especially polysorbate 20 and polysorbate 80; copolymers of ethylene oxide (EO), propylene oxide (PO), and/or butylene oxide (BO), sold under the DOWFAX™ tradename, such as linear EO/PO block copolymers; octoxynols, which can vary in the number of repeating ethoxy (oxy-1,2-ethanediyl) groups, with octoxynol-9 (Triton X-100, or t-octylphenoxypolyethoxyethanol) being of particular interest; (octylphenoxy)polyethoxyethanol (IGEPAL CA-630/NP-40); phospholipids such as phosphatidylcholine (lecithin); nonylphenol ethoxylates, such as the Tergitol™ NP series; polyoxyethylene fatty ethers derived from lauryl, cetyl, stearyl and oleyl alcohols (known as Brij surfactants), such as triethyleneglycol monolauryl ether (Brij 30); and sorbitan esters (commonly known as the SPANs), such as sorbitan trioleate (Span 85) and sorbitan monolaurate. A preferred surfactant for including in the emulsion is Tween 80 (polyoxyethylene sorbitan monooleate).

Mixtures of surfactants can be used, e.g. Tween 80/Span 85 mixtures. A combination of a polyoxyethylene sorbitan ester such as polyoxyethylene sorbitan monooleate (Tween 80) and an octoxynol such as t-octylphenoxypolyethoxyethanol (Triton X-100) is also suitable. Another useful combination comprises laureth 9 plus a polyoxyethylene sorbitan ester and/or an octoxynol.

Preferred amounts of surfactants (% by weight) are: polyoxyethylene sorbitan esters (such as Tween 80) 0.01 to 1%, in particular about 0.1 %; octyl- or nonylphenoxy

Petitioner Merck Ex-1004, 0626

polyoxyethanols (such as Triton X-100, or other detergents in the Triton series) 0.001 to 0.1 %, in particular 0.005 to 0.02%; polyoxyethylene ethers (such as laureth 9) 0.1 to 20 %, preferably 0.1 to 10 % and in particular 0.1 to 1 % or about 0.5%.

In another embodiment, the pharmaceutical composition is delivered in a controlled release system. For example, the agent can be administered using intravenous infusion, a transdermal patch, liposomes, or other modes of administration. In another embodiment, polymeric materials are used; e.g. in microspheres in or an implant.

Also comprehended by the invention are compounds modified by the covalent attachment of water-soluble polymers such as polyethylene glycol, copolymers of polyethylene glycol and polypropylene glycol, carboxymethyl cellulose, dextran, polyvinyl alcohol, polyvinylpyrrolidone or polyproline. Such modifications may increase the compound's solubility in aqueous solution, eliminate aggregation, enhance the physical and chemical stability of the compound, and greatly reduce the reactogenicity of the compound. In another embodiment, the desired *in vivo* biological activity is achieved by the administration of such polymer-compound abducts less frequently or in lower doses than with the unmodified compound.

In a preferred embodiment, the vaccine composition is formulated in L-histidine buffer with sodium chloride.

Having described various embodiments of the invention with reference to the accompanying description and drawings, it is to be understood that the invention is not limited to those precise embodiments, and that various changes and modifications may be effected therein by one skilled in the art without departing from the scope or spirit of the invention as defined in the appended claims.

The following examples illustrate, but do not limit the invention.

EXAMPLES

EXAMPLE 1: Preparation of *S. Pneumoniae* Capsular Polysaccharides

Methods of culturing pneumococci are well known in the art. See, e.g., Chase, 1967, Methods of Immunology and Immunochemistry 1:52. Methods of preparing pneumococcal capsular polysaccharides are also well known in the art. See, e.g., European Patent No. EP0497524. Isolates of pneumococcal subtypes are available from the ATCC.

Petitioner Merck Ex-1004, 0627

The bacteria are identified as encapsulated, non-motile, Gram-positive, lancet-shaped diplococci that are alpha-hemolytic on blood-agar. Subtypes are differentiated on the basis of Quelling reaction using specific antisera. See, e.g., U.S. Pat. No. 5,847,112.

Cell Banks

Cell banks representing each of the *S. pneumococcus* serotypes present in PCV-15 were obtained from the Merck Culture Collection (Rahway, NJ) in a frozen vial.

Inoculation

A thawed seed culture was transferred to the seed fermentor containing an appropriate pre-sterilized growth media.

Seed Fermentation

The culture was grown in the seed fermentor with temperature and pH control. The entire volume of the seed fermentor was transferred to the production fermentor containing pre-sterilized growth media.

Production Fermentation

The production fermentation was the final cell growth stage of the process. Temperature, pH and the agitation rate was controlled.

Inactivation

The fermentation process was terminated via the addition of an inactivating agent. After inactivation, the batch was transferred to the inactivation tank where it was held at controlled temperature and agitation.

Purification

Cell debris was removed using a combination of centrifugation and filtration. The batch was ultrafiltered and diafiltered. The batch was then subjected to solvent-based fractionations that remove impurities and recover polysaccharide.

EXAMPLE 2: Preparation of Pneumococcal Polysaccharide-CRM_{197} Conjugates

Activation Process

The different serotype saccharides are individually conjugated to the purified CRM_{197} carrier protein using a common process flow. In this process the saccharide is dissolved, sized to a target molecular mass, chemically activated and buffer-exchanged by ultrafiltration. The purified CRM_{197} is then conjugated with the activated saccharide and the resulting conjugate is purified by ultrafiltration prior to a final 0.2 μm membrane filtration. Several process

Petitioner Merck Ex-1004, 0628

parameters within each step, such as pH, temperature, concentration, and time are serotype-specific as described in this example.

Step 1: Dissolution

Purified polysaccharide was dissolved in water to a concentration of 2 – 3 mg/mL. The dissolved polysaccharide was passed through a mechanical homogenizer with pressure preset from 0-1000 bar. Following size reduction, the saccharide was concentrated and diafiltered with sterile water on a 10 kDa MWCO ultrafilter. The permeate was discarded and the retentate was adjusted to a pH of 4.1 with a sodium acetate buffer, 50 mM final concentration. For serotypes 4 and 5, 100 mM sodium acetate at pH 5.0 was used. For serotype 4, the solution was incubated at 50° ± 2° C. Hydrolysis was stopped by cooling to 20 - 24° C.

Step 2: Periodate Reaction

The required sodium periodate molar equivalents for pneumococcal saccharide activation was determined using total saccharide content. With thorough mixing, the oxidation was allowed to proceed between 3 – 20 hours at 20 - 24° C for all serotypes except 5, 7F, and 19F for which the temperature was 2 - 6° C.

Step 3: Ultrafiltration

The oxidized saccharide was concentrated and diafiltered with 10 mM potassium phosphate, pH 6.4 (10 mM sodium acetate, pH 4.3 for serotype 5) on a 10 kDa MWCO ultrafilter. The permeate was discarded and the retentate was adjusted to a pH of 6.3 – 8.4 by addition of 3 M potassium phosphate buffer.

Conjugation Process

Step 1: Conjugation Reaction

The concentrated saccharide was mixed with CRM_{197} carrier protein in a 0.2 – 2 to 1 charge ratio. The blended saccharide-CRM_{197} mixture was filtered through a 0.2 μm filter.

The conjugation reaction was initiated by adding a sodium cyanoborohydride solution to achieve 1.8 – 2.0 moles of sodium cyanoborohydride per mole of saccharide. The reaction mixture was incubated for 48 – 120 hours at 20 - 24° C (8 - 12° C for serotypes 3, 5, 6A, 7F, 19A, and 19F).

Petitioner Merck Ex-1004, 0629

Step 2: Borohydride Reaction

At the end of the conjugation incubation the reaction mixture was adjusted to 4 - 8° C, and a pH of 8 – 10 with either 1.2 M sodium bicarbonate buffer or 3 M potassium phosphate buffer (except serotype 5). The conjugation reaction was stopped by adding the sodium borohydride solution to achieve 0.6 – 1.0 moles of sodium borohydride per mole of saccharide (0 moles of borohydride added for serotype 5). The reaction mixture was incubated for 45 – 60 minutes.

Step 3: Ultrafiltration Steps

The reaction mixture was diafiltered on a 100 kDa MWCO ultrafilter with a minimum of 20 volumes of 100 mM potassium phosphate, pH 8.4 buffer. The retentate from the 100 kDa ultrafilter was diafiltered on a 300 kDa MWCO ultrafilter with a minimum of 20 diavolumes of 150 mM sodium chloride at 20 - 24° C. The permeate was discarded.

Step 4: Sterile Filtration

The retentate from the 300 kDa MWCO diafiltration was filtered through a 0.2 μm filter and filled into borosilicate glass containers at appropriate volumes for release testing, in-process controls, and formulation (except serotype 19F). The serotype 19F conjugate was passed through a 0.2 μm filter into a holding tank and incubated at 20 - 24°C. Following incubation, the conjugate was diafiltered on a 300 kDa MWCO ultrafilter with a minimum of 20 diavolumes of 150 mM sodium chloride at 20 - 24° C. The permeate was discarded, and the retentate was filtered through a 0.2 μm filter and filled into borosilicate glass containers at appropriate volumes for release testing, in-process controls, and formulation. The final bulk concentrates were stored at 2 - 8° C.

EXAMPLE 3: Formulation of a 15-valent Pneumococcal Conjugate Vaccine

The required volumes of bulk concentrates were calculated based on the batch volume and the bulk saccharide concentrations. The combined 15 conjugates were further diluted to a target adsorption concentration by the addition of a sodium chloride and L-histidine, pH 5.8, containing buffer. After sufficient mixing, the blend was sterile filtered through a 0.2 μm membrane. The sterile formulated bulk was mixed gently during and following its blending with bulk aluminum phosphate. The formulated vaccine was stored at 2 – 8° C.

In an alternate process, the combined 15 conjugates were further diluted to a target concentration by the addition of a sodium chloride and L-histidine, pH 5.8, containing buffer. Polysorbate 80 was added to a final concentration of 0.005%, to the diluted buffered conjugate

Petitioner Merck Ex-1004, 0630

mixture prior to sterile filtration. Following sterile filtration, the formulated vaccine was stored at 2 – 8° C.

Table 1 shows the final composition of the adjuvanted and non-adjuvanted form of PCV-15.

Table 1: Composition of Adjuvanted and Non-Adjuvanted 15—valent Pneumococcal Conjugate Vaccine Formulations

Description of Ingredients		Clinical Formulations, unit/0.5 mL dose	
		Adjuvanted PCV-15	Non-adjuvanted PCV-15
Active Ingredients	Pneumococcal polysaccharide antigens	32 µg of total polysaccharide (2 µg of each of the following polysaccharide serotypes 1, 3, 4, 5, 6A, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F, 33F; 4 µg of serotype 6B polysaccharide)	32 µg of total polysaccharide (2 µg of each of the following polysaccharide serotypes 1, 3, 4, 5, 6A, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F, 33F; 4 µg of serotype 6B polysaccharide))
	Carrier protein CRM_{197}	~ 32 µg	~32µg
Other Ingredients	Aluminum (µg) [a]	125	0
	Polysorbate-80 (µg)	0	2.5
	L-histidine (mM)	20	20
	Sodium Chloride (mM)	150	150
	Water for Injection	Q.S. [b]	Q.S. [b]
[a] Quantity of elemental aluminum in APA. [b] Quantity sufficient to 0.5 mL.			

EXAMPLE 4: Immunogenicity Studies

Experiments were designed to evaluate the immunogenicity of multiple formulations of pneumococcal conjugate vaccines in the infant rhesus monkeys (IRM) and New Zealand White Rabbits (NZWR) animal models. Experiments in infant rhesus monkeys were designed to closely match the recommended schedule for pneumococcal conjugate vaccine in United States, with the infant series given at 2, 4, and 6 months of age. Thus, infant rhesus

Petitioner Merck Ex-1004, 0631

monkeys were immunized starting at 2-3 months of age and administered vaccine at 2-month intervals. The 4th dose, which is also part of the recommended schedule for U.S. children was not administered. Adult rabbits (NZWR) were used to evaluate multiple vaccine formulations. NZWR studies were performed using two vaccine doses given in an accelerated (2-week interval) immunization regimen. For the preclinical evaluation of immune responses, a full human dose was delivered to rabbits whereas infant monkeys received a half-human dose. The rationale for selecting a half-human dose for infant monkeys was due to limitations in the volume that can be administered to infant rhesus monkeys in a single intramuscular site.

Assessment of Serotype-specific IgG Responses

A multiplexed electrochemiluminescence (ECL) assay was developed for use with rabbit and rhesus monkey serum based on a human assay using Meso Scale Discovery (MSD) technology which utilizes a SULFO-TAG™ label that emits light upon electrochemical stimulation. See Marchese *et al.*, 2009, Clin Vaccine Immunol 16:387-96. Using a dedicated ECL plate reader, an electrical current is placed across the plate-associated electrodes resulting in a series of electrically induced reactions leading to luminescent signal. The multi-spot configuration used in development and validation was 10 spots/well in a 96-well plate – format, and each well was coated with 5 ng pneumococcal (Pn) polysaccharide (Ps) per spot. Two plate formats were used to ensure that crossreacting polysaccharides (i.e., 6A and 6B, and 19A and 19F) were tested in separate plates. Plate format 1 contained serotypes 3, 4, 6B, 9V, 14, 18C, 19F, and 23F whereas plate format 2 contained serotypes 1, 5, 6A, 7F, 19A, 22F and 33F. Each well also contained two bovine serum albumin (BSA) spots which were used to assess the background reactivity of the assay (i.e., the response associated with serum and labeled secondary antibody in the absence of PnPs). Assay standard (89SF-2), controls, and test sera were diluted to appropriate levels in phosphate buffered saline (PBS) containing 0.05% Tween 20, 1% BSA, 5 µg/ml C-polysaccharide (CPs), 10 µg/ml serotype 25 polysaccharide (PnPs25) and 10 µg/ml serotype 72 polysaccharide (PnPs72) and incubated overnight at 4°C (2 to 8°C) or at ambient temperature for 45 minutes. Human antibody reagents and standards were used when testing the infant monkey samples whereas SULFO-TAG™-labeled anti-rabbit IgG was used as the secondary antibody when testing rabbit serum samples. Each antigen coated plate was incubated at ambient temperature for 1 hour on a shaker platform with blocking agent. Plates were washed with 0.05% PBS-T and 25 µL per well of the pre-adsorbed and diluted test sera was added and incubated for 45 min at ambient temperature on a shaker platform. Plates were washed with 0.05% PBS-T and then MSD SULFO-TAG™ labeled-goat anti-human IgG

Petitioner Merck Ex-1004, 0632

secondary antibody (for rhesus monkey serum) and labeled goat anti-rabbit IgG secondary antibody (for rabbit serum) was added to each well and incubated 1 hour at ambient temperature on a shaker platform. Plates were washed with 0.05% PBS-T and 150µL of MSD Read Buffer-T 4X (with surfactant) diluted 1:4 in water added to each well. The plates were read using a MSD Sector Imager Model No. 2400 or 6000. For rabbit studies, the results are presented as geometric mean titers (GMTs) or ratios of GMTs. For infant rhesus monkey studies, the results were expressed as geometric mean concentrations read from a standard curve using the serotype-specific IgG concentrations assigned to the human reference standard (89 SF-2).

Assessment of Functional (Opsonophagocytic) Responses

Samples from infant rhesus monkey study 2 were tested in a 4-plexed MOPA assay (MOPA-4). See Burton *et al.*, 2006, Clin Vaccine Immunol 13:1004-9. The assay uses bacterial strains selected to be resistant to one of 4 antibiotics so that the first part of the assay (opsonization and uptake into differentiated HL-60 cells) can be performed with up to 4 serotypes at a time. The read-out for bacterial killing is done in parallel in the presence of each of the 4 antibiotics to which the corresponding strains are resistant in order to determine killing titers for each specific serotype. Results are expressed as the reciprocal dilution at which 50% killing is observed (after interpolation).

Statistical Methodology for Preclinical Studies

Both animal models have limitations related to sample size. In general, 8 infant monkeys or 8 rabbits were used per study arm. With 8 animals per arm a critical fold difference in geometric mean titer between treatment arms of 2.5 fold was regarded as a meaningful response threshold. The 2.5-fold difference was determined based on the assumption that for each serotype, the standard deviation of the natural log transformed titers within a treatment arm is $ln(2)$. Letting $\bar{Y}_i$ denote the mean of the ln transformed titers in the ith treatment arm, n_i the number of animals within the ith treatment arm, σ_i^2 the known variance of the ln transformed titers among animals within the ith treatment arm, and setting $n_i = 8$ and $\sigma_i^2 = (ln(2))^2$ for all i, then the value of 2.5 is obtained by solving for $e^{\bar{Y}_j - \bar{Y}_k}$ where $\frac{\bar{Y}_j - \bar{Y}_k}{\sqrt{\frac{\sigma_j^2}{n_j} + \frac{\sigma_k^2}{n_k}}} = Z_{0.995}$, and $Z_{0.995}$ denotes the inverse of the standard normal cumulative distribution, with a probability of 0.995 (i.e., $Z_{0.995} = 2.576$). Note that the calculated value of 2.44 is rounded to 2.5 as 2.5 also provides for a convenient reciprocal in 0.4.

Petitioner Merck Ex-1004, 0633

Serotype-specific IgG Response of Infant Rhesus Monkeys (IRMs) to PCV-15

A pilot immunogenicity study (IRM-1) was conducted to determine whether infant rhesus monkeys (IRMs) would be a good model in which to evaluate Pn polysaccharide CRM_{197} conjugate vaccines. The primary goal of the experiment was to determine whether IRMs (like human infants) would be unresponsive to free Pn polysaccharides but respond well to conjugate vaccines. Groups of 5 IRMs were injected starting at 2-3 months of age with either Pn polysaccharide, Prevnar® or PCV-15. Three doses of vaccine were administered intramuscularly (IM) at 2 month intervals, and serotype-specific IgG responses were measured prior to the first dose and at 1 month postdose 2 and at 1 month postdose 3 using a multiarray electrochemiluminescence (ECL) assay (data not shown).

The results indicated that IRMs responded poorly, if at all, to free Pn polysaccharide but very well to the conjugate vaccines. The results indicated that induction of an IgG response to Pn polysaccharides in infant rhesus monkeys was dependent upon conjugation of the polysaccharides to a carrier protein and therefore was a classic T-cell dependent response. Thus, the IRM model was determined to be suitable for evaluating PCV-15 formulations.

A second study (IRM-2) was conducted to evaluate a formulation of PCV-15 using a bulk conjugation process that minimized free (unconjugated) polysaccharide and unconjugated CRM_{197}. Figure 1 shows the postdose 2 (PD-2) and postdose 3 (PD-3) IgG responses to PCV-15 versus Prevnar® for the 7 serotypes contained in Prevnar® (4, 6B, 9V, 14, 18C, 19F, 23F). PD-2 responses to PCV-15 were equivalent or slightly lower than the corresponding responses to Prevnar® whereas PD-3 responses to PCV-15 were somewhat higher than those elicited by Prevnar® for nearly all serotypes.

IRM responses to the non-Prevnar serotypes in PCV-15 are shown in Figure 2. PD-2 responses to the non-Prevnar serotypes in PCV-15 were all at least 10-fold higher than baseline (pre-vaccination) IgG concentrations, and titers continued to rise at PD-3.

The results indicate that antibody responses to PCV-15 and Prevnar® were comparable for the 7 common serotypes and that post-vaccination responses to PCV-15 were > 10-fold higher than baseline for the 8 added serotypes.

Functional (Opsonophagocytic) Immune Response of IRMs to PCV-15

In order to determine whether PCV-15 induced functional antibody responses in infant monkeys, an opsonophagocytic killing (OPA) assay was performed on sera from IRM-2. Pre-vaccination, PD-2, and PD-3 responses to PCV-15 and Prevnar® are shown in Table 2. The results shown are the GMTs from serum samples from 7-8 monkeys per time point assayed in

Petitioner Merck Ex-1004, 0634

duplicate. Also shown are the percent responders (i.e., those with OPA titers ≥ 8) at the PD-3 time point. PCV-15 induced a high PD-2 GMT for all serotypes except types 1 and 33F. After 3 vaccine doses, PCV-15 induced high OPA GMTs to each serotype and a 100% OPA response rate for all 15 serotypes contained in the vaccine. Of note, PCV-15 also induced a good crossreactive OPA response to serotype 6C, which is not in the vaccine. Prevnar® induced high OPA titers and a 100% response rate for all serotypes contained in that vaccine, but it induced only a weak crossreactive response to serotypes 6A and 6C in a fraction of monkeys.

Table 2: Serotype-Specific OPA GMTs in Infant Rhesus Monkeys after Vaccination with PCV-15 or Prevnar®
(Pre-vaccination, PD-2, and PD-3 geometric mean titers and PD-3 percent responders with a titer ≥8)

	Prevnar				PCV-15			
Serotype	**Pre**	**PD-2**	**PD-3**	**PD-3 Responders (titers ≥ 8)**	**Pre**	**PD-2**	**PD-3**	**PD-3 Responders (titers ≥ 8)**
1	n.d.	n.d.	n.d.	n.d.	4	65	340	100%
3	n.d.	n.d.	n.d.	n.d.	5	1442	1548	100%
4	4	11459	5004	100%	4	5280	3453	100%
5	4	4	4	0%	4	1879	1719	100%
6A	4	21	113	57%	4	1188	7807	100%
6B	8	8294	6043	100%	4	2477	9601	100%
6C	*4*	*11*	*16*	*29%*	*4*	*1038*	*5134*	*100%*
7F	4	4	71	43%	4	7541	10092	100%
9V	4	1779	748	100%	4	625	1297	100%
14	8	12395	7782	100%	4	11366	9891	100%
18C	4	5571	1718	100%	4	1934	1701	100%
19A	4	15	4	0%	4	2210	1895	100%
19F	4	1365	432	100%	4	2555	4021	100%
22F	n.d.	n.d.	n.d.	n.d.	4	2489	7298	100%
23F	4	1789	2126	100%	4	3093	2465	100%
33F	n.d.	n.d.	n.d.	n.d.	4	14	11548	100%

Note: Serotypes contained in Prevnar are bolded. Results for serotype 6C are shown in italics since that serotype is not contained in PCV-15.
n.d. not determined

Evaluation of PCV-15 Formulations in New Zealand White Rabbits

PCV-15 formulations were evaluated in 4 studies in adult New Zealand White Rabbits (NZWRs) using a compressed immunization regimen in which rabbits received a full human dose of vaccine at day 0 and day 14, and serum was collected at day 0, 14 and 28 for

Petitioner Merck Ex-1004, 0635

analysis. All studies were benchmarked with Prevnar®, and as summarized in Table 3 (NZWR Experiments 1-4).

Results are shown in Table 3 for Post-dose 2 responses of New Zealand white rabbits expressed as a ratio of the geometric mean IgG responses to Merck PCV-15 over Prevnar® for serotypes in common between the vaccines.

Table 3

Post-dose 2 IgG Response Ratios (PCV-15:Prevnar™) of Lead PCV-15 Formulations Tested in NZWR

Serotype	NZWR-1	NZWR-2	NZWR-3	NZWR-4
4	0.70	0.59	0.63	1.06
6B	1.35	0.49	1.53	0.45
9V	2.07	1.79	1.70	1.31
14	2.37	0.58	2.32	2.55
18C	0.87	0.6	0.52	0.27
19F	0.66	0.76	2.70	1.25
23F	0.36	0.30	1.22	0.41

Serotype-specific IgG responses were generally within 2.5-fold of the corresponding responses to Prevnar®. An exception was serotype (23F), which was > 2.5-fold lower than that to Prevnar® in 2 of 4 experiments. The fold-rise in antibody levels to the non-Prevnar® serotypes from Day 0 to Day 28 (Post-dose 2, PD-2) are summarized in Table 4.

Table 4

Fold-rise (Post-dose 2:Pre-dose 1) in IgG Responses to Non-Prevnar™ Serotypes of PCV-15 Lead Formulations Tested in NZWR

Serotype	NZWR-1	NZWR-2	NZWR-3	NZWR-4
1	14.9	30.5	55.1	59.9
3	33.6	16.2	61.5	28.5
5	12.8	70.2	112.0	134.0
6A	21.3	77.8	143.0	123.0
7F	42.0	83.8	194.0	108.0
19A	40.5	79.1	450.0	314.0
22F	45.7	87.8	243.0	135.0

Petitioner Merck Ex-1004, 0636

33F	21.7	47.9	98.8	69.4

Effect of Polysaccharide Conjugate Vaccine Dose on Immunogenicity in NZWRs

The immunogenicity of an increased dose (double dose, 2x) of polysaccharide conjugates was also evaluated for all serotypes contained in PCV-15 compared with the planned human dose (1x) of the vaccine. For the 2x polysaccharide conjugate formulation, the APA concentration was increased to 1.5x in order to assure that most of the conjugate would be bound to the aluminum adjuvant. As shown in Table 5, there did not appear to be a significant benefit in increasing the amount of polysaccharide-conjugate in the vaccine. Differences across all serotypes were within 2-fold., and the geometric mean fold-ratio (1x PCV-15/2X PCV-15 +1.5X APA) was 1.1.

Table 5

Post-dose 2 Geometric Mean IgG titers (95% confidence intervals) with Prevnar®, 1x Human dose of PCV-15* or 2x Human Dose of PCV-15† in NZWR

	Treatment Arm			Fold-Difference
Serotype	Prevnar™ (n=8)	1x PCV-15 (n=8)	2x PCV-15 (n=8)	Ratio of 2x PCV-15/1x PCV-15
4	**736,400** (483200, 1122400)	**436,000** (199700, 951700)	**472,100** (246800, 902900)	**1.1**
6B	**363,600** (205000, 644800)	**176,500** (72800, 427800)	**196,900** (85900, 451400)	**1.1**
9V	**298,200** (173800, 511700)	**534,700** (362800, 788100)	**580,600** (366300, 920200)	**1.1**
14	**345,200** (200200, 595000)	**198,900** (94500, 418700)	**273,600** (229500, 326100)	**1.4**
18C	**954,500** (815700, 1116800)	**573,000** (396900, 827400)	**455,900** (245100, 848000)	**0.8**
19F	**720,100** (475700, 1090200)	**548,000** (367700, 816800)	**544,300** (269000, 1101400)	**1.0**
23F	**816,300** (565100, 1179100)	**246,200** (117200, 517100)	**188,500** (78100, 454800)	**0.8**
1	**5,300**	**91,500**	**72,200**	**0.8**

Petitioner Merck Ex-1004, 0637

	(3100, 9200)	(62600, 133600)	(46700, 111600)	
3	**12,000**	**32,300**	**23,600**	**0.7**
	(8600, 16800)	(19600, 53000)	(14100, 39400)	
5	**5,700**	**245,600**	**224,600**	**0.9**
	(4100, 7900)	(114200, 528200)	(136700, 369100)	
6A	**525,900**	**186,700**	**251,800**	**1.3**
	(275000, 1005600)	(71300, 488600)	(102300, 620100)	
7F	**4,600**	**326,900**	**212,200**	**0.6**
	(4000, 5200)	(238000, 449000)	(134200, 335500)	
19A	**432,800**	**260,900**	**276,100**	**1.1**
	(237800, 787800)	(145500, 468000)	(153200, 497600)	
22F	**6,000**	**359,800**	**345,300**	**1.0**
	(4400, 8100)	(239000, 541700)	(221200, 539000)	
33F	**6,600**	**177,400**	**138,500**	**0.8**
	(4700, 9300)	(118300, 266200)	(68300, 280900)	

*Formulated with 1x aluminum adjuvant (APA)

† Formulated with 1.5x APA

Effect of Aluminum Adjuvant on Immunogenicity of PCV-15 in NZWRs

The impact of aluminum adjuvant (APA) on antibody responses was evaluated in one rabbit study. PCV-15 formulated with the planned human dose of APA (PCV-15 1x APA), with double the planned human dose of APA (PCV-15 2x APA), and without any aluminum adjuvant (PCV-15 0x APA), were tested. A Prevnar® group was also included in the study.

The PD-2 results indicated that doubling the concentration of APA had little impact on the serotype-specific IgG response to PCV-15. The fold-difference in titer (1x APA/2x APA) ranged from 0.6 (serotype 6B) to 2.3 (serotype 22F) and geometric mean fold-ratio across the 15 serotypes was 1.1. In the absence of aluminum adjuvant antibody titers appeared lower for many of the serotypes relative to PCV-15 with 1x APA. The fold-difference in titer (1x/0x) ranged from 0.5 (serotype 5) to 2.9 (serotype 23F) and the geometric mean fold-ratio across the 15 serotypes was 1.4. Overall, there does not appear to be a genuine advantage to doubling the level of aluminum adjuvant and there appears to be a disadvantage to eliminating the adjuvant (Table 6) in this animal model.

The PD-2 results indicated that there was a decrease in antibody titers for many of the serotypes in the arm that did not contain Aluminum Phosphate Adjuvant (APA) when compared to PCV-15 containing APA (Figure 3) indicating a requirement for the inclusion of an

Petitioner Merck Ex-1004, 0638

aluminum adjuvant for optimal PCV-15 immunogenicity in rabbits. In addition, no benefit was found when double the amount of APA was included in the vaccine (data not shown).

Petitioner Merck Ex-1004, 0639

Table 6

Post-dose 2 Geometric Mean IgG Titers (95% confidence intervals) of PCV-15 Formulated with 1x, 2x or 0x Aluminum Adjuvant (APA) in NZWR

	Treatment Arm				Fold Difference Relative to PCV-15 (1x APA)	
Sero	Prevnar™ (n=8)	PCV-15 1x APA (n=8)	PCV-15 2x APA (n=8)	PCV-15 0x APA (n=6)	1x/2x APA	1x/0x APA
4	**380,700** (226100, 641000)	**268,400** (205100, 351200)	**196,200** (84000, 458800)	**404,600** (105500, 1550700)	**1.4**	**0.7**
6B	**105,900** (59800, 187400)	**143,300** (90700, 226300)	**236,200** (102000, 547100)	**93,800** (46300, 189700)	**0.6**	**1.5**
9V	**271,600** (177400, 415700)	**562,000** (382700, 825400)	**595,600** (390200, 908900)	**569,200** (239900,1350500)	**0.9**	**1.0**
14	**142,500** (75200, 269700)	**337,600** (174000, 654900)	**453,000** (237400, 864400)	**167,600** (65100, 431700)	**0.7**	**2.0**
18C	**323,000** (250400, 416500)	**282,200** (210400, 378500)	**248,700** (193900, 319000)	**175,200** (91800, 334000)	**1.1**	**1.6**
19F	**302,500** (232700, 393200)	**199,800** (132200, 302100)	**243,400** (87600, 676600)	**127,700** (35100, 463700)	**0.8**	**1.6**
23F	**328,200** (178200, 604300)	**117,200** (60300, 227700)	**77,600** (31300, 192300)	**40,200** (10100, 160900)	**1.5**	**2.9**

Petitioner Merck Ex-1004, 0640

1	**5,600**	**91,700**	**67,800**	**53,300**	**1.4**	**1.7**
	(4400, 7200)	(46800, 179800)	(38900, 118100)	(14800, 191600)		
3	**6,300**	**156,100**	**155,700**	**104,000**	**1.0**	**1.5**
	(4800, 8100)	(104800, 232500)	(75300, 322200)	(45000, 240000)		
5	**4,800**	**81,700**	**60,700**	**176,100**	**1.3**	**0.5**
	(4000, 5700)	(50800, 131300)	(29100, 126400)	(72100, 430300)		
6A	**84,500**	**163,600**	**226,800**	**99,300**	**0.7**	**1.6**
	(50300, 141900)	(114000, 234900)	(92500, 556200)	(48800, 202000)		
7F	**5,200**	**216,800**	**282,100**	**212,200**	**0.8**	**1.0**
	(3300, 8400)	(141500, 332000)	(158200, 503200)	(64200, 701900)		
19A	**95,100**	**238,300**	**207,400**	**125,700**	**1.1**	**1.9**
	(54300, 166700)	(161000, 352700)	(69800, 615900)	(34000, 465100)		
22F	**6,500**	**348,900**	**149,400**	**336,800**	**2.3**	**1.0**
	(4900, 8500)	(267200, 455500)	(90100, 247600)	(184200, 615700)		
33F	**7,100**	**235,600**	**163,600**	**222,000**	**1.4**	**1.1**
	(5000, 10100)	(106500, 521300)	(92700, 288800)	(125100, 393800)		

Petitioner Merck Ex-1004, 0641

Discussion and Conclusions

The preclinical data demonstrate that a formulation of PCV-15 (formulated on APA) is highly immunogenic in two species (infant rhesus monkeys and rabbits). Serotype-specific responses to PCV-15 were comparable to those elicited by Prevnar® for the 7 serotypes in common between the vaccines. For the 8 new serotypes in PCV-15, there was a robust response elicited in both infant rhesus monkeys and in rabbits, with ≥10-fold rise in IgG responses for all serotypes after 2 vaccine doses in both species. Limited dose-ranging experiments indicated that a 2-fold increase in the amount of polysaccharide conjugates did not result in an increased antibody response. Similarly, a 2-fold increase in aluminum adjuvant concentration did not appear to significantly improve the immunogenicity profile of PCV-15. Elimination of the adjuvant did, however, result in lower responses to some serotypes suggesting the potential need for an adjuvant in humans. Functional (OPA) antibody responses were elicited by PCV-15 to all 15 serotypes in the vaccine as well as to Serotype 6C, which is not a component of PCV-15.

Petitioner Merck Ex-1004, 0642

WHAT IS CLAIMED IS:

1. An immunogenic composition comprising:

(1) a multivalent polysaccharide-protein conjugate mixture consisting of capsular polysaccharides from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F, and 33F of *Streptococcus pneumoniae* conjugated to a carrier protein; and

(2) a pharmaceutically acceptable carrier.

2. The immunogenic composition of claim 1, wherein the carrier protein is CRM_{197}.

3. The immunogenic composition of claim 1, further comprising an adjuvant.

4. The immunogenic composition claim 3, wherein the adjuvant is an aluminum-based adjuvant.

5. The immunogenic composition of claim 4, wherein the adjuvant is selected from the group consisting of aluminum phosphate, aluminum sulfate and aluminum hydroxide.

6. The immunogenic composition of claim 5, wherein the adjuvant is aluminum phosphate.

7. A method of inducing an immune response to a *Streptococcus pneumoniae* capsular polysaccharide, comprising administering to a human an immunologically effective amount of the immunogenic composition of claim 1.

8. The method of claim 7, wherein the immunogenic composition administered is a single 0.5 mL dose formulated to contain: 2 μg of each saccharide, except for 6B at 4 μg; about 32 μg CRM_{197} carrier protein; 0.125 mg of elemental aluminum (0.5 mg aluminum phosphate) adjuvant; 150 mM sodium chloride and 20 mM L-histidine buffer.

Petitioner Merck Ex-1004, 0643

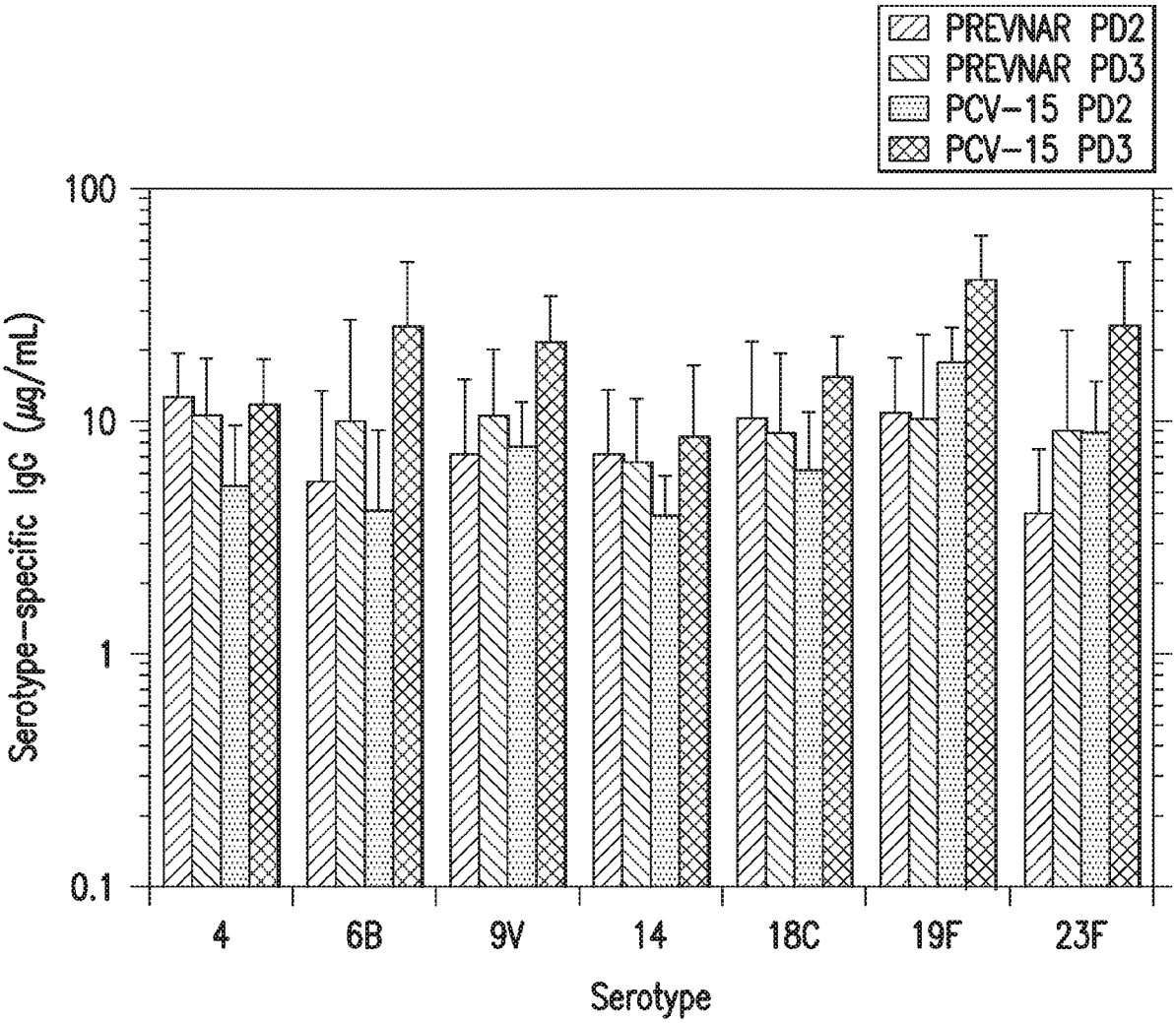

PREVNAR PD2
PREVNAR PD3
PCV-15 PD2
PCV-15 PD3
100
10
1
0.1
Serotype-specific IgG (μg/mL)
4
6B
9V
14
18C
19F
23F
Serotype

FIG. 1

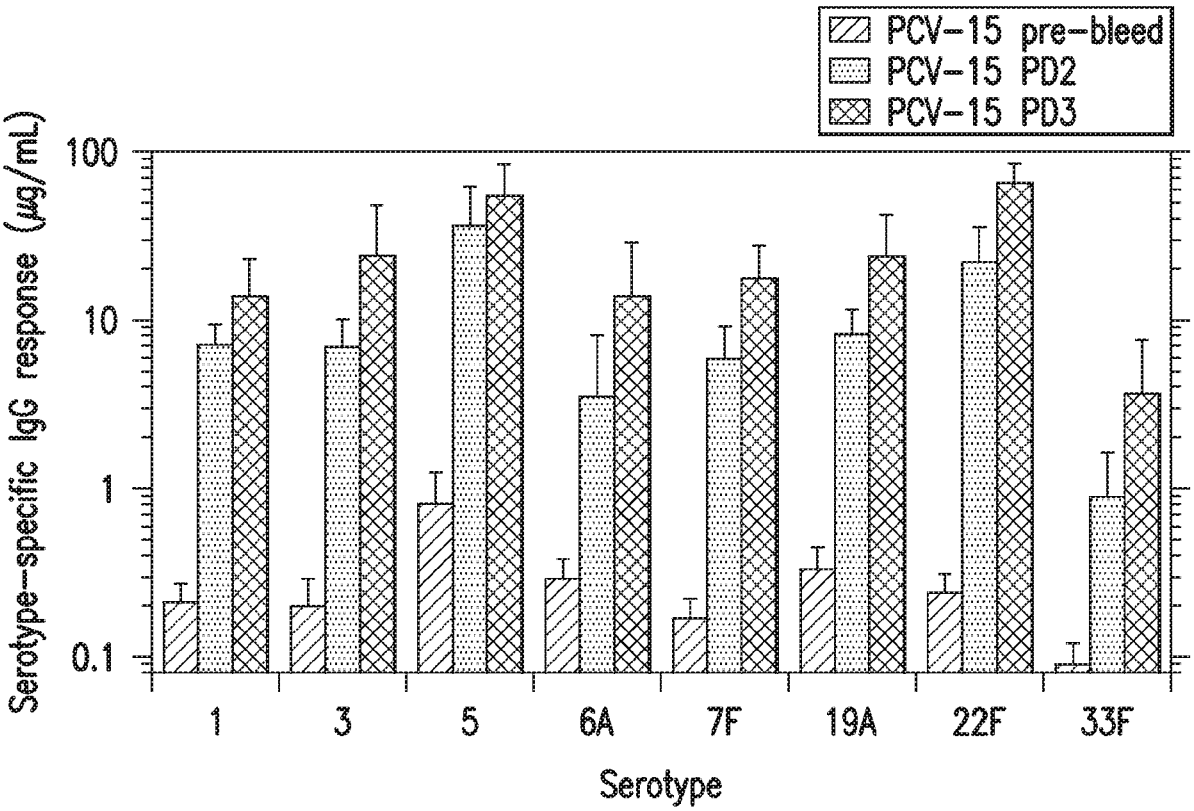

PCV-15 pre-bleed
PCV-15 PD2
PCV-15 PD3
Serotype-specific IgG response (μg/mL)
100
10
1
0.1
1
3
5
6A
7F
19A
22F
33F
Serotype

FIG. 2

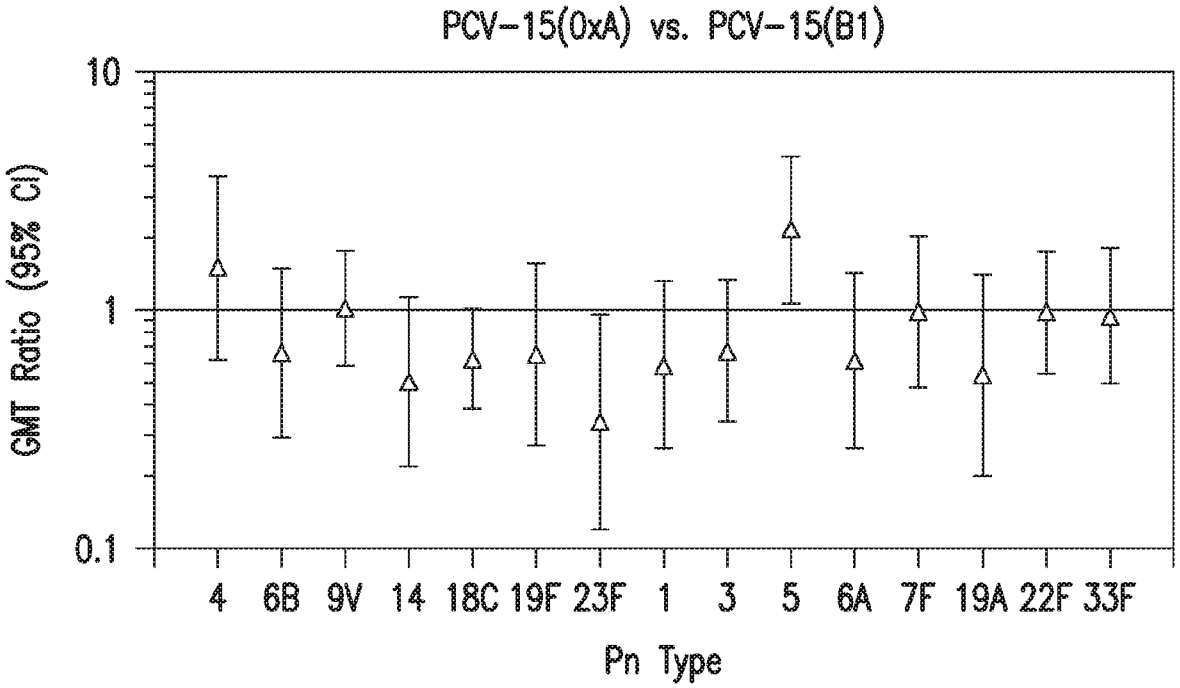

PCV-15(0xA) vs. PCV-15(B1)
GMT Ratio (95% CI)
10
1
0.1
4
6B
9V
14
18C
19F
23F
1
3
5
6A
7F
19A
22F
33F
Pn Type

FIG.3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 11/23526

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/00 (2011.01)
USPC - 424/184.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC (8) - A61K 39/00 (2011.01)
USPC - 424/184.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 424/184.1,244,1,203,1,197.11 (see search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST (PGPB,USPT,USOC,EPAB,JPAB); Google
Search Terms Used: multivalent, capsular, polysaccharide, conjugate, serotype, Streptococcus pneumoniae, CRM197, aluminum phosphate, histidine, buffer, 33F, 6B

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2009/0010959 A1 (Biemans et al.) 08 January 2009 (08.01.2009), para [0023]-[0028], [0063], [0084], [0129], [0143], [0212]	1-7 --- 8
Y	US 2009/0035326 A1 (Contorni et al.) 05 February 2009 (05.02.2009), para [0078]-[0089]	8

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 March 2011 (21.03.2011)	04 APR 2011
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

Petitioner Merck Ex-1004, 0647

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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
29 December 2016 (29.12.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/207905 A2

(51) International Patent Classification:
A61K 39/09 (2006.01) *C07K 16/12* (2006.01)
A61K 47/48 (2006.01)

(21) International Application Number:
PCT/IN2016/000157

(22) International Filing Date:
21 June 2016 (21.06.2016)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
3140/CHE/2015 23 June 2015 (23.06.2015) IN

(71) Applicant: **BIOLOGICAL E LIMITED** [IN/IN]; 18/1 & 3, Azamabad, Hyderabad - 500020, Telangana (IN).

(72) Inventors: **RAMESH, Venkat, Matur**; Biological E Ltd., 18/1 & 3, Azamabad, Hyderabad - 500020, Telangana (IN). **THIRUMENI, Nagarajan**; Biological E Ltd., 18/1 & 3, Azamabad, Hyderabad - 500020, Telangana (IN). **RAJENDAR, Burke**; Biological E Ltd., 18/1 & 3, Azamabad, Hyderabad - 500020, Telangana (IN). **NARENDER, Dev, Mantena**; Biological E Ltd., 18/1 & 3, Azamabad, Hyderabad - 500020, Telangana (IN). **MAHIMA, Datla**; Biological E Ltd., 18/1 & 3, Azamabad, Hyderabad - 500020, Telangana (IN).

(74) Agent: **MANDHANE, Ramachandra**; c/o. M/s. Kayser & Company, Raja Bahadur Mansion, 2nd floor, 20, Ambalal Doshi Marg, Mumbai 400001, Maharashtra (IN).

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

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

(54) Title: MULTIVALENT PNEUMOCOCCAL CONJUGATE VACCINE

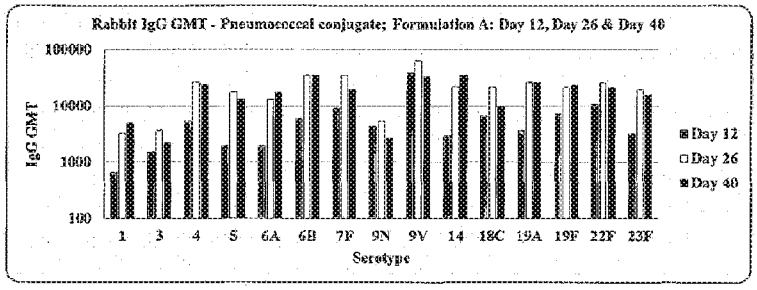


Figure 1

(57) Abstract: The present invention relates to a multivalent Pneumococcal conjugate vaccine (PCV) composition comprising: 1) at least 12 capsular polysaccharides selected from serotypes 1, 3, 4, 5, 6B, 7F, 9N, 9V, 15B, 14, 18C, 19A, 19F, 22F, 23F and 33F of *S. pneumoniae* activated with CDAP and conjugated to carrier protein selected from CRM_{197}, pneumococcal surface protein A (PspA), pneumococcal adhesin protein (PsaA) or combination thereof and 2) a pharmaceutically acceptable carrier, wherein the composition does not contain capsular polysaccharide from serotype 6A.

WO 2016/207905 A2

Petitioner Merck Ex-1004, 0648

Multivalent Pneumococcal Conjugate Vaccine

Field of Invention

The present invention relates to novel multivalent Pneumococcal conjugate vaccine composition and a method for the production thereof.

Background of Invention

Streptococcus pneumoniae (pneumococcus) is a Gram-positive bacteria responsible for considerable morbidity and mortality (particularly in the young and aged), causing invasive diseases such as pneumonia, bacteremia and meningitis, and diseases associated with colonization, such as acute Otitis media. The rate of pneumococcal pneumonia in the U.S. for persons over 60 years of age is estimated to be 3 to 8 per 100,000. In 20% of cases this leads to bacteremia, and other manifestations such as meningitis, with a mortality rate close to 30% even with antibiotic treatment.

Pneumococcus is encapsulated with a chemically linked polysaccharide which confers serotype specificity. There are more than 90 known serotypes of pneumococci, and capsule is the principal virulence determinant for pneumococci, as the capsule not only protects the inner surface of the bacteria from complement, but is itself poorly immunogenic.

Pneumococcal vaccines includes pneumococcal polysaccharide vaccine and pneumococcal conjugate vaccines. It is generally accepted that the protective efficacy of the commercialized pneumococcal polysaccharide vaccine is more or less related to the concentration of antibody induced upon vaccination; indeed, the 23 polysaccharides were approved and marketed under the trade name Pneumovax®23 by Merck solely upon the immunogenicity of each component polysaccharide. Pneumovax®23 comprises unconjugated polysaccharides belonging to serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F and 33F.

Petitioner Merck Ex-1004, 0649

The multivalent pneumococcal polysaccharide vaccines that have been licensed for many years were proved valuable in preventing pneumococcal disease in adults, particularly, the elderly and those at high-risk. However, infants and young children respond poorly to unconjugated pneumococcal polysaccharides. The pneumococcal conjugate vaccine, Prevnar®, containing the 7 most frequently isolated serotypes (4, 6B, 9V, 14, 18C, 19F and 23F) causing invasive pneumococcal disease (IPD) in young children and infants at the same time, was first licensed in the United States in February 2000. Following universal use of Prevnar®-7 in the United States, there has been a significant reduction in IPD in children due to the serotypes present in Prevnar®-7. Due to the limitations in serotype coverage with Prevnar®-7 in certain regions of the world, a 13-valent conjugate vaccine was developed and approved under the trade name Prevenar-13® containing serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F conjugated to CRM_{197}. The activation of the polysaccharides is accomplished by partial oxidation of adjacent (vicinal) hydroxyl groups in the carbohydrate repeat units using sodium periodate or periodic acid.

Ten valent pneumococcal vaccine was approved under the trade name Synflorix® containing polysaccharide serotypes 1, 4, 5, 6B, 7, 9, 14, 23F conjugated to protein D (PD), serotype 18C conjugated to tetanus toxoid (TT) and serotype 19F conjugated to diphtheria toxoid (DT). For conjugate preparation, coupling of each of the *S. pneumoniae* serotype polysaccharides to either PD, DT or TT is done utilizing CDAP (1-cyano-4-dimethylamino-pyridinium tetrafluoroborate) as chemical reagent under controlled pH.

U.S. Patent 5,360,897 disclosed immunogenic conjugate comprising reductive amination product of an intact capsular polymer of the bacterial pathogen *S. pneumoniae* having at least two carbonyl groups and a bacterial toxin or toxoid, said conjugate comprising a cross-linked conjugate in which there is a direct covalent linkage between the capsular polymer and the toxin or toxoid.

Petitioner Merck Ex-1004, 0650

U.S. Patent 7,862,823 claims a multivalent conjugate vaccine composition with at least two different carrier proteins.

U.S. Patent 7,955,605 disclosed a process of making immunogenic conjugate consisting 19A where the activated serotype 19A polysaccharide and carrier protein are re-suspended in dimethyl sulfoxide (DMSO) to form conjugate.

U.S. Patent 8,603,484 disclosed a method of making multivalent immunogenic composition where serotype 3 is reacted with a mild acid to hydrolyze it, this hydrolyzed serotype is reacted with oxidizing agent in the presence of bivalent cations resulting in an activated serotype 3 and then it is conjugated with a carrier protein, which involves reacting this activated products with a reducing agent resulting in a serotype 3 polysaccharide: carrier protein conjugate.

U.S. Patent 8,808,708 B2 disclosed a 13-valent immunogenic composition consisting polysaccharide-protein conjugates where serotypes consist of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F, and wherein the carrier protein is CRM_{197}.

U.S. Patent publication 2009/0017059 A1 disclosed an immunogenic composition where serotypes 19A and 19F are conjugated to different bacterial toxoids.

U.S. Patent publication 2010/0074922 A1 disclosed immunogenic composition containing 10 or more serotypes wherein 19F capsular saccharide is conjugated to diphtheria toxoid (DT), serotype 18C capsular saccharide is conjugated to tetanus toxoid and serotypes 1, 4, 5, 6B, 7F, 9V, 14 and 23F capsular saccharides are conjugated to Protein D from *Haemophilus influenzae*.

U.S. patent publication US 2010/0239604 disclosed composition comprising multivalent *S. pneumoniae* capsular saccharide conjugates wherein serotype 19A is conjugated to a first bacterial toxoid and 19F is conjugated to a second bacterial toxoid and 2-9 of the *S. pneumoniae* capsular saccharides are conjugated to protein D.

Petitioner Merck Ex-1004, 0651

US Patent publication 2012/321658 A1 (2010) disclosed an immunogenic composition wherein serotypes 1, 3, 19A and 19F linked to a protein carrier(s) either directly or indirectly through a chemistry other than reductive amination, and one or more different saccharides is/are selected from a second group consisting of serotypes 4, 5, 6A, 6B, 7F, 9V, 14, 18C and 23F which is/are linked to a protein carrier(s) by reductive amination.

U.S. Patent 8,192,746 disclosed a multivalent immunogenic composition having capsular polysaccharides from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F, and 33F conjugated to CRM_{197}.

WO 2013/191459 A1 disclosed a conjugated 15 valent composition comprising different serotypes of *S. pneumoniae* derived from a capsular polysaccharide 1, 2, 3, 4, 5, 6A, 6B, 7F, 9N, 9V, 14, 18C, 19A, 19F and 23F.

WO 2014/092378 A1 disclosed an immunogenic conjugate composition where 12 serotypes are selected from 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F and remaining one from 22F or 33F.

WO 2014/092377 A1 disclosed a 13 valent composition wherein 12 serotypes are selected from 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F and the last serotype is either 12 or 9N.

Chinese Patent Application Publication No. CN 101590224 described a 14-valent pneumococcal polysaccharide-protein conjugate vaccine containing serotypes 1, 2, 4, 5, 6A, 6B, 7F, 9N, 9V, 14, 18C, 19A, 19F and 23F.

Chinese Patent Application Publication No. CN 103623401 disclosed 14 multivalent pneumococcal capsular polysaccharide - protein conjugate composition wherein said 14 different blood serotype is 1, 3, 4, 5,6A, 6B, 9V, 14,18C, 19A, 19F, 22F, 23F and 33F.

Petitioner Merck Ex-1004, 0652

Chinese Patent Application Publication No. CN 103656632 A disclosed multivalent pneumococcal capsular polysaccharide composition containing serotype 6A and at least one extra serotype selected from the group consisting of 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F. The multivalent pneumococcal capsular polysaccharide composition provided herein can be used for inducing an organism to generate humoral immunity and can generate a relatively good protecting effect for infectious diseases caused by the 24 common serotype of pneumococci.

Chinese Patent Application Publication No. CN 103656631 A disclosed multivalence pneumococcus capsular polysaccharide-protein conjugate composition and a preparation method thereof. The conjugate composition is prepared from capsular polysaccharides of pneumococcus of 24 different serotypes and a carrier protein in a covalent linkage manner, wherein the 24 different serotypes are 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.

Chinese Patent Application Publication No. CN 104069488 A disclosed multivalent pneumococcus capsular polysaccharides of 14 different serotypes and carrier protein, wherein the 14 serotypes include 1, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F.

Cyanylating agents are well known in art (Wilcheck et al., Affinity chromatography. Meth. Enzymol., 104C:3-55; Wakelsman et al., J.C.S Chem. Comm., 1976:21 (1976). It was reported as a mild reagent that can be used for modifying protein cysteine groups. Whereas it was Kohn et al., (Anal. Biochem, 115:375, 1981) who compared CDAP, N-cuanotriethyl-ammonium tetrafluoroborate (CTEA) and p-nitrophenylcyanate (Pnpc). This comparison was done to compare the activation of agarose by these agents. CDAP has also been used to activate insoluble particles such as Sepharose and glyceryl-controlled pore glass (Carpenter et al., Journal of Chromatography, 573:132-135, 1992)

Petitioner Merck Ex-1004, 0653

U.S. Patent 5,693,326 gives a generalised method for preparing a conjugate vaccine wherein for activating viral, fungal or bacterial polysaccharides, organic cyanylating agent is used selected from the group 1-cyano-4-(dimethylamino)-pyridinium tetrafluoroborate, N-cyanotriethyl-ammonium tetrafluoroborate, and p-nitrophenylcyanate, to form an activated carbohydrate and then coupled to the protein or carrier protein.

U.S. Patent 8,465,749 disclosed a method for preparing a conjugate vaccine by reacting a polysaccharide with CDAP and reacting a protein with hydrazine or adipic acid dihydrazide with specific pH range.

U.S. Patent 8,557,250 B2 disclosed a method which comprises contacting the mixture of the plurality of cyanate activated immunogenic distinct polysaccharides with at least one hydrazide activated protein.

Anderson P et al, (2003, Vaccine; 21 (13-14):1554-9) discloses a comparative study of tetravalaent conjugate vaccines with each polysaccharide types 6A, 14, 19F, and 23F separately coupled to tetanus toxoid or diphtheria CRM_{197} or a mixture of halved doses of polysaccharide types 6A, 14, 19F, and 23F separately coupled to tetanus toxoid and diphtheria CRM_{197}.

Anticipating multivalent polysaccharide conjugate vaccines of the future to be used in infancy, this strategy would have two hypothetical advantages worth further investigation—avoiding "carrier epitopic overload" by reducing each carrier dosage and recruiting T-helper activity by both carriers for each polysaccharide.

Wuorimaa et al. (2001, The Paediatric Infectious Disease Journal, Volume 20(3), pp 272-277) discloses a study to assess the tolerability and immunogenicity in healthy toddlers of an 11-valent pneumococcal conjugate vaccine that uses both tetanus and diphtheria toxoids as carriers.

Petitioner Merck Ex-1004, 0654

Gatchalian et al. (2001, 17th Annual Meeting of the Eur. Soc. Paed. Inf. Dis (ESPID), poster number 4, P1A Poster Session 1, Istanbul Turkey) discloses OPA results from infants who had received doses of the 11-valent vaccine failed to show antibody responses for serotype 3 at levels comparable to other tested serotypes.

Nurkka et al. (2004, Ped. Inf. Dis. J., 23:1008-1014) discloses a study of the immunogenicity and safety of an 11-valent pneumococcal protein D conjugate vaccine where no priming effect was observed for serotype 3 in infants who had received three doses of the vaccine followed by a booster dose of either the same vaccine or a pneumococcal polysaccharide vaccine.

The above mentioned references disclosed polysaccharides belonging to various serotypes and their conjugation with carrier proteins following different methods. But there is a need to have a new serotype composition in view of the serotype prevalence of a given region and their production in a simple and efficient manner. The inventors of the present invention found that the combination of serotypes 22F and 33F together with other serotypes without 6A improves the immunogenicity towards serotypes.

Objective of the Invention

The main objective of the present invention is to provide a novel multivalent polysaccharide conjugate vaccine.

Yet another objective of the present invention is to provide a process for preparing the novel multivalent polysaccharide conjugate vaccine.

Summary of Invention

The present invention relates to a multivalent Pneumococcal conjugate vaccine (PCV) composition comprising:

1) at least 12 capsular polysaccharides selected from serotypes 1, 3, 4, 5, 6B, 7F, 9N, 9V, 15B, 14, 18C, 19A, 19F, 22F, 23F and 33F of *S. pneumoniae* activated with CDAP and conjugated to carrier protein selected from CRM_{197},

Petitioner Merck Ex-1004, 0655

pneumococcal surface protein A (PspA), pneumococcal adhesin protein (PsaA) or combination thereof and

2) a pharmaceutically acceptable carrier, wherein the composition does not contain capsular polysaccharide from serotype 6A.

The present invention relates to a multivalent Pneumococcal conjugate vaccine (PCV) composition comprising:

(1) at least 14 capsular polysaccharides selected from serotypes 1, 3, 4, 5, 6B, 7F, 9N, 9V, 14, 15B, 18C, 19A, 19F, 22F, 23F and 33F of *S. pneumoniae* activated with CDAP and conjugated to CRM_{197}, PspA, PsaA or combination thereof and

(2) a pharmaceutically acceptable carrier, wherein the composition does not contain capsular polysaccharide from serotype 6A.

Brief description of the Drawings

Figure 1. Titre of IgG to individual pneumococcal capsular polysaccharides induced by Formulation A in rabbits as measured by Indirect ELISA.

Figure 2. Titre of IgG to individual pneumococcal capsular polysaccharides induced by Formulation B in rabbits as measured by Indirect ELISA.

Figure 3. Titre of IgG to individual pneumococcal capsular polysaccharides induced by Formulation C in rabbits as measured by Indirect ELISA.

Figure 4. Titre of IgG to individual pneumococcal capsular polysaccharides induced by Prevnar 13™ in rabbits as measured by Indirect ELISA.

Figure 5. Titre of IgG to individual pneumococcal capsular polysaccharides induced by Placebo in rabbits as measured by Indirect ELISA.

Petitioner Merck Ex-1004, 0656

Detailed Description of the Invention

The present invention provides an immunogenic multivalent serotype composition, more specifically a conjugated pneumococcal vaccine composition comprising at least 13 pneumococcal polysaccharide serotypes which are individually conjugated to a pharmaceutically acceptable carrier protein CRM_{197}, wherein the composition does not contain capsular saccharide from serotype 6A. Serotypes 6A and 6B are structurally and serologically similar. Capsules of serotypes 6A and 6B are isopolymers differing only in the rhamnose-ribitol linkage, because of which antibodies specific to serotype 6B capsular polysaccharide cross-react with serotype 6A capsular polysaccharide. Therefore, exclusion of serotype 6A, provides a leverage for including an additional epidemiologically relevant serotype for broadening the protection against pneumococcal disease without having to expand the valence of multivalent PCV.

The present invention also provides an immunogenic multivalent serotype composition, wherein the capsular polysaccharides from serotypes 1, 3, 4, 5, 6B, 7F, 9N, 9V, 14, 15B, 18C, 19A, 19F, 22F, 23F and 33F of *S. pneumoniae* are conjugated to a carrier protein CRM_{197}, wherein the composition does not contain capsular polysaccharide from serotype 6A. Further, the present invention may additionally contain one or more serotypes selected from 6C, 8, 10A, 11A, 12F, 15A, 23A, 23B and 35B of *S. pneumoniae.*

Preferably, the present invention provides 13 valent, 14 valent or 15 valent immunogenic composition, wherein the composition does not contain capsular polysaccharide from serotype 6A.

Serotype 22F and serotype 3 are the most commonly found serotypes causing IPD due to expansion of certain clonal types. Serotype 33F is also amongst the most commonly encountered serotypes.

CRM_{197} is a variant of diphtheria toxin and is by itself non-toxic (i.e., toxoid) for use in vaccines. CRM_{197} is isolated from cultures of *Corynebacterium diphtheriae* strain C7

Petitioner Merck Ex-1004, 0657

(β197) grown in casamino acids and yeast extract-based medium. CRM_{197} may be prepared recombinantly in accordance with the methods described in U.S. Pat. No. 5,614,382. Alternatively, CRM_{197} is prepared recombinantly in accordance with the methods known in the literature or according to the method disclosed in our PCT publication WO 2016/079755. CRM_{197} may be purified by ultrafiltration, ammonium sulphate precipitation, and ion-exchange chromatography, methods well known in art.

The polysaccharide may be extracted from the microorganism according to conventional methods and purified likewise. This polysaccharide may be used in the native form after extraction/purification. Alternatively, it may be fragmented in order to obtain a polysaccharide having an average molecular weight less than that of the polysaccharide originally extracted.

The polysaccharide thus derived are then activated by CDAP and then conjugated with carrier proteins such as CRM_{197}, PspA, PsaA or combination thereof.

In another embodiment, the conjugation method depends on activation of the saccharide with 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 haloacetylated carrier protein (for example using iodoacetimide [e.g. ethyl iodoacetimide HCl] or N-succinimidyl bromoacetate or SIAB, or SIA, or SBAP). Preferably, the cyanate ester is coupled with hexane diamine or adipic acid dihydrazide (ADH) and the amino-derivatised saccharide is conjugated to the carrier protein using carbodiimide (e.g. EDAC or EDC) chemistry *via* a carboxyl group on the protein carrier. Such conjugates are described in WO 93/15760, WO 95/08348 and WO 96/29094; and Chu et al., 1983, Infect. Immunity 40:245-256.

Petitioner Merck Ex-1004, 0658

After conjugation of the capsular polysaccharide to the carrier protein, the polysaccharide-protein conjugates are purified (enriched with respect to the amount of polysaccharide-protein conjugate) by a variety of techniques. These techniques include concentration/diafiltration operations, precipitation/elution, column chromatography, and depth filtration.

After the individual glycoconjugates are purified, they are compounded to formulate the immunogenic composition of the present invention, which can be used as a vaccine.

In a preferred embodiment, the invention provides a multivalent Pneumococcal conjugate vaccine (PCV) composition comprising

a) at least 13 capsular polysaccharides selected from serotypes 1, 3, 4, 5, 6B, 7F, 9N, 9V, 14, 15B, 18C, 19A, 19F, 22F, 23F and 33F of *Streptococcus pneumoniae* activated with CDAP and conjugated to CRM_{197}, PspA, PsaA or combination thereof and
b) A pharmaceutically acceptable carrier, wherein the composition does not contain capsular saccharide from serotype 6A.

In another embodiment, amount of polysaccharide from each of the serotypes 1, 3, 4, 5, 6B, 7F, 9N, 9V, 14, 15B, 18C, 19A, 19F, 22F, 23F and 33F is 1 to 10 µg, preferably, 1 to 5 µg conjugated to 3 to 30 µg of CRM_{197} carrier protein. The ratio of polysaccharide to carrier protein is 0.3: 3.

A composition according to the invention may be manufactured conventionally. In particular, it may be formulated with a pharmaceutically acceptable diluent or vehicle, e.g. water or a saline solution. In addition, the composition may contain ingredients such as a buffer, a preservative or a stabilizer, polysorbate, an adjuvant such as an aluminum compound, e.g. an aluminium hydroxide, an aluminium phosphate or an aluminium hydroxyphosphate, and, a lyophilization excipient. In general, these products may be selected as a function of the mode and route of administration and based on standard pharmaceutical practices.

Petitioner Merck Ex-1004, 0659

The composition of the present invention can be formulated in a form of a unit dose vial, multiple dose vial, or pre-filled syringe. The composition may further comprise of one or more preservative(s) selected from thiomersal, 2-phenoxyethanol and the like. The amount of preservative may range from 4 to 20 mg/mL.

A composition according to the invention may be administered by any conventional route which is used in the field of vaccines, in particular by the systemic, i.e. parenteral route, e.g. by the subcutaneous, intramuscular, intradermal or intravenous route, or by the mucosal route, e.g. by the oral or nasal route.

"Effective amount" of a composition of the invention refers to a dose required to elicit antibodies that significantly reduce the likelihood or severity of infectivity of *S. pneumoniae* during a subsequent challenge.

In one embodiment, the present invention further provides an immunogenic composition administered as a single 0.5 mL dose formulated to contain: 2 µg of each polysaccharide; 30-40µg CRM_{197} carrier protein; 0.2 to 1 mg of aluminum phosphate adjuvant; sodium chloride and buffer as excipients.

In yet another preferred embodiment, the present invention provides a serotype composition for formulating a 13 valent PCV comprising;

- conjugation of the pneumococcal capsular polysaccharides of known size belonging to 13 serotypes activated utilizing CDAP, individually to an immunogenic carrier protein CRM_{197},
- diafiltration of the individual monovalent pneumococcal conjugates followed by purification using size exclusion chromatography,
- analysis of the fractions by SEC-HPLC and pooling of fractions containing monovalent pneumococcal conjugates before filter sterilization using 0.2µm filter and
- formulation of the 13 valent PCV using 13 monovalent pneumococcal conjugates; 4.4µg for serotype 6B; 2.2µg for rest of the serotypes and Adju-

Petitioner Merck Ex-1004, 0660

Phos® adjuvant together with appropriate excipient and buffer followed by aseptic filling.

The serotype composition of PCV 13 contains serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F and 33F.

In yet another preferred embodiment, the present invention provides a serotype composition for formulating a 14 valent PCV comprising;

- conjugation of pneumococcal capsular polysaccharides of known size belonging to 13 serotypes activated utilizing CDAP, individually to an immunogenic carrier protein CRM_{197},
- diafiltration of the individual monovalent pneumococcal conjugates followed by purification using size exclusion chromatography,
- analysis of the fractions by SEC-HPLC and pooling of fractions containing monovalent pneumococcal conjugates before filter sterilization using 0.2µm filter and
- formulation of the 14 valent PCV using monovalent pneumococcal conjugates; 4.4µg for serotype 6B; 2.2µg for rest of the serotypes and Adju-Phos® adjuvant together with appropriate excipient and buffer followed by aseptic filling.

The serotype composition of PCV 14 contains serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F.

Carrier proteins are preferably proteins that are non-toxic and non-reactogenic and obtainable in sufficient amount and purity. A carrier protein can be conjugated or joined with a *S. pneumoniae* polysaccharide to enhance immunogenicity of the polysaccharide. Carrier proteins should be amenable to standard conjugation procedures. In a particular embodiment of the present invention, CRM_{197} is used as the carrier protein. In one embodiment, each capsular polysaccharide is conjugated to a single carrier protein. In

Petitioner Merck Ex-1004, 0661

another embodiment, the capsular polysaccharides are conjugated to two or more carrier proteins.

In another embodiment, the capsular polysaccharides from *S. pneumoniae* may be conjugated to one or more carrier proteins such as inactivated bacterial toxins such as tetanus toxoid, pertussis toxoid; cholera toxoid, exotoxin A from *Pseudomonas aeruginosa*, bacterial outer membrane proteins such as outer membrane complex c (OMPC), porins, transferrin binding proteins, pneumolysin, pneumococcal surface protein A (PspA), pneumococcal adhesin protein (PsaA), C5a peptidase from Group A or Group B streptococcus, or *Haemophilus influenzae* protein D. Other proteins, such as ovalbumin, keyhole limpet hemocyanin, (KLH), bovine serum albumin (BSA) or purified protein derivative of tuberculin (PPD) may also be used as carrier proteins.

The amount of conjugate in each vaccine dose is selected as an amount that induces an immunoprotective response without significant, adverse effects. Such an amount may vary depending upon the pneumococcal serotype. Generally, each vaccine dose will comprise 0.1 to 50 μg of each polysaccharide, preferably 0.1 to 10 μg, and more preferably 1 to 5 μg.

In a preferred embodiment of the present invention, the PCV is a 14 valent sterile liquid formulation consisting of capsular polysaccharides of serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F individually conjugated to CRM_{197}. Each 0.5 mL dose is formulated to contain: 2 μg of each polysaccharide, except for 6B at 4 μg; about 32 μg CRM_{197} carrier protein; 0.125 mg of elemental aluminum (0.5 mg aluminum phosphate) adjuvant; and sodium chloride and L-histidine buffer.

In yet another preferred embodiment of the present invention, the PCV is a 13 valent sterile liquid formulation consisting of capsular polysaccharides of serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F and 33F individually conjugated to CRM_{197}. Each 0.5 mL dose is formulated to contain: 2 μg of each polysaccharide, except for 6B at 4

Petitioner Merck Ex-1004, 0662

μg; about 32 μg CRM_{197} carrier protein; 0.125 mg of elemental aluminum (0.5 mg aluminum phosphate) adjuvant; and sodium chloride and L-histidine buffer.

In yet another preferred embodiment of the present invention, the PCV is a 13 valent sterile liquid formulation consisting of capsular polysaccharides of serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F and 33 individually conjugated to CRM_{197}. Each 0.5 mL dose is formulated to contain: 2 μg of each polysaccharide, except for 6B at 4 μg; about 32 μg CRM_{197} carrier protein; 0.125 mg of elemental aluminum (0.5 mg aluminum phosphate) adjuvant; and sodium chloride and L-histidine buffer.

According to the methods of the present invention, the subject is human. In certain embodiments, the human patient is an infant (less than 1 year of age), toddler (approximately 12 to 24 months), or young child (approximately 2 to 5 years). In other embodiments, the human patient is an elderly patient (> 65 years). The compositions of this invention are also suitable for use with older children, adolescents and adults (e.g., aged 18 to 45 years or 18 to 65 years).

The present invention also provides a method of inducing an immune response to *a S. pneumoniae* capsular polysaccharide conjugate, comprising administering to a human an immunologically effective amount of the above multivalent immunogenic composition.

Effective amount here refers to a dose which is sufficient or most likely to elicit antibodies such that the immunized subject has reduced severity of infection.
The following examples are provided to illustrate the invention and are merely for illustrative purpose only and should not be construed to limit the scope of the invention.

Example 1

Conjugation of individual pneumococcal polysaccharide to carrier protein to form Polysaccharide-CRM_{197} Conjugates

Petitioner Merck Ex-1004, 0663

Polysaccharide size reduction:

In the first step, the polysaccharide (Ps) solution was passed through a high pressure homogenizer to reduce the molecular size of the polysaccharide followed by a concentrating and diafiltration step, using 100 kDa MWCO membrane. The sized polysaccharide produced thus was used for conjugation.

Activation of polysaccharide and conjugation with carrier protein:

Approximately, 1:1 ratio of polysaccharide and (6.0 mL of Ps, concentration of 10 mg/mL) CDAP (100 mg/mL in Acetonitrile (w/v)) was mixed in a glass vial and stirred for 1 min. Adjusted the pH of polysaccharide to 9.25 with 0.2M Triethylamine and stirred for 3 min at room temperature (RT). Slowly added (4.0 mL of conc. 15.0 mg/mL) CRM_{197} to the activated polysaccharide to a ratio of 1:1.5 (Ps: Carrier protein). Adjusted the pH of the reaction to ~9.05 with 0.2M Triethylamine and continued the reaction under stirring for 5 hours at RT and finally quenched the reaction by adding excess concentration of glycine.

The reaction mixture was diafiltered using 100 kDa MWCO membrane and purified by size-exclusion chromatography. The fractions were analysed by SEC-HPLC and fractions containing conjugates were pooled and sterile filtered with 0.2µm filters. This material was called monovalent conjugate bulk. All monovalent conjugate bulks for 14 serotypes were generated with similar process.

Example 2

Formulation of multivalent Polysaccharide-CRM_{197} into Vaccine

Three individual formulations namely, formulation A (13 valent: 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F and 23F), formulation B (13 valent: 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F and 33F) and formulation C (14 valent: 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F) were prepared. In the above formulations, according to their composition 2.2 µg of each monovalent conjugate sterile bulk of 13 serotypes (1, 3, 4, 5, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F) and 4.4 µg of 6B monovalent conjugate sterile bulks were accurately measured. Each monovalent bulk conjugate, was

Petitioner Merck Ex-1004, 0664

slowly transferred (drop wise) one by one to the blending vessel, under continuous stirring (~150 rpm) and continued the stirring for 2 minutes at pH 6.5.

To the above mix Adju-Phos® was accurately measured and slowly transferred drop wise to the above blending vessel, under continuous stirring (~ 150 rpm) to the final concentration of Al^{+3} to 1 mg/mL. After complete addition of the Adju-Phos®, the system was left under stirring for 2 minutes. The final pH of the solution was adjusted to 5.8±0.2 and continued stirring for 2 hours at around 150 rpm/5°±3°C in a refrigerator. After completion of stirring, the vaccine was aseptically filled into vials inside a laminar air flow unit and stored at 5°±3°C chamber.

Example 3

Immunogenicity studies

Immunogenicity of pneumococcal conjugate formulations prepared according to the present invention was assessed in rabbit model of immunity. Five groups of female rabbits (adult, nulliparous and non-pregnant; 2.0 to 2.5 kg body weight) with a group size of seven were recruited for this study. Three formulations *viz.* Formulation A, Formulation B, Formulation C; a comparator vaccine (Prevnar 13™) and placebo were included in this study (Table 1). The rabbits were given single human dose equivalent irrespective of valence of the formulations with a dose volume of 0.5 ml. Each group of rabbits received 3 doses at an interval of 14 days, using the respective formulation through intramuscular route on days 1, 15 and 29 after appropriately preparing the site. They were humanely test bled on days 0, 12, 26 and 40, precisely 11 days after the administration of formulations. The serum samples were separated from clotted blood samples before storing them at -20°C as aliquots. The serum samples were designated as preimmune serum (PIS; day 0), immune serum – post dose 1 (PD1; day 12), immune serum – post dose 2 (PD2; day 26) and immune serum – post dose 3 (PD3; day 40).

Petitioner Merck Ex-1004, 0665

Table 1. Details of rabbit treatment groups chosen for immunogenicity assessment.

S.No	Formulation	Group	Group size	Schedule	
				Immunization	Bleeding
1	Formulation A	I	7	Day 1, Day 15 & Day 29	Day 0, Day 12, Day 26 & Day 40
2	Formulation B	II	7		
3	Formulation C	III	7		
4	Prevnar 13™	IV	7		
5	Placebo	V	7		

Titre of IgG to individual pneumococcal capsular polysaccharides (CPS) was measured by using ELISA, essentially following the standardized WHO ELISA protocol with slight modifications. Nunc Maxisorp® plates were used for immobilizing purified pneumococcal CPS sourced from ATCC, USA / Statens serum Institut, Denmark. The 007sp international pneumococcal reference serum known to contain anti-CPS IgG for 23 serotypes of 23 valent pneumococcal polysaccharide vaccine was used as a positive control. The non-functional cell wall polysaccharide (CWPS) reactive IgG was depleted by adsorbing the test and control serum samples with CWPS Multi™ as per the manufacturer's instruction before testing them by Indirect ELISA. Recombinant protein A/G peroxidase conjugate which combines the binding specificity of protein A and protein G was used for universal detection of rabbit and human IgG. Anti-CPS IgG titre for individual rabbits from a given group was determined based on cut-off which was calculated using the formula, [Mean OD of Blank × 2]. Finally, the geometric mean titre (GMT) of anti-CPS IgG induced by Formulation A, Formulation B, Formulation C, Prevnar 13™ and Placebo was calculated (Figures 1 - 5).

Petitioner Merck Ex-1004, 0666

Claims :

1. A multivalent Pneumococcal conjugate vaccine (PCV) composition comprising:
   a) at least 12 capsular polysaccharides selected from serotypes 1, 3, 4, 5, 6B, 7F, 9N, 9V, 15B, 14, 18C, 19A, 19F, 22F, 23F and 33F of *S. pneumoniae* activated with CDAP and conjugated to carrier protein selected from CRM_{197}, pneumococcal surface protein A (PspA), pneumococcal adhesin protein (PsaA) or combination thereof and
   b) a pharmaceutically acceptable carrier, wherein the composition does not contain capsular polysaccharide from serotype 6A.

2. A multivalent Pneumococcal conjugate vaccine (PCV) composition comprising:
   a) at least 14 capsular polysaccharides selected from serotypes 1, 3, 4, 5, 6B, 7F, 9N, 9V, 14, 15B, 18C, 19A, 19F, 22F, 23F and 33F of *S. pneumoniae* activated with CDAP and conjugated to CRM_{197}, PspA, PsaA or combination thereof and
   b) a pharmaceutically acceptable carrier, wherein the composition does not contain capsular polysaccharide from serotype 6A.

3. The composition as claimed in claims 1 and 2, further comprises one or more serotypes selected from 6C, 8, 10A, 11A, 12F, 15A, 23A, 23B and 35B of *S. pneumoniae.*

4. The composition as claimed in claims 1 and 2, wherein the amount of polysaccharide from each of the serotypes is 1 to 10 µg.

5. The composition as claimed in claims 1 and 2, wherein the amount of carrier protein conjugated to polysaccharide is 3 to 30 µg.

6. The composition as claimed in claim 1, wherein the single 0.5 mL dose contain 2 µg of each polysaccharide; 30-40µg CRM_{197} carrier protein; 0.2 to 1 mg of aluminum phosphate adjuvant; sodium chloride and buffer.

Petitioner Merck Ex-1004, 0667

7. A 14 valent sterile liquid Pneumococcal conjugate vaccine (PCV) formulation comprising 2 µg of each capsular polysaccharides of serotypes 1, 3, 4, 5, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F, 4 µg of serotype 6B each individually conjugated to about 32 µg CRM_{197}; 0.125 mg of elemental aluminum (0.5 mg aluminum phosphate) adjuvant; and sodium chloride and L-histidine buffer, wherein the formulation does not contain capsular polysaccharide from serotype 6A.

8. A 13 valent sterile liquid Pneumococcal conjugate vaccine (PCV) formulation comprising 2 µg of each capsular polysaccharides of serotypes 1, 3, 4, 5, 7F, 9V, 14, 18C, 19A, 19F, 22F and 33F, 4 µg of serotype 6B each individually conjugated to about 32 µg CRM_{197}; 0.125 mg of elemental aluminum (0.5 mg aluminum phosphate) adjuvant; and sodium chloride and L-histidine buffer, wherein the formulation does not contain capsular polysaccharide from serotype 6A.

9. A 13 valent sterile liquid Pneumococcal conjugate vaccine (PCV) formulation comprising 2 µg of each capsular polysaccharides of serotypes 1, 3, 4, 5, 7F, 9V, 14, 18C, 19A, 19F, 23F and 33F, 4 µg of serotype 6B each individually conjugated to about 32 µg of CRM_{197}; 0.125 mg of elemental aluminum (0.5 mg aluminum phosphate) adjuvant; and sodium chloride and L-histidine buffer, wherein the formulation does not contain capsular polysaccharide from serotype 6A.

10. A serotype composition for formulating a 13 valent PCV comprising;

a) conjugation of the pneumococcal capsular polysaccharides of known size from serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F and 33F activated utilizing CDAP, individually to an immunogenic carrier protein CRM_{197},
b) diafiltration of the individual monovalent pneumococcal conjugates followed by purification using size exclusion chromatography,
c) analysis of the fractions by SEC-HPLC and pooling of fractions containing monovalent pneumococcal conjugates before filter sterilization using 0.2µm filter,

Petitioner Merck Ex-1004, 0668

d) formulation of the 13 valent PCV using 13 monovalent pneumococcal conjugates; 4.4μg for serotype 6B; 2.2μg for rest of the serotypes and Adju-Phos® adjuvant together with appropriate excipient and buffer followed by aseptic filling,

e) wherein the said 13 valent PCV does not contain capsular polysaccharide from serotype 6A.

11. A serotype composition for formulating a 14 valent PCV comprising;

a) conjugation of pneumococcal capsular polysaccharides of known size from serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F activated utilizing CDAP, individually to an immunogenic carrier protein CRM_{197},

b) diafiltration of the individual monovalent pneumococcal conjugates followed by purification using size exclusion chromatography,

c) analysis of the fractions by SEC-HPLC and pooling of fractions containing monovalent pneumococcal conjugates before filter sterilization using 0.2μm filter and

d) formulation of the 14 valent PCV using monovalent pneumococcal conjugates; 4.4μg for serotype 6B; 2.2μg for rest of the serotypes and Adju-Phos® adjuvant together with appropriate excipient and buffer followed by aseptic filling.

Petitioner Merck Ex-1004, 0669

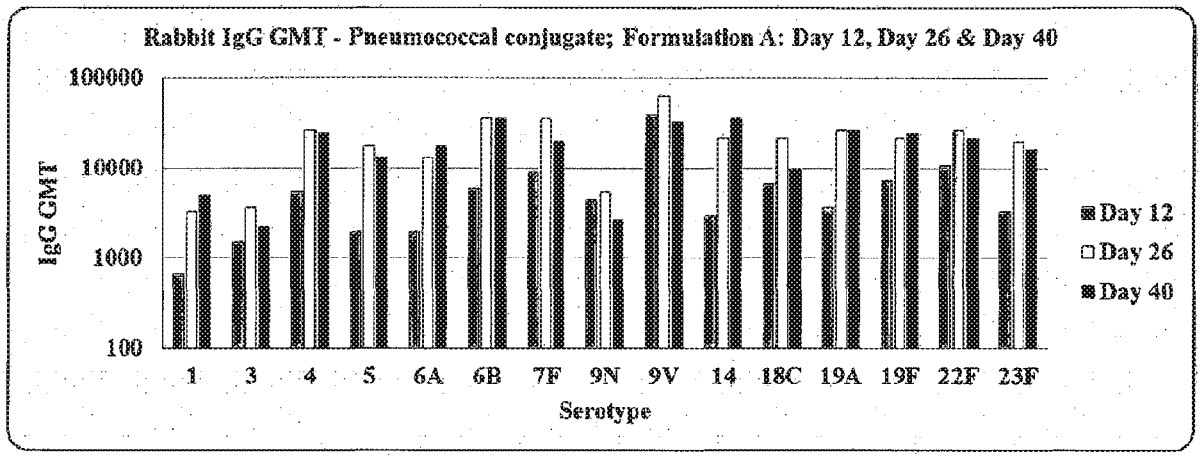


Figure 1

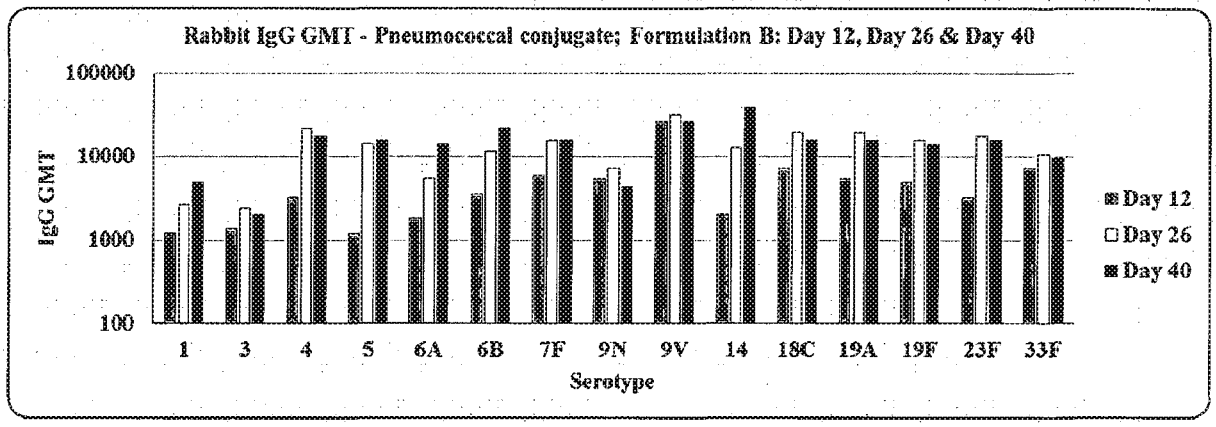


Figure 2

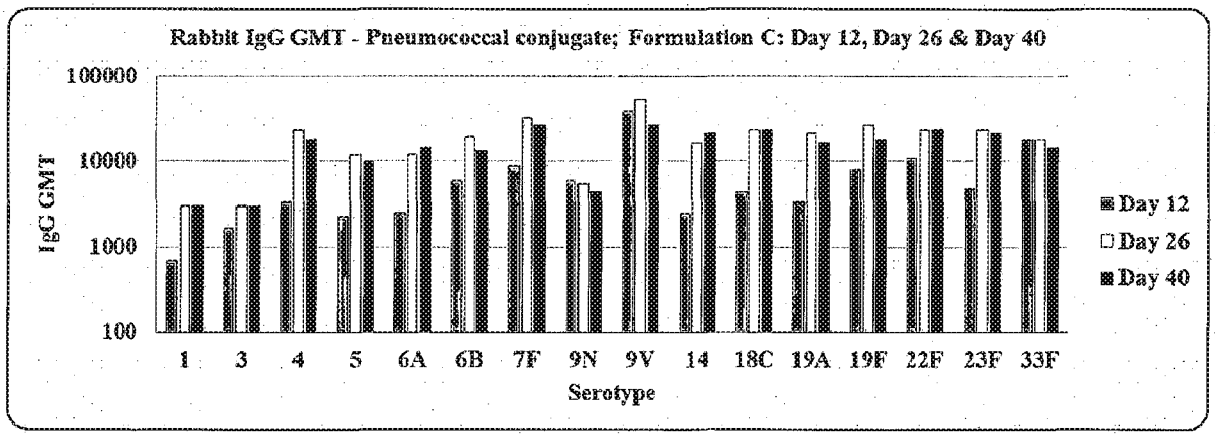


Figure 3

Petitioner Merck Ex-1004, 0670

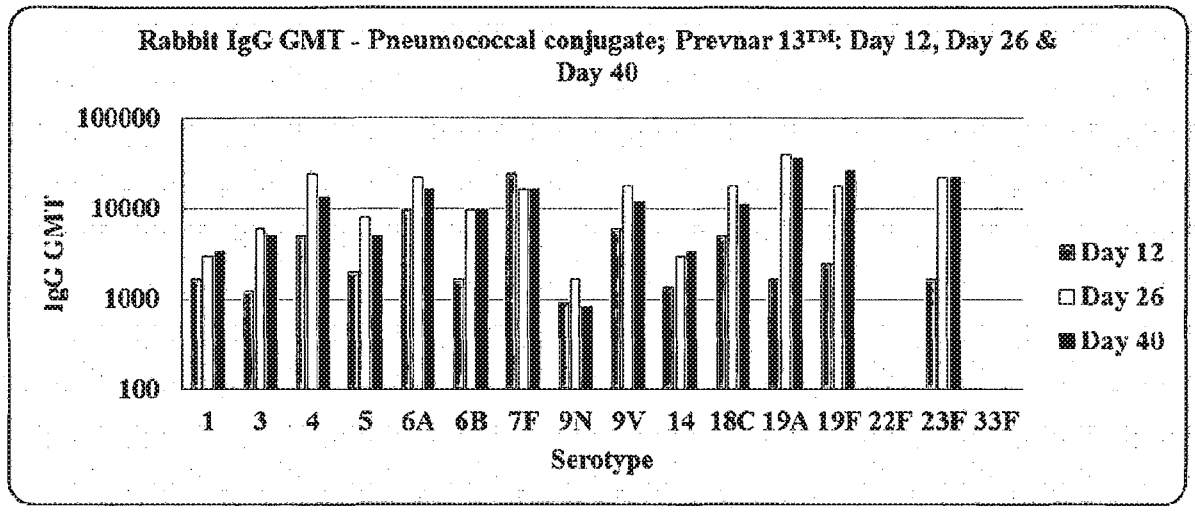


Figure 4

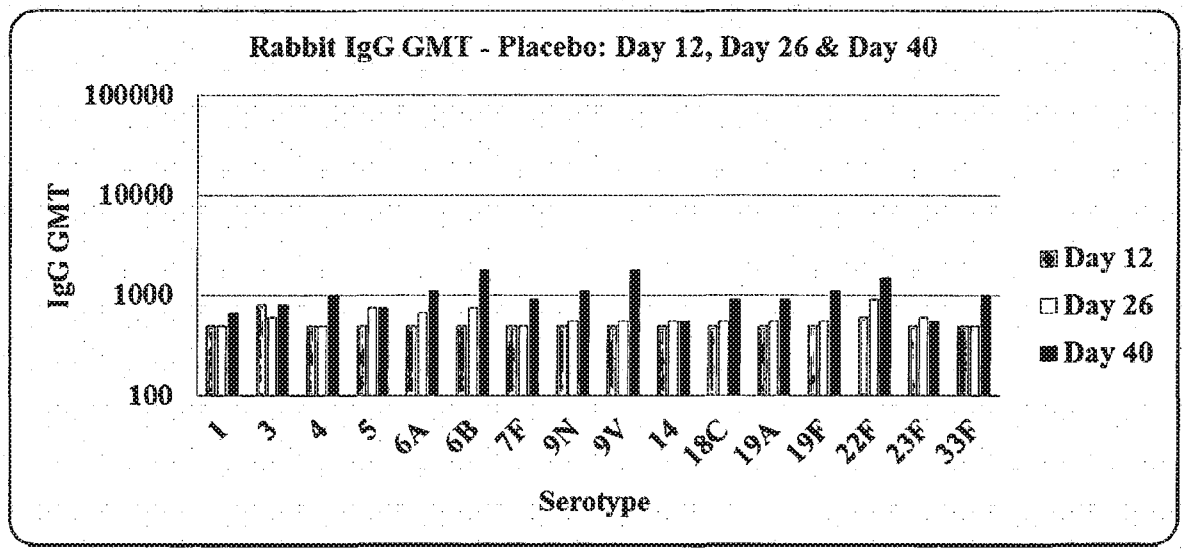


Figure 5

Petitioner Merck Ex-1004, 0671

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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
05 April 2018 (05.04.2018)

WIPO | PCT

(10) International Publication Number
WO 2018/064444 A1

(51) International Patent Classification:
A61K 39/09 (2006.01) *A61P 31/04* (2006.01)
A61K 39/385 (2006.01) *A61K 39/00* (2006.01)
A61K 39/39 (2006.01)

(21) International Application Number:
PCT/US2017/054237

(22) International Filing Date:
29 September 2017 (29.09.2017)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
201641033563 30 September 2016 (30.09.2016) IN

(71) Applicant: **BIOLOGICAL E LIMITED** [IN/IN]; 18/1 & 3, Azamabad, Hyderabad, Telangana 500 020 (IN).

(72) Inventors; and
(71) Applicants *(for US only)*: **MATUR, Ramesh, Venkat** [US/IN]; Biological E Limited, 18/1 & 3, Azamabad, Hyderabad, Telangana 500 020 (IN). **MANTENA, Narender, Dev** [US/IN]; Biological E Limited, 18/1 & 3, Azamabad, Hyderabad, Telangana 500 020 (IN).

(72) Inventors: **SRIRAMAN, Rajan**; Biological E Limited, 18/1 & 3, Azamabad, Hyderabad, Telangana 500 020 (IN). **CHAKKA, Deviprasanna**; Biological E Limited, 18/1 & 3, Azamabad, Hyderabad, Telangana 500 020 (IN). **SUREDDI, Satyam, Naidu**; Biological E Limited, 18/1 & 3, Azamabad, Hyderabad, Telangana 500 020 (IN). **BURKI, Rajendar**; Biological E Limited, 18/1 & 3, Azamabad, Hyderabad, Telangana 500 020 (IN). **GANTI, Sreenivasa, Rao**; Biological E Limited, 18/1 & 3, Azamabad, Hyderabad, Telangana 500 020 (IN). **DATLA, Mahima**; Biological E Limited, 18/1 & 3, Azamabad, Hyderabad, Telangana 500 020 (IN).

(74) Agent: **FOURNIER, David, B.** et al., Perkins Coie LLP, P.O. Box 1247, Seattle, WA 98111-1247 (US).

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

(54) Title: MULTIVALENT PNEUMOCOCCAL VACCINE COMPOSITIONS COMPRISING POLYSACCHARIDE-PROTEIN CONJUGATES

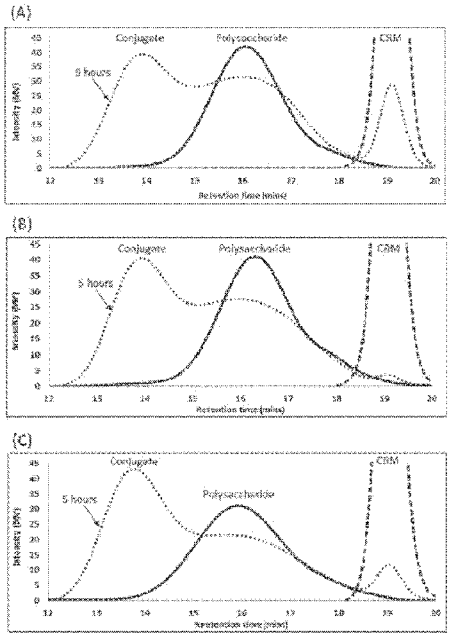


FIG. 1

(57) Abstract: The present disclosure relates to multivalent pneumococcal vaccine compositions comprising capsular pneumococcal polysaccharide serotypes each individually conjugated to carrier proteins. When conjugated, the combination of the capsular pneumococcal polysaccharide serotype and the carrier protein is referred to herein as a polysaccharide-protein conjugate. The pneumococcal vaccine compositions may further comprise one or more of the following: a pharmaceutically acceptable carrier, a pharmaceutically acceptable diluent, a buffer, a preservative, a stabilizer, an adjuvant, and/or a lyophilization excipient. Methods of making and administering the pneumococcal vaccine compositions described herein are also provided.

WO 2018/064444 A1

[Continued on next page]

Petitioner Merck Ex-1004, 0672

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

Petitioner Merck Ex-1004, 0673

MULTIVALENT PNEUMOCOCCAL VACCINE COMPOSITIONS COMPRISING POLYSACCHARIDE-PROTEIN CONJUGATES

CROSS-REFERENCE TO RELATED APPLICATION

[0001] The present application claims priority to Indian Provisional Application No. 201641033563 filed on September 30, 2016, and incorporated herein by reference in its entirety.

TECHNICAL FIELD

[0002] The present disclosure relates to multivalent pneumococcal vaccine compositions comprising capsular pneumococcal polysaccharide of one or more *Streptococcus pneumoniae* serotypes conjugated to one or more carrier proteins. When conjugated, the capsular pneumococcal polysaccharide and the carrier protein is referred to herein as a polysaccharide-protein conjugate.

BACKGROUND

[0003] *Streptococcus pneumoniae* ("pneumococcus") is a gram-positive bacteria that causes invasive diseases, such as pneumonia, bacteremia and meningitis, and diseases associated with colonization, such as acute otitis media (e.g., colonization of middle ear). These pneumococcus-induced diseases result in morbidity and mortality, particularly in persons less than 24 months old and greater than 60 years old. The rate of pneumococcal pneumonia in the U.S. for persons over 60 years of age is estimated to be 3 to 8 per 100,000. In 20% of cases, pneumococcal pneumonia leads to bacteremia and meningitis collectively having a mortality rate close to 30% despite antibiotic treatment.

[0004] Proteins such as pneumolysin and the pneumococcal surface protein A (PspA) have been evaluated for their suitability as carrier protein candidates in pneumococcal vaccines. While both are partially protective in mice (Paton et al. "Effect of immunization with pneumolysin on survival time of mice challenged with Streptococcus pneumoniae." Infect Immun. 1983 May; 40(2): 548–552 and McDaniel et al. "PspA, a surface protein of Streptococcus pneumoniae, is capable of eliciting protection against pneumococci of more than one capsular type" Infect Immun. 1991

Petitioner Merck Ex-1004, 0674

Jan; 59(1): 222–228.), there are disadvantages to their use as immunogens in vaccines. Pneumolysin, although well conserved among pneumococci, has toxic effects in its native state (AlonsoDeVelasco et al. "Streptococcus pneumoniae: Virulence factors, pathogenesis, and vaccines." Microbiol Rev. 1995 Dec; 59(4): 591–603.). On the other hand, PspA is serologically and structurally heterogeneous. (Crain et al. "Pneumococcal surface protein A) is serologically highly variable and is expressed by all clinically important capsular serotypes of Streptococcus pneumoniae." Infect Immun. 1990 Oct; 58(10): 3293–3299). Its use in vaccine formulations would require multiple PspA types, thus increasing the complexity of vaccine preparation.

[0005] Pneumococcal surface adhesion A ("PsaA") is a multi-functional lipoprotein and is involved in host cell adherence and colonization. It is a highly conserved surface protein and 97% homologous across known serotypes of *S. pneumoniae*. PsaA is immunogenic and anti-PsaA antibodies are known to increase during natural nasopharyngeal colonization of pneumococci.

[0006] Pneumococcal vaccines may be administered to prevent infections. Current vaccines include multivalent pneumococcal polysaccharide vaccines (comprises pneumococcal polysaccharides from two or more serotypes) and pneumococcal conjugate vaccines. The protective efficacy of the pneumococcal polysaccharide vaccine is known to be related to the concentration of antibody generated against a capsular polysaccharide. Pneumococcus cells are encapsulated with a polysaccharide giving rise to more than 90 different pneumococcus serotypes. The capsule is the principal virulence determinant for pneumococci - it not only protects the cell's inner surface from complement mediated cell lysis, it is also poorly immunogenic.

[0007] Merck's Pneumovax®23 is a multivalent pneumococcal polysaccharide vaccine and contains unconjugated capsular polysaccharides from 23 pneumococcal serotypes including serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F and 33F. In addition to Pneumovax®23, the multivalent pneumococcal polysaccharide vaccines that have been licensed thus far proved valuable in preventing pneumococcal disease in adults, particularly, the elderly

Petitioner Merck Ex-1004, 0675

and those at high-risk. However, infants and young children respond poorly to these unconjugated pneumococcal polysaccharide vaccines.

[0008] Prevnar®-7 is a pneumococcal polysaccharide-protein conjugate vaccine and includes the seven most frequently isolated polysaccharide serotypes (e.g., 4, 6B, 9V, 14, 18C, 19F, and 23F conjugated to CRM_{197}). Since use of Prevnar®-7 began in the United States in 2000, there has been a significant reduction in invasive pneumococcal disease (IPD) in children. A 13-valent conjugate vaccine Prevenar-13®, containing thirteen serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F conjugated to CRM_{197}, was developed and approved due to the limitations in serotype coverage with Prevnar®-7 in certain regions of the world.

[0009] Synflorix® is a pneumococcal vaccine that includes ten polysaccharide serotypes – serotypes 1, 4, 5, 6B, 7, 9, 14, 23F – conjugated to protein D (PD), serotype 18C conjugated to tetanus toxoid (TT) and serotype 19F conjugated to diphtheria toxoid (DT). Each of the serotype polysaccharides are coupled utilizing 1-cyano-4-dimethylamino-pyridinium tetrafluoroborate (CDAP) under controlled pH.

[0010] Despite these vaccines, there is a need for additional multivalent pneumococcal vaccines comprising alternative polysaccharide serotypes and carrier proteins, as well as simple and efficient production thereof.

[0011] Pneumococcal vaccines have been described in additional references. For example, U.S. Patent No. 5,360,897 discloses an immunogenic conjugate comprising a reductive amination product of an intact capsular polymer of the bacterial pathogen *S. pneumoniae* having at least two carbonyl groups and a bacterial toxin or toxoid, said conjugate comprising a cross-linked conjugate in which there is a direct covalent linkage between the capsular polymer and the toxin or toxoid.

[0012] U.S. Patent No. 5,693,326 provides a generalized method for preparing a conjugate vaccine wherein for activating viral, fungal or bacterial polysaccharides, an organic cyanylating agent is used selected from the group 1-cyano-4-(dimethylamino)-pyridinium tetrafluoroborate, N-cyanotriethyl-ammonium tetrafluoroborate, and p-nitrophenylcyanate, to form an activated carbohydrate and is subsequently coupled to the protein or carrier protein.

Petitioner Merck Ex-1004, 0676

[0013] U.S. Patent No. 5,854,416 discloses amino acid and DNA sequences of 37-kDa protein from *S. pneumonia* known as PsaA (Pneumococcal surface adhesion A) and U.S. Patent No. 7,862,823 discloses a multivalent conjugate vaccine composition comprising pneumococcal capsular polysaccharides with at least two different carrier proteins, such as DT and TT.

[0014] U.S. Patent No. 7,955,605 describes a process of making immunogenic conjugate consisting 19A where the activated serotype 19A polysaccharide and carrier protein are re-suspended in dimethyl sulfoxide (DMSO) to form a conjugate.

[0015] U.S. Patent No. 8,192,746 discloses a 15-valent pneumococcal polysaccharide-protein conjugate vaccine composition having capsular polysaccharides from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F, and 33F conjugated to CRM_{197}.

[0016] U.S. Patent No. 8,465,749 provides a method for preparing a conjugate vaccine by reacting a polysaccharide with CDAP and reacting a protein with hydrazine or adipic acid dihydrazide with specific pH range.

[0017] U.S. Patent No. 8,557,250 B2 discloses a method comprising contacting a mixture of a plurality of cyanate activated immunogenic distinct polysaccharides with at least one hydrazide activated protein.

[0018] U.S. Patent No. 8,808,708, 7,955,605 and U.S. Patent No. 8,603,484 describe a 13-valent immunogenic composition consisting polysaccharide-protein conjugates where serotypes consist of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F, and carrier protein CRM_{197}.

[0019] U.S. Patent Publication No. 2009/0017059 A1 discloses an immunogenic composition wherein serotypes 19A and 19F are conjugated to different bacterial toxoids including tetanus toxoid, diphtheria toxoid, pertussis toxoid, bacterial cytolysins and/or pneumolysin.

[0020] U.S. Patent Publication No. 2010/0074922 A1 discloses an immunogenic composition containing 10 or more serotypes wherein 19F capsular saccharide is conjugated to DT, serotype 18C capsular saccharide is conjugated to tetanus toxoid and serotypes 1, 4, 5, 6B, 7F, 9V, 14 and 23F capsular saccharides are conjugated to Protein D isolated from *Haemophilus influenzae.*

Petitioner Merck Ex-1004, 0677

[0021] U.S. Patent Publication No. 2010/0239604 describes an immunogenic composition comprising multivalent *S. pneumoniae* capsular saccharide conjugates from serotypes 19A and 19F wherein serotype 19A is conjugated to a first bacterial toxoid and 19F is conjugated to a second bacterial toxoid and 2-9 of the *S. pneumoniae* capsular saccharides are conjugated to protein D.

[0022] U.S. Patent Publication No. 2012/321658 A1 discloses an immunogenic composition wherein serotypes 1, 3, 19A and 19F linked to protein carrier(s) either directly or indirectly through a chemistry other than reductive amination, and one or more different saccharides is/are selected from a second group consisting of serotypes 4, 5, 6A, 6B, 7F, 9V, 14, 18C and 23F which is/are linked to a protein carrier(s) by reductive amination.

[0023] IN 140/DEL/2011 provides a *S. pneumonia* vaccine comprising either of (a) 7 or more (b) 10 or more polysaccharides from different serotypes conjugated to at least 2 or more carrier proteins selected from a group comprising DT, diphtheria toxoid, CRM_{197}, and tetanus toxoid.

[0024] WO Publication No. 2013/191459 A1 discloses a conjugated 15 valent composition comprising different serotypes of *S. pneumoniae* derived from a capsular polysaccharide 1, 2, 3, 4, 5, 6A, 6B, 7F, 9N, 9V, 14, 18C, 19A, 19F and 23F conjugated to CRM_{197}.

[0025] WO Publication No. 2014/092377 A1 discloses a 13 valent composition wherein 12 serotypes are selected from 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F and the last serotype is either 12 or 9N conjugated to CRM_{197}.

[0026] WO Publication No. 2014/092378 A1 describes an immunogenic conjugate composition where 12 serotypes are selected from 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F and remaining one from 22F or 33F conjugated to CRM_{197}.

[0027] WO 2016207905 A2 discloses a multivalent Pneumococcal conjugate vaccine (PCV) composition comprising: 1) at least 12 capsular polysaccharides selected from serotypes 1, 3, 4, 5, 6B, 7F, 9N, 9V, 15B, 14, 18C, 19A, 19F, 22F, 23F and 33F of S. pneumoniae activated with CDAP and conjugated to carrier protein

Petitioner Merck Ex-1004, 0678

CRM197, and 2) a pharmaceutically acceptable carrier, wherein the composition does not contain capsular polysaccharide from serotype 6A.

[0028] Chinese Patent Application Publication No. CN 101590224 describes a 14 valent pneumococcal polysaccharide-protein conjugate vaccine containing serotypes 1, 2, 4, 5, 6A, 6B, 7F, 9N, 9V, 14, 18C, 19A, 19F and 23F conjugated to CRM_{197}.

[0029] Chinese Patent Application Publication No. CN 103623401 discloses a 14 multivalent pneumococcal capsular polysaccharide - protein conjugate composition wherein said 14 different serotype are 1, 3, 4, 5, 6A, 6B, 9V, 14,18C, 19A, 19F, 22F, 23F and 33F conjugated to CRM_{197}.

[0030] Chinese Patent Application Publication No. CN 103656632 discloses a multivalent pneumococcal capsular polysaccharide composition containing serotype 6A and at least one extra serotype selected from the group consisting of 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F conjugated to CRM_{197}.

[0031] Chinese Patent Application Publication No. CN 103656631 provides a multivalent pneumococcus capsular polysaccharide-protein conjugate composition and a preparation method thereof. The conjugate composition is prepared from capsular polysaccharides of pneumococcus of 24 different serotypes and a carrier protein in a covalent linkage manner, wherein the 24 different serotypes are 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F conjugated to CRM_{197}.

[0032] Chinese Patent Application Publication No. CN 104069488 discloses a multivalent pneumococcus capsular polysaccharides of 14 different serotypes and carrier protein, wherein the 14 serotypes include 1, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F conjugated to CRM_{197}.

[0033] Deborah et al, (1996, Volume 21, Issue 1, Pages 17-22) describes PsaA as having significant homology with fimbrial adhesion proteins. Immunization of CBA/CaHNJ Xid mice with PsaA using either complete Freund's or TiterMax™ adjuvants significantly protected mice against heterologous intravenous challenge with type 3 pneumococcal strain WU2 at doses up to 45 times the LD_{50}.

Petitioner Merck Ex-1004, 0679

[0034] Miyaji E N et al, (2001, Vaccine; 20 (5-6): 805-12) discloses *S. pneumoniae* as one of the most important human pathogens. DNA vaccine vectors containing either the full-length PsaA or a truncated PspA gene were constructed. Both constructs showed transient expression of the antigens in vertebrate cells and induced significant antibody response to the pneumococcal antigens in BALB/c mice injected intramuscularly.

[0035] Wuorimaa et al. (2001, The Paediatric Infectious Disease Journal, Volume 20(3), pp 272-277) discloses a study to assess the tolerability and immunogenicity in healthy toddlers of an 11-valent pneumococcal conjugate vaccine that uses both TT and DT as carriers.

[0036] Gatchalian et al. (2001, 17th Annual Meeting of the Eur. Soc. Paed. Inf. Dis (ESPID), poster number 4, P1A Poster Session 1, Istanbul Turkey) discloses an opsanophagocytic assay (OPA)results from infants who had received doses of the 11-valent vaccine failed to show antibody responses for serotype 3 at levels comparable to other tested serotypes.

[0037] Anderson P et al, (2003, Vaccine; 21 (13-14):1554-9) discloses a comparative study of tetravalent conjugate vaccines with each polysaccharide types 6A, 14, 19F, and 23F separately coupled to tetanus toxoid or diphtheria CRM_{197} or a mixture of halved doses of polysaccharide types 6A, 14, 19F, and 23F separately coupled to tetanus toxoid and diphtheria CRM_{197}.

[0038] Nurkka et al. (2004, Ped. Inf. Dis. J., 23:1008-1014) discloses a study of the immunogenicity and safety of an 11-valent pneumococcal protein D conjugate vaccine where no priming effect was observed for serotype 3 in infants who had received three doses of the vaccine followed by a booster dose of either the same vaccine or a pneumococcal polysaccharide vaccine.

[0039] The above mentioned references disclose, amongst other compositions, methods, and the like, multivalent pneumococcal vaccines comprising polysaccharides from one or more serotypes as well as conjugation of these polysaccharides with carrier proteins such as CRM_{197}, protein D, DT, and TT. In view of the different serotypes that are prevalent across various regions, there is a need for additional multivalent pneumococcal vaccines comprising novel conjugates of

Petitioner Merck Ex-1004, 0680

polysaccharide serotypes with improved immune response, as well as simple and efficient production thereof.

SUMMARY

[0040] In several embodiments, the present disclosure provides a pneumococcal vaccine composition, the composition comprising two or more capsular pneumococcal polysaccharide serotypes each individually conjugated to pneumococcal surface adhesion protein A (PsaA) or combination of PsaA and CRM_{197} as carrier proteins.

[0041] In one embodiment, the present disclosure provides a pneumococcal vaccine composition that is a 10 valent, 14 valent, 15 valent, 17 valent, 18 valent, 19 valent, 20 valent, 22 valent, 23 valent, 24 valent, or 25 valent pneumococcal vaccine composition.

[0042] In another embodiment, the present disclosure provides a pneumococcal vaccine composition comprising pneumococcal polysaccharides that are each serotypes selected from pneumococcal serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 23F, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, 39, and 45.

[0043] In another embodiment, the present disclosure provides a pneumococcal vaccine composition comprising pneumococcal polysaccharides from each serotypes selected from pneumococcal serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 23F, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, 39, and 45 conjugated with pneumococcal surface adhesion protein A (PsaA) as a carrier protein.

[0044] In another embodiment, the present disclosure provides a pneumococcal vaccine composition comprising pneumococcal polysaccharides from serotypes selected from 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 23F, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, 39, and 45 conjugated with pneumococcal surface adhesion protein A (PsaA) or combination of PsaA and CRM_{197} as carrier proteins.

[0045] In another embodiment, the present disclosure provides a pneumococcal vaccine composition comprising pneumococcal polysaccharides from serotypes selected from 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B,

Petitioner Merck Ex-1004, 0681

15C, 16F, 17F, 18C, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 23F, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, 39, and 45 wherein at least 3 pneumococcal polysaccharides are conjugated with pneumococcal surface adhesion protein A (PsaA).

[0046] In another embodiment, the present disclosure provides a pneumococcal vaccine composition comprising pneumococcal polysaccharides from serotypes 3, 6A, 6B conjugated with pneumococcal surface adhesion protein A (PsaA).

[0047] In another embodiment, the present disclosure provides a pneumococcal vaccine composition comprising at least 10 pneumococcal polysaccharides selected from serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 23F, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, 39 and 45 and the carrier proteins comprise PsaA, or combination of PsaA and CRM_{197}.

[0048] In another embodiment, the present disclosure provides a pneumococcal vaccine composition comprising at least 14 pneumococcal polysaccharide conjugates.

[0049] In another embodiment, the present disclosure provides a pneumococcal vaccine composition comprising at least 17 pneumococcal polysaccharide conjugates.

[0050] In another embodiment, the present disclosure provides a pneumococcal vaccine composition comprising at least 19 pneumococcal polysaccharide conjugates.

[0051] In another embodiment, the present disclosure provides a pneumococcal vaccine composition comprising at least 20 pneumococcal polysaccharide conjugates.

[0052] In another embodiment, the present disclosure provides a pneumococcal vaccine composition comprising at least 22 pneumococcal polysaccharide conjugates.

[0053] In another embodiment, the present disclosure provides a pneumococcal vaccine composition comprising at least 24 pneumococcal polysaccharide conjugates.

[0054] In another embodiment, the present disclosure provides a pneumococcal vaccine composition comprising pneumococcal polysaccharides of two or more serotypes and a carrier protein, wherein the serotypes are selected from 3, 5, 6A, 6B, 9V and 18C, and the carrier protein is PsaA.

[0055] In another embodiment, the present disclosure provides a pneumococcal vaccine composition comprising pneumococcal polysaccharides of two or more

Petitioner Merck Ex-1004, 0682

serotypes and a carrier protein, wherein the serotypes are selected from 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19F, 19A and 22F and the carrier protein is PsaA.

[0056] In another embodiment, the present disclosure provides a pneumococcal vaccine composition comprising pneumococcal polysaccharides of two or more serotypes and a carrier protein, wherein the serotypes are selected from 1, 3, 4, 5, 6A, 6B, 7F, 9V, 10A, 12F, 14, 15A, 15B, 18C, 19F, 19A, 22F, 23F, 33F, 34, 35, 38 and 45, and the carrier protein is PsaA.

[0057] In another embodiment, the present disclosure provides a pneumococcal vaccine composition comprising pneumococcal polysaccharides of one or more serotypes and a carrier protein, wherein the serotypes are 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 23F, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, 39 and 45, and the carrier protein is PsA.

[0058] In another embodiment, the present disclosure provides a pneumococcal vaccine composition comprising pneumococcal polysaccharides of one or more serotypes and a carrier protein wherein the pneumococcal polysaccharides are each individually conjugated to PsaA or combination of PsaA and CRM_{197}.

[0059] In another embodiment, the present disclosure provides a pneumococcal vaccine composition comprising at least six pneumococcal polysaccharides each individually conjugated to PsaA, and one or more pneumococcal polysaccharides each individually conjugated to CRM_{197}.

[0060] In another embodiment, the present disclosure provides a pneumococcal vaccine composition comprising at least five pneumococcal polysaccharides each individually conjugated to PsaA, and at least five pneumococcal polysaccharides each individually conjugated to CRM_{197}.

[0061] In another embodiment, the present disclosure provides a 14 valent pneumococcal vaccine comprising at least five pneumococcal polysaccharides each individually conjugated to PsaA, and at least five pneumococcal polysaccharides each individually conjugated to CRM_{197}.

[0062] In another embodiment, the present disclosure provides a 17 valent pneumococcal vaccine comprising at least five serotypes of pneumococcal

Petitioner Merck Ex-1004, 0683

polysaccharides each individually conjugated to PsaA, and at least five serotypes of pneumococcal polysaccharides each individually conjugated to CRM_{197}.

[0063] In another embodiment, the present disclosure provides a 20 valent pneumococcal vaccine comprising at least five serotypes of pneumococcal polysaccharides each individually conjugated to PsaA, and at least five serotypes of pneumococcal polysaccharides each individually conjugated to CRM_{197}.

[0064] In another embodiment, the present disclosure provides a 22 valent pneumococcal vaccine comprising at least five serotypes of pneumococcal polysaccharides each individually conjugated to PsaA, and at least five serotypes of pneumococcal polysaccharides each individually conjugated to CRM_{197}.

[0065] In another embodiment, the present disclosure provides a 24 valent pneumococcal vaccine comprising at least five serotypes of pneumococcal polysaccharides each individually conjugated to PsaA, and at least five serotypes of pneumococcal polysaccharides each individually conjugated to CRM_{197}.

[0066] In another embodiment, the present disclosure provides a 14 valent pneumococcal vaccine comprising pneumococcal polysaccharide serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F, wherein at least serotypes 3, and 6B are conjugated to PsaA and one or more serotypes 1, 4, 5, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F are conjugated to CRM_{197}.

[0067] In another embodiment, the present disclosure provides a 14 valent pneumococcal vaccine comprising pneumococcal polysaccharide serotypes 1, 3, 4, 5, 6A, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F, wherein at least serotypes 3, and 6A are conjugated to PsaA and one or more serotypes 1, 4, 5, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F are conjugated to CRM_{197}.

[0068] In another embodiment, the present disclosure provides a 15 valent pneumococcal vaccine comprising pneumococcal polysaccharide serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F wherein at least serotypes 3, 6A and 6B are conjugated to PsaA and one or more serotypes 1, 4, 5, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F are conjugated to CRM_{197}.

[0069] In another embodiment, the present disclosure provides a 17 valent pneumococcal vaccine comprising pneumococcal polysaccharide serotypes 1, 3, 4, 5,

Petitioner Merck Ex-1004, 0684

6A, 6B, 7F, 9V, 12F,14, 18C, 19A, 19F, 22F, 23F, 33F and 35B wherein at least serotypes 3, 6A and 6B are conjugated to PsaA and one or more serotypes 1, 4, 5, 7F, 9V, 12F, 14, 18C, 19A, 19F, 22F, 23F, 33F and 35B are conjugated to CRM_{197}.

[0070] In another embodiment, the present disclosure provides a 20 valent pneumococcal vaccine comprising pneumococcal polysaccharide serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, 33F, 35B and 45 wherein at least serotypes 3, 6A and 6B are conjugated to PsaA and one or more serotypes 1, 4, 5, 7F, 9V, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, 33F, 35B and 45 are conjugated to CRM_{197}.

[0071] In another embodiment, the present disclosure provides a 22 valent pneumococcal vaccine comprising pneumococcal polysaccharide serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 10A, 12F, 14, 15A, 15B, 18C, 19A, 19F, 22F, 23F, 33F, 34, 35B and 38 wherein at least serotypes 3, 6A and 6B are conjugated to PsaA and one or more serotypes 1, 4, 5, 7F, 9V, 10A, 12F, 14, 15A, 15B, 18C, 19A, 19F, 22F, 23F, 33F, 34, 35B and 38 are conjugated to CRM_{197}.

[0072] In another embodiment, the present disclosure provides a 22 valent pneumococcal vaccine comprising pneumococcal polysaccharide serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 10A, 12F, 14, 15A, 15B, 18C, 19A, 19F, 22F, 23F, 33F, 34, 35B and 38 wherein serotypes 1, 3, 6A, 10A, 12F, 15A 15B, 22F, 34, 35B, 38 are conjugated to PsaA and serotypes 14, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F and 33F are conjugated to CRM_{197}.

[0073] In another embodiment, the present disclosure provides a 24 valent pneumococcal vaccine comprising pneumococcal polysaccharide serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 12F, 14, 15A, 15B, 16F, 18C, 19A, 19F, 22F, 23F, 33F, 34, 35B and 38 wherein at least serotypes 3, 6A and 6B are conjugated to PsaA and one or more serotypes 1, 4, 5, 7F, 8, 9V, 10A, 12F, 14, 15A, 15B, 16F, 18C, 19A, 19F, 22F, 23F, 33F, 34, 35B and 38 are conjugated to CRM_{197}.

[0074] In another embodiment, the present disclosure provides a 24 valent pneumococcal vaccine comprising pneumococcal polysaccharide serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 12F, 14, 15A, 15B, 16F 18C, 19A, 19F, 22F, 23F, 33F, 34, 35B and 38 wherein serotypes 1, 3, 6A, 8, 10A, 12F, 15A 15B, 16F 22F, 34, 35, 38

Petitioner Merck Ex-1004, 0685

are conjugated to PsaA and one or more serotypes 14, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F and 33F are conjugated to CRM_{197}.

[0075] In another embodiment, the present disclosure provides a pneumococcal vaccine composition formulated into a dosage unit.

[0076] In another embodiment, the present disclosure provides a pneumococcal vaccine composition formulated into a dosage unit wherein the dosage unit comprises about 0.1 µg to about 50 µg of each polysaccharide, about 0.1 µg to about 10 µg, or about 1 µg to about 5 µg of each polysaccharide, wherein each polysaccharide is each individually conjugated to the carrier protein from about 1.5 µg to about 5 µg of carrier protein.

[0077] In another embodiment, the present disclosure provides a pneumococcal vaccine composition comprising a pharmaceutically acceptable diluent, buffer, preservative, stabilizer, adjuvant, and/or a lyophilization excipient.

[0078] In another embodiment, the present disclosure provides a pneumococcal vaccine composition formulated into a dosage unit that is supplied as a unit dose vial, a multiple dose vial, or a pre-filled syringe.

[0079] In another embodiment, the present disclosure provides a pneumococcal vaccine composition as a single 0.5 mL dose, the single dose comprising about 2.2 to 4.4 µg of one or more pneumococcal polysaccharides; about 1 µg to about 10 µg of PsaA conjugated to each of the one or more pneumococcal polysaccharides; about 1 µg to about 10 µg of CRM_{197} conjugated to each of the one or more pneumococcal polysaccharides; about 0.2 mg to about 1 mg of aluminum phosphate adjuvant; and an excipient.

[0080] In another embodiment, the present disclosure provides a 13 valent pneumococcal vaccine comprising pneumococcal polysaccharide serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F and 33F each individually conjugated to PsaA or combination of PsaA and CRM_{197}, wherein the pneumococcal vaccine composition is about a 0.5 mL dose formulated as a sterile liquid comprising about 2.2 µg to about 4.4 µg of each polysaccharide, about 25 µg to about 30 µg PsaA and CRM_{197}, about 0.125 mg of elemental aluminum as about 0.5 mg aluminum phosphate, sodium chloride, and a L-histidine buffer.

Petitioner Merck Ex-1004, 0686

[0081] In another embodiment, the present disclosure provides a 14 valent pneumococcal vaccine comprising pneumococcal polysaccharide serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F each individually conjugated to PsaA or combination of PsaA and CRM_{197}, wherein the pneumococcal vaccine composition is about a 0.5 mL dose formulated as a sterile liquid comprising, about 2.2 µg to about 4.4 µg of each polysaccharide, about 30 µg to about 35 µg PsaA and CRM_{197}, about 0.125 mg of elemental aluminum as about 0.5 mg aluminum phosphate, sodium chloride, and a L-histidine buffer.

[0082] In another embodiment, the present disclosure provides a 14 valent pneumococcal vaccine comprising pneumococcal polysaccharide serotypes 1, 3, 4, 5, 6A, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F each individually conjugated to PsaA or combination of PsaA and CRM_{197}, wherein the pneumococcal vaccine composition is about a 0.5 mL dose formulated as a sterile liquid comprising, about 2.2 to 4.4 µg of each polysaccharide, about 30 µg to about 35 µg PsaA and CRM_{197}, about 0.125 mg of elemental aluminum as about 0.5 mg aluminum phosphate, sodium chloride, and a L-histidine buffer.

[0083] In another embodiment, the present disclosure provides a 15 valent pneumococcal vaccine comprising pneumococcal polysaccharide serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V,14, 18C, 19A, 19F, 22F, 23F and 33F wherein at least serotypes 3, 6A and 6B are conjugated to PsaA and one or more serotypes 1, 4, 5, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F are conjugated to CRM_{197}, wherein the pneumococcal vaccine composition is about a 0.5 mL dose formulated as a sterile liquid comprising, about 2.2 µg to about 4.4 µg of each polysaccharide, about 5 µg to about 20 µg of PsaA, about 20 µg to about 40 µg of CRM_{197}, about 0.125 mg of elemental aluminum as about 0.5 mg aluminum phosphate, sodium chloride, and a L-histidine buffer.

[0084] In another embodiment, the present disclosure provides a 17 valent pneumococcal vaccine comprising pneumococcal polysaccharide serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 12F,14, 18C, 19A, 19F, 22F, 23F, 33F and 35B wherein at least serotypes 3, 6A and 6B are conjugated to PsaA and one or more serotypes 1, 4, 5, 7F, 9V, 12F,14, 18C, 19A, 19F, 22F, 23F, 33F and 35B are conjugated to CRM_{197}, wherein the pneumococcal vaccine composition is about a 0.5 mL dose formulated as a sterile liquid comprising, about 2.2 µg to about 4.4 µg of each polysaccharide, about

Petitioner Merck Ex-1004, 0687

5 µg to about 20 µg of PsaA, about 20 µg to about 40 µg of CRM_{197}, about 0.125 mg of elemental aluminum as about 0.5 mg aluminum phosphate, sodium chloride, and a L-histidine buffer.

[0085] In another embodiment, the present disclosure provides a 20 valent pneumococcal vaccine comprising pneumococcal polysaccharide serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, 33F, 35B and 45 wherein at least serotypes 3, 6A and 6B are conjugated to PsaA and one or more serotypes 1, 4, 5, 7F, 9V, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, 33F, 35B and 45 are conjugated to CRM_{197}, wherein the pneumococcal vaccine composition is about a 0.5 mL dose formulated as a sterile liquid comprising, about 2.2 µg to about 4.4 µg of each polysaccharide, about 5 µg to about 20 µg of PsaA, about 20 µg to about 50 µg of CRM_{197}, about 0.125 mg of elemental aluminum as about 0.5 mg aluminum phosphate, sodium chloride, and a L-histidine buffer.

[0086] In another embodiment, the present disclosure provides a 22 valent pneumococcal vaccine comprising pneumococcal polysaccharide serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 10A, 12F, 14, 15A, 15B, 18C, 19A, 19F, 22F, 23F, 33F, 34, 35B and 38 wherein at least serotypes 3, 6A and 6B are conjugated to PsaA and one or more serotypes 1, 4, 5, 7F, 9V, 10A, 12F, 14, 15A, 15B, 18C, 19A, 19F, 22F, 23F, 33F, 34, 35B and 38 are conjugated to CRM_{197}, wherein the pneumococcal vaccine composition is about a 0.5 mL dose formulated as a sterile liquid comprising, about 2.2 µg to about 4.4 µg of each polysaccharide, about 5 µg to about 20 µg of PsaA, about 20 µg to about 50 µg of CRM_{197}, about 0.125 mg of elemental aluminum as about 0.5 mg aluminum phosphate, sodium chloride, and a L-histidine buffer.

[0087] In another embodiment, the present disclosure provides a 22 valent pneumococcal vaccine comprising pneumococcal polysaccharide serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 10A, 12F, 14, 15A, 15B, 18C, 19A, 19F, 22F, 23F, 33F, 34, 35B and 38 wherein serotypes 1, 3, 6A, 10A, 12F, 15A 15B, 22F, 34, 35B, 38 are conjugated to PsaA and serotypes 14, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F and 33F are conjugated to CRM_{197}, wherein the pneumococcal vaccine composition is about a 0.5 mL dose formulated as a sterile liquid comprising, about 2.2 µg to about 4.4 µg of each polysaccharide, about 20 µg to about 40 µg of PsaA, about 20 µg to about 40 µg

Petitioner Merck Ex-1004, 0688

of CRM_{197}, about 0.125 mg of elemental aluminum as about 0.5 mg aluminum phosphate, sodium chloride, and a L-histidine buffer.

[0088] In another embodiment, the present disclosure provides a 24 valent pneumococcal vaccine comprising pneumococcal polysaccharide serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 12F, 14, 15A, 15B, 16F, 18C, 19A, 19F, 22F, 23F, 33F, 34, 35B and 38 wherein at least serotypes 3, 6A and 6B are conjugated to PsaA and one or more serotypes 1, 4, 5, 7F, 8, 9V, 10A, 12F, 14, 15A, 15B, 16F, 18C, 19A, 19F, 22F, 23F, 33F, 34, 35B and 38 are conjugated to CRM_{197}, wherein the pneumococcal vaccine composition is about a 0.5 mL dose formulated as a sterile liquid comprising, about 2.2 µg to about 4.4 µg of each polysaccharide, about 5 µg to about 20 µg of PsaA, about 20 µg to about 50 µg of CRM_{197}, about 0.125 mg of elemental aluminum as about 0.5 mg aluminum phosphate, sodium chloride, and a L-histidine buffer.

BRIEF DESCRIPTION OF THE DRAWINGS

[0089] Figure 1: An SEC-HPLC chromatogram illustrates conjugation reaction kinetics of serotype 7F (A), serotype 14 (B), serotype 19F (C), serotype 3 (D), serotype 6A (E), and serotype 6B (F).

[0090] Figure 2: An SEC-HPLC chromatogram illustrates conjugation reaction kinetics of serotype 5 (A), serotype 9V (B), serotype 18C (C), serotype 3 (D), serotype 6A (E), and serotype 6B (F).

[0091] Figure 3: Immune response to different serotypes in rabbits immunized with a composition of the present disclosure as described in Example 3.

[0092] Figure 4: Immune response to different serotypes in rabbits immunized with Prevnar 13®.

[0093] Figure 5: Immune response to different serotypes in rabbits immunized with another composition of the present disclosure as described in Example 4.

DETAILED DESCRIPTION

[0094] In an embodiment, the present disclosure provides a pneumococcal vaccine composition comprising pneumococcal polysaccharides where one or more of the pneumococcal polysaccharides are native pneumococcal polysaccharides.

Petitioner Merck Ex-1004, 0689

[0095] In another embodiment, the present disclosure provides a pneumococcal vaccine composition comprising pneumococcal polysaccharides where one or more of the pneumococcal polysaccharides are fragmented, each fragmented pneumococcal polysaccharide having an average molecular weight less than that of a native pneumococcal polysaccharide.

[0096] In another embodiment, the present disclosure provides a pneumococcal vaccine composition comprising pneumococcal polysaccharides where each of the pneumococcal polysaccharides is activated with 1-cyano-4-dimethylamino-pyridinium tetrafluoroborate (CDAP) to form a cyanate ester prior to conjugation to the carrier protein.

[0097] In another embodiment, the present disclosure provides a pneumococcal vaccine composition comprising pneumococcal polysaccharides where one or more of the pneumococcal polysaccharides are directly coupled to an amino group of the carrier protein or are coupled to the amino group by a spacer.

[0098] In another embodiment, the present disclosure provides a pneumococcal vaccine composition comprising pneumococcal polysaccharides wherein the spacer is cystamine, cysteamine, hexane diamine, adipic acid dihydrazide (ADH), EDAC or EDC.

[0099] In another embodiment, the present disclosure provides a pneumococcal vaccine composition comprising pneumococcal polysaccharides of one or more serotypes and a carrier protein wherein the PsaA carrier protein is a modified PsaA and does not include wild-type hydrophobic N-terminal leader peptide.

[0100] In another embodiment, the present disclosure provides a pneumococcal vaccine composition comprising pneumococcal polysaccharides of one or more serotypes and a carrier protein wherein the PsaA carrier protein includes 290 amino acids.

[0101] The present disclosure provides a pneumococcal vaccine composition comprising two or more capsular pneumococcal polysaccharide serotypes each individually conjugated to a carrier protein, referred to herein as polysaccharide-protein conjugates and/or conjugates. When included in the pneumococcal vaccine composition described herein, pneumococcal vaccine is a multivalent pneumococcal

Petitioner Merck Ex-1004, 0690

polysaccharide-protein conjugate vaccine (also referred to herein as multivalent conjugate vaccine, conjugate vaccine, and/or polysaccharide-protein conjugate vaccine). In addition to the multivalent conjugate vaccine, the present disclosure provides a process for preparing and/or administering the same to a subject in need thereof.

[0102] In some embodiments, the pneumococcal vaccine composition is a multivalent immunogenic composition comprising one or more conjugates. For example, the conjugates may include two or more pneumococcal polysaccharides, each of the pneumococcal polysaccharides selected from pneumococcal polysaccharides of serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 23F, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, 39, and 45.

[0103] In some embodiments, the pneumococcal vaccine composition is multivalent and comprises five pneumococcal polysaccharides (5 valent), ten pneumococcal polysaccharides (10 valent), eleven pneumococcal polysaccharides (11 valent), twelve pneumococcal polysaccharides (12 valent), thirteen pneumococcal polysaccharides (13 valent), fourteen pneumococcal polysaccharides (14 valent), fifteen pneumococcal polysaccharides (15 valent), sixteen pneumococcal polysaccharides (16 valent), seventeen pneumococcal polysaccharides (17 valent), eighteen pneumococcal polysaccharides (18 valent), nineteen pneumococcal polysaccharides (19 valent), twenty pneumococcal polysaccharides (20 valent), twenty-one pneumococcal polysaccharides (21 valent), twenty-two pneumococcal polysaccharides (22 valent), twenty-three pneumococcal polysaccharides (23 valent), twenty-four pneumococcal polysaccharides (24 valent), twenty-five pneumococcal polysaccharides (25 valent), twenty-six pneumococcal polysaccharides (26 valent), twenty-seven pneumococcal polysaccharides (27 valent), twenty-eight pneumococcal polysaccharides (28 valent), twenty-nine pneumococcal polysaccharides (29 valent), or thirty pneumococcal polysaccharides (30 valent). In other embodiments, the pneumococcal vaccine composition is a 10 valent, 14 valent, 15 valent, 17 valent, 18 valent, 19 valent, 20 valent, 22 valent, 23 valent, 24 valent or 25 valent pneumococcal vaccine composition.

Petitioner Merck Ex-1004, 0691

[0104] Surprisingly, the multivalent pneumococcal conjugate vaccine compositions of the present disclosure offer an improved immune response compared to multivalent pneumococcal vaccines comprising pneumococcal polysaccharides not conjugated to carrier proteins. More specifically, the multivalent pneumococcal conjugate vaccine compositions of the present disclosure were surprisingly most effective when the pneumococcal polysaccharides from one or more serotypes of *Streptococcus. pneumoniae* are conjugated to PsaA and/or CRM_{197}.

[0105] Carrier proteins are non-toxic and non-reactogenic proteins that are obtainable in a sufficient amount and purity. In some embodiments, the present disclosure provides a pneumococcal conjugate vaccine composition comprising one or more carrier proteins conjugated to one or more *S. pneumoniae* polysaccharides (also referred to herein as "pneumococcal polysaccharides"). By conjugating a pneumococcal polysaccharide to a carrier protein, the pneumococcal polysaccharide has increased immunogenicity compared to an unconjugated pneumococcal polysaccharide. Carrier proteins useful with the present disclosure should be amenable to standard conjugation procedures.

[0106] CRM_{197} is a variant of diphtheria toxin and is non-toxic for use in vaccines. CRM_{197} may be isolated from cultures of *Corynebacterium diphtheriae* strain C7 (β197) grown in casamino acids and yeast extract-based medium. CRM_{197} may be prepared recombinantly in accordance with the methods described in U.S. Pat. No. 5,614,382. Alternatively, CRM_{197} may be prepared recombinantly in accordance with the methods known in the literature or according to the method disclosed in PCT publication WO 2016/079755. CRM_{197} may be purified by ultrafiltration, ammonium sulphate precipitation, and ion-exchange chromatography or other methods well known in art.

[0107] In some embodiments, the pneumococcal polysaccharides of the present disclosure are conjugated to one or more carrier proteins. For example, a pneumococcal polysaccharide is each individually conjugated to a carrier protein. In other embodiments, more than one pneumococcal polysaccharide is conjugated to a carrier protein. For example, two pneumococcal polysaccharides, three pneumococcal polysaccharides, four pneumococcal polysaccharides, five pneumococcal polysaccharides, six pneumococcal polysaccharides, seven

Petitioner Merck Ex-1004, 0692

pneumococcal polysaccharides, eight pneumococcal polysaccharides, nine pneumococcal polysaccharides, or ten pneumococcal polysaccharides are conjugated to a carrier protein. In some embodiments, a pneumococcal polysaccharide is conjugated to more than one carrier protein. For example, a pneumococcal polysaccharide is conjugated to one carrier protein, two carrier proteins, three carrier proteins, and/or four carrier proteins.

[0108] In some embodiments, the carrier protein is PsaA. In additional embodiments, a combination of the carrier protein used, which includes two or more carrier proteins, such as PsaA and CRM_{197} which are each individually conjugated to each pneumococcal polysaccharide. In further embodiments, the carrier protein is two carrier proteins and includes PsaA and CRM_{197}.

[0109] In some embodiments, the carrier protein is selected from one or more of the following carrier proteins, PsaA, CRM_{197}, inactivated bacterial toxins such as tetanus toxoid, pertussis toxoid; cholera toxoid, exotoxin A from *Pseudomonas aeruginosa*, bacterial outer membrane proteins such as outer membrane complex c (OMPC), porins, transferrin binding proteins, pneumolysin, PspA, C5a peptidase from Group A or Group B streptococcus, or *Haemophilus influenzae* protein D, ovalbumin, keyhole limpet hemocyanin, (KLH), bovine serum albumin (BSA) and purified protein derivative of tuberculin (PPD).

[0110] Furthermore, one or more of the conjugates may include native (e.g., wild-type) carrier proteins and/or one or more of the conjugates may include carrier proteins modified from their native form to a non-native form (e.g., engineered to lack one or more amino acids). In some embodiments, carrier proteins may be engineered to eliminate one or more protein domains, such as a leader peptide, or other domains that might have properties which are undesirable for conjugates of the present disclosure. For example, PsaA may be a 290 amino acid carrier protein engineered to lack a hydrophobic N-terminal leader peptide having hydropathy index of 2.052.

[0111] In some embodiments, the present disclosure provides a pneumococcal polysaccharide-protein conjugate vaccine composition comprising pneumococcal polysaccharides (e.g., capsular pneumococcal polysaccharides) selected from serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 23F, 24B, 24F, 31, 33F, 34, 35B,

Petitioner Merck Ex-1004, 0693

35F, 38, 39 and 45. The selected pneumococcal polysaccharides are each individually conjugated to carrier protein PsaA or a first portion of the selected pneumococcal polysaccharides are conjugated to PsaA and a second portion of the selected pneumococcal polysaccharides are conjugated to CRM_{197}. In any of these embodiments, the pneumococcal polysaccharide-protein conjugate vaccine composition is an immunogenic multivalent pneumococcal polysaccharide-protein conjugate composition, such as a 10 valent, 13 valent, 14 valent, 15 valent, 17 valent, 18 valent, 19 valent, 20 valent, 22 valent, 23 valent, 24 valent or 25 valent immunogenic multivalent pneumococcal polysaccharide-protein conjugate composition.

[0112] In some embodiments, the present disclosure provides a pneumococcal polysaccharide-protein conjugate vaccine composition comprising pneumococcal polysaccharides (e.g., capsular pneumococcal polysaccharides) selected from serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 23F, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, 39 and 45. The selected pneumococcal polysaccharides are each individually conjugated to carrier protein PsaA. In other embodiments, a first portion of the selected pneumococcal polysaccharides are conjugated to PsaA and a second portion of the selected pneumococcal polysaccharides are conjugated to CRM_{197}. In any of these embodiments, the pneumococcal polysaccharide-protein conjugate vaccine composition is an immunogenic multivalent pneumococcal polysaccharide-protein conjugate composition, such as a 10 valent, 13 valent, 14 valent, 15 valent, 17 valent, 18 valent, 19 valent, 20 valent, 22 valent, 23 valent, 24 valent or 25 valent immunogenic multivalent pneumococcal polysaccharide-protein conjugate composition.

[0113] In further embodiments, the present disclosure provides a pneumococcal polysaccharide-protein conjugate vaccine composition comprising pneumococcal polysaccharides (e.g., capsular pneumococcal polysaccharides) selected from serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 23F, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, 39 and 45. In these embodiments, at least three of the selected pneumococcal polysaccharides are each individually conjugated to carrier protein PsaA and additional pneumococcal polysaccharides are each individually conjugated

Petitioner Merck Ex-1004, 0694

to carrier protein CRM_{197}. In any of these embodiments, the pneumococcal polysaccharide-protein conjugate vaccine composition is an immunogenic multivalent pneumococcal polysaccharide-protein conjugate composition, such as a 10 valent, 13 valent, 14 valent, 15 valent, 17 valent, 18 valent, 19 valent, 20 valent, 22 valent, 23 valent, 24 valent or 25 valent immunogenic multivalent pneumococcal polysaccharide-protein conjugate composition.

[0114] In further embodiments, the present disclosure provides a pneumococcal polysaccharide-protein conjugate vaccine composition comprising pneumococcal polysaccharides (e.g., capsular pneumococcal polysaccharides) selected from serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 23F, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, 39 and 45. In these embodiments, at least a first five of the selected pneumococcal polysaccharides are each individually conjugated to carrier protein PsaA and at least a second five polysaccharides are each individually conjugated to carrier protein CRM_{197}. The first five selected pneumococcal polysaccharides are selected from serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 23F, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, 39 and 45 and the second five selected from serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 23F, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, 39 and 45. In other embodiments, a first portion of the selected pneumococcal polysaccharides are conjugated to PsaA and a second portion of the selected pneumococcal polysaccharides are conjugated to CRM_{197}. In any of these embodiments, the pneumococcal polysaccharide-protein conjugate vaccine composition is an immunogenic multivalent pneumococcal polysaccharide-protein conjugate composition, such as a 10 valent, 14 valent, 15 valent, 17 valent, 18 valent, 19 valent, 20 valent, 22 valent, 23 valent, 24 valent or 25 valent immunogenic multivalent pneumococcal polysaccharide-protein conjugate composition.

[0115] In further embodiments, the present disclosure provides a 14 valent pneumococcal polysaccharide-protein conjugate vaccine composition comprising at least three pneumococcal polysaccharides (e.g., capsular pneumococcal polysaccharides) selected from serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 19F, 19A, 20A, 20B, 22F, 23A, 23B,

Petitioner Merck Ex-1004, 0695

23F, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, 39 and 45, and are conjugated to PsaA. Moreover, the 14 valent pneumococcal polysaccharide-protein conjugate vaccine composition further comprises at least five pneumococcal polysaccharides selected from serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 23F, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, 39 and 45, and are conjugated to CRM_{197}.

[0116] In further embodiments, the present disclosure provides a 15 valent pneumococcal polysaccharide-protein conjugate vaccine composition comprising at least three pneumococcal polysaccharides (e.g., capsular pneumococcal polysaccharides) selected from serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 23F, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, 39 and 45, and are conjugated to PsaA. Moreover, the 15 valent pneumococcal polysaccharide-protein conjugate vaccine composition further comprises at least five pneumococcal polysaccharides selected from serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 23F, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, 39 and 45, and are conjugated to CRM_{197}.

[0117] In some embodiments, the present disclosure provides a 17 valent pneumococcal polysaccharide-protein conjugate vaccine composition comprising at least three pneumococcal polysaccharides (e.g., capsular pneumococcal polysaccharides) selected from serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 23F, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, 39 and 45, and are conjugated to PsaA. Moreover, the 17 valent pneumococcal polysaccharide-protein conjugate vaccine composition further comprises at least five pneumococcal polysaccharides selected from serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 23F, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, 39 and 45, and are conjugated to CRM_{197}.

[0118] In other embodiments, the present disclosure provides a 20 valent pneumococcal polysaccharide-protein conjugate vaccine composition comprising at least three pneumococcal polysaccharides (e.g., capsular pneumococcal polysaccharides) selected from serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A,

Petitioner Merck Ex-1004, 0696

11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 23F, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, 39 and 45, and are conjugated to PsaA. Moreover, the 20 valent pneumococcal polysaccharide-protein conjugate vaccine composition further comprises at least five pneumococcal polysaccharides selected from serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 23F, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, 39 and 45, and are conjugated toCRM_{197}.

[0119] In additional embodiments, the present disclosure provides a 22 valent pneumococcal polysaccharide-protein conjugate vaccine composition comprising at least five pneumococcal polysaccharides (e.g., capsular pneumococcal polysaccharides) selected from serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 23F, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, 39 and 45, and are conjugated to PsaA . Moreover, the 22 valent pneumococcal polysaccharide-protein conjugate vaccine composition further comprises at least five pneumococcal polysaccharides selected from serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 23F, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, 39 and 45, and are conjugated to CRM_{197}.

[0120] In some embodiments, the present disclosure provides a 24 valent pneumococcal polysaccharide-protein conjugate vaccine composition comprising at least five pneumococcal polysaccharides (e.g., capsular pneumococcal polysaccharides) selected from serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 23F, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, 39 and 45, and are conjugated to PsaA. Moreover, the 24 valent pneumococcal polysaccharide-protein conjugate vaccine composition further comprises at least five pneumococcal polysaccharides selected from serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 23F, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, 39 and 45, and are conjugated to CRM_{197}.

[0121] In further embodiments, the present disclosure provides a 25 valent pneumococcal polysaccharide-protein conjugate vaccine composition comprising at least five pneumococcal polysaccharides (e.g., capsular pneumococcal

Petitioner Merck Ex-1004, 0697

polysaccharides) selected from serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 23F, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, 39 and 45, and are conjugated to PsaA . Moreover, the 25 valent pneumococcal polysaccharide-protein conjugate vaccine composition further comprises at least five pneumococcal polysaccharides selected from serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 23F, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, 39 and 45, and are conjugated to CRM_{197}.

[0122] In other embodiments, the present disclosure provides a 14 valent pneumococcal polysaccharide-protein conjugate vaccine composition comprising pneumococcal polysaccharides (e.g., capsular pneumococcal polysaccharides) selected from serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F. Of the selected serotypes, at least serotype 3, and serotype 6B are conjugated to PsaA, and each of one or more serotypes 1, 4, 5, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F are conjugated to CRM_{197}.

[0123] In other embodiments, the present disclosure provides a 14 valent pneumococcal polysaccharide-protein conjugate vaccine composition comprising pneumococcal polysaccharides (e.g., capsular pneumococcal polysaccharides) selected from serotypes 1, 3, 4, 5, 6A, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F. Of the selected serotypes, at least serotype 3, and serotype 6A are conjugated to PsaA, and each of one or more serotypes 1, 4, 5, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F are conjugated to CRM_{197}.

[0124] In additional embodiments, the present disclosure provides a 15 valent pneumococcal polysaccharide-protein conjugate vaccine composition comprising pneumococcal polysaccharides (e.g., capsular pneumococcal polysaccharides) selected from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V,14, 18C, 19A, 19F, 22F, 23F and 33F. Of the selected serotypes, at least serotypes 3, 6A, and 6B are conjugated to PsaA, and each of one or more serotypes 1, 4, 5, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F are conjugated to CRM_{197}.

[0125] In some embodiments, the present disclosure provides a 17 valent pneumococcal polysaccharide-protein conjugate vaccine composition comprising pneumococcal polysaccharides (e.g., capsular pneumococcal polysaccharides)

Petitioner Merck Ex-1004, 0698

selected from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 12F,14, 18C, 19A, 19F, 22F, 23F, 33F and 35B. Of the selected serotypes, at least serotypes 3, 6A, and 6B are conjugated to PsaA, and each of one or more serotypes 1, 4, 5, 7F, 9V, 12F,14, 18C, 19A, 19F, 22F, 23F, 33F and 35B are conjugated to CRM_{197}.

[0126] In some embodiments, the present disclosure provides a 20 valent pneumococcal polysaccharide-protein conjugate vaccine composition comprising pneumococcal polysaccharides (e.g., capsular pneumococcal polysaccharides) selected from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, 33F, 35B and 45. Of the selected serotypes, at least serotypes 3, 6A, and 6B are conjugated to PsaA, and each of one or more serotypes 1, 4, 5, 7F, 9V, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, 33F, 35B and 45 are conjugated to CRM_{197}.

[0127] In other embodiments, the present disclosure provides a 22 valent pneumococcal polysaccharide-protein conjugate vaccine composition comprising pneumococcal polysaccharides (e.g., capsular pneumococcal polysaccharides) selected from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 10A, 12F, 14, 15A, 15B, 18C, 19A, 19F, 22F, 23F, 33F, 34,35B and 38. Of the selected serotypes, at least serotypes 3, 6A, and 6B are conjugated to PsaA, and each of one or more serotypes 1, 4, 5, 7F, 9V, 10A, 12F, 14, 15A, 15B, 18C, 19A, 19F, 22F, 23F, 33F, 34, 35B and 38 are conjugated to CRM_{197}.

[0128] In other embodiments, the present disclosure provides a 22 valent pneumococcal polysaccharide-protein conjugate vaccine composition comprising pneumococcal polysaccharides (e.g., capsular pneumococcal polysaccharides) selected from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 10A, 12F, 14, 15A, 15B, 18C, 19A, 19F, 22F, 23F, 33F, 34, 35B and 38. Of the selected serotypes, serotypes 1, 3, 6A, 10A, 12F, 15A 15B, 22F, 34, 35, and 38 are conjugated to PsaA, and each of serotypes 14, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F and 33F are conjugated to CRM_{197}.

[0129] In other embodiments, the present disclosure provides a 24 valent pneumococcal polysaccharide-protein conjugate vaccine composition comprising pneumococcal polysaccharides (e.g., capsular pneumococcal polysaccharides) selected from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 12F, 14, 15A, 15B, 16F, 18C, 19A, 19F, 22F, 23F, 33F, 34, 35B and 38. Of the selected serotypes, serotypes

Petitioner Merck Ex-1004, 0699

1, 3, 6A, 8, 10A, 12F, 15A 15B, 16F, 22F, 34, 35, and 38 are conjugated to PsaA, and each of serotypes 14, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F and 33F are conjugated to CRM_{197}.

[0130] The pneumococcal polysaccharide-protein conjugate compositions of the present disclosure further comprise one or more of the following: a pharmaceutically acceptable carrier, a pharmaceutically acceptable diluent, a buffer, a preservative, a stabilizer, an adjuvant, and/or a lyophilization excipient. For example, the pneumococcal polysaccharide-protein conjugate compositions of the present disclosure may comprise a pharmaceutically acceptable carrier. In some embodiments, the pneumococcal polysaccharide-protein conjugate compositions of the present disclosure comprise at least 10 pneumococcal polysaccharides selected from serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 23F, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, 39 and 45. The selected serotypes are each individually conjugated to PsaA or a combination of PsaA and CRM_{197}, and a pharmaceutically acceptable carrier.

[0131] In some embodiments, the pneumococcal polysaccharides useful in the compositions of the present disclosure may be extracted from one or more microorganisms (e.g. Streptococcus pneumoniae) according to conventional methods. For example, pneumococcal polysaccharides may be prepared according to known procedures. Furthermore, purification of the pneumococcal polysaccharides may be performed according to the procedure described in PCT publication WO 2016/174683 A1.

[0132] The extracted pneumococcal polysaccharides may be purified according to conventional methods and may be used in its native form. In other embodiments, the extracted and purified pneumococcal polysaccharides may be fragmented to obtain one or more portions of the pneumococcal polysaccharide, each portion of the pneumococcal polysaccharide having an average molecular weight less than that of the extracted and purified pneumococcal polysaccharides.

[0133] In another embodiment, the present disclosure provides a pneumococcal vaccine composition comprising pneumococcal polysaccharides, each pneumococcal polysaccharide having a molecular between about 150 kDa and 450 kDa.

Petitioner Merck Ex-1004, 0700

[0134] In another embodiment, the present disclosure provides a pneumococcal vaccine composition comprising one or more capsular pneumococcal polysaccharide serotype each individually conjugated to a carrier protein, such as a polysaccharide-protein conjugate wherein each polysaccharide-protein conjugate has a molecular weight of about 1,500 kDa to about 15,000 kDa.

[0135] In other embodiments, the extracted and purified pneumococcal polysaccharides may be activated prior to conjugation to one or more carrier proteins. For example, the extracted and purified pneumococcal polysaccharides may be activated (e.g., chemically) prior to conjugation to one or more carrier proteins. Each activated pneumococcal polysaccharide may be each individually conjugated to a carrier protein forming a polysaccharide-protein conjugate (e.g., a glycoconjugate). In other embodiments, one or more of the activated pneumococcal polysaccharides may be conjugated to an individual carrier protein and/or an activated pneumococcal polysaccharide may be conjugated to an individual carrier protein. The conjugates may be prepared by known techniques.

[0136] In some embodiments, the pneumococcal polysaccharides may be chemically activated and subsequently conjugated to carrier proteins according to known techniques, such as those described in U.S. Pat. Nos. 4,365,170, 4,673,574 and 4,902,506. For example, pneumococcal polysaccharides can be activated by oxidation of a terminal hydroxyl group to an aldehyde with an oxidizing agent, such as periodate (e.g., sodium periodate, potassium periodate, or periodic acid) by random oxidative cleavage of one or more vicinal hydroxyl groups of the carbohydrates and formation of one or more reactive aldehyde groups.

[0137] The pneumococcal polysaccharides may also be activated by CDAP (1-cyano-4-dimethylamino-pyridinium tetrafluoroborate) and subsequently conjugated to one or more carrier proteins such as PsaA, CRM_{197}, PspA, or combination thereof. In other embodiments, pneumococcal polysaccharides activated with CDAP to form a cyanate ester may be directly conjugated to one or more carrier proteins or conjugated using a spacer (e.g., linker). The spacer may couple to an amino group on the carrier protein. In some embodiments, the spacer may be cystamine or cysteamine, which generates a thiolated polysaccharide that may be coupled to the carrier protein through a thioether linkage to a maleimide-activated carrier protein

Petitioner Merck Ex-1004, 0701

(e.g., using GMBS) or a haloacetylated carrier protein (e.g., using iodoacetimide, ethyl iodoacetimide HCl, SIAB, SIA, SBAP, and/or N-succinimidyl bromoacetate. In other embodiments, the cyanate ester is coupled using hexane diamine or adipic acid dihydrazide (ADH) and an amino-derivitized saccharide is conjugated to a carrier protein using carbodiimide (e.g. EDAC or EDC) chemistry via a carboxyl group on the protein carrier. Such conjugates are described in International Patent Application Publication No. WO 93/15760, International Patent Application Publication No. WO 95/08348, International Patent Application Publication No. WO 96/29094, and Chu et al., 1983, Infect. Immunity 40:245-256.

[0138] Other suitable activation and/or coupling techniques for use with the polysaccharide-protein conjugates and vaccine compositions of the present disclosure include use of carbodiimides, hydrazides, active esters, norborane, p-nitrobenzoic acid, N-hydroxysuccinimide, S—NHS, EDC, TSTU, and other methods described in International Patent Application Publication No. WO 98/42721. For example, conjugation may involve a carbonyl linker which may be formed by reaction of a free hydroxyl group of the saccharide with CDI (See Bethell et al., 1979, J. Biol. Chem. 254:2572-4; Hearn et al., 1981, J. Chromatogr. 218:509-18) followed by coupling with a protein to form a carbamate linkage. In some embodiments, the anomeric terminus may be reduced 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.

[0139] For example, another suitable activation and/or coupling techniques for use with the polysaccharide-protein conjugates and vaccine compositions of the present disclosure include the following method: sized pneumococcal polysaccharides (e.g., about 6 mL of sized polysaccharide at a concentration of about 10 mg/mL) and CDAP (e.g., about 100 mg/mL in acetonitrile (w/v)) can be mixed in a glass vial in a ratio of about 1 to about 1 (e.g., by stirring for about 1 minute). The pH of the polysaccharide solution may be adjusted as necessary (e.g., to about 9.25 with about 0.2M triethylamine and stirred for 3 min at room temperature). In addition, PsaA (e.g., about 4 mL of a solution having a concentration of about 15 mg/mL) may be added slowly to the activated pneumococcal polysaccharides (e.g., in a ratio of about 1 to about 1 (Ps: Carrier protein)). The pH of the reaction may be adjusted (e.g., to about

Petitioner Merck Ex-1004, 0702

9.05 using 0.2M trimethylamine) and the reaction may be continued (e.g., by stirring for 5 hours at room temperature). The reaction mixture may be quenched (e.g., by addition of an excess concentration of glycine).

[0140] In some embodiments, the reaction mixture may be diafiltered using a membrane (e.g., a 100 K MWCO membrane) and may be purified by size-exclusion chromatography. The diafiltered and purified fractions may be analyzed using SEC-MALLS, and an anthrone method. The analyzed fractions containing conjugates may be pooled and sterile filtered (e.g., using 0.2 µm filters).

[0141] Following conjugation of pneumococcal polysaccharides to one or more carrier proteins, the polysaccharide-protein conjugates may be purified (e.g., enriched with respect to the amount of polysaccharide-protein conjugate) by a variety of techniques. These techniques include, but are not limited to concentration/diafiltration operations, precipitation/elution, column chromatography, and depth filtration. For example, after the conjugates are purified, the conjugates may be compounded to formulate the pneumococcal polysaccharide-protein conjugate compositions of the present disclosure, which may be used as vaccines.

[0142] In some embodiments, the present disclosure provides a method for preparing a polysaccharide-protein conjugate of the pneumococcal vaccine composition described herein wherein the method further comprises formulating the polysaccharide-protein conjugate into the pneumococcal vaccine composition including an adjuvant, an excipient, and a buffer.

[0143] In some embodiments, the present disclosure provides a method for preparing a polysaccharide-protein conjugate of the pneumococcal vaccine composition described herein wherein the adjuvant is aluminum phosphate.

[0144] In some embodiments, the present disclosure provides a method of treating a subject in need thereof comprising, administering a pneumococcal vaccine composition described herein to the subject in need thereof.

[0145] In some embodiments, the subject has a disease mediated by *streptococcus pneumoniae,* such as invasive pneumococcal disease (IPD).

[0146] In one embodiment, the subject is a human, such as an infant (less than about 1 year of age), a toddler (about 12 months to about 24 months of age), a young

Petitioner Merck Ex-1004, 0703

child (about 2 years to about 5 years of age), an older child (about 5 years to about 13 years of age), an adolescent (about 13 years to about 18 years of age), an adult (about 18 years to about 65 years of age), or an elder (more than about 65 years of age).

[0147] In some embodiments, the present disclosure provides a method of inducing an immune response to an *S. pneumoniae* capsular polysaccharide conjugate comprising administering an immunologically effective amount of the pneumococcal vaccine composition described herein to a subject.

[0148] In one embodiment, method of inducing an immune response to an *S. pneumoniae* capsular polysaccharide conjugate, comprising administering the pneumococcal vaccine composition described herein to the subject systemically, subcutaneously, and/or mucousally.

[0149] In some embodiments, an amount of each conjugate in a dose of the vaccine compositions of the present disclosure is an amount sufficient to induce an immunoprotective response, such as an immunoprotective response without significant, adverse effects. While the amount of each conjugate may vary depending upon the pneumococcal serotype, each dose of the vaccine compositions may comprise about 0.1 µg to about 50 µg of each pneumococcal polysaccharide, about 0.1 µg to about 10 µg, or about 1 µg to about 5 µg of each pneumococcal polysaccharide conjugated to each carrier protein comprising about 1.5 µg to about 5 µg of carrier protein.

[0150] In another embodiment, the present disclosure provides a pneumococcal vaccine composition comprising pneumococcal polysaccharides and carrier proteins, the pneumococcal vaccine composition having a percent ratio of protein to polysaccharide (protein/PS) of about 0.5 to about 2.0 protein/PS, preferably, 0.7 to 1.2.

[0151] In some embodiments, the present disclosure provides pneumococcal polysaccharide-protein conjugate vaccine compositions comprising pneumococcal polysaccharides having molecular weights ranging between about 100 kDa to about 400 kDa, about 125 kDa to about 425 kDa, about 150 kDa to about 450 kDa, about 175 kDa to about 475 kDa, about 200 kDa to about 500 kDa, about 250 kDa to about 550 kDa, or about 300 kDa to about 600 kDa.

Petitioner Merck Ex-1004, 0704

[0152] In other embodiments, the present disclosure provides pneumococcal polysaccharide-protein conjugate vaccine compositions comprising one or more polysaccharide-protein conjugates having a molecular weight ranging between about 1,000 kDa to about 10,000 kDa, about 1,500 kDa to about 15,000 kDa, about 2,000 kDa to about 20,000 kDa, about 2,500 kDa to about 25,000 kDa, or about 3,000 kDa to about 30,000 kDa.

[0153] The pneumococcal polysaccharide-protein conjugate vaccine compositions of the present disclosure may be manufactured using known methods. For example, the pneumococcal polysaccharide-protein conjugate vaccine compositions may be formulated with a pharmaceutically acceptable diluent or vehicle, e.g. water or a saline solution. In addition, the pneumococcal polysaccharide-protein conjugate vaccine compositions may further include one or more of the following: a buffer, a preservative or a stabilizer, polysorbate, an adjuvant such as an aluminum compound, e.g. an aluminium hydroxide, an aluminium phosphate or an aluminium hydroxyphosphate, and/or a lyophilization excipient. Inclusion of any one of the above compounds in the pneumococcal polysaccharide-protein conjugate vaccine compositions of the present disclosure may be selected as a function of the mode and route of administration to a subject in need thereof and may further be based on standard pharmaceutical practices.

[0154] In some embodiments, the present disclosure provides a method for preparing a fourteen valent pneumococcal polysaccharide-protein conjugate composition comprising pneumococcal polysaccharides selected from serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F, wherein at least serotypes 3, and 6B are conjugated to PsaA and one or more serotypes 1, 4, 5, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F are conjugated to CRM_{197}. The method for preparing the fourteen valent pneumococcal polysaccharide-protein conjugate composition comprises the steps of;

(a) individually conjugating one or more of the fourteen pneumococcal polysaccharides (e.g., activated utilizing CDAP) to an immunogenic carrier protein, such as PsaA and/or CRM_{197},

(b) diafiltering and purifying the conjugates using size exclusion chromatography,

Petitioner Merck Ex-1004, 0705

(c) analyzing the purified fractions using SEC-MALLS, pooling fractions containing each of the fourteen conjugates, and filter sterilizing the monovalent conjugate fractions, and

(d) formulating the fourteen conjugates (e.g., about 2.2 to 4.4 µg for each serotype, about 5 µg to about 10 µg of PsaA, and about 15 µg to about 36 µg of CRM_{197}), an adjuvant (such as aluminum phosphate), an excipient, and buffer into the fourteen valent pneumococcal polysaccharide-protein conjugate composition.

In some embodiments, the fourteen valent pneumococcal polysaccharide-protein conjugate composition may be filtered (e.g., aseptically).

[0155] In some embodiments, the present disclosure provides a method for preparing a fourteen valent pneumococcal polysaccharide-protein conjugate composition comprising pneumococcal polysaccharides selected from serotypes 1, 3, 4, 5, 6A, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F, wherein at least serotypes 3, and 6A are conjugated to PsaA and one or more serotypes 1, 4, 5, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F are conjugated to CRM_{197}. The method for preparing the fourteen valent pneumococcal polysaccharide-protein conjugate composition comprises the steps of;

(a) individually conjugating one or more of the fourteen pneumococcal polysaccharides (e.g., activated utilizing CDAP) to an immunogenic carrier protein, such as PsaA and/or CRM_{197},

(b) diafiltering and purifying the conjugates using size exclusion chromatography,

(c) analyzing the purified fractions using SEC-MALLS, pooling fractions containing each of the fourteen conjugates, and filter sterilizing the monovalent conjugate fractions, and

(d) formulating the fourteen conjugates (e.g., about 2.2 to 4.4µg for each serotype, about 5 µg to about 10 µg of PsaA, and about 15 µg to about 36 µg of CRM_{197}), an adjuvant (such as aluminum phosphate), an excipient, and buffer into the fourteen valent pneumococcal polysaccharide-protein conjugate composition.

Petitioner Merck Ex-1004, 0706

In some embodiments, the fourteen valent pneumococcal polysaccharide-protein conjugate composition may be filtered (e.g., aseptically).

[0156] In some embodiments, the present disclosure provides a method for preparing a fifteen valent pneumococcal polysaccharide-protein conjugate composition comprising pneumococcal polysaccharides selected from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V,14, 18C, 19A, 19F, 22F, 23F and 33F wherein at least serotypes 3, 6A and 6B are conjugated to PsaA and one or more serotypes 1, 4, 5, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F are conjugated to CRM_{197}. The method for preparing the fifteen valent pneumococcal polysaccharide-protein conjugate composition comprises the steps of;

(a) individually conjugating one or more of the fifteen pneumococcal polysaccharides (e.g., activated utilizing CDAP) to an immunogenic carrier protein, such as PsaA and/or CRM_{197},

(b) diafiltering and purifying the conjugates using size exclusion chromatography,

(c) analyzing the purified fractions using SEC-MALLS, pooling fractions containing each of the fifteen conjugates, and filter sterilizing the monovalent conjugate fractions, and

(d) formulating the fifteen conjugates (e.g., about 2.2 to 4.4 µg for each serotype, about 5 µg to about 20 µg of PsaA, and about 20 µg to about 40 µg of CRM_{197}), an adjuvant (such as aluminum phosphate), an excipient, and buffer into the fifteen valent pneumococcal polysaccharide-protein conjugate composition.

In some embodiments, the fifteen valent pneumococcal polysaccharide-protein conjugate composition may be filtered (e.g., aseptically).

[0157] In some embodiments, the present disclosure provides a method for preparing a seventeen valent pneumococcal polysaccharide-protein conjugate composition comprising pneumococcal polysaccharides selected from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 12F,14, 18C, 19A, 19F, 22F, 23F, 33F and 35B wherein at least serotypes 3, 6A and 6B are conjugated to PsaA and one or more serotypes 1, 4, 5, 7F, 9V, 12F,14, 18C, 19A, 19F, 22F, 23F, 33F and 35B are conjugated to CRM_{197}.

Petitioner Merck Ex-1004, 0707

The method for preparing the seventeen valent pneumococcal polysaccharide-protein conjugate composition comprises the steps of;

(a) individually conjugating one or more of the seventeen pneumococcal polysaccharides (e.g., activated utilizing CDAP) to an immunogenic carrier protein, such as PsaA and/or CRM_{197},

(b) diafiltering and purifying the conjugates using size exclusion chromatography,

(c) analyzing the purified fractions using SEC-MALLS, pooling fractions containing each of the seventeen conjugates, and filter sterilizing the monovalent conjugate fractions, and

(d) formulating the seventeen conjugates (e.g., about 2.2 to 4.4 µg for each serotype, about 5 µg to about 20 µg of PsaA, and about 20 µg to about 40 µg of CRM_{197}), an adjuvant (such as aluminum phosphate), an excipient, and buffer into the seventeen valent pneumococcal polysaccharide-protein conjugate composition.

In some embodiments, the seventeen valent pneumococcal polysaccharide-protein conjugate composition may be filtered (e.g., aseptically).

[0158] In some embodiments, the present disclosure provides a method for preparing a twenty valent pneumococcal polysaccharide-protein conjugate composition comprising pneumococcal polysaccharides selected from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, 33F, 35B and 45 wherein at least serotypes 3, 6A and 6B are conjugated to PsaA and one or more serotypes 1, 4, 5, 7F, 9V, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, 33F, 35B and 45 are conjugated to CRM_{197}. The method for preparing the twenty valent pneumococcal polysaccharide-protein conjugate composition comprises the steps of;

(a) individually conjugating one or more of the twenty pneumococcal polysaccharides (e.g., activated utilizing CDAP) to an immunogenic carrier protein, such as PsaA and/or CRM_{197},

(b) diafiltering and purifying the conjugates using size exclusion chromatography,

Petitioner Merck Ex-1004, 0708

(c) analyzing the purified fractions using SEC-MALLS, pooling fractions containing each of the twenty conjugates, and filter sterilizing the monovalent conjugate fractions, and

(d) formulating the twenty conjugates (e.g., about 2.2 to 4.4 µg for each serotype, about 5 µg to about 20 µg of PsaA, and about 20 µg to about 50 µg of CRM_{197}), an adjuvant (such as aluminum phosphate), an excipient, and buffer into the twenty valent pneumococcal polysaccharide-protein conjugate composition.

In some embodiments, the twenty valent pneumococcal polysaccharide-protein conjugate composition may be filtered (e.g., aseptically).

[0159] In some embodiments, the present disclosure provides a method for preparing a twenty-two valent pneumococcal polysaccharide-protein conjugate composition comprising one or more pneumococcal polysaccharides selected from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 10A, 12F, 14, 15A, 15B, 18C, 19A, 19F, 22F, 23F, 33F, 34, 35B and 38wherein at least serotypes 3, 6A and 6B are conjugated to PsaA and one or more serotypes 1, 4, 5, 7F, 9V, 10A, 12F, 14, 15A, 15B, 18C, 19A, 19F, 22F, 23F, 33F, 34, 35B and 38 are conjugated to CRM_{197}. The method for preparing the twenty-two valent pneumococcal polysaccharide-protein conjugate composition comprises the steps of;

(a) individually conjugating one or more of the twenty-two pneumococcal polysaccharides (e.g., activated utilizing CDAP) to an immunogenic carrier protein, such as PsaA and/or CRM_{197},

(b) diafiltering and purifying the conjugates using size exclusion chromatography,

(c) analyzing the purified fractions using SEC-MALLS, pooling fractions containing each of the twenty-two conjugates, and filter sterilizing the monovalent conjugate fractions, and

(d) formulating the twenty-two conjugates (e.g., about 2.2 to 4.4 µg for each serotype, about 5 µg to about 20 µg of PsaA, and about 20 µg to about 50 µg of CRM_{197}), an adjuvant (such as aluminum phosphate),

Petitioner Merck Ex-1004, 0709

an excipient, and buffer into the twenty-two valent pneumococcal polysaccharide-protein conjugate composition.

In some embodiments, the twenty-two valent pneumococcal polysaccharide-protein conjugate composition may be filtered (e.g., aseptically).

[0160] The compositions of the present disclosure may be formulated into a unit dose, for example, a unit dose vial, into a multiple dose, for example, a multiple dose vial, or a pre-filled syringe. The compositions of the present disclosure may further comprise of one or more preservative(s) selected from thiomersal, 2-phenoxyethanol and the like, in an amount which may range from about 4 mg/mL to about 20 mg/mL.

[0161] In some embodiments, the present disclosure also provides an immunogenic composition (e.g., a vaccine), such as a pneumococcal polysaccharide-protein conjugate composition, administered as a single dose of about 0.5 mL formulated to contain at least the following: about 2.2 to 4.4 µg of two or more pneumococcal polysaccharide serotypes, about 1 µg to about 10 µg of PsaA per serotype, about 1 µg to about 10 µg of CRM_{197} for each serotype, about 0.2 mg to about 1 mg of an adjuvant (e.g., aluminum phosphate), and one or more excipients (e.g., sodium chloride, and/or a buffer).

[0162] In other embodiments, the present disclosure also provides a thirteen valent immunogenic composition (e.g., a vaccine) in a sterile liquid formulation, such as a thirteen valent pneumococcal polysaccharide-protein conjugate composition, administered as a single dose of about 0.5 mL formulated to contain at least the following: pneumococcal polysaccharide serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F and 33F each individually conjugated to PsaA or combination of PsaA and CRM_{197}.

[0163] The thirteen valent vaccine may be further formulated into one or more doses of about 0.5 mL dose, each 0.5 mL dose comprising about 2.2 µg to about 4.4 µg of each of the thirteen serotypes, about 25 µg to about 30 µg PsaA and CRM_{197}, about 0.125 mg of an adjuvant (e.g., elemental aluminum such as about 0.5 mg aluminum phosphate), sodium chloride, and L-histidine buffer.

[0164] In some embodiments, the present disclosure also provides a fourteen valent immunogenic composition (e.g., a vaccine) in a sterile liquid formulation, such

Petitioner Merck Ex-1004, 0710

as a fourteen valent pneumococcal polysaccharide-protein conjugate composition, administered as a single dose of about 0.5 mL formulated to contain at least the following: pneumococcal polysaccharide serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F each individually conjugated to PsaA or combination of PsaA and CRM_{197}.

[0165] In some embodiments, the present disclosure also provides a fourteen valent immunogenic composition (e.g., a vaccine) in a sterile liquid formulation, such as a fourteen valent pneumococcal polysaccharide-protein conjugate composition, administered as a single dose of about 0.5 mL formulated to contain at least the following: pneumococcal polysaccharide serotypes 1, 3, 4, 5, 6A, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F each individually conjugated to PsaA or combination of PsaA and CRM_{197}.

[0166] The fourteen valent vaccine may be further formulated into one or more doses of about 0.5 mL dose, each 0.5 mL dose comprising about 2.2 µg to about 4.4 µg of each of the fourteen serotypes, about 20 µg to about 35 µg PsaA and CRM_{197}, about 0.125 mg of an adjuvant (e.g., elemental aluminum such as about 0.5 mg aluminum phosphate), sodium chloride, and L-histidine buffer.

[0167] In other embodiments, the present disclosure also provides a fifteen valent immunogenic composition (e.g., a vaccine) in a sterile liquid formulation, such as a fifteen valent pneumococcal polysaccharide-protein conjugate composition, administered as a single dose of about 0.5 mL formulated to contain at least the following: pneumococcal polysaccharide serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V,14, 18C, 19A, 19F, 22F, 23F and 33F, wherein at least serotypes 3, 6A and 6B are each individually conjugated to PsaA and one or more serotypes 1, 4, 5, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F are each individually conjugated to CRM_{197}.

[0168] The fifteen valent vaccine may be further formulated into one or more doses of about 0.5 mL dose, each 0.5 mL dose comprising about 2.2 µg to about 4.4 µg of each of the fifteen serotypes, about 5 µg to about 20 µg PsaA, about 20 µg to 40 µg of CRM_{197}, about 0.125 mg of an adjuvant (e.g., elemental aluminum such as about 0.5 mg aluminum phosphate), sodium chloride, and L-histidine buffer.

[0169] In additional embodiments, the present disclosure also provides a seventeen valent immunogenic composition (e.g., a vaccine) in a sterile liquid

Petitioner Merck Ex-1004, 0711

formulation, such as a seventeen valent pneumococcal polysaccharide-protein conjugate composition, administered as a single dose of about 0.5 mL formulated to contain at least the following: pneumococcal polysaccharide serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 12F,14, 18C, 19A, 19F, 22F, 23F, 33F and 35B, wherein at least serotypes 3, 6A and 6B are each individually conjugated to PsaA and one or more serotypes 1, 4, 5, 7F, 9V, 12F,14, 18C, 19A, 19F, 22F, 23F, 33F and 35B are each individually conjugated to CRM_{197}.

[0170] The seventeen valent vaccine may be further formulated into one or more doses of about 0.5 mL dose, each 0.5 mL dose comprising about 2.2 µg to about 4.4 µg of each of the seventeen serotypes, about 5 µg to about 20 µg PsaA, about 20 µg to 40 µg of CRM_{197}, about 0.125 mg of an adjuvant (e.g., elemental aluminum such as about 0.5 mg aluminum phosphate), sodium chloride, and L-histidine buffer.

[0171] In some embodiments, the present disclosure also provides a twenty valent immunogenic composition (e.g., a vaccine) in a sterile liquid formulation, such as a twenty valent pneumococcal polysaccharide-protein conjugate composition, administered as a single dose of about 0.5 mL formulated to contain at least the following: pneumococcal polysaccharide serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, 33F, 35B and 45, wherein at least serotypes 3, 6A and 6B are each individually conjugated to PsaA and one ore more serotypes 1, 4, 5, 7F, 9V, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, 33F, 35B and 45 are each individually conjugated to CRM_{197}.

[0172] The twenty valent vaccine may be further formulated into one or more doses of about 0.5 mL dose, each 0.5 mL dose comprising about 2.2 µg to about 4.4 µg of each of the twenty serotypes, about 5 µg to about 20 µg PsaA, about 20 µg to 50 µg of CRM_{197}, about 0.125 mg of an adjuvant (e.g., elemental aluminum such as about 0.5 mg aluminum phosphate), sodium chloride, and L-histidine buffer.

[0173] In some embodiments, the present disclosure also provides a twenty-two valent immunogenic composition (e.g., a vaccine) in a sterile liquid formulation, such as a twenty-two valent pneumococcal polysaccharide-protein conjugate composition, administered as a single dose of about 0.5 mL formulated to contain at least the following: pneumococcal polysaccharide serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 10A, 12F, 14, 15A, 15B, 18C, 19A, 19F, 22F, 23F, 33F, 34, 35B and 38, wherein at least

Petitioner Merck Ex-1004, 0712

serotypes 3, 6A and 6B are each individually conjugated to PsaA and one or more serotypes 1, 4, 5, 7F, 9V, 10A, 12F, 14, 15A, 15B, 18C, 19A, 19F, 22F, 23F, 33F, 34, 35B and 38 are each individually conjugated to CRM_{197}.

[0174] The twenty-two valent vaccine may be further formulated into one or more doses of about 0.5 mL dose, each 0.5 mL dose comprising about 2.2 µg to about 4.4 µg of each of the twenty-two serotypes, about 5 µg to about 20 µg PsaA, about 20 µg to 50 µg of CRM_{197}, about 0.125 mg of an adjuvant (e.g., elemental aluminum such as about 0.5 mg aluminum phosphate), sodium chloride, and L-histidine buffer.

[0175] In some embodiments, the present disclosure also provides a twenty-four valent immunogenic composition (e.g., a vaccine) in a sterile liquid formulation, such as a twenty-four valent pneumococcal polysaccharide-protein conjugate composition, administered as a single dose of about 0.5 mL formulated to contain at least the following: pneumococcal polysaccharide serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 12F, 14, 15A, 15B, 16F, 18C, 19A, 19F, 22F, 23F, 33F, 34, 35B and 38, wherein at least serotypes 3, 6A and 6B are each individually conjugated to PsaA and one or more serotypes 1, 4, 5, 7F, 8, 9V, 10A, 12F, 14, 15A, 15B, 16F, 18C, 19A, 19F, 22F, 23F, 33F, 34, 35B and 38 are each individually conjugated to CRM_{197}.

[0176] The twenty-four valent vaccine may be further formulated into one or more doses of about 0.5 mL dose, each 0.5 mL dose comprising about 2.2 µg to about 4.4 µg of each of the twenty-two serotypes, about 5 µg to about 20 µg PsaA, about 20 µg to 50 µg of CRM_{197}, about 0.125 mg of an adjuvant (e.g., elemental aluminum such as about 0.5 mg aluminum phosphate), sodium chloride, and L-histidine buffer.

[0177] Compositions of the present disclosure may be administered to a subject in need thereof by any number of conventional routes used in the field of vaccines. For example, compositions of the present disclosure may be administered systemically, such as parenterally (e.g. subcutaneously, intramuscularly, intradermally and/or intravenously) or mucosally (e.g., orally and/or nasally).

[0178] In some embodiments, the present disclosure also provides methods of inducing an immune response in a subject in need thereof to one or more *s. pneumoniae* capsular polysaccharides conjugated to one or more carrier proteins. The methods for inducing the immune response comprise administering an

Petitioner Merck Ex-1004, 0713

immunologically effective amount of the compositions described herein to the subject in need thereof.

[0179] According to the methods of the present disclosure, the subject to whom the compositions described herein is a human, such as an infant (less than about 1 year of age), a toddler (about 12 months to about 24 months of age), a young child (about 2 years to about 5 years of age), an older child (about 5 years to about 13 years of age), an adolescent (about 13 years to about 18 years of age), an adult (about 18 years to about 65 years of age), or an elder (more than about 65 years of age).

[0180] As used herein, an "effective amount" of the compositions described in the present disclosure refers to an amount required to elicit an immune response in the subject to which the composition was administered. The immune response is characterized by the presence of one or more *S. pneumoniae* antigen-specific antibodies in the host that significantly reduce the likelihood or severity of infection of *S. pneumoniae* during a subsequent challenge.

EXAMPLES

[0181] The following examples are provided to illustrate the disclosure and are merely for illustrative purpose only and should not be construed to limit the scope of the disclosure.

Example 1: Conjugation of individual pneumococcal polysaccharide to carrier protein to form Polysaccharide-PsaA Conjugates

A) *PsaA Preparation:*

[0182] The PsaA gene was PCR amplified from *Streptococcus pneumoniae* Serotype 4, without its hydrophobic leader peptide sequence. The gene was sequence verified and cloned into *E. coli* using a vector constructed in-house (pBE66) for higher expression.

[0183] Glycerol stock culture encoding the PSaA gene was revived on a 20 mL LB Media containing 1 mL of Glycerol Stock in a 150 mL conical flask. The culture was incubated for about 6 hrs at 37° C under 200 rpm to a final OD_{600nm} of 3.5 OD. The revived culture was transferred to 1L seed culture in a 5 L conical flask. The culture was grown for about 10 hrs at 37° C under 200 rpm to a final OD 600nm of 3.

Petitioner Merck Ex-1004, 0714

[0184] The seed culture was transferred aseptically to a 20 L fermenter containing the following media components, HyPeptone 6 g/L , Yeast extract 12/L, di Potassium Hydrogen ortho phosphate 13.5 g/L , ammonium phosphate di basic 4 g/L , Citric acid 1.7 g/L , $MgSO_4.7H_2O$ 1.2 g/L , Glucose 4 g/L , thamine HCL 10 mg/L along with 1 mL/L trace elements (e.g., trace elements for 100mL composition $FeCl_3$ 2.7 g, $ZnCl_2$ 0.2g, $CoCl_2.6H_2O$ 0.2g, $Na_2MoO_4.2H_2O$ 0.2 g, $CuSO_4$ $5H_2O$ 0.1 g, Boric Acid 0.05 g, $CaCl_2$ $2H_2O$ 0.1g, Conc., HCL 10mL.) The initial fermentation started with OD $_{600nm}$ 0.2 OD. The pH was maintained at 7± 0.2 throughout the fermentation with 20% ortho-phosphoric acid and 12.5 % ammonium hydroxide.

[0185] When the glucose level falls below 0.5 g/L the feed batch was initiated at a steady rate of 3 – 4 g/L/hr, the DO% was maintained > 20% throughout the fermentation with oxygen enrichment.

[0186] Cells were grown in the fermentor and the cell pellet was harvested by centrifugation. The cells were lysed using cell-disruption device (Panda). The lysate was centrifuged at 10000g, the clarified supernatant was subject to purification.

[0187] PsaA purification was performed similar to the procedure described in Larentis et.al, 2011 (Protein expression and Purification 78 (2011) 38). Purification was further optimized by using mixed mode chromatography (Ceramic Hydroxyapatite Type-II) after DEAE to achieve higher purity of PsaA.

[0188] *Anion exchange chromatography:* 30 mL of DEAE Sepharose (GE) resin was packed in XK16/20 column. The resin was washed with 5 column volumes of sterile distilled water followed by 10 column volumes of 20mM Tris, 1mM EDTA, pH 8.0 (Equilibration buffer). 30 mL of supernatant was diluted to 100 mL with equilibration buffer and loaded onto column and flow through was collected. The column was washed with 5 volumes of equilibration buffer. PsaA was eluted with 12 volumes of linear gradient of (0-100%B). (Buffer A containing 20 mM Tris, 1 mM EDTA pH 8.0; Buffer B-20 mM Tris, 1 mM EDTA, 250 mM NaCl pH 8.0.) This was followed by washing the columns with 20 mM Tris, 1mM EDTA, 1 M NaCl pH8.0.

[0189] *Mixed mode Chromatography:* 25ml of Ceramic Hydroxyapatite Type II (CHT-II) was packed in column. The resin was washed with volumes of sterile distilled water followed by 10 volumes of 20 mM Tris pH 6.8. Elution fractions from DEAE resin that showed clear major visible band of approximately 37 KD good

Petitioner Merck Ex-1004, 0715

concentration of PsaA on SDS PAGE were pooled and loaded onto CHT-II resin. The flow through was collected and the column was washed with 5 column volumes of equilibration buffer. Protein was eluted with 5 column volumes step gradients of (15%B, 20%B, 50%B and 100%B). Buffer A contains 20 mM Tris pH 6.8, while the Buffer B contains 250 mM Phosphate buffer pH 6.8.

[0190] All the elution fractions showing a clean band at the expected size of PsaA were pooled, concentrated by 10 kDa MWCO cassette and diafiltered against 20mM Phosphate buffer pH 7.5. The purified protein was loaded on SDS-PAGE gel to assess purity.

B) *Activation and Conjugation of Pneumococcal Polysaccharide Serotype 3 To PsaA*

[0191] The size reduced polysaccharide of serotype 3 (concentration of 5 mg/mL) and 1.5 mL of CDAP (100 mg/mL in acetonitrile (w/v)) was mixed in a glass bottle in the ratio of 1:0.5 (PS: CDAP) and stirred for 1 min. The pH of the polysaccharide solution was adjusted to 9.0 with 3.5 mL of 0.2M triethylamine and stirred for 1 min at room temperature (RT). 210 mg of PsaA (14.0 mL of 15.0 mg/mL concentration) was added slowly to the activated polysaccharide in a ratio of 1:0.7 (Ps: Carrier protein).

[0192] The pH of the reaction was adjusted to about 9.01 with 0.7 mL of 0.2M triethylamine and the reaction was continued under stirring for 5 hours at room temperature followed by quenching of the reaction by adding excess concentration of glycine (100 mM). The conjugation kinetics of reactions were monitored using SEC-HPLC at each hour of the reaction.

[0193] The reaction mixture was diafiltered and concentrated using 100 kDa MWCO TFF membrane. Concentrate was purified by size-exclusion chromatography. The fractions were analyzed by SEC-MALLS, anthrone method and fractions containing conjugates were pooled and sterile filtered with 0.2 µm filters.

C) *Activation and Conjugation of Pneumococcal Polysaccharide Serotype 6A To PsaA*

[0194] The size reduced polysaccharide Type 6A (concentration of 14.6 mg/mL) and 400 µL of CDAP (100 mg/mL in Acetonitrile (w/v)) was mixed in a glass bottle in

Petitioner Merck Ex-1004, 0716

the ratio of 1: 1 (PS:CDAP) and stirred for 1 min. The pH of the polysaccharide solution was adjusted to 9.5 with 800 µL of 0.2M triethylamine and stirred for 1 min at room temperature (RT). 40 mg of PsaA (3.78 mL of 11.0 mg/mL concentration) was added slowly to the activated polysaccharide in a ratio of 1:1 (Ps: Carrier protein).

[0195] The pH of the reaction was adjusted to about 9.01 with 0.7mL of 0.2M triethylamine and the reaction was continued under stirring for 5 hours at room temperature followed by quenching of the reaction by adding excess concentration of glycine (100 mM). The conjugation kinetics of reactions were monitored using SEC-HPLC at each hour of the reaction.

[0196] The reaction mixture was diafiltered and concentrated using 100 kDa MWCO TFF membrane. Concentrate was purified by size-exclusion chromatography. The fractions were analyzed by SEC-MALLS, anthrone method and fractions containing conjugates were pooled and sterile filtered with 0.2µm filters.

D) *Activation And Conjugation Of Pneumococcal Polysaccharide Serotype 6B To PsaA*

[0197] The size reduced polysaccharide Type 6B (concentration of 14.97 mg/mL) and 4.0 mL of CDAP (100 mg/mL in acetonitrile (w/v)) was mixed in a glass bottle in the ratio of 1: 2 (PS: CDAP) and stirred for 1 min. The pH of the polysaccharide solution was adjusted to 9.1 with 8.0 mL of 0.2M Triethylamine and stirred for 1 min at room temperature (RT). 340 mg of PsaA (22.66 mL of 15.0 mg/mL concentration) was added slowly to the activated polysaccharide in a ratio of 1:1.7 (Ps: Carrier protein).

[0198] The pH of the reaction was adjusted to about 9.01 with 0.7mL of 0.2M triethylamine and the reaction was continued under stirring for 5 hours at room temperature followed by quenching of the reaction by adding excess concentration of glycine (100 mM). The conjugation kinetics of reactions were monitored using SEC-HPLC at each hour of the reaction.

[0199] The reaction mixture was diafiltered and concentrated using 100 kDa MWCO TFF membrane. Concentrate was purified by size-exclusion chromatography. The fractions were analyzed by SEC-MALLS, anthrone method and fractions containing conjugates were pooled and sterile filtered with 0.2µm filters.

Petitioner Merck Ex-1004, 0717

[0200] SEC-HPLC chromatogram for conjugation reaction kinetics of Serotype 3 (D), 6A (E) and 6B (F) were performed. The following symbols were used for all three chromatograms; polysaccharide (solid line), PsaA (dashed line), 3 or 5 hour reaction (dotted line). As shown in Figure 2 for all the chromatograms, the consumption of PsaA is indicated based on the reduction or disappearing the peak belongs PsaA at retention time of about 19 minutes. The conjugation is confirmed based on the formation of a new peak at about 13.5 minutes to about 14 minutes.

[0201] Conjugates of pneumococcal polysaccharides from serotype 5, 9V, 12F, 10A, 15B, 18C and 45 with PsaA were prepared using the procedure similar to the one described above. SEC-HPLC chromatogram for conjugation reaction kinetics of Serotype 5 (A), 9V (B) and 18C (C) was carried out. As shown in Figure 2, all three chromatograms Polysaccharide (solid line), PsaA (dashed line), 3 or 5 hour reaction (dotted line). In all the chromatograms the consumption of PsaA is indicated based on the reduction or disappearing the peak belongs PsaA at retention time of about 19 mins. The conjugation is confirmed based on the formation of a new peak at about 13.5 minutes to about 14 minutes.

Example 2: Preparation of Pneumococcal Capsular Polysaccharide-CRM_{197} Conjugates

A) *Activation of Polysaccharide and Conjugation With Carrier Protein:*

[0202] Sized polysaccharide (6.0 mL of Ps, concentration of 10 mg/mL) and CDAP (100 mg/mL in Acetonitrile (w/v)) was mixed in a glass vial in the ratio of 1:1 and stirred for 1 min. The pH of the polysaccharide solution was adjusted to 9.25 with 0.2M triethylamine and stirred for 3 min at room temperature (RT). CRM_{197} (4.0 mL of conc. 15.0 mg/mL) was added slowly to the activated polysaccharide in a ratio of 1:1 (Ps: Carrier protein).

[0203] The pH of the reaction were adjusted to about 9.05 with 0.2M triethylamine and the reaction was continued under stirring for 5 hours at room temperature and finally the reaction was quenched by adding excess concentration of glycine.

[0204] The reaction mixture was diafiltered using 100 K MWCO membrane and purified by size-exclusion chromatography. The fractions were analyzed by SEC-

Petitioner Merck Ex-1004, 0718

MALLS, anthrone method and fractions containing conjugates were pooled and sterile filtered with 0.2µ filters. This material is called monovalent conjugate bulk.

[0205] The reaction kinetics of the conjugates prepared from serotypes 7F (A), 14 (B) and 19F (C) with CRM_{197} were prepared and the reaction kinetics are shown in Figure 1. In all three chromatograms polysaccharide (solid line), CRM_{197} (dashed line), 5 hour reaction (dotted line). In all the chromatograms the consumption of CRM_{197} is indicated based on the reduction or disappearing the peak belongs CRM_{197} at retention time of about 19 minutes. The conjugation is confirmed based on the formation of a new peak at about 13.5 minutes to about 14 minutes.

[0206] The reaction kinetics of the conjugates prepared from serotypes 3 (D), 6A (E) and 6B (F) with CRM_{197} were prepared and the reaction kinetics are shown in Figure 1. In all three chromatograms polysaccharide (solid line), CRM_{197} (dashed line), 5 hour reaction (dotted line). In all the chromatograms the consumption of CRM_{197} is indicated based on the reduction or disappearing the peak belongs CRM_{197} at retention time of about 19 minutes. The conjugation is confirmed based on the formation of a new peak at about 13.5 minutes to about 14 minutes.

Example 3: Formulation of Pneumococcal Capsular Polysaccharide-protein conjugate vaccine

[0207] A 15 valent conjugated vaccine was formulated as 0.5 mL dose containing 2.2 µg of each pneumococcal polysaccharide from serotypes 1, 4, 5, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F & 33F conjugated to ~28 µg CRM_{197} Protein and 2.2 µg of each polysaccharide from serotypes 3, 6A and 6B conjugated to about 7 µg PsaA Protein. All the conjugates were adsorbed on to aluminum phosphate gel equivalent to 0.5 mg Al^{3+} per dose of 0.5 mL. The 0.9% W/V saline was used as diluent and vehicle for the formulation and the final formulation pH was adjusted to pH 6 using 1N hydrochloric acid. For effective adsorption post adjusting the pH, the formulation was mixed for 2 hours under constant stirring. After 2 hours of blending, the formulated blend was aseptically filled at 0.58 mL fill volume per vial into the 3 mL sterile nonsiliconized vials, closed with sterile 13 mm rubber stoppers and sealed with 13 mm sterile pink colored flip off aluminum seals, followed by optical inspection and labelling of filled vials. From the lot, some vials randomly picked were sent for

Petitioner Merck Ex-1004, 0719

analyzing the appearance, pH, Osmolality, total poly and protein content (µg/SHD), %Adsorption, aluminum content (mg/SHD).

Example 4: Formulation of Pneumococcal Capsular Polysaccharide-protein conjugate vaccine

[0208] A 15 valent conjugated vaccine was formulated as 0.5 mL dose containing 2.2 µg of each polysaccharide from serotypes 1, 4, 5, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F & 33F conjugated to ~28 µg CRM_{197} protein and 4.4 µg of each polysaccharide from serotypes 3, 6A and 6B conjugated to ~14 µg PsaA Protein. All the conjugates were adsorbed on to aluminum phosphate gel equivalent to 0.5 mg Al^{3+} per dose of 0.5 mL. The 0.9% w/v saline was used as diluent and vehicle for the formulation and the final formulation pH was adjusted to pH 6 using 1N hydrochloric acid. For effective adsorption post adjusting the pH, the formulation was mixed for 2 hours under constant stirring. After 2 hours of blending, the formulated blend was aseptically filled at 0.58 mL fill volume per vial into the 3 mL sterile nonsiliconized vials, closed with sterile 13 mm rubber stoppers and sealed with 13 mm sterile pink colored flip off aluminum seals, followed by optical inspection and labelling of filled vials. From the lot, some vials randomly picked were sent for analyzing the appearance, pH, Osmolality, Total poly and protein content (µg/SHD), % Adsorption, aluminum content (mg/SHD).

A) *Immunization of Rabbits With the Formulated Vaccine*

[0209] Healthy rabbits 1.5 to 2 kg each were bred and reared in a specific pathogen free contained facility. Rabbits were immunized with the aforementioned formulation with the following schedule.

[0210] Group 1 consisted of 7 rabbits that were immunized with the 15 valent conjugate vaccine (Example 3). The 15 valent vaccine comprised of 2.2µg each of the *S. pneumoniae* polysaccharides of serotypes 1, 4, 5, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F, 33F conjugated to CRM_{197} and 2.2µg each *S. pneumoniae* polysaccharides of serotypes 3, 6A and 6B conjugated to PsaA. Rabbits were immunized on days 1, 15 and 29; blood samples were collected on days 0, 15 and 36. Serum was separated from the blood sample and stored at -80°C.

Petitioner Merck Ex-1004, 0720

[0211] Group 2 consists of 7 rabbits that were immunized with the 13 valent conjugate vaccine – Prevnar13®. Rabbits were immunized on days 1, 15 and 29; blood samples were collected on days 0, 12, 26 and 40. Data from day 12 and day 40 was used to plot for comparison with the PsaA study (Figure 4). Serum was separated from the blood sample and stored at -80°C.

[0212] Group 3 consists of 7 rabbits that were immunized with the 15 valent conjugate vaccine (Example 4). The 15 valent vaccine comprised of 2.2 µg of polysaccharide for each serotypes 1, 4, 5, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F & 33F conjugated to ~28 µg CRM_{197} protein and 4.4 µg of each polysaccharide for Serotypes 3, 6A and 6B conjugated to ~14 µg PsaA protein. Rabbits were immunized on days 1, 15 and 29; blood samples were collected on days 0, 15 and 36. Serum was separated from the blood sample and stored at -80°C. Serum obtained from the immunized rabbits were analyzed for serotype specific immune response using ELISA.

[0213] The ELISA was performed as per the WHO suggested protocol. Briefly, Maxisorp™ ELISA plates were coated with PnCPS of given serotype (1µg/50µL/well using PBS; sterile endotoxin free, with 0.02% sodium azide). Plates were placed in a box with moistened paper towels for humidification and incubated at 37°C ± 2°C for 5hrs, the plates were then stored at 5°C ± 3°C until use.

[0214] The test sera were pre-adsorbed to CWPS Mlti™ to eliminate background reactivity originating from cell-wall polysaccharide. To achieve this 2µL of test and positive control serum were diluted using 998µL preadsorption solution (1mL-1µL CWPS Multi™ in 999µL of 10% SuperBlock™ in PBST) to get final dilution of 1:500. The diluted samples were incubated at room temperature (25°C ± 5°C) for 1 hr with continuous shaking. The unbound PnCPS were removed by flicking the plate and the free sites in the wells were blocked by adding 200µL of blocker (20% SuperBlock™ in PBS). The plate were incubated at room temperature (25°C ± 5°C) for 1 hr without shaking.

B) *Test Samples and Controls Addition*

[0215] 50µL diluent (10% SuperBlock™ in PBST) were added to all wells except A1 to A12. Following this 100µL/well preadsorbed test sera samples were added to A1 to A10, control sera samples were added to A11 and A12. Two fold serial dilution

Petitioner Merck Ex-1004, 0721

of test samples was performed by transferring 50µL from 1st to 2nd and so on i.e. from A1-A10 to H1-H10. Similarly, serial dilution of control samples (007SP) from A11 and 12 to E11 and E12 were performed. F11 and F12 to H11 and H12 without dilution were set up as blank.

[0216] The plates were incubated at room temperature (25°C ± 5°C) for 2 hrs without shaking. Following the incubation step the contents were discarded and the plates were washed thrice with PBST (~250µL/well) manually or with plate washer.

C) *Primary Antibody Addition*

[0217] 50µL/well recombinant protein A/G peroxidase (diluted 1:20000 using 10% SuperBlock™ in PBST) was added to all wells and the plates were incubated for 1hr at room temperature (25°C ± 5°C) without shaking. Following this, the plates were washed thrice with PBST (250µL/well) manually or with plate washer.

D) *Development and Reading*

[0218] Chromogenic reaction was developed by adding 50µL/well TMB substrate and incubated for 15mins at room temperature (25°C ± 5°C) without shaking. The reaction was stopped by adding 50µL/well 1.25M Sulphuric acid. The OD at 450nm was measured.

E) *Titer Estimation*

[0219] Antibody titer in the immunized animals were assigned as inverse of dilution factor. The highest dilution showing OD_{450nm} as twice the pre-immune titer (approximately 0.2) was reported as titer. The titer of serum antibody to each serotype was plotted and compared with different treatment groups.

[0220] The immune response data of animals from Group 1 shows dose dependent rise in serotype specific rise in serum antibody titer i.e. the level of antibody titer increased with each subsequent dose in the vaccinated animals. The immune response to Serotype 3 (Figure 5) was about 1:10000 when the animals were immunized with conjugates of PsaA containing 4.4 µg of polysaccharide (group 3). Further, the serum antibody titer to serotypes 3, 6A and 6B were at least twice as much when compared to the immune response obtained from Prevnar®13 immunized rabbits. The serum IgG titer after first immunization in rabbits with Prevnar®13 does not show rise to the same extent as observed with the 15 valent formulation of the

Petitioner Merck Ex-1004, 0722

present disclosure (Figure 3 & 4). Even though the concentration of polysaccharide for serotype 6B in Prevnar®13 is twice as much (4.4µg), when compared to the concentration of serotype 3 polysaccharide conjugated to PsaA (Figure 3), is twice the level of IgG response. The titer of Serotype 3 antibody barely reaches 8000 in formulations containing 2.2 µg of Serotype 3 conjugated to CRM_{197}. This titer shows a notable improvement (16000) when conjugated to 4.4 µg of PsaA.. This is an improvement in the immune response due to conjugation of serotype 3, 6A and 6B polysaccharides to PsaA. Serum antibody titer to serotype 14 in Prevnar®13 vaccinated animals were lower when compared to the formulations of the present disclosure. IgG response to other serotypes in animal vaccinated either with the vaccine formulations of the present disclosure or with Prevnar®13 were similar. Serotypes 3, 6A and 6B titers were improved upon conjugation to PsaA even at 2.2 µg concentration.

[0221] The use of numerical values specified in this application, unless expressly indicated otherwise, are stated as approximations through the minimum and maximum values specified within the stated ranges, and preceded by the word "about." The disclosure of ranges is intended as a continuous range including every value between the minimum and maximum values recited as well as any ranges that may be formed through such values. The numerical values presented in this application represent various embodiments of the present disclosure.

[0222] Throughout this disclosure, the singular terms "a," "an," and "the" include plural referents unless the context clearly indicates otherwise. Similarly, unless the word "or" is expressly limited to mean only a single item exclusive from the other items in reference to a list of two or more items, then the use of "or" in such a list is to be interpreted as including (a) any single item in the list, (b) all of the items in the list, or (c) any combination of the items in the list. Additionally, the terms "comprising" and the like are used throughout this disclosure to mean including at least the recited feature(s) such that any greater number of the same feature(s) and/or one or more additional types of features are not precluded. Reference herein to "one embodiment," "an embodiment," or similar formulations means that a particular feature of a composition, a composition, a method, or a characteristic described in connection with the embodiment may be included in at least one embodiment of the present technology. Thus, the appearances of such phrases or formulations herein are not

Petitioner Merck Ex-1004, 0723

necessarily all referring to the same embodiment. Furthermore, various particular features, compositions, methods, or characteristics may be combined in any suitable manner in one or more embodiments.

[0223] This disclosure is not intended to be exhaustive or to limit the present technology to the precise forms disclosed herein. Although specific embodiments are disclosed herein for illustrative purposes, various equivalent modifications are possible without deviating from the present technology, as those of ordinary skill in the relevant art will recognize. In some cases, well-known structures and functions have not been shown and/or described in detail to avoid unnecessarily obscuring the description of the embodiments of the present technology. Although steps of methods may be presented herein in a particular order, in alternative embodiments the steps may have another suitable order. Similarly, certain embodiments of the present technology disclosed in the context of particular embodiments may be combined or eliminated in other embodiments. Furthermore, while advantages associated with certain embodiments may have been disclosed in the context of those embodiments, other embodiments may also exhibit such advantages, and not all embodiments need necessarily exhibit such advantages or other advantages disclosed herein to fall within the scope of the present technology. Accordingly, this disclosure and associated technology may encompass other embodiments not expressly shown and/or described herein.

[0224] From the foregoing, it will be appreciated that specific embodiments of the disclosure have been described herein for purposes of illustration, but that various modifications may be made without deviating from the scope of the disclosure. Accordingly, the disclosure is not limited except as by the appended claims.

Petitioner Merck Ex-1004, 0724

CLAIMS

What is claimed is:

1. A pneumococcal vaccine composition, the composition comprising two or more capsular pneumococcal polysaccharide serotypes each individually conjugated to a carrier protein pneumococcal surface adhesion protein A (PsaA) or combination of PsaA and CRM_{197} as a carrier proteins.

2. The pneumococcal vaccine composition of claim 1 wherein the pneumococcal vaccine composition is a 10 valent, 13 valent, 14 valent, 15 valent, 17 valent, 18 valent, 19 valent, 20 valent, 22 valent, 23 valent, 24 valent or 25 valent pneumococcal vaccine composition.

3. The pneumococcal vaccine composition of claims 1 or 2 wherein each of the pneumococcal polysaccharides include one or more serotypes selected from 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 23F, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, 39 and 45.

4. The pneumococcal vaccine composition of any one of the preceding claims having a percent ratio of carrier protein to pneumococcal polysaccharide (protein/PS) of about 0.5 to about 2.0 protein/PS.

5. The pneumococcal vaccine composition of any one of the preceding claims wherein the pneumococcal vaccine composition further comprises a pharmaceutically acceptable carrier.

6. The pneumococcal vaccine composition of any one of the preceding claims wherein the pneumococcal polysaccharides comprise at least 10 pneumococcal polysaccharides, each pneumococcal polysaccharide selected from serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 23F, 24B, 24F, 31, 33F, 34, 35B,

Petitioner Merck Ex-1004, 0725

35F, 38, 39 and 45 and the carrier proteins comprise PsaA, or a combination of PsaA and CRM_{197}.

7. The pneumococcal vaccine composition of any one of the preceding claims wherein the pneumococcal vaccine composition comprises at least 14 pneumococcal polysaccharides.

8. The pneumococcal vaccine composition of any one of the preceding claims wherein the pneumococcal vaccine composition comprises at least 17 pneumococcal polysaccharides.

9. The pneumococcal vaccine composition of any one of the preceding claims wherein the pneumococcal vaccine composition comprises at least 19 pneumococcal polysaccharides.

10. The pneumococcal vaccine composition of any one of the preceding claims wherein the pneumococcal vaccine composition comprises at least 20 pneumococcal polysaccharides.

11. The pneumococcal vaccine composition of any one of the preceding claims wherein the pneumococcal vaccine composition comprises at least 22 pneumococcal polysaccharides.

12. The pneumococcal vaccine composition of any one of the preceding claims wherein the pneumococcal vaccine composition comprises at least 24 pneumococcal polysaccharides.

13. The pneumococcal vaccine composition of any one of the preceding claims wherein each of the pneumococcal polysaccharides are selected from serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 23F, 24B, 24F, 31, 33F, 34, 35B, 35F, 38, 39 and 45, and the carrier protein is PsA.

Petitioner Merck Ex-1004, 0726

14. The pneumococcal vaccine composition of any one of the preceding claims wherein the pneumococcal vaccine composition is a 14 valent pneumococcal vaccine composition comprising pneumococcal polysaccharides selected from serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F, wherein at least serotypes 3, and 6B are conjugated to PsaA and one or more serotypes 1, 4, 5, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F are conjugated to CRM_{197}.

15. The pneumococcal vaccine composition of any one of the preceding claims wherein the pneumococcal vaccine composition is a 14 valent pneumococcal vaccine composition comprising pneumococcal polysaccharides selected from serotypes 1, 3, 4, 5, 6A, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F, wherein at least serotypes 3, and 6A are conjugated to PsaA and one or more serotypes 1, 4, 5, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F are conjugated to CRM_{197}.

16. The pneumococcal vaccine composition of any one of the preceding claims wherein the pneumococcal vaccine composition is a 15 valent pneumococcal vaccine composition comprising pneumococcal polysaccharides selected from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V,14, 18C, 19A, 19F, 22F, 23F and 33F wherein at least serotypes 3, 6A and 6B are conjugated to PsaA and one or more serotypes 1, 4, 5, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F are conjugated to CRM_{197}.

17. The pneumococcal vaccine composition of any one of the preceding claims wherein the pneumococcal vaccine composition is a 17 valent pneumococcal vaccine composition comprising pneumococcal polysaccharides selected from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 12F,14, 18C, 19A, 19F, 22F, 23F, 33F and 35B wherein at least serotypes 3, 6A and 6B are conjugated to PsaA and one or more serotypes 1, 4, 5, 7F, 9V, 12F,14, 18C, 19A, 19F, 22F, 23F, 33F and 35B are conjugated to CRM_{197}.

18. The pneumococcal vaccine composition of any one of the preceding claims wherein the pneumococcal vaccine composition is a 20 valent pneumococcal vaccine composition comprising pneumococcal polysaccharides selected from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F,

Petitioner Merck Ex-1004, 0727

33F, 35B and 45 wherein at least serotypes 3, 6A and 6B are conjugated to PsaA and one or more serotypes 1, 4, 5, 7F, 9V, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, 33F, 35B and 45 are conjugated to CRM_{197}.

19. The pneumococcal vaccine composition of any one of the preceding claims wherein the pneumococcal vaccine composition is a 22 valent pneumococcal vaccine comprising pneumococcal polysaccharides selected from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 10A, 12F, 14, 15A, 15B, 18C, 19A, 19F, 22F, 23F, 33F, 34, 35B, and 38 wherein at least serotypes 3, 6A and 6B are conjugated to PsaA and one or more serotypes 1, 4, 5, 7F, 9V, 10A, 12F, 14, 15A, 15B, 18C, 19A, 19F, 22F, 23F, 33F, 34, 35B and 38 are conjugated to CRM_{197}.

20. The pneumococcal vaccine composition of any one of the preceding claims wherein the pneumococcal vaccine composition is a 22 valent pneumococcal vaccine comprising pneumococcal polysaccharides selected from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 10A, 12F, 14, 15A, 15B, 18C, 19A, 19F, 22F, 23F, 33F, 34, 35B and 38 wherein serotypes 1, 3, 6A, 10A, 12F, 15A 15B, 22F, 34, 35B, 38 are conjugated to PsaA and serotypes 14, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F and 33F are conjugated to CRM_{197}.

21. The pneumococcal vaccine composition of any one of the preceding claims wherein the pneumococcal vaccine composition is a 24 valent pneumococcal vaccine comprising pneumococcal polysaccharides selected from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A,12F, 14, 15A, 15B, 16F, 18C, 19A, 19F, 22F, 23F, 33F, 34, 35B and 38 wherein at least serotypes 3, 6A and 6B are conjugated to PsaA and one or more serotypes 1, 4, 5, 7F, 8, 9V, 10A, 12F, 14, 15A, 15B, 16F, 18C, 19A, 19F, 22F, 23F, 33F, 34, 35B and 38 are conjugated to CRM_{197}.

22. The pneumococcal vaccine composition of any one of the preceding claims wherein the pneumococcal vaccine composition is a 24 valent pneumococcal vaccine comprising pneumococcal polysaccharides selected from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 12F, 14, 15A, 15B, 16F 18C, 19A, 19F, 22F, 23F, 33F, 34, 35B and 38 wherein serotypes 1, 3, 6A, 8, 10A, 12F, 15A 15B, 16F 22F, 34, 35B, 38

Petitioner Merck Ex-1004, 0728

are conjugated to PsaA and serotypes 14, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F and 33F are conjugated to CRM_{197}.

23. The pneumococcal vaccine composition comprising pneumococcal polysaccharides of two or more serotypes and a carrier protein, wherein the serotypes are selected from 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19F, 19A and 22F and the carrier protein is PsaA.

24. The pneumococcal vaccine composition comprising pneumococcal polysaccharides of two or more serotypes and a carrier protein, wherein the serotypes are selected from 1, 3, 4, 5, 6A, 6B, 7F, 9V, 10A, 12F, 14, 15A, 15B, 18C, 19F, 19A, 22F, 23F, 33F, 34, 35, 38 and 45, and the carrier protein is PsaA.

25. The pneumococcal vaccine composition of any one of the preceding claims wherein the pneumococcal vaccine composition is formulated into a dosage unit.

26. The pneumococcal vaccine composition of any one of the preceding claims wherein the dosage unit comprises about 0.1 µg to about 50 µg of each pneumococcal polysaccharide, about 0.1 µg to about 10 µg, or about 1 µg to about 5 µg of each pneumococcal polysaccharide, wherein each pneumococcal polysaccharide is each individually conjugated to the carrier protein from about 1.5 µg to about 5 µg of carrier protein.

27. The pneumococcal vaccine composition of any one of the preceding claims further comprising a pharmaceutically acceptable diluent, buffer, preservative, stabilizer, adjuvant, and/or a lyophilization excipient.

28. The pneumococcal vaccine composition of any one of the preceding claims wherein the dosage unit is supplied as a unit dose vial, a multiple dose vial, or a pre-filled syringe.

Petitioner Merck Ex-1004, 0729

29. The pneumococcal vaccine composition of any one of the preceding claims wherein the pneumococcal vaccine composition is a single 0.5 mL dose comprising:

about 2.2 to 4.4 µg of one or more pneumococcal polysaccharides;

about 1 µg to about 10 µg of PsaA conjugated to each of the one or more pneumococcal polysaccharides;

about 1 µg to about 10 µg of CRM_{197} conjugated to each of the one or more pneumococcal polysaccharides;

about 0.2 mg to about 1 mg of aluminum phosphate adjuvant; and

an excipient.

30. The pneumococcal vaccine composition of any one of the preceding claims wherein the pneumococcal vaccine composition is a 13 valent pneumococcal vaccine composition comprising:

pneumococcal polysaccharide selected from serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F and 33F, each pneumococcal polysaccharide each individually conjugated to PsaA or combination of PsaA CRM_{197},

wherein the pneumococcal vaccine composition is about a 0.5 mL dose formulated as a sterile liquid comprising:

about 2.2 µg to about 4.4 µg of each pneumococcal polysaccharide,

about 25 µg to about 30 µg PsaA and CRM_{197},

about 0.125 mg of elemental aluminum as about 0.5 mg aluminum phosphate,

sodium chloride, and

a L-histidine buffer.

31. The pneumococcal vaccine composition of any one of the preceding claims wherein the pneumococcal vaccine composition is a 14 valent pneumococcal vaccine composition comprising:

pneumococcal polysaccharides selected from serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F, each pneumococcal

Petitioner Merck Ex-1004, 0730

polysaccharide each individually conjugated to PsaA or combination of PsaA CRM_{197},

wherein the pneumococcal vaccine composition is about a 0.5 mL dose formulated as a sterile liquid comprising:

about 2.2 µg to about 4.4 µg of each pneumococcal polysaccharide,

about 20 µg to about 35 µg PsaA and CRM_{197},

about 0.125 mg of elemental aluminum as about 0.5 mg aluminum phosphate,

sodium chloride, and

a L-histidine buffer.

32. The pneumococcal vaccine composition of any one of the preceding claims wherein the pneumococcal vaccine composition is a 14 valent pneumococcal vaccine composition comprising:

pneumococcal polysaccharides selected from serotypes 1, 3, 4, 5, 6A, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F, each pneumococcal polysaccharide each individually conjugated to PsaA or combination of PsaA CRM_{197},

wherein the pneumococcal vaccine composition is about a 0.5 mL dose formulated as a sterile liquid comprising:

about 2.2 to 4.4 µg of each pneumococcal polysaccharide,

about 20 µg to about 35 µg PsaA and CRM_{197},

about 0.125 mg of elemental aluminum as about 0.5 mg aluminum phosphate,

sodium chloride, and

a L-histidine buffer.

33. The pneumococcal vaccine composition of any one of the preceding claims wherein the pneumococcal vaccine composition is a 15 valent pneumococcal vaccine composition comprising:

pneumococcal polysaccharides selected from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V,14, 18C, 19A, 19F, 22F, 23F and 33F wherein at least serotypes 3,

Petitioner Merck Ex-1004, 0731

6A and 6B are conjugated to PsaA and one or more serotypes 1, 4, 5, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F are conjugated to CRM_{197},

wherein the pneumococcal vaccine composition is about a 0.5 mL dose formulated as a sterile liquid comprising:

about 2.2 µg to about 4.4 µg of each pneumococcal polysaccharide,

about 5 µg to about 20 µg of PsaA;

about 20 µg to about 40 µg of CRM_{197},

about 0.125 mg of elemental aluminum as about 0.5 mg aluminum phosphate,

sodium chloride, and

a L-histidine buffer.

34. The pneumococcal vaccine composition of any one of the preceding claims wherein the pneumococcal vaccine composition is a 17 valent pneumococcal vaccine composition comprising:

pneumococcal polysaccharides selected from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 12F,14, 18C, 19A, 19F, 22F, 23F, 33F and 35B wherein at least serotypes 3, 6A and 6B are conjugated to PsaA and one or more serotypes 1, 4, 5, 7F, 9V, 12F, 14, 18C, 19A, 19F, 22F, 23F, 33F and 35B are conjugated to CRM_{197},

wherein the pneumococcal vaccine composition is about a 0.5 mL dose formulated as a sterile liquid comprising:

about 2.2 µg to about 4.4 µg of each pneumococcal polysaccharide,

about 5 µg to about 20 µg of PsaA,

about 20 µg to about 40 µg of CRM_{197},

about 0.125 mg of elemental aluminum as about 0.5 mg aluminum phosphate,

sodium chloride, and

a L-histidine buffer.

Petitioner Merck Ex-1004, 0732

35. The pneumococcal vaccine composition of any one of the preceding claims wherein the pneumococcal vaccine composition is a 20 valent pneumococcal vaccine composition comprising:

pneumococcal polysaccharides selected from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, 33F, 35B and 45 wherein at least serotypes 3, 6A and 6B are conjugated to PsaA and one or more serotypes 1, 4, 5, 7F, 9V, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, 33F, 35B and 45 are conjugated to CRM_{197},

wherein the pneumococcal vaccine composition is about a 0.5 mL dose formulated as a sterile liquid comprising:

about 2.2 µg to about 4.4 µg of each pneumococcal polysaccharide,

about 5 µg to about 20 µg of PsaA,

about 20 µg to about 50 µg of CRM_{197},

about 0.125 mg of elemental aluminum as about 0.5 mg aluminum phosphate,

sodium chloride, and

a L-histidine buffer.

36. The pneumococcal vaccine composition of any one of the preceding claims wherein the pneumococcal vaccine composition is a 22 valent pneumococcal vaccine composition comprising:

pneumococcal polysaccharides selected from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 10A, 12F, 14, 15A, 15B, 18C, 19A, 19F, 22F, 23F, 33F, 34, 35B and 38 wherein at least serotypes 3, 6A and 6B are conjugated to PsaA and one or more serotypes 1, 4, 5, 7F, 9V, 10A, , 12F, 14, 15A, 15B, 18C, 19A, 19F, 22F, 23F, 33F, 34, 35B and 38 are conjugated to CRM_{197},

wherein the pneumococcal vaccine composition is about a 0.5 mL dose formulated as a sterile liquid comprising:

about 2.2 µg to about 4.4 µg of each pneumococcal polysaccharide,

about 5 µg to about 20 µg of PsaA,

about 20 µg to about 50 µg of CRM_{197},

Petitioner Merck Ex-1004, 0733

about 0.125 mg of elemental aluminum as about 0.5 mg aluminum phosphate,
sodium chloride, and
a L-histidine buffer.

37. The pneumococcal vaccine composition of any one of the preceding claims wherein the pneumococcal vaccine composition is a 24 valent pneumococcal vaccine composition comprising:

pneumococcal polysaccharides selected from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 12F, 14, 15A, 15B, 16F, 18C, 19A, 19F, 22F, 23F, 33F, 34, 35B and 38 wherein at least serotypes 3, 6A and 6B are conjugated to PsaA and one or more serotypes 1, 4, 5, 7F, 8, 9V, 10A, 12F, 14, 15A, 15B, 16F, 18C, 19A, 19F, 22F, 23F, 33F and 35B are conjugated to CRM_{197},

wherein the pneumococcal vaccine composition is about a 0.5 mL dose formulated as a sterile liquid comprising:

about 2.2 µg to about 4.4 µg of each pneumococcal polysaccharide,
about 5 µg to about 20 µg of PsaA,
about 20 µg to about 50 µg of CRM_{197},
about 0.125 mg of elemental aluminum as about 0.5 mg aluminum phosphate,
sodium chloride, and
a L-histidine buffer.

38. A method of treating a subject in need thereof comprising, administering the pneumococcal vaccine composition of any one of the preceding claims to the subject in need thereof.

39. The method of claim 38 wherein the subject has a disease mediated by *streptococcus pneumoniae.*

Petitioner Merck Ex-1004, 0734

40. The method of claim 39 wherein the disease mediated by *streptococcus pneumoniae* is invasive pneumococcal disease (IPD).

41. The method of any one of claims 38-40 wherein the subject is a human.

42. The method of claim 41 wherein the human is an infant (less than about 1 year of age), a toddler (about 12 months to about 24 months of age), a young child (about 2 years to about 5 years of age), an older child (about 5 years to about 13 years of age), an adolescent (about 13 years to about 18 years of age), an adult (about 18 years to about 65 years of age), or an elder (more than about 65 years of age).

43. A method of inducing an immune response to a *S. pneumoniae* capsular polysaccharide conjugate, comprising administering an immunologically effective amount of the pneumococcal vaccine composition of any one of claims 1-37 to a subject in need thereof.

44. The method of claim 43 wherein the method further comprises formulating the polysaccharide-protein conjugate into the pneumococcal vaccine composition including an adjuvant, an excipient, and a buffer.

45. The method of claim 44 wherein the adjuvant is aluminum phosphate.

Petitioner Merck Ex-1004, 0735

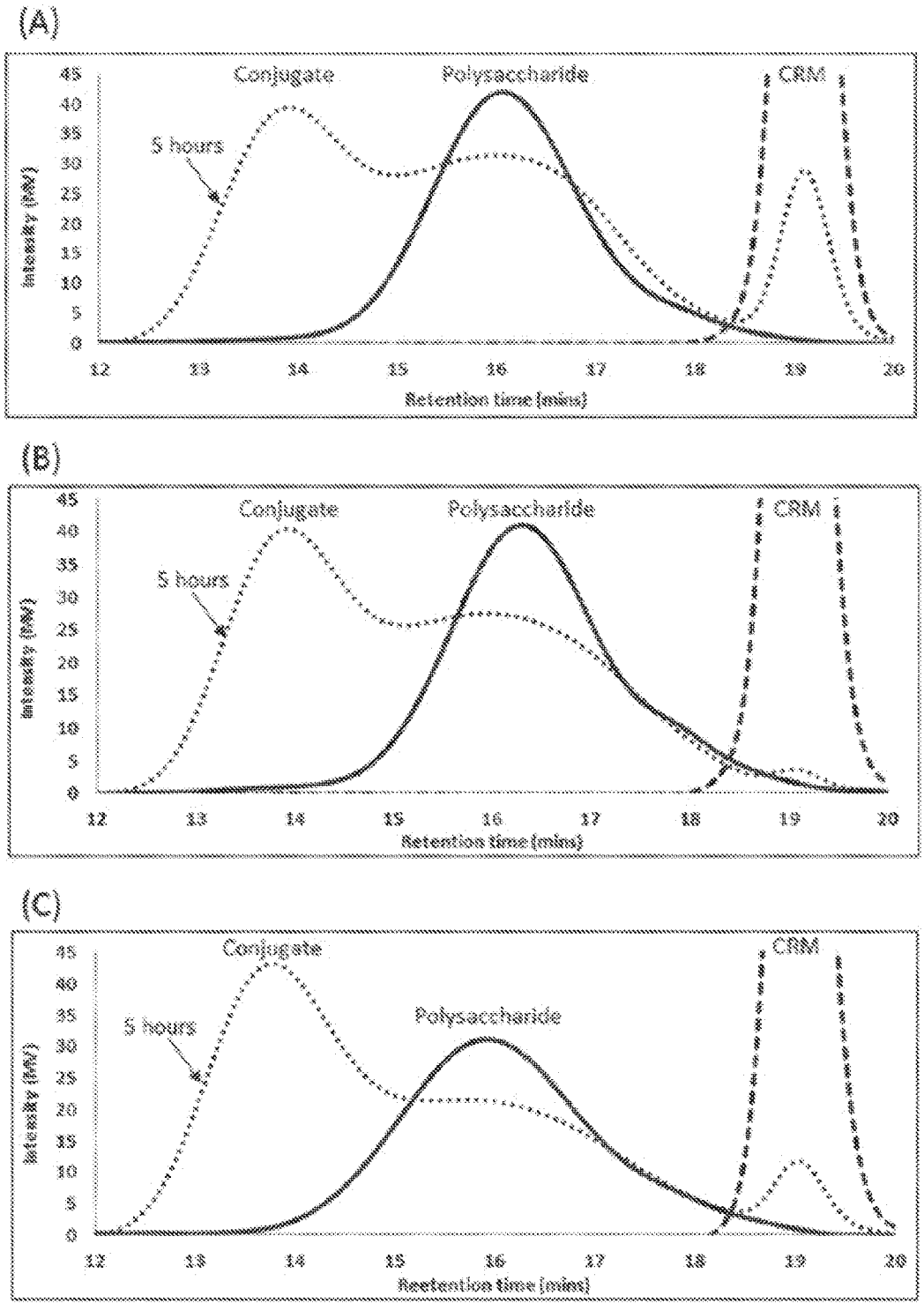
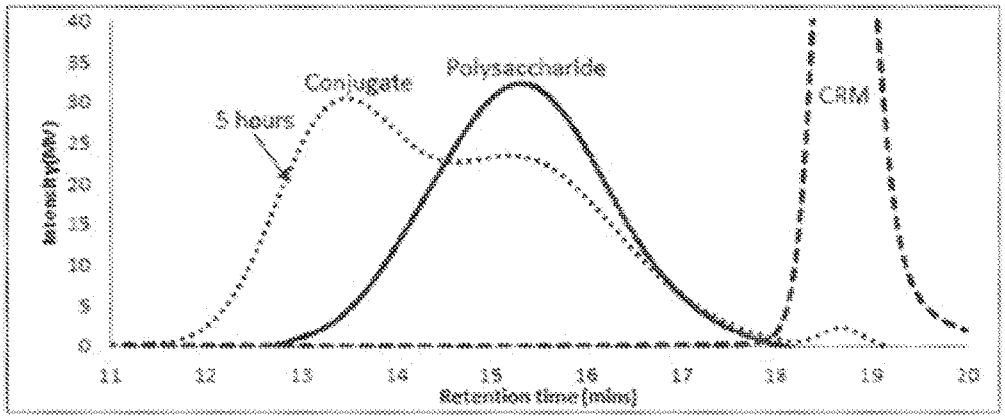
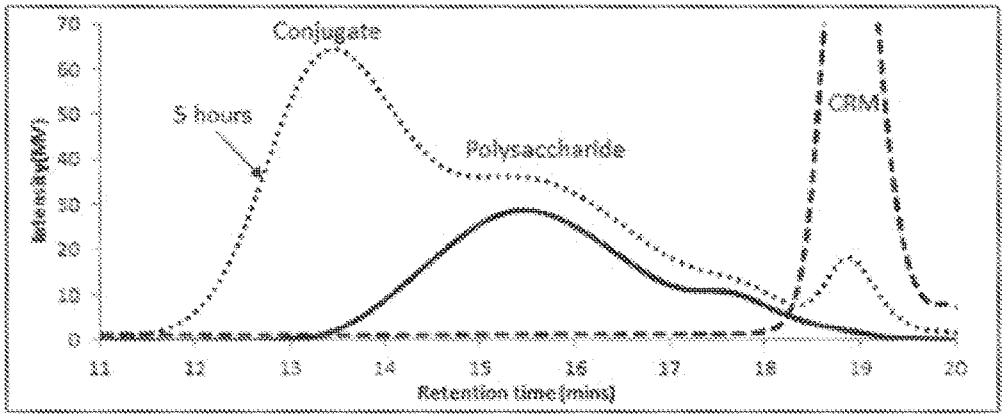

(A)
Conjugate
Polysaccharide
CRM
5 hours
Intensity (MV)
Retention time (mins)
(B)
Conjugate
Polysaccharide
CRM
5 hours
Intensity (MV)
Retention time (mins)
(C)
Conjugate
CRM
5 hours
Polysaccharide
Intensity (MV)
Retention time (mins)

FIG. 1

(D)



(E)



(F)

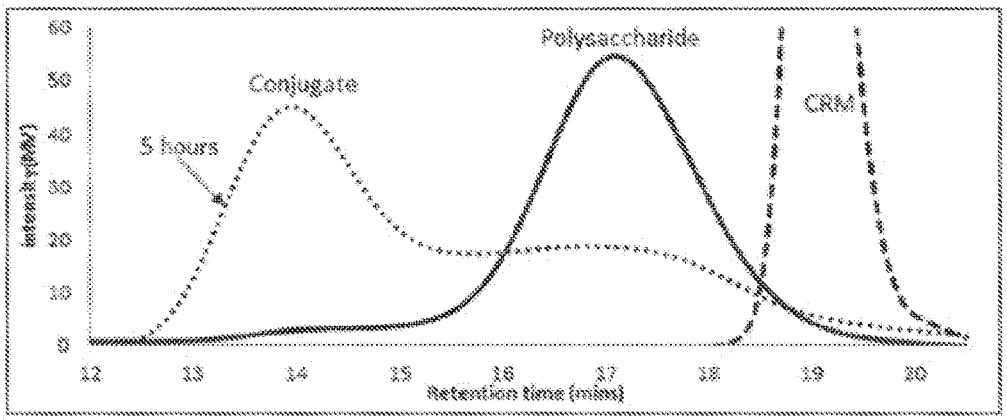


FIG. 1 (contd.)

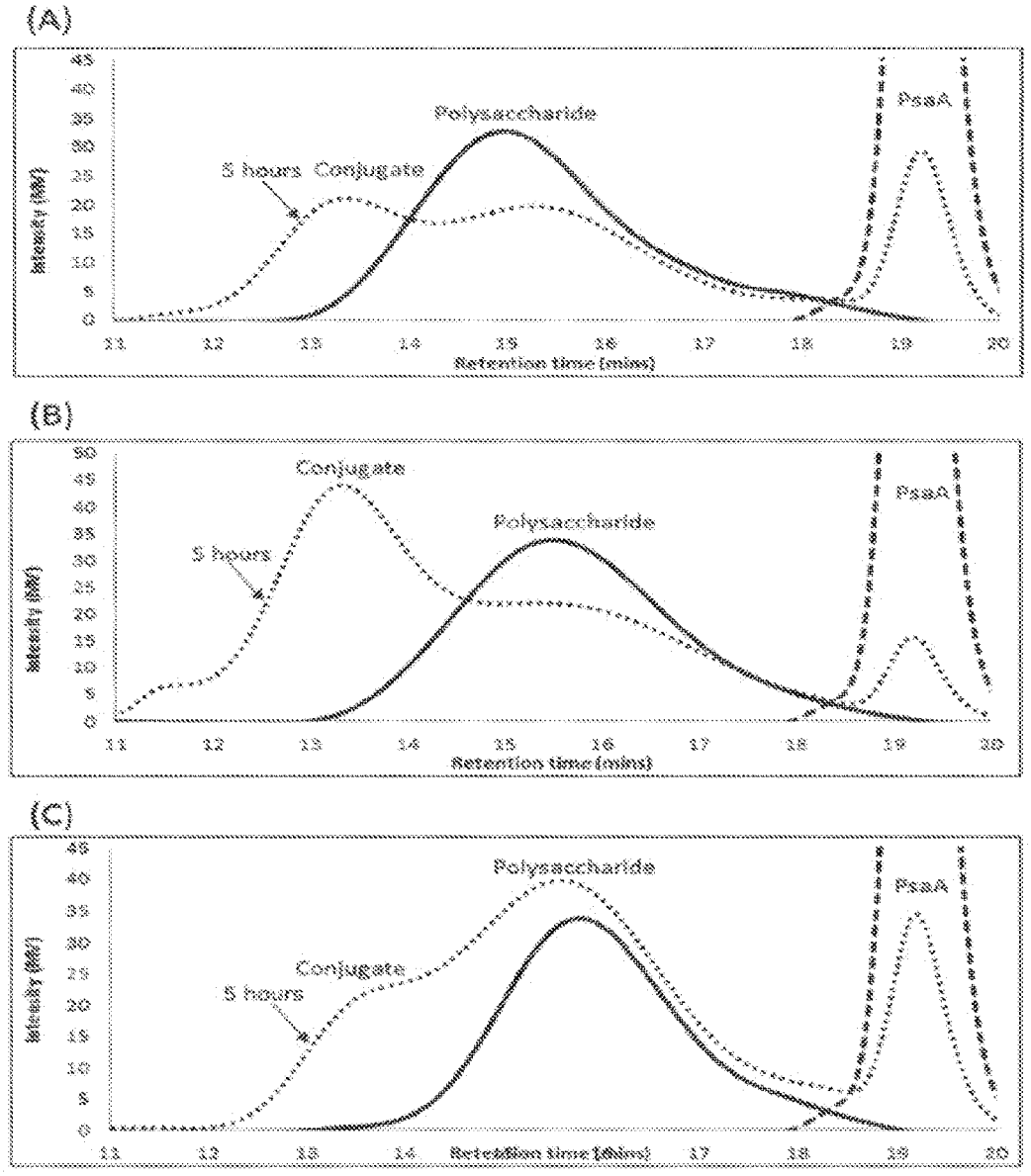
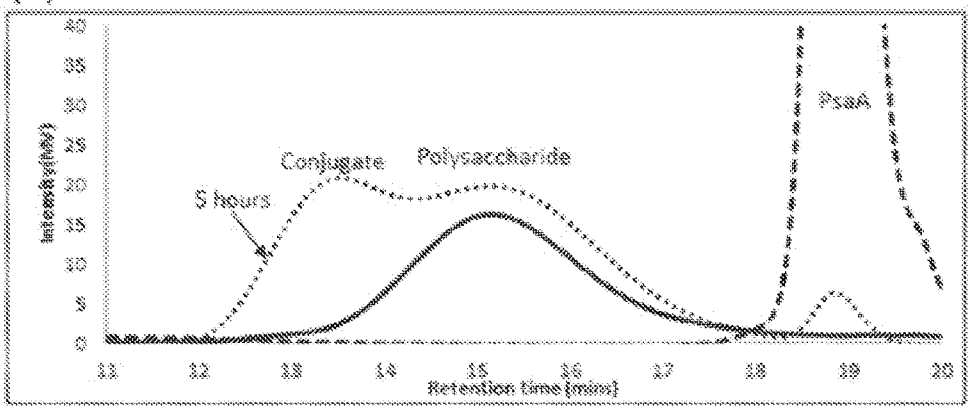
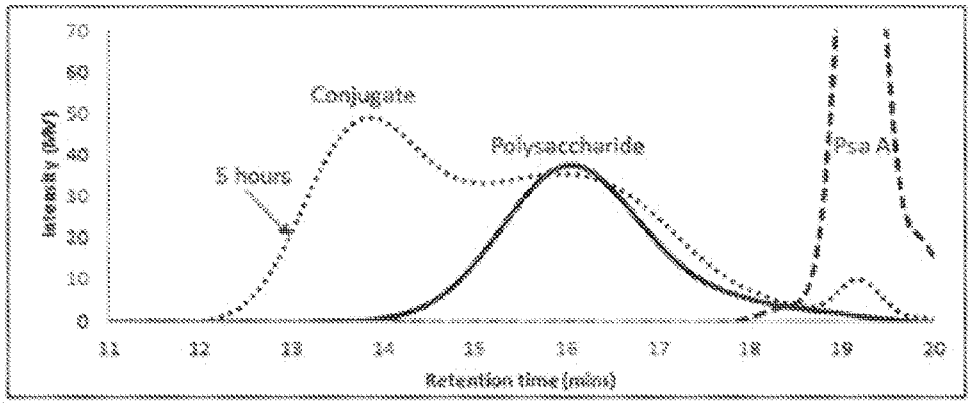

(A)
Polysaccharide
PsaA
5 hours
Conjugate
Intensity (MV)
Retention time (mins)
(B)
Conjugate
PsaA
Polysaccharide
5 hours
Intensity (MV)
Retention time (mins)
(C)
Polysaccharide
PsaA
Conjugate
5 hours
Intensity (MV)

FIG. 2

(D)



(E)



(F)

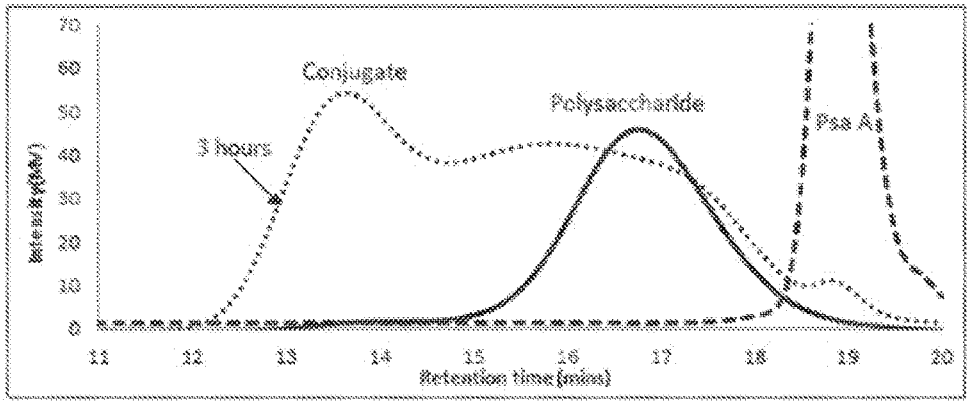


FIG. 2 (contd.)

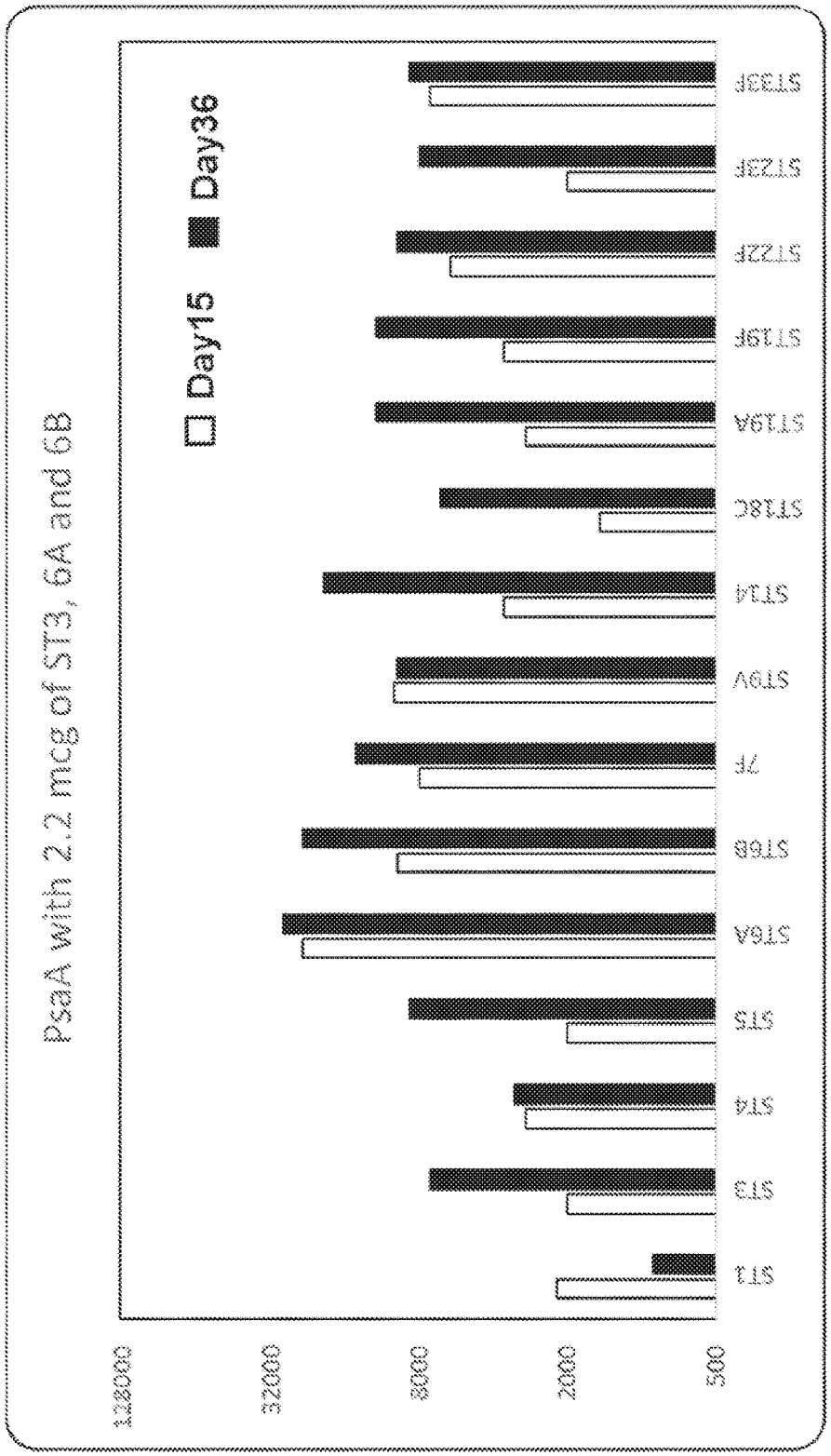

PsaA with 2.2 mcg of ST3, 6A and 6B
Day15
Day36
128000
32000
8000
2000
500
ST1
ST3
ST4
ST5
ST6A
ST6B
7F
ST9V
ST14
ST18C
ST19A
ST19F
ST22F
ST23F
ST33F

FIG. 3

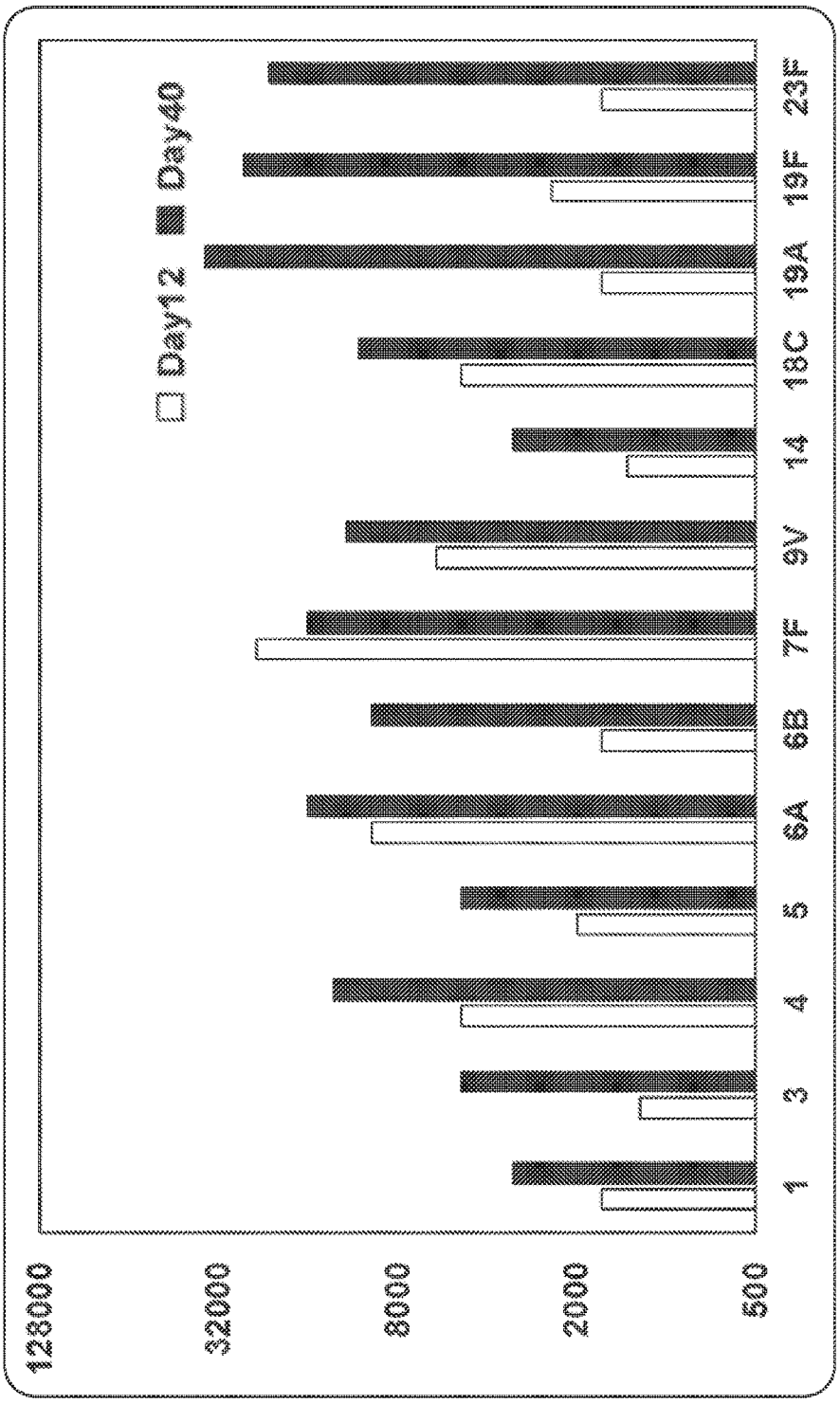

Day12
Day40
128000
32000
8000
2000
500
1
3
4
5
6A
6B
7F
9V
14
18C
19A
19F
23F

FIG. 4

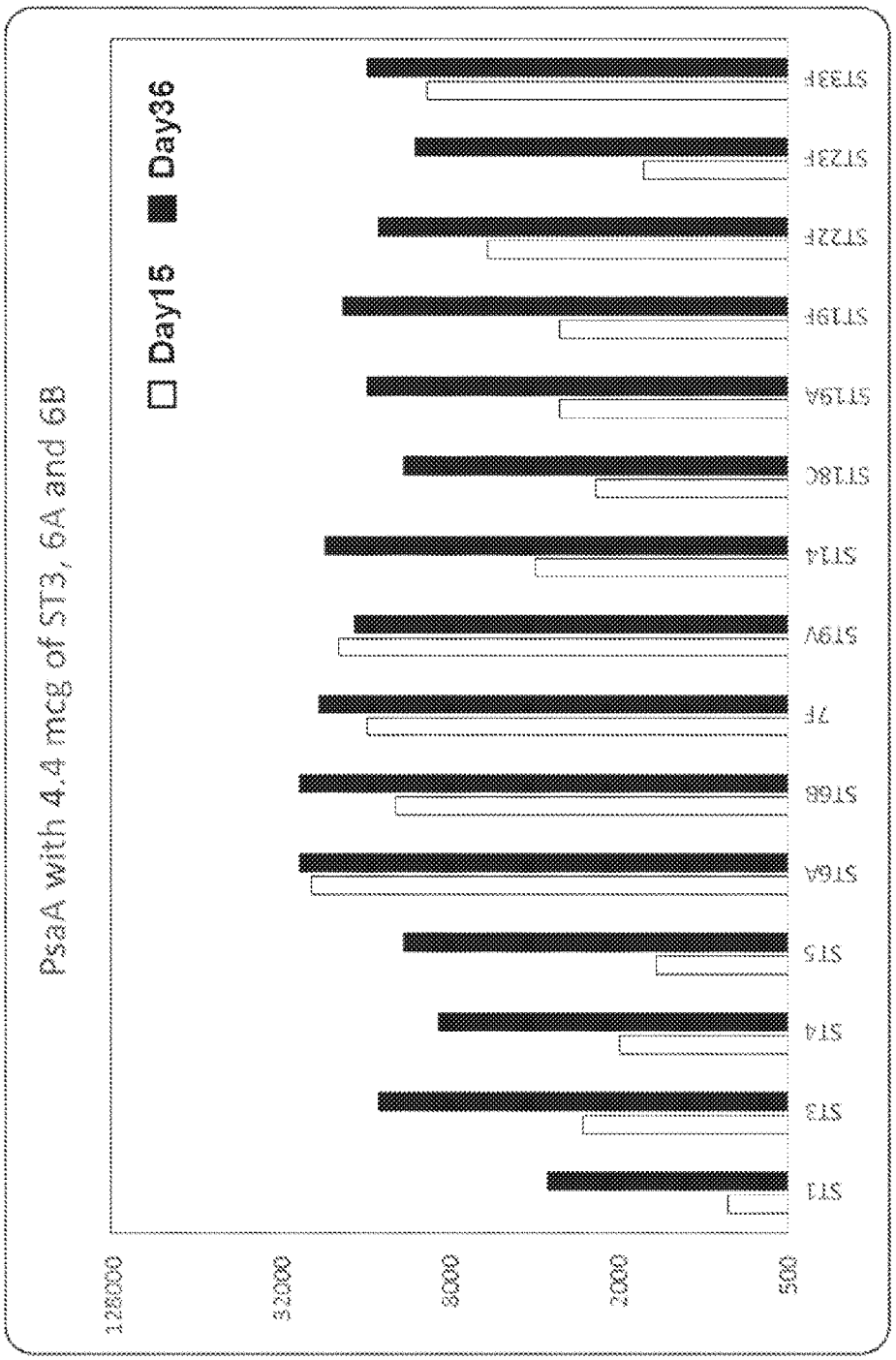

PsaA with 4.4 mcg of ST3, 6A and 6B
Day15
Day36
128000
32000
8000
2000
500
ST1
ST3
ST4
ST5
ST6A
ST6B
7F
ST9V
ST14
ST18C
ST19A
ST19F
ST22F
ST23F
ST33F

FIG. 5

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/054237

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/09 A61K39/385 A61K39/39 A61P31/04 A61K39/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, SCISEARCH, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LIN H ET AL: "Preparation and immunogenicity of capsular polysaccharide-surface adhesin A (PsaA) conjugate of Streptococcuspneumoniae", IMMUNOBIOLOGY, URBAN UND FISCHER VERLAG, DE, vol. 215, no. 7, 1 July 2010 (2010-07-01), pages 545-550, XP027066167, ISSN: 0171-2985, DOI: 10.1016/J.IMBIO.2009.08.008 [retrieved on 2009-10-31] table 1 page 548, paragraph bridging - page 549 -----	1-45
Y	EP 2 425 856 A1 (WYETH LLC [US]) 7 March 2012 (2012-03-07) examples 15,16 -----	1-45
	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 November 2017	28/11/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Domingues, Helena

Form PCT/ISA/210 (second sheet) (April 2005)

Petitioner Merck Ex-1004, 0743

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/054237

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ALLANA J SUCHER ET AL.: "Prevnar 13, the New 13-Valent Pneumococcal Conjugate Vaccine", THE ANNALS OF PHARMACOTHERAPY, vol. 45, no. 12, 1 December 2011 (2011-12-01), pages 1516-1524, XP055411056, the whole document	1-45
Y	WO 2010/120921 A1 (UNIV HOWARD [US]; US OF AMERICA DEPT OF HEALTH A [US]; TAI STANLEY SHI) 21 October 2010 (2010-10-21) page 16 - page 19	1-45
Y,P	WO 2016/207905 A2 (BIOLOGICAL E LTD [IN]) 29 December 2016 (2016-12-29) page 7 - page 8 claims 1,2	1-45

1

Form PCT/ISA/210 (continuation of second sheet) (April 2005)

Petitioner Merck Ex-1004, 0744

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2017/054237

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 2425856 A1	07-03-2012	AR 053354 A1	02-05-2007
		AT 548051 T	15-03-2012
		AU 2006235013 A1	19-10-2006
		BR P10607025 A2	28-07-2009
		CA 2604363 A1	19-10-2006
		CN 101180079 A	14-05-2008
		CN 102716480 A	10-10-2012
		CN 104815327 A	05-08-2015
		CY 1112777 T1	10-02-2016
		DK 1868645 T3	10-04-2012
		EP 1868645 A1	26-12-2007
		EP 2425851 A1	07-03-2012
		EP 2425852 A1	07-03-2012
		EP 2425853 A1	07-03-2012
		EP 2425854 A1	07-03-2012
		EP 2425855 A1	07-03-2012
		EP 2425856 A1	07-03-2012
		ES 2382048 T3	04-06-2012
		HK 1120416 A1	23-11-2012
		HK 1213184 A1	30-06-2016
		IL 186367 A	30-09-2013
		JP 4472770 B2	02-06-2010
		JP 5173920 B2	03-04-2013
		JP 5730261 B2	03-06-2015
		JP 2008535838 A	04-09-2008
		JP 2009161567 A	23-07-2009
		JP 2013006881 A	10-01-2013
		KR 20070118700 A	17-12-2007
		KR 20130048262 A	09-05-2013
		KR 20150061018 A	03-06-2015
		KR 20150061019 A	03-06-2015
		KR 20150061020 A	03-06-2015
		KR 20150061021 A	03-06-2015
		KR 20170086139 A	25-07-2017
		MY 145150 A	30-12-2011
		NZ 562406 A	25-09-2009
		PL 1868645 T3	31-07-2012
		PT 1868645 E	17-05-2012
		RS 52249 B	31-10-2012
		SI 1868645 T1	30-04-2012
		TW I386222 B	21-02-2013
		TW 201212937 A	01-04-2012
		TW 201438734 A	16-10-2014
		US 2006228380 A1	12-10-2006
		US 2009130137 A1	21-05-2009
		US 2012237542 A1	20-09-2012
		US 2014314805 A1	23-10-2014
		US 2016158345 A1	09-06-2016
		WO 2006110381 A1	19-10-2006
		ZA 200709483 B	25-03-2009
WO 2010120921 A1	21-10-2010	CA 2758921 A1	21-10-2010
		EP 2419130 A1	22-02-2012
		JP 5744842 B2	08-07-2015
		JP 2012524099 A	11-10-2012
		US 2010266625 A1	21-10-2010
		US 2012009213 A1	12-01-2012
		US 2015182613 A1	02-07-2015

Petitioner Merck Ex-1004, 0745

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2017/054237

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
		WO 2010120921 A1	21-10-2010
WO 2016207905 A2	29-12-2016	NONE	

Petitioner Merck Ex-1004, 0746

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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
11 April 2019 (11.04.2019)

WIPO | PCT

(10) International Publication Number
WO 2019/070994 A1

(51) International Patent Classification:
A61K 39/09 (2006.01) *A61K 39/00* (2006.01)

(21) International Application Number:
PCT/US2018/054391

(22) International Filing Date:
04 October 2018 (04.10.2018)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/568,164 04 October 2017 (04.10.2017) US
62/732,260 17 September 2018 (17.09.2018) US

(71) Applicant: **LIFFEY BIOTECH LIMITED** [IE/IE]; c/o Mathesons, 70 Sir John Rogerson's Quay, Dublin 2, D02 R296 (IE).

(72) Inventors; and
(71) Applicants: **FORREST, Bruce D.** [US/US]; 135 Piermont Avenue, South Nyack, New York 10960 (US). **LOVE, Jack D.** [US/US]; 4111 W. Inman Avenue, Tampa, Florida 33609 (US).

(74) Agent: **HOLZAPFEL, Keli**; Wilson Sonsini goodrich & Rosati, 650 Page Mill road, Palo Alto, California 94304 (US).

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

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

WO 2019/070994 A1

(54) Title: SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF

(57) Abstract: Saccharide-polypeptide conjugates, compositions containing these, methods of making and using the conjugates and compositions, and kits containing these, are disclosed.

Petitioner Merck Ex-1004, 0747

SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF

CROSS REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/568,164, filed October 4, 2017, and United States Provisional Application No. 62/732,260, filed September 17, 2018, all of which are incorporated herein by reference in their entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on October 1, 2018, is named 50308-706_601_SL.txt and is 5,037 bytes in size.

SUMMARY

[0003] Provided herein is a brief summary. This summary can be used to provide an understanding of the embodiments provided herein, but the embodiments are not limited to what is provided in this summary.

[0004] Provided herein is a pharmaceutical composition in unit dose form that can comprise a plurality of at least eight unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 2, 6C, 7C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35B, 35F, and 38, and wherein a total amount of capsular polysaccharide, fragment thereof, or combination thereof, individually for each *Streptococcus pneumoniae* serotype in the pharmaceutical composition in unit dose form, ranges from about 0.5 μg to about 5 μg. A pharmaceutical composition in unit dose form can comprises a plurality of at least 29 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 1, 2, 3, 4, 5, 6A, 6B, 7F,

Petitioner Merck Ex-1004, 0748

7C, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 35F, 34, 35B, and 38, and wherein a total amount of capsular polysaccharide, fragment thereof, or combination thereof, individually for each *Streptococcus pneumoniae* serotype in the pharmaceutical composition in unit dose form, ranges from about 0.5 μg to about 5 μg. A pharmaceutical composition in unit dose form can comprise at least 2 immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype group, wherein one serotype is 6C and at least one additional serotype is selected from the group consisting of 1, 2, 3, 4, 5, 6A, 6B, 7F, 7C, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35B, and 38, and wherein a total amount of capsular polysaccharide, fragment thereof, or combination thereof, individually for each *Streptococcus pneumoniae* serotype in the pharmaceutical composition in unit dose form, ranges from about 0.5 μg to about 5 μg. A pharmaceutical composition in unit dose form can comprise at least 2 immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype group, wherein one serotype is 20B and at least one additional serotype is selected from the group consisting of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 7C, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35B, and 38, and wherein a total amount of capsular polysaccharide, fragment thereof, or combination thereof, individually for each *Streptococcus pneumoniae* serotype in the pharmaceutical composition in unit dose form, ranges from about 0.5 μg to about 5 μg. A pharmaceutical composition can comprise an immunogenic saccharide-polypeptide conjugate comprising, individually, at least two unique capsular polysaccharides, fragments thereof, or combinations thereof conjugated to a polypeptide, wherein the at least two unique capsular polysaccharides, fragments thereof, or combinations thereof are from a *Streptococcus pneumoniae* serotype selected from a group consisting of 1, 2, 3, 4, 5, 6A, 6B, 7F, 7C, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35F, 35B, and 38. A vaccine in unit dose form can comprise at

Petitioner Merck Ex-1004, 0749

least one immunogenic saccharide-polypeptide conjugate comprising a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a *Streptococcus pneumoniae* serotype selected from a group consisting of 23A and 23B, or a combination thereof.

[0005] Provided herein is a method that can comprise administering to a subject a first composition, wherein the first composition is one of any pharmaceutical composition or vaccine as described herein. Further provided herein is a method that can comprise administering to the subject the first composition occurs at least about four weeks before or at least about four weeks after an administration of a second composition comprising an immunogenic saccharide-polypeptide conjugate comprising a second polypeptide to the subject, wherein the second polypeptide has at least about 70% length homology, at least about 70% sequence homology, or a combination thereof, to the polypeptide of the first composition.

[0006] Provided herein is a method that can comprise: administering a second immunogenic saccharide-polypeptide conjugate composition to a subject at least four weeks after a first immunogenic saccharide-polypeptide conjugate composition was administered; wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a first polypeptide that has at least about 70% length homology, at least about 70% sequence homology, or a combination thereof, to a second polypeptide of the second immunogenic saccharide-polypeptide conjugate composition; and wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a first serotype; wherein the second immunogenic saccharide-polypeptide conjugate composition comprises a second serotype, and wherein the first serotype and the second serotype are different; wherein the first immunogenic saccharide-polypeptide conjugate composition or the second immunogenic saccharide-polypeptide conjugate composition is the vaccine of any one of claims 159-249.

[0007] Further provided herein is a method of making a composition that can comprise: contacting the immunogenic saccharide-polypeptide conjugate of any one of the described pharmaceutical compositions or vaccines with an excipient, an adjuvant, or any combination thereof.

[0008] Provided herein is a kit that can comprise any pharmaceutical composition or vaccine described herein contained in a container described herein.

Petitioner Merck Ex-1004, 0750

[0009] Provided herein are methods of making pharmaceutical compositions or vaccines, methods of immunizing with pharmaceutical compositions or vaccines, and methods of vaccinating with pharmaceutical compositions or vaccines.

INCORPORATION BY REFERENCE

[0010] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE FIGURES

[0011] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present disclosure will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the disclosure are utilized, and the accompanying drawings of which:

[0012] FIG. 1 illustrates a flow diagram of the general process for manufacture of a saccharide peptide conjugate vaccine.

[0013] FIG. 2 illustrates a flow diagram of a process for cell banking of a bacterial strain.

[0014] FIG. 3 illustrates a flow diagram of a process for producing, isolating, and purifying CRM_{197}.

[0015] FIG. 4 illustrates a flow diagram of a fermentation process for isolating a polysaccharide from a strain of *Streptococcus pneumoniae*.

[0016] FIG. 5 illustrates a flow diagram of a conjugation process for conjugating a polysaccharide and CRM_{197}.

[0017] FIG. 6 illustrates a flow diagram for a process for formulating a polysaccharide polypeptide conjugate vaccine.

[0018] FIG. 7 illustrates the projected expanded coverage provided by a *Streptococcus pneumoniae* polypeptide conjugate vaccine comprising *Streptococcus pneumoniae* serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B (ePCV; LiffeyVax Serotypes) against invasive pneumococcal disease (IPD) in United States and Canada when this vaccine is administered in a vaccination regime with Prevnar 13 (PCV13). Prevnar 13 alone only covers about 46.1% of IPD compared to an overall coverage of 90% when administered in a vaccination regime with ePCV.

Petitioner Merck Ex-1004, 0751

[0019] FIG. 8 illustrates the projected expanded coverage provided by a *Streptococcus pneumoniae* polypeptide conjugate vaccine comprising *Streptococcus pneumoniae* serotypes 6C, 9N, 15A, 15C, 16F, 23A, 23B, and 35B (Liffey/Vax ePCV8) against invasive pneumococcal disease (IPD) in United States and Canada when this vaccine is administered in a vaccination regime with Prevnar 13 (PCV13) or a vaccine comprising the Prevnar 13 serotypes plus seven additional serotypes (8, 10A, 11A, 12F, 15B, 22F, and 33F) (PCV20). Prevnar 13 alone only covers about 46.1% of IPD, but the addition of the seven serotypes in the PCV20 is predicted to cover about 21% more of IPD. The further addition of the eight serotypes in the Liffey/Vax ePCV8 is predicted to cover about 23% more of IPD when administered in a vaccination regime with PCV20.

[0020] FIG. 9 illustrates the projected expanded coverage provided by a *Streptococcus pneumoniae* polypeptide conjugate vaccine comprising *Streptococcus pneumoniae* serotypes 6C, 9N, 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 34, 35F, 35B, and 38 (ePCV8+7) against invasive pneumococcal disease (IPD) in United States and Canada when this vaccine is administered in a vaccination regime with Prevnar 13 (PCV13) or a vaccine comprising the Prevnar 13 serotypes plus seven additional serotypes (8, 10A, 11A, 12F, 15B, 22F, and 33F) (PCV20). Prevnar 13 alone only covers about 46.1% of IPD, but the addition of the seven serotypes in the PCV20 is predicted to cover about 21% more of IPD. The further addition of seven serotypes in the Liffey/Vax ePCV8 (Liffey/Vax ePCV8+7) (see **FIG. 8** for ePCV8) is predicted to cover about 29% more of IPD when administered in a vaccination regime with PCV20.

[0021] FIGS. 10A, 10B, 10C, 10D, 10E, and 10F illustrate the annotated capsular polysaccharide (*cps*) locus for various *S. pneumoniae* serotypes (Bentley et al., Genetic Analysis of the Capsular Biosynthetic Locus from all 90 Pneumococcal Serotypes. *PloS Genet* 2(3): 31 (2006)).

DETAILED DESCRIPTION

[0022] Several aspects are described below with reference to example applications for illustration. It should be understood that numerous specific details, relationships, and methods are set forth to provide a full understanding of the features described herein. One having ordinary skill in the relevant art, however, will readily recognizes that the features described herein can be practiced without one or more of the specific details or with other methods. The features described herein are not limited by the illustrated ordering of acts or events, as some acts can

Petitioner Merck Ex-1004, 0752

occur in different orders and/or concurrently with other acts or events. Furthermore, not all illustrated acts or events are required to implement a methodology in accordance with the features described herein.

[0023] The terminology used herein is for the purpose of describing particular cases only and is not intended to be limiting. As used herein, the singular forms "a", "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise. Furthermore, to the extent that the terms "including", "includes", "having", "has", "with", or variants thereof are used in either the detailed description and/or the claims, such terms are intended to be inclusive in a manner similar to the term "comprising".

[0024] The term "about" or "approximately" can mean within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *i.e.*, the limitations of the measurement system. For example, "about" can mean within 1 or more than 1 standard deviation, per the practice in the art. Alternatively, "about" can mean a range covering plus or minus up to 20%, up to 10%, up to 5%, or up to 1% of a given value. Where particular values are described in the application and claims, unless otherwise stated the term "about" can mean within an acceptable error range for the particular value should be assumed. When a conjugate is present in µg, "about" can mean +/- 0.5 µg, 0.75 µg, 1.0 µg, 1.25 µg, 1.5 µg, 1.75 µg, or 2 µg for each conjugate.

[0025] A "unit dose" when used in reference to a pharmaceutical composition or vaccine can refer to units suitable as unitary dosage for humans, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect. In some instance the unit dose can contain a diluent; *i.e.*, carrier, or vehicle. In some instances, the unit dose can be a physically discrete.

[0026] The term "saccharide" can refer to a saccharide, oligosaccharide or polysaccharide. For example, saccharide can mean a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, a hexasaccharide, a heptasaccharide, an octasaccharide, a nonasaccharide, or a decasaccharide. An oligosaccharide can refer to two to ten saccharides. A polysaccharide can refer to more than ten saccharides.

[0027] The term "polypeptide" can refer to a single linear chain of amino acids held together by amide bonds. A polypeptide can refer to at least two amino acids. A polypeptide can be an oligopeptide, wherein the term "oligopeptide" can refer to a single chain of from two to twenty

Petitioner Merck Ex-1004, 0753

amino acids joined by amide bonds. A polypeptide can be a protein, wherein the term "protein" can refer to a single chain of fifty or more amino acids held together by amide bonds.

[0028] The term an "immune response" can refer to the production of cytokines and/or antibodies with a degree of specificity for an antigen after administering the antigen to a subject. In some instances, the term "immune response" can refer to any change in immune cells or change caused by immune cells after administering the antigen to a subject. An immune response can be T cell independent, T cell dependent, or both.

[0029] The term "homology" can mean percent sequence identity between a particular nucleic acid or amino acid sequence and another nucleic acid or amino acid sequence. First, a nucleic acid or amino acid sequence can be compared another sequence using the BLAST 2 Sequences (Bl2seq) program from the stand- alone version of BLASTZ containing BLASTN version 2.0.14 and BLASTP version 2.0.14. BLASTN can be used to compare nucleic acid sequences, while BLASTP can be used to compare amino acid sequences. Once aligned, the number of matches is determined by counting the number of positions where an identical nucleotide or amino acid residue is presented in both sequences. The percent sequence identity or homology can be determined by dividing the number of matches either by the length of the sequence set forth in the identified sequence, or by an articulated length (*e.g.*, 100 consecutive nucleotides or amino acid residues from a sequence set forth in an identified sequence), followed by multiplying the resulting value by 100%.

[0030] The term "length homology" can mean percent length identity between the length of a particular polypeptide and the length of another polypeptide. "Length homology" can in some instances be calculated by dividing the number of amino acids in a first peptide chain by the number of amino acids in a second peptide chain and multiplying the result by 100%. Chain amino acids can be those forming the backbone of the peptide.

[0031] The term "length" in reference to a polypeptide can refer to counting the number of amino acids along the amino acid chain.

[0032] The term "immunogenic fragment" can be a fragment that is conjugated to a polypeptide, administered to a subject, and which elicits an immune response. In some instances, the fragment can be a fragment of a capsular polysaccharide. In other instances, the polypeptide can be CRM_{197}.

Petitioner Merck Ex-1004, 0754

[0033] Immunogenic, which can but need not be obligated to be preceded any term herein, can mean that which, when administered to a subject, which can be a human or an animal, for example a dog, cat, rat, mouse, sheep, or monkey, produces an immune response in the animal. For example, a dog can be *Canis lupis familiarius*, a cat can be *Felis catus*, a rat can be *Rattus norvegicus*, a mouse can be *Mus musculus*, a sheep can be *Ovis aries*, or a monkey can be *Simia inuus*. The administration can be, for example, subcutaneous, intramuscular, or rectal, for example in the form of a suppository.

[0034] In some embodiments, a unique immunogenic saccharide-polypeptide conjugate can be one that differs from other unique immunogenic saccharide-polypeptide conjugates, for example in a plurality of these, by at least one aspect. For example, the unique immunogenic saccharide-polypeptide conjugate can differ by the saccharide or fragment thereof it contains - in that this can render the unique immunogenic saccharide-polypeptide conjugate unique, or by the polypeptide it contains, or both. In some instances, unique immunogenic saccharide-polypeptide conjugates, to be unique, simply need not be 100% identical.

[0035] The term "partly mitigated" in reference to the toxin activity of a polypeptide can refer to a decreased toxicity of the polypeptide as compared to a wild type version of the polypeptide.

[0036] The term "molecular weight" can be a number average molecular weight or a weight average molecular weight.

[0037] The term "plurality" can mean two or more.

[0038] The term "subject" can refer to a human or an animal. The subject can be a subject in need thereof.

[0039] Bacteria can incorporate saccharides into their surface structure. For example, *Streptococcus pneumoniae* (*S. pneumoniae*) can contain saccharides in its surface structure. The saccharides can be antigens that can induce an immune response in host, such as an animal or human. For example, B cells can produce antibodies against a saccharide. This response can be a T cell independent immune response. An immune response can be induced with full-length, native form polysaccharides, oligosaccharides, or fragments thereof. An immune response can be induced with full-length, native form capsular polysaccharides, or fragments thereof. Other bacteria that can contain saccharides are *Neisseria meningitidis (N. meningitidis)*, *Salmonella enterica (S. enterica)*, and *Haemophilus influenzae (H. influenzae)*.

Petitioner Merck Ex-1004, 0755

[0040] The saccharides on bacteria can be used to classify the bacteria into a subclass or serotype. More specifically, a serotype can refer to the type of a microorganism determined by its constituent antigens. The constituent antigens can be different saccharides, such as different capsular polysaccharides or immunogenic fragments thereof. Some non-limiting examples of *S. pneumoniae* serotypes can include, but are not limited to 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 7C, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 19F, 19A, 22F, 23F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35F, 35B, 38, and Serogroup 20 (comprising serotypes 20A and 20B).

[0041] By conjugating a saccharide to a polypeptide, the immune response to the saccharide can be enhanced by inducing a T cell dependent response. A saccharide alone may not stimulate T cells because, for example, a saccharide may not be loaded onto the Major Histocompatibility Complex (MHC) of antigen presenting cells. However, peptides can be loaded onto MHC. Therefore, by conjugating a saccharide to a polypeptide, the saccharide-polypeptide conjugate can then be loaded onto the MHC, recognized by the T cell, and stimulate a T cell response. T cells can then stimulate a more vigorous immune response and also promote a more rapid and long-lasting immunologic memory. Provided herein are saccharide-polypeptide conjugates that can be used to induce an immune response, which can be referred to as immunogenic saccharide-polypeptide conjugates. In some instances, the immunogenic saccharide-polypeptide conjugates can provide immune protection against a saccharide or organism containing the saccharides. In other instances, the immunogenic saccharide-polypeptide conjugates can be part of a vaccine.

Compositions of Saccharide-Polypeptide Conjugate

[0042] An immunogenic saccharide-polypeptide conjugate composition can be a saccharide conjugated to a polypeptide. An immunogenic saccharide-polypeptide conjugate composition can be a capsular polysaccharide conjugated to a polypeptide. An immunogenic saccharide-polypeptide conjugate composition can be a capsular polysaccharide or fragment of the capsular polysaccharide conjugated to a polypeptide. An immunogenic saccharide-polypeptide conjugate can comprise a saccharide antigen coupled to one or more polypeptides. An immunogenic saccharide-polypeptide conjugate can comprise one unique saccharide antigen coupled a polypeptide. An immunogenic saccharide-polypeptide conjugate can comprise two or more unique saccharide antigens coupled a polypeptide. A saccharide antigen can elicit an immune response.

Petitioner Merck Ex-1004, 0756

[0043] A saccharide can be from any bacteria and fungi that can incorporate saccharides into their surface structure. For example, saccharides can be from *S. pneumoniae*. Further examples include saccharides from *N. meningitidis*, *S. enterica*, or *H. influenzae*. A saccharide can be a full-length, native form polysaccharide, oligosaccharide, or fragments thereof. A saccharide can be in any form that induces an immune response. A saccharide can be a capsular polysaccharide or fragment thereof. A saccharide can be from a subclass or serotype. Some non-limiting examples of saccharides from a *S. pneumoniae* serotype include, but are not limited to 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 7C, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 19F, 19A, 22F, 23F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35F, 35B, 38, and Serogroup 20. Serogroup 20 can comprise serotype 20A and serotype 20B. *S. pneumoniae* that can produce serotype 20A polysaccharide can be identified by a mutated *whaF* allele as compared to the intact *whaF* allele of *S. pneumoniae* that can produce serotype 20B polysaccharide. **TABLE 1** depicts exemplary repeat unit polysaccharide structures of various *S. pneumoniae* serotypes. **FIGS. 10A, 10B, 10C, 10D**, **10E**, and **10F** depict annotated the capsular polysaccharide (*cps*) locus for various *S. pneumoniae* serotypes (Bentley *et al.* Genetic Analysis of the Capsular Biosynthetic Locus from all 90 Pneumococcal Serotypes. *PloS Genet* 2(3): 31 (2006)).

Petitioner Merck Ex-1004, 0757

TABLE 1. Exemplary *S. pneumoniae* Polysaccharide Serotypes

Serotype	Polysaccharide Structure (Repeat Unit)
1	→3)-α-Sug*p*-(1→4)-α-D-Gal*p*A-(1→3)-α-D-Gal*p*A-(1→]$_n$ Sug*p*: 2-acetamido-2,6-dideoxy-D-*xylo*-hexos-4-ulose
2	→4)-β-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-β-L-Rha*p*-(1→]$_n$ 2 ↓ 1 α-D-Glc*p*A-(1→6)-α-D-Glc*p*
3	→3)-β-D-GlcA*p*-(1→4)-β-D-Glc*p*-(1→]$_n$
4	→3)-β-D-Man*p*NAc-(1→3)-α-L-Fuc*p*NAc-(1→3)-α-D-Gal*p*NAc-(1→4)-α-Gal*p*2,3(S)Pyr-(1→]$_n$
5	→4)-β-D-Glc*p*-(1→4)- α -L-Fuc*p*NAc-(1→3)-β-D-Sug*p*-(1→]$_n$ 3 ↑ 1 α-L-Pne*p*NAc-(1-2)-β-D-Glc*p*A L-PneNAc: 2-acetamido-2,6-dideoxy-l-talose (pneumosamine) Sug*p*: 2-acetamido-2,6-dideoxy-D-*xylo*-hexos-4-ulose
6A	→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→3)-D-Ribitol-(5→P→]$_n$
6B	→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→4)-D-Ribitol-(5→P→]$_n$
6C	→2)-α-D-Glc*p*-(1→3)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→3)-D-Ribitol-(5→P→]$_n$

Petitioner Merck Ex-1004, 0758

Serotype	Polysaccharide Structure (Repeat Unit)
7F	→6)-α-D-Gal*p*-(1→3)-β-L-Rha*p*2Ac-(1→4)-β-D-Glc*p*-(1→3)-β-D-Gal*p*NAc-(1→ 4 ↑ 1 β-D-Gal*p* α-D-Gal*p*NAc-(1→2)-α-L-Rha*p*
7C	→6)-α-D-Glc*p*NAc-(1→2)-α-L-Rha*p*-(1→2)-β-L-Rha*p*-(1→4)-β-D-Glc*p*-(1→4)-α-D-Gal*p*NAc-(1→ 3 ↑ 1 β-D-Rib*f*-(1→4)-α-L-Rha*p*
8	→4)-β-D-Glc*p*-(1→4)-α-D-Gal*p*-(1→4)-α-D-GlcA*p*-(1→4)-β-D-Glc*p*-(1→]$_n$
9N	→4)-α-D-Glc*p*NAc-(1→4)-α-D-Glc*p*A-(1→3)-α-D-Glc*p*-(1→3)-β-D-Man*p*NAc-(1→4)-β-D-Glc*p*-(1→]$_n$
9V	→4)-α-D-Glc*p*A-(1→3)-α-D-Glc*p*-(1→3)-β-D-Man*p*NAc-(1→4)-β-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→]$_n$ ↑ ↑ 2Ac (17%) 4Ac (6%) 2Ac (3%) 3Ac (25%) 6Ac (55%) 3Ac (4%)
10A	Galβ1 ↓ 6 →4)-GalNAc-β-(1→3)-Gal-α-(1→2)-ribitol-5-PO4$^-$-5Gal*f*β-(1→3)-Galβ-(1→]$_n$ 3 ↑ Gal*f*β1

Petitioner Merck Ex-1004, 0759

Serotype	Polysaccharide Structure (Repeat Unit)
11A	
12F	
14	
15A	

Petitioner Merck Ex-1004, 0760

Serotype	Polysaccharide Structure (Repeat Unit)
15B	→6)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→]$_n$ 4 ↑ 1 α -D-Gal*p*-(1→2)-β-D-Gal*p*-3-PO_4—R 80% R = H 20% R = choline Note: On average 70% of the repeating units is O acetylated. The site of the O acetylation is unknown (Jansson *et al.* Carbohydr. Res. (1987) 162:111-116), although Venkateswaran *et al.* Infect. Dis. (1983) 147:1041-1054 suggested that it is linked to a galactose moiety.
15C	→6)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→]$_n$ 4 ↑ 1 α-D-Gal*p*-(1→2)-β-D-Gal*p*-3-PO_4—R 80% R = H 20% R = choline
17F	OAc ↓ 2 -[3)-L-Rha*p*-β-(1→4)-D-Glc*p*-β-(1→3)-D-Gal*p*-α-(1→3)-L-Rha*p*-β-(1→4)-L-Rha*p*-α-(1→2)-D-Arabinitol-1-PO_4→]$_n$ 4 ↑ 4 β-D-Gal*p* and

Petitioner Merck Ex-1004, 0761

Serotype	Polysaccharide Structure (Repeat Unit)
	OAc ↓ 2 -[3)-L-Rha*p*-β-(1→4)-D-Glc*p*-β-(1→3)-D-Gal*p*-α-(1→3)-L-Rha*p*-β-(1→4)-L-Rha*p*-α-(1→2)-D-Arabinitol-1-PO$_4$→]$_n$ 1 ↑ 4 β-D-Gal*p*
18C	α-D-Glc*p* 1 ↓ 2 -[4)-β-D-Glc*p*-(1→4)-β-D-Gal*p*-(1→4)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→]n 3 ↑ PO4-1-glycerol
19F	→2)-α-L-Rha-(1→5-PO4$^-$-5→4)-β-D-ManNAc-(1→4)-β-D-Glc-(1→]$_n$
19A	→3)-α-L-Rha-(1→5-PO$_4^-$-5→4)-β-D-ManNAc-(1→4)-β-D-Glc-(1→]$_n$

Petitioner Merck Ex-1004, 0762

Serotype	Polysaccharide Structure (Repeat Unit)
20A	
20B	
22F	

Petitioner Merck Ex-1004, 0763

Serotype	Polysaccharide Structure (Repeat Unit)
23F	α-L-Rha*p* 1 ↓ 2 →4)-β-D-Glc*p*-(1→4)-β-D-Gal*p*-(1→4)-β-L-Rha*p*-(1→]$_n$ 3 ↑ PO_4-1-glycerol
23A	α-L-Rha*p* 1 ↓ 2 PO_4-1-glycerol-(2→P→3)-β-D-Gal*p*-(1 ↓ 4 →4)-β-D-Glc*p*-(1→3)-β-L-Rha*p*-(1→]$_n$
23B	→4)-β-D-Glc*p*-(1→4)-β-D-Gal*p*-(1→4)-β-L-Rha*p*-(1→]$_n$ 3 ↑ PO_4-1-glycerol
31	→2)-β-L-Rha*p*-(1→3)-β-D-Gal*f*-(1→3)-β-Rha*p*-(1→4)-β-D-Glc*p*A-(1→3)-β-D-Gal*f*-(1→]$_n$
33F	→3)-β-D-Gal*p*-(1→3)-α-D-Gal*p*-(1→3)– β-D-Gal*f*-(1→3)-β-D-Glc*p*-(1→5)-β-D-Gal*f*-(1→]$_n$ 2 2 ↑ ↑ 1 $OAc_{0.4}$ α-D-Gal*p*

Petitioner Merck Ex-1004, 0764

Serotype	Polysaccharide Structure (Repeat Unit)
33B	Ac 2 ↓ 5)-β-D-Gal*f*-(1→3)-β-D-Gal*p*-NAc-(1→3)-α-D-Gal*p*-(1→4)-Ribitol-5-P→2)- α-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→]$_n$ 2 ↑ 1 α-D-Gal*p*
34	→6)-Gal-β-(1→3)-Gal-α-(1→2)-Rib-5-PO_4^--6-Gal*f*-β-(1→3)-Gal-β-1→]$_n$ ↓ ↓ 2OAc 3,5OAc
34	→3)-β-D-Gal*f*-(1→3)-α-D-Glc*p*-(1→2)-β-D-Gal*p*6$Ac_{0.5}$-(1→3)-α-D-Gal*p*-(1→2)-D-Rib-ol-(5→*P*→
35F	→6)-Gal*f*-β-(1→3)-Gal-α-(1→2)-Rib-5-PO_4^--3-Gal*f*-β-(1→3)-Gal-β-1→]$_n$ ↓ 2OAc
35B	$OAc_{0.7}$ ↓ 2 →4)-β-D-Gal*p*NAc-(1→6)-β-D-Gal*f*-(1→3)-β-D-Glc*p*-(1→6)-β-D-Gal*f*-(1→1)-Rib-(5-PO_4→]$_n$

Petitioner Merck Ex-1004, 0765

[0044] Saccharides can be isolated from bacteria. Saccharides can be isolated from bacteria and then conjugated to a polypeptide to form a saccharide-polypeptide conjugate. Any method for isolating the unconjugated polysaccharide can be used. For example, a polysaccharide serotype can be grown in a soy-based medium. The individual polysaccharides can then be purified by steps including centrifugation, precipitation, and ultra-filtration. *See* U.S. Patent Application Publication No. 2008/028638 and U.S. Pat. No. 5,847,112. Other processes for purifying polysaccharides are described in U.S. Pat. No. 4,686,102; 4,242,501; 4221,906; 5623,057; and 5,847,112. Alternatively or additionally, polysaccharides may be isolated simultaneously from more than one serotype, which can produce a mixture of polysaccharide serotypes with the desired polysaccharide already combined. Furthermore, these isolated polysaccharides can be sized by microfluidization. The polysaccharides can be sized in order to reduce viscosity in polysaccharide samples and/or to improve filterability for conjugated sample. A saccharide-polypeptide conjugate can include a saccharide or a fragment thereof which is at least partially embedded in one or more polypeptides. The one or more polypeptides can have one or more crosslinks. The at least partially embedded saccharide or fragment thereof can, but need not be, covalently bound to at least one of the polypeptides when at least partially embedded therein.

[0045] Any of the *S. pneumoniae* strains for isolating and purifying the polysaccharides as described herein can be provided by either the CDC, United States or CCUG, Gothenburg. Alternatively, any of the polysaccharides described herein can be isolated and purified from any source (e.g., any laboratory) in which serotyping or other genetic sequencing is used to identify the unique strain from which the polysaccharides are isolated and purified. A vial of a strain of *S. pneumoniae* can be seeded and then can be grown overnight on an agar plate. A single colony can be picked from this plate and streaked onto a new agar plate for overnight growth. This process can be repeated three times to purify the strain before a flask can be seeded and incubated. After allowing the seeded flask sufficient time for growth, the flask contents can be harvested by centrifugation. The centrifugation product can be resuspended in a medium of about 20% glycerol and then can be dispensed into cryogenic vials. The cell bank can be contained in the cryogenic vials, which can be stored at -70°C until use. The cell bank of the strain can then be used for isolation and purification of the polysaccharides of that strain using a fermentation process.

[0046] Polysaccharides of a *S. pneumoniae* strain can be isolated and purified from a cell bank of a strain. The strain can be provided by either the CDC, United States or CCUG, Gothenburg, and then can be used to produce a cell bank as described above. Alternatively,

Petitioner Merck Ex-1004, 0766

strain can be provided by any source (e.g., any laboratory) in which serotyping or other genetic sequencing is used to identify the serotype of the strain. A vial of a cell bank can be produced as described above is used to inoculate a flask, which is allowed to grow overnight. This culture is used to seed a new culture for fermentation in a fermenter. The culture is allowed to grow under the appropriate conditions for that strain. After fermentation, the cells are inactivated and then are treated with deoxycholate to break open the cells and release the capsular polysaccharide. Subsequently, debris is removed by centrifugation, which is followed by a depth filter step through a 0.45 μm filter and the cell free broth is collected. The product is then purified and the polysaccharide size is determined. Quality control tests are performed on the product, such as a purity assay and confirmation of strain inactivation (no growth after plating). Any polysaccharides described herein can be isolated and purified using this method.

[0047] The saccharides or polysaccharides of the invention are depolymerized (sized) to a final range from 100-500 kDa.

[0048] Saccharides that can be used in the saccharide-polypeptide conjugate can also be obtained from commercial sources. For example, individual polysaccharide serotypes can be obtained in a powder form. These polysaccharides can be dissolved in water and incubated with a salt to dissociate residual impurities, which can then be removed by a filtration step. The purified polysaccharides serotypes can then be conjugated to a polypeptide.

[0049] A polypeptide of an immunogenic saccharide-polypeptide conjugate can be an oligopeptide. A polypeptide of can be a carrier protein. A polypeptide can be any polypeptide that allows for conjugation or coupling of saccharide and results in the display of the conjugate or coupling in a way that induces a protective immune response against the saccharide. A polypeptide can be any polypeptide that allows for conjugation or coupling of a capsular polysaccharide or fragment of the capsular polysaccharide and can result in the display of the conjugate or coupling in a way that can induce a protective immune response against the capsular polysaccharide or fragment of the capsular polysaccharide. A polypeptide can be CRM_{197}, tetanus toxoid, a diphtheria toxoid, a cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, a non-typable *H. influenzae* P4 protein, a non-typable *H. influenzae* P6 protein, *M catarrhalis* uspA, a keyhole limpet haemocyanin (KLH), OMPC from *N. meningitidis*, the purified protein derivative of tuberculin(PPD), protein D from *H. influenzae*, PspA, or any fragment thereof. The polypeptide can be a polypeptide in which the

Petitioner Merck Ex-1004, 0767

toxin activity of the polypeptide can be at least partially mitigated. Examples of suitable polypeptides for use as in a saccharide-polypeptide conjugate are described in U.S. patent 8,808,707 and can include: polylysine, polyglutamic acid, polyaspartic acid, co-polymers thereof, and mixed polymers of these amino acids and others, *e.g.*, serines, threonines, or tyrosines, to confer desirable solubility properties on the resultant loaded carrier and conjugate. A polypeptide can be a CRM_{197} with 100% sequence homology with GADDVVDSSKSFVMENFSSYHGTKPGYVDSIQKGIQKPKSGTQGNYDDDWKEFYST DNKYDAAGYSVDNENPLSGKAGGVVKVTYPGLTKVLALKVDNAETIKKELGLSLTE PLMEQVGTEEFIKRFGDGASRVVLSLPFAEGSSSVEYINNWEQAKALSVELEINFETR GKRGQDAMYEYMAQACAGNRVRRSVGSSLSCINLDWDVIRDKTKTKIESLKEHGPI KNKMSESPNKTVSEEKAKQYLEEFHQTALEHPELSELKTVTGTNPVFAGANYAAWA VNVAQVIDSETADNLEKTTAALSILPGIGSVMGIADGAVHHNTEEIVAQSIALSSLMV AQAIPLVGELVDIGFAAYNFVESIINLFQVVHNSYNRPAYSPGHKTQPFLHDGYAVS WNTVEDSIIRTGFQGESGHDIKITAENTPLPIAGVLLPTIPGKLDVNKSKTHISVNGRKI RMRCRAIDGDVTFCRPKSPVYVGNGVHANLHVAFHRSSSEKIHSNEISSDSIGVLGYQ KTVDHTKVNSKLSLFFEIKS (SEQ ID NO: 1). A polypeptide can be a CRM_{197} with at least 80%, 90%, 95%, or 98% sequence homology with SEQ ID NO: 1. A polypeptide can be a modified CRM_{197} as disclosed in US Patent No. 9,359,400. A polypeptide can be CRM_{197} with non-natural amino acid substitutions as disclosed in US Patent No.9,764,039.

[0050] Moreover, suitable polypeptide carriers can include bacterial toxins, toxoids, or inactivated toxin. A toxoid can be a bacterial toxin whose toxicity has been weakened or suppressed while other properties, typically immunogenicity, are maintained. As a class, bacterial toxins and derivatives thereof can to be highly immunogenic. Polypeptides derived from bacterial toxins can be effective at eliciting an immune response to saccharides of the conjugate. Steps can be taken (*e.g.*, by chemical and/or genetic means) to render the toxins non-toxic and safe for administration to mammals. Examples of such bacterial toxin-derived polypeptides which may currently commonly used in vaccine compositions, and can be used in the immunogenic saccharide-polypeptide conjugate described herein, include the diphtheria and tetanus toxoids, and variants thereof (*e.g.*, DT, CRM_{197},TT), cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, non-typable *H. influenzae* P4 protein, non-typable *H. influenzae* P6 protein, *M catarrhalis* uspA, keyhole limpet haemocyanin (KLH), OMPC from *N. meningitidis*, the purified protein derivative of tuberculin(PPD), protein D from *H.*

Petitioner Merck Ex-1004, 0768

influenzae, PspA, or any fragment thereof. Polypeptide fragments can be used to elicit a protective immune response against the capsular polysaccharide or fragment of the capsular polysaccharide of the immunogenic saccharide-polypeptide conjugate. These fragments can encompass T-helper epitopes.

[0051] CRM_{197} is a non-toxic mutant of diphtheria toxin with a single amino acid substitution of glutamic acid for glycine at position 52, with the amino acid sequence of GADDVVDSSKSFVMENFSSYHGTKPGYVDSIQKGIQKPKSGTQGNYDDDWKEFYST DNKYDAAGYSVDNENPLSGKAGGVVKVTYPGLTKVLALKVDNAETIKKELGLSLTE PLMEQVGTEEFIKRFGDGASRVVLSLPFAEGSSSVEYINNWEQAKALSVELEINFETR GKRGQDAMYEYMAQACAGNRVRRSVGSSLSCINLDWDVIRDKTKTKIESLKEHGPI KNKMSESPNKTVSEEKAKQYLEEFHQTALEHPELSELKTVTGTNPVFAGANYAAWA VNVAQVIDSETADNLEKTTAALSILPGIGSVMGIADGAVHHNTEEIVAQSIALSSLMV AQAIPLVGELVDIGFAAYNFVESIINLFQVVHNSYNRPAYSPGHKTQPFLHDGYAVS WNTVEDSIIRTGFQGESGHDIKITAENTPLPIAGVLLPTIPGKLDVNKSKTHISVNGRKI RMRCRAIDGDVTFCRPKSPVYVGNGVHANLHVAFHRSSSEKIHSNEISSDSIGVLGYQ KTVDHTKVNSKLSLFFEIKS (SEQ ID NO: 1). CRM_{197} can be from 58-59 kDa in size. CRM_{197} can be 58.4 kDa in size. CRM_{197} can be produced, isolated, and purified from a cell bank. A vial from a cell bank of CRM_{197} can be used to seed a new flask and allowed to ferment. The fermentation culture can be centrifuged and the contents can be harvested, clarified, and filtered using a 0.2 μm filter. The filtrate can then be concentrated and diafiltered. The CRM_{197} can be further purified by filtration using microtubes or membranes of the appropriate pore size. Then anion exchange chromatography can be used in the isolation and purification of the CRM_{197}, which can be followed by ammonium sulfate precipitation, and another filtration using a 0.45 μm filter. CRM_{197} can be further isolated and purified from this product by hydrophobic interaction chromatography, after which CRM_{197} can be concentrated and diafiltered into the desired buffer. A final step can be filtration with 0.2 μm filter. The isolated and purified CRM_{197} can be stored at ≤ -70°C until use in conjugation with a saccharide or polysaccharide. Quality control tests can be performed on the isolated and purified CRM_{197}.

[0052] A polypeptide can be conjugated to an immunogenic serotype 1 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 2 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 3 capsular polysaccharide, fragment thereof, or

Petitioner Merck Ex-1004, 0769

combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 4 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 5 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 6A capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 6B capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 6C capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 7F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 7C capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 8 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 9N capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 9V capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 10A capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 11A capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 12F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 14 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 15A capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 15B capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 15C capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 16F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 17F capsular polysaccharide,

Petitioner Merck Ex-1004, 0770

fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 18C capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 19A capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 19F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 20A capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 20B capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 22F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 23A capsular polysaccharide or fragment thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 23B capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 23F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 24F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 24B capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 31 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 33F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 33B capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 34 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 35F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 35B capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. A polypeptide can be conjugated to an immunogenic serotype 38 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*.

Petitioner Merck Ex-1004, 0771

[0053] In some embodiments, CRM_{197} can be conjugated to an immunogenic serotype 1 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 2 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 3 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 4 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 5 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 6A capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 6B capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 6C capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 7F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 7C capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*.CRM_{197} can be conjugated to an immunogenic serotype 8 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 9N capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 9V capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 10A capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 11A capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 12F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 14 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 15A capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 15B capsular polysaccharide, fragment thereof, or combination thereof isolated from *S.*

Petitioner Merck Ex-1004, 0772

pneumoniae. CRM_{197} can be conjugated to an immunogenic serotype 15C capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 16F capsular polysaccharide, fragment thereof, or combination thereof from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 17F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 18C capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 19A capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 19F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 20A capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 20B capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 22F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 23A capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 23B capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 23F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 24F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 24B capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 31 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 33F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 33B capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 34 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 35F capsular polysaccharide, fragment thereof, or combination thereof isolated from *S.*

Petitioner Merck Ex-1004, 0773

pneumoniae. CRM_{197} can be conjugated to an immunogenic serotype 35B capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*. CRM_{197} can be conjugated to an immunogenic serotype 38 capsular polysaccharide, fragment thereof, or combination thereof isolated from *S. pneumoniae*.

[0054] In further embodiments, any of the saccharides as described herein can be conjugated to any polypeptides described herein. Any of the immunogenic saccharide-polypeptide conjugates described herein can be isolated and purified.

Methods of Conjugating a Saccharide and Polypeptide to Produce a Saccharide-Polypeptide Conjugate

[0055] The saccharides can be conjugated to the polypeptide by, *e.g.*, chemical or biological synthesis. A saccharide can be a polysaccharide, a capsular polysaccharide, or an immunogenic fragment thereof. A capsular polysaccharide or fragment of the capsular polysaccharide can be conjugated to the polypeptide by chemical or biological synthesis.

[0056] For example, the capsular polysaccharide or immunogenic fragment thereof can be chemically activated and then reacted with the polypeptide. For example, capsular polysaccharides or fragments of the capsular polysaccharides can be reacted with either ammonia or diaminoethane to generate a free terminal amino group. The amino group can then be reacted with an excess of disuccinimidyl ester of adipic acid, which can introduce an active succininmidyl ester group. This can then be reacted to a free amino group on the polypeptide to form covalent amide bonds.

[0057] A polysaccharide and polypeptide can be conjugated using 1-cyano-dimethylaminopyridinium tetrafluoroborate (CDAP) chemistry. CDAP can be used to activate a broad range of acidic, neutral, and other classes of polysaccharides, which can then be derivatized with diamines, dihydrazides or other functional groups in preparation for coupling to proteins. In CDAP, the cynanylating reagent 1-cyano-dimethylaminopyridinium tetrafluoroborate can be used for the synthesis of polysaccharide-protein conjugates. The cyanilation reaction can be performed under relatively mild conditions, which can avoid hydrolysis of the alkaline sensitive polysaccharides. This synthesis can allow for direct coupling to a carrier. The polysaccharide can be solubilized in water or a saline solution. CDAP can be dissolved in acetonitrile and can be added immediately to the polysaccharide solution. The CDAP can reacts with the hydroxyl groups of the polysaccharide to form a cyanate ester. This cyanate ester can be highly reactive, which can result in an activated polysaccharide. This can be done at a pH of 9–10. After this activation step, the polypeptide

Petitioner Merck Ex-1004, 0774

can be added. Amino groups of lysine of a polypeptide can react with the activated polysaccharide to form an isourea covalent link. This conjugation step can occur under mildly alkaline conditions, such as a pH of 7–9. In some cases, there can be a strong pH dependence on the epsilon amines of lysine to form a stable O-alkyl-isourea linkage. After the coupling reaction, a large excess of an amino-containing reagent such as glycine can then be added to quench the residual activated functional groups. The CDAP activation can take only a few minutes and conjugation can be completed in a few hours. The conjugate can then be passed through a gel permeation column to remove unreacted polypeptide and residual reagents.

[0058] In some embodiments, CDAP can be made up at 100 mg/ml in acetonitrile and stored at -20°C for up to 1 month. CDAP can be slowly pipetted into a vortexed solution of polysaccharide in water (rapid addition of the organic co-solvent precipitates the polysaccharide) and 20 s later a volume of aqueous 0.2M triethylamine (TEA) equal to volume of CDAP used can be added. At 2.5 min, a large molar excess of a 0.5 M hexanediamine solution in either 0.1 M sodium borate at pH 9.3 or 0.75 M HEPES at pH 7.5, can be added. The reaction mixture can be allowed to stand overnight at 4°C, can be desalted on either a P-6 DG or a P-6 cartridge, can be equilibrated with saline and then can be further dialyzed into saline. The extent of derivatization with hexanediamine can be determined using a TNBS assay for primary amines; absorbance can be measured at 366nm, using an extinction coefficient of 11000 M^{-1}. Polysaccharide concentrations can be determined as described by Monsigny et al., using the corresponding polysaccharide as the standard.

[0059] In some embodiments, polysaccharide can be solubilized in saline at 10 mg/ml. At t = 0, CDAP (100 mg/ml in acetonitrile) can be added (1 mg CDAP/ mg polysaccharide) while vortexing. At 30 s, 0.2 M TEA can be added (10 µl/mg polysaccharide) to raise the pH. At 2.5 min the pH can be readjusted to the coupling pH and the amine or hydrazide reagent or the polypeptide can be added, in the appropriate buffer. After an overnight reaction, the solution can be dialyzed and/or desalted on a P6DG column. Polysaccharide polypeptide conjugates can be fractionated on an S400HR column, which can be equilibrated with either saline or PBS. HPLC can be performed on HPLC size exclusion columns using a Waters 626 pump, monitored at 280 nm and with a refractive index detector. Samples can be filtered before chromatography using a Millex Ultrafree MC device.

[0060] In some embodiments, for low pH coupling, amines on polypeptide can be functionalized with hydrazides using the two-step method as follows. Five mg of polypeptide at 12.5 mg/ml in 0.19 M HEPES, 2.5 mM EDTA, pH 7.3 can be iodoacetylated by the addition of a 15-fold molar excess of SIA over moles of polypeptide. After 2 h reaction in the

Petitioner Merck Ex-1004, 0775

dark, the solution can be desalted on a 1 x 15 cm P6DG column, can be equilibrated with 2 mM MES, 0.2 M NaCl, pH 5.9 and the void volume fractions, which can be concentrated with a Centricon 50 device. DTPH (30 µl) at 0.1 M in water, can be reduced to 3-mercapto-propionyl hydrazide by addition of 25 µl 0.1 M TCEP in water, and can then be added to the iodoacetylated polypeptide with a pH that can be adjusted to about 5. After a 2 h reaction, reagent can be removed by desalting again and concentrating with a Centricon 50 device to 10 mg/ml. TNBS can be used to determined number of hydrazide per moles of polypeptide. To couple the hydrazide-derivatized polypeptide, 3 mg of polysaccharide at 10 mg/ml in saline can be activated by the addition of 15 µl CDAP at 100 mg/ml in acetonitrile, followed 30 s later by 30 µl of 0.2 M TEA. At 2.5 min, the pH can be reduced to 5.5 by the addition of 100 µl 1 M MES, pH 5.5 and the activated polysaccharide can be added to 3.8 mg of the polypeptide-hydrazide. After an overnight reaction at 4°C, the reaction can be quenched by the addition of 50 µl 1 M ethanolamine. The solution can be applied to an S400HR column, which can be equilibrated with PBS, and the void volume fractions can be pooled. Sterile filtration can be done by passage through a Millex GB 0.2 µm filter.

[0061] In some embodiments, CDAP can be used to conjugate a polysaccharide and CRM_{197}. The polysaccharide (10 mg) can be dissolved in 1.0 mL of 0.1M sodium borate buffer pH 9.0 and can be activated by addition of 50 µl of a solution of CDAP dissolved in acetone:water (6:4). Immediately, the pH can be adjusted and maintained at 9.0 by continuous dropwise addition of a 0.1 M NaOH solution. The mixture can be stirred for 5 min and then a volume of 1.4 mL of 0.05 M HCl can be added, stirred for a while and then left to rest during 1 ½ min more. The mixture can be rapidly passed through a PD-10 column equilibrated in 0.1 M sodium bicarbonate buffer pH 8.3. An aliquot of this can be used to determine cyanate ester formation and the rest of the preparation can be immediately used for coupling purposes. Polypeptide (15 mg) can be dissolved in 1.0 mL of 0.1 M $NaHCO_3$ buffer pH 8.3 and added to the activated polysaccharide solution. The mixture can be gently stirred during 8 h at room temperature. A volume of 1.0 mL 0.1 M Tris-HCl buffer pH 8.6 can be added to block any remaining activation sites on the unreacted polysaccharide and incubated during 1 h more. The final solution can be lyophilized and stored until used for the purification procedure. The progress of the conjugation can be analyzed by high-performance size exclusion chromatography (HPSEC) using a Zorbax GF-250 column and monitored at 280 nm and 210 nm. For example, aliquots of the reaction mixture can be taken at 10 min and at every hour up to 4 h. The column can be equilibrated in 0.025 M phosphate buffer pH 7.0 supplemented with 0.13 M NaCl and be calibrated with the following proteins: thyroglobulin(669 kDa),

Petitioner Merck Ex-1004, 0776

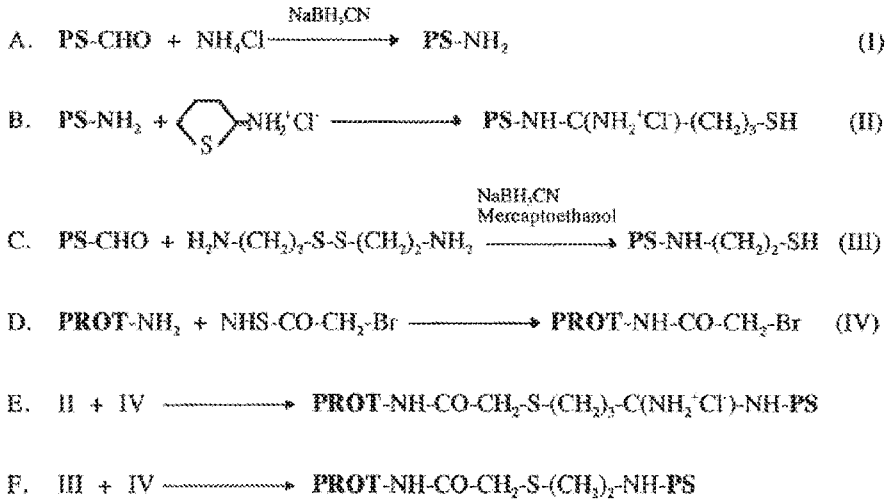
ferritin (440 kDa), catalase (232 kDa), aldolase (158 kDa), BSA (67 kDa), ovalbumin (43 kDa), and ribonuclease (13.7 kDa). The void volume can be determined with Blue dextran. Samples (10 µl aliquots) can be injected into the column and a flow rate of 0.5 mL/min can be used. Prior and after the analysis the column can be washed with the equilibration buffer. The conjugation reaction progress (%) can be measured by comparing the conjugate peak area related to the free protein peak area using detection at wavelength 280 nm according to the following equation: (conjugate peak area/(conjugate peak area + free protein peak area)) x 100.

[0062] In some embodiments, the polysaccharide can be activated via CDAP chemistry in a CDAP solution. The polysaccharide can be mixed with CDAP solution, which can then be incubated at the appropriate temperature for polysaccharide activation. A calculated amount of CRM_{197} can be added to the activated polysaccharides. The reaction can then be quenched using glycine or any other suitable quenching reagent. The conjugation reaction mixture can be filtered, diafiltered, and concentrated for the polysaccharide-CRM_{197} conjugates. The polysaccharide-CRM_{197} conjugates can then be purified by gel filtration chromatography. The fractions containing the polysaccharide-CRM_{197} conjugates can be pooled, concentrated, and then filtered through a 0.2 µm filter. The product can be stored at 2-8°C.

[0063] As another example, the capsular polysaccharide or immunogenic fragment thereof can activated by periodate oxidation. Sodium periodate oxidizes diols (two adjacent carbons with hydroxyl groups) can be converted into aldehydes (C=O) and in the process breaks C-C bonds. Thus, depending upon the structure, periodate activation can fragment a capsular polysaccharide or immunogenic fragment thereof and open the ring structures of sugars. When the diol is within a ring, the ring sugar can be opened possibly altering the polysaccharide conformation. When the diol is in a glycerol or ribitol side chain, the side chain can disappear. The reactive aldehyde groups can then condense with free amino groups on the polypeptide in the presence of sodium cyanoborohydride to form a stable secondary amine. Condensation of the aldehyde with the epsilon amino group on lysine can be a slow process and can take a few days. The remaining aldehyde groups can then be quenched using sodium borohydride, which can convert them back to hydroxyls.

[0064] Another example of coupling a polypeptide to a polysaccharide is based on thioether bonding of polysaccharides activated with 2-iminothiolane (2-IT), or alternatively, with cystamine, to bromoacetylated polypeptides such as tetanus toxoid.

Petitioner Merck Ex-1004, 0777



Schematic I

[0065] The above Schematic I represents end-point coupling of polysaccharides (PS) to proteins (PROT) through thioether bond. (A) Amination of polysaccharide through reducing terminal using ammonium chloride in the presence of cyanoborohydride ion. (B) Thiolation of aminated polysaccharide (I) with 2-iminothiolane (2-IT). (C) Alternative thiolation of polysaccharide through reductive amination with cystamine with concomitant cleavage of disulfide bond. (D) Bromoacetylation of amino groups of protein using N-hydroxysuccimidyl bromoacetate. (E) Coupling of polysaccharide derivatized with 2-IT (II) to bromoacetylated protein (IV). (F) Alternative coupling of polysaccharide derivatized with cystamine (III) to bromocacetylated protein (IV).

[0066] As another example, the immunogenic saccharide-polypeptide conjugate can be produced using bacteria, such as *Escherichia coli* (*E. coli*). The immunogenic saccharide-polypeptide can comprise the capsular polysaccharide at least partially embedded in the polypeptide. The immunogenic saccharide-polypeptide can comprise the capsular polysaccharide chemically cross-linked to the polypeptide. The immunogenic saccharide-polypeptide can comprise the capsular polysaccharide at least partially chemically cross-linked to the polypeptide. A capsular polysaccharide or fragment of the capsular polysaccharide of a serotype from *S. pneumoniae* can be conjugated to a polypeptide by chemical or biological synthesis. One saccharide can be conjugated to one polypeptide. Two or more saccharides can be conjugated to one polypeptide. The saccharides can be the same serotype. The saccharides can be different serotypes. One capsular polysaccharide or immunogenic fragment thereof can be conjugated to one polypeptide. Two or more capsular polysaccharides or fragments thereof can be conjugated to one polypeptide. The capsular

Petitioner Merck Ex-1004, 0778

polysaccharides or fragments thereof can be the same serotype. The capsular polysaccharides or fragments thereof can be different serotypes.

[0067] After conjugation, the polysaccharide polypeptide conjugate can be purified. For example, different chromatographic strategies can be used to purify a polysaccharide-polypeptide conjugate: SEC (using a column packed with Sepharose CL-6B), a combination of ion-exchange chromatography (DEAE-Sepharose) can be followed by HPSEC (Sepharose CL-6B and hydrophobic interaction chromatography (HIC) (column packed with Sephadex LH-20). For HPSEC purification on a Sepharose CL-6B column, an amount of 4.0 mg of the lyophilized mixture containing the polysaccharide polypeptide conjugate, free polysaccharide and free polypeptide, can be dissolved in 0.1 M formic acid buffer (ammonium salt) pH 6.0 and can be applied to the column (60 cm x 4.0 cm) equilibrated with the same buffer, at a flow of 0.5 mL/min. Isocratic elution can be performed with the buffer, and the process can be continuously monitored using a differential refractometric detector. Fractions can be collected, can be lyophilized, and can be used for further analysis. For the second strategy, an amount of 4.0 mg of the lyophilized reaction mixture can be dissolved in 500 µL of 0.1 M phosphate-buffered saline (PBS) pH 7.0 and can be applied to the DEAE-Sepharose column, previously equilibrated in the same buffer. The pass-through of the column can be collected and after washing with the buffer, elution can be performed using a gradient (0.25-1.0 M) of NaCl. The presence of the polysaccharide can be analyzed in each fraction by the Phenol sulfuric technique. The eluted fractions can be pooled, can be lyophilized, can be resuspended in 0.1 M ammonium formate buffer pH 6.0, and can be applied to a Sepharose CL-6B column. Fractions of 5 mL can be collected, can be dialyzed, can be lyophilized, and can be analyzed for protein and carbohydrate contents. In a third chromatographic approach, an amount of 4.0 mg of the lyophilized reaction mixture can be dissolved in 1.0 mL of distilled water and applied to a column packed with Sephadex LH-20 previously equilibrated with distilled water, at a flow rate of 0.5 mL/min. Elution can be performed isocratically with distilled water, and the process can be monitored as described above. Fractions can be collected, can be lyophilized, and can be used for further analysis.

[0068] Furthermore, 'click' chemistry can be used to conjugate a saccharide with a polypeptide as disclosed in US Patent No. 8,753,645 and in Hu et al., Towards the next generation of biomedicines by site selective conjugation, *Chem. Soc. Rev.*, 2016, 45, 1691. 'Click' chemistry can be used to conjugate modified CRM_{197} or CRM_{197} with non-natural amino acids to polysaccharides.

Petitioner Merck Ex-1004, 0779

Pharmaceutical Compositions and Vaccines

[0069] The saccharide-polypeptide conjugates described herein can be in the form of a pharmaceutical composition. The saccharide-polypeptide conjugates described herein can be in the form of a pharmaceutical composition in unit dose form. The saccharide-polypeptide conjugates described herein can be in the form of a vaccine. The saccharide-polypeptide conjugates described herein can be in the form of a vaccine in unit dose form. A pharmaceutical composition or vaccine can comprise an immunogenic saccharide-polypeptide conjugate. A pharmaceutical composition or vaccine can comprise an immunogenic capsular polysaccharide-polypeptide conjugate. A pharmaceutical composition or vaccine can be in unit dose form and can comprise an immunogenic capsular polysaccharide-polypeptide conjugate. A pharmaceutical composition or vaccine can comprise an immunogenic saccharide-polypeptide conjugate wherein the capsular polysaccharide or immunogenic fragment thereof of the saccharide-polypeptide conjugate can be one serotype. In another embodiment, a pharmaceutical composition or vaccine can comprise an immunogenic saccharide-polypeptide conjugates wherein the capsular polysaccharides or immunogenic fragments thereof can be from different serotypes, creating a mixture of different immunogenic saccharide-polypeptide conjugates. A pharmaceutical composition or vaccine can comprise an immunogenic saccharide-polypeptide conjugates wherein the polypeptide is the same polypeptide in all the immunogenic saccharide-polypeptide conjugates. In other embodiments, a pharmaceutical composition or vaccine can comprise immunogenic saccharide-polypeptide conjugates wherein the polypeptide(s) in the saccharide-polypeptide conjugates are a mixture of different polypeptide(s).

[0070] For example, a pharmaceutical composition or vaccine can comprise an immunogenic saccharide-polypeptide conjugate comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *S. pneumoniae* selected from the group comprising 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise an immunogenic saccharide-polypeptide conjugate comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *S. pneumoniae* selected from the group comprising 2, 6C, 7C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35F, 35B, 38, and Serogroup 20

Petitioner Merck Ex-1004, 0780

(comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of at least two immunogenic saccharide-polypeptide conjugate comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *S. pneumoniae* selected from the group comprising 2, 6C, 7C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of at least two immunogenic saccharide-polypeptide conjugate comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *S. pneumoniae* selected from the group comprising 2, 6C, 7C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35F, 35B, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of at least eight immunogenic saccharide-polypeptide conjugate comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *S. pneumoniae* selected from the group comprising 2, 6C, 7C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of at least 20 immunogenic saccharide-polypeptide conjugate comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *S. pneumoniae* selected from the group comprising 3, 6C, 7C, 8, 9N, 10A, 11A, 12F, 15A, 16F, 19A, 19F, 22F, 23A, 23B, 31, 33F, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of at least 20 immunogenic saccharide-polypeptide conjugate comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *S. pneumoniae* selected from the group comprising 3, 6C, 7F, 7C, 8, 9N, 11A, 12F, 15A, 16F, 19A, 19F, 22F, 23A, 23B, 31, 33F, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of at least 15 immunogenic saccharide-polypeptide conjugate comprising individually a capsular polysaccharide, fragment thereof,

Petitioner Merck Ex-1004, 0781

or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *S. pneumoniae* selected from the group comprising 3, 6C, 7F, 7C, 8, 9N, 10A, 11A, 12F, 15A, 16F, 19A, 19F, 22F, 23A, 23B, 31, 33F, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of at least 16 immunogenic saccharide-polypeptide conjugate comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *S. pneumoniae* selected from the group comprising 3, 6C, 7F, 7C, 8, 9N, 10A, 11A, 12F, 15A, 16F, 19A, 19F, 22F, 23A, 23B, 31, 33F, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of at least 20 immunogenic saccharide-polypeptide conjugate comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *S. pneumoniae* selected from the group comprising 3, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 16F, 19A, 19F, 22F, 23A, 23B, 31, 33F, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of at least 20 immunogenic saccharide-polypeptide conjugate comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *S. pneumoniae* selected from the group comprising 3, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 19A, 22F, 23A, 23B, 24F, 33F, 35B, and 38. A pharmaceutical composition or vaccine can comprise a plurality of at least 10 immunogenic saccharide-polypeptide conjugate comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *S. pneumoniae* selected from the group comprising 3, 8, 9N, 12F, 15A, 19A, 22F, 23A, 23B, and 24F. A pharmaceutical composition or vaccine can comprise a plurality of at least 11 immunogenic saccharide-polypeptide conjugate comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *S. pneumoniae* selected from the group comprising 3, 8, 9N, 10A, 12F, 15A, 19A, 22F, 23A, 23B, and 24F. A pharmaceutical composition or vaccine can comprise a plurality of at least 24 immunogenic saccharide-polypeptide conjugate comprising individually a capsular polysaccharide, fragment thereof,

Petitioner Merck Ex-1004, 0782

or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *S. pneumoniae* selected from the group comprising 3, 4, 6C, 7C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 19A, 22F, 23A, 23B, 24F, 31, 33F, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of at least 20 immunogenic saccharide-polypeptide conjugate comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *S. pneumoniae* selected from the group comprising 3, 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 19A, 19F, 22F, 23A, 23B, 31, 33F, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of at least 20, 21, 22, 23, 24, 25, 26, or 27 immunogenic saccharide-polypeptide conjugate comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *S. pneumoniae* selected from the group comprising 3, 6C, 7C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 19A, 19F, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a saccharide-polypeptide conjugate comprising a capsular polysaccharide or immunogenic fragment thereof from a serotype of *S. pneumoniae* conjugated to a polypeptide, and wherein the serotype is not any one of, or not at least any one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F; and the serotype is at least one of 2, 6C, 7C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35F, 35B, 38, and Serogroup 20 (comprising serotypes 20A and 20B); or a combination thereof. A pharmaceutical composition or vaccine can comprise a saccharide-polypeptide conjugate comprising a capsular polysaccharide or immunogenic fragment thereof from a serotype of *S. pneumoniae* conjugated to a polypeptide, and wherein the serotype is not any one of, or not at least any one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F; and the serotype is at least one of 2, 6C, 7C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B); or a combination thereof. A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae*

Petitioner Merck Ex-1004, 0783

serotype selected from a group consisting of at least 1, 2, 3, 4, 5, 6, 7, 8, or 9 serotypes selected from the group consisting of 2, 6C, 7C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of at least 1, 2, 3, 4, 5, 6, 7, 8, or 9 serotypes selected from the group consisting of 2, 6C, 7C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise at least 10, 11, 12, 13, 14, 15, or 16 serotypes selected from the group consisting of 2, 6C, 7C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35F, 35B, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise at least 17, 18, 19, 20, 21, 22, 23, or 24 serotypes selected from the group consisting of 2, 6C, 7C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35F, 35B, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise at least 10, 11, 12, 13, 14, 15, or 16 serotypes selected from the group consisting of 2, 6C, 7C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise at least 17, 18, 19, 20, 21, 22, 23, or 24 serotypes selected from the group consisting of 2, 6C, 7C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 24F, 33F, and 35B. A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B. A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition or

Petitioner Merck Ex-1004, 0784

vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 20B, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B, and 38. A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B. A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B and 38. A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 23A, 23B, and 35B. A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 23A, 23B, 24F, and 35B. A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 23A, 23B, 24F, and 35B. A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 10A, 11A, 15A, 15B, 15C, 23A, 24B, 35B, and 38. A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 23A, 23B, and 35B. A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 23A, 23B, and 35B. A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 23A, 23B, 24F, and 35B. A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising 6C, 10A, 11A, 15A, 15B, 15C, 23A, 24B, 35B and 38. A pharmaceutical composition or vaccine can comprise a plurality of

Petitioner Merck Ex-1004, 0785

saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 23A, 23B, and 35B. A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 23A, 23B, and 35B. A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 9N, 15A, 15C, 16F, 23A, 23B, and 35B. A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 15A, 15C, 16F, 23A, 23B, and 35B. A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 24F, 31, 33F, 34, 35F, and 35B. A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 20A, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 15A, 15C, 16F, 20B, 23A, 23B, 34F, 31, 33F, 34, 35F, and 35B. A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20A, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising at least 13 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7C, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising at least 15 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7C, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising at least 16 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7C, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B 31, 33F, 33B, 35B, 34, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype comprising 1, 2, 3, 4, 5, 6A, 6B, 7C, 7F, 9V, 14, 18C, 19A, 19F, 20B, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide

Petitioner Merck Ex-1004, 0786

conjugates comprising the serotype 6C and at least one additional serotype comprising 2, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 16F, 17F, 22F, 23A, 23B, 24B, 31, 33F, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype comprising 1, 3, 4, 6A, 6B, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 22F, 23A, 23B, 24F, 33F, 35F, 35B, 38 or Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 1, 3, 4, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 22F, 23A, 23B, 23F, 24F, 33F, 35F, 35B, 38 or Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising at least 13 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising at least 15 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 34, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising at least 16 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype comprising 2, 8, 9N, 10A, 11A, 12F, 15A, 15B, 16F, 17F, 22F, 23A, 23B, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype comprising 1, 3, 4, 6A, 6B, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 22F, 23A, 23B, 24F, 33F, 34, 35F, 35B, 38, and Serogroup

Petitioner Merck Ex-1004, 0787

20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 1, 3, 4, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 22F, 23A, 23B, 23F, 24F, 33F, 34, 35F, 35B, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 3, 4, 7F, 8, 9N, 11A, 12F, 15A, 15B, 15C, 16F, 19A, 22F, 23A, 23B, and 35B. A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 1, 3, 4, 6A, 7F, 8, 9N, 10A, 11A, 12F, 14, 19A, 19F, 22F, 23A, 23B, 23F, 24F, and 33F. A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 3, 4, 6B, 10A, 15A, 15B, 19A, 19F, 22F, 23F, 24F, 35B. A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least two additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least three additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least four additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least five additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least six additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F,

Petitioner Merck Ex-1004, 0788

17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least seven additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least eight additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least nine additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least ten additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least eleven additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least twelve additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 34, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least thirteen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 34, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least fourteen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F,

Petitioner Merck Ex-1004, 0789

9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least fifteen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least sixteen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least seventeen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least eighteen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least nineteen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least twenty additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least twenty-one additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide

Petitioner Merck Ex-1004, 0790

conjugates comprising the serotype 6C and at least twenty-two additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 34, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least twenty-three additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least twenty-four additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35B, 34, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least twenty-five additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least 1, 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, or 37 additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 7C, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B), or a combination thereof. A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least two additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least three additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least four additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A,

Petitioner Merck Ex-1004, 0791

19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least five additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least six additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least seven additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least eight additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least nine additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least ten additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least eleven additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least

Petitioner Merck Ex-1004, 0792

twelve additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least thirteen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least fourteen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least fifteen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least sixteen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least seventeen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least eighteen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least nineteen additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and

Petitioner Merck Ex-1004, 0793

20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least twenty additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least twenty-one additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least twenty-two additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least twenty-three additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least twenty-four additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least twenty-five additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least 1, 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, or 37 additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 7C, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, 38, and Serogroup 20 (comprising serotypes 20A and 20B), or a combination thereof.

Petitioner Merck Ex-1004, 0794

[0071] A vaccine can comprise immunogenic saccharide-polypeptide conjugates that can each comprise a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof can be from a *Streptococcus pneumoniae* serotype selected from a group consisting of 23A and 23B. A vaccine can comprise at least 2 immunogenic saccharide-polypeptide conjugates that can each comprise a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof can be from a *Streptococcus pneumoniae* serotype group, wherein one serotype can be 23A and at least one additional serotype can be selected from the group consisting of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 20B, 22F, 23B, 24F, 24B, 31, 33F, 33B, 34, 35B, 35F, and 38. A vaccine can comprising at least 2 immunogenic saccharide-polypeptide conjugates that can each comprise a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof can be from a *Streptococcus pneumoniae* serotype group, wherein one serotype can be 23B and at least one additional serotype can be selected from the group consisting of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 20B, 22F, 23A, 24F, 24B, 31, 33F, 33B, 34, 35B, 35F, and 38.

[0072] The immunogenic saccharide-polypeptide conjugate(s) can be individually, respectively, or collectively present in an amount of about: 0.001, 0.01, 0.1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, or 100%, by weight, based on the weight of the pharmaceutical composition or vaccine. The immunogenic saccharide-polypeptide conjugate(s) can include excipients that can be individually, respectively, or collectively present in an amount of about: 0.001, 0.01, 0.1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, or 100%, by weight, based on the weight of the pharmaceutical composition or vaccine. The immunogenic saccharide-polypeptide conjugate(s) can include adjuvants that can be individually, respectively, or collectively present in an amount of about: 0.001, 0.01, 0.1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, or 100%, by weight, based on the weight of the pharmaceutical composition or vaccine. The pharmaceutical composition or vaccine can be administered to one of a plurality of humans, which elicits at least one of the following side effects in less than about 5%, 10%, 20% or 30% of the plurality of humans: fatigue, headache, muscle pain, joint pain, decreased appetite, chills, or rash.

Petitioner Merck Ex-1004, 0795

[0073] In other embodiments, a pharmaceutical composition or vaccine can comprise a saccharide-polypeptide conjugate wherein the saccharide is from *N. meningitidis*. A pharmaceutical composition can comprise a saccharide-polypeptide conjugate wherein the saccharide can be from *S. enterica*. A pharmaceutical composition or vaccine can comprise a saccharide-polypeptide conjugate wherein the saccharide can be from *H. influenzae*. A pharmaceutical composition can comprise a plurality of saccharide-polypeptide conjugates comprising saccharides from *Neisseria meningitidis*. A pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising saccharides from *S. enterica*. A pharmaceutical composition or vaccine can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising saccharides from *H. influenzae*.

[0074] The polypeptide of any of the plurality of saccharide-polypeptide conjugates can be the same polypeptide. For example, the polypeptide of any of the plurality of saccharide-polypeptide conjugates can be CRM_{197}. The plurality of saccharide-polypeptide conjugates can comprise a mixture of different polypeptides. For example, the plurality of saccharide-polypeptide conjugates can comprise a mixture of CRM_{197}, tetanus toxoid, a diphtheria toxoid, or any fragment thereof.

[0075] Any pharmaceutical composition or vaccine described herein can be in unit dose form. Additionally, the pharmaceutical composition or vaccine can further comprise an adjuvant. In some embodiments, the adjuvant can be an aluminum-cation comprises adjuvant. In other embodiments, the adjuvant can be aluminum phosphate, aluminum sulfate, or aluminum hydroxide. More examples of suitable adjuvants for use in the pharmaceutical composition or vaccine include: aluminum-based salts and variants thereof; emulsions (either water-in-oil or oil-in-water) and variants thereof, *e.g.*, Freund's Incomplete Adjuvant, MF59® (Glaxosmithkline plc); PRR ligands and variants thereof, *e.g.*, pathogen associated molecular patterns (PAMPs); TLR3 and RLR ligands or variants thereof, *e.g.*, Synthetic analogs of dsRNA, poly(I:C), etc.; TLR4 ligands or variants thereof, *e.g.*, bacterial flagellin, Glucopyranosyl Lipid Adjuvant (GLA), monophosphoryl lipid A, etc.; TLR5 ligands or variants thereof, *e.g.*, imiquimod, gardiquimod, R848, etc.; TLR9 ligands or variants thereof, *e.g.*, Oxydeoxynucleotides containing CpG motifs (CpG ODNs such as ODN1826 and ODN2006); NOD2 ligands or variants thereof, *e.g.*, fragments of bacterial cell walls (such as muramyl dipeptide [MDP]); saponins including synthetic derivatives or variants thereof, e.g., QS21, TQL1055, etc.; or any combination of above. An adjuvant can be 3M-052.

Petitioner Merck Ex-1004, 0796

[0076] A pharmaceutical composition or vaccine can further comprise an excipient. An excipient can be Acacia, Acesulfame Potassium, Acetic Acid, Glacial Acetone, Acetyltributyl Citrate, Acetyltriethyl Citrate, Agar, Albumin, Alcohol, Alginic Acid, Aliphatic Polyesters, Alitame, Almond Oil, Alpha Tocopherol, Aluminum Hydroxide Adjuvant, Aluminum Oxide, Aluminum Phosphate Adjuvant, Aluminum Stearate, Ammonia Solution, Ammonium Alginate, Ascorbic Acid, Ascorbyl Palmitate, Aspartame, Attapulgite, Bentonite, Benzalkonium Chloride, Benzethonium Chloride, Benzoic Acid, Benzyl Alcohol, Benzyl Benzoate, Boric Acid, Bronopol, Butylated Hydroxyanisole, Butylated Hydroxytoluene, Butylparaben, Calcium Alginate, Calcium Carbonate, Dibasic Anhydrous Calcium Phosphate, Dibasic Dihydrate Calcium Phosphate, Tribasic Calcium Phosphate, Calcium Stearate, Calcium Sulfate, Canola Oil, Carbomer, Carbon Dioxide, Carboxymethylcellulose Calcium, Carboxymethylcellulose Sodium, Carrageenan, Castor Oil, Hydrogenated Castor Oil, Microcrystalline Cellulose, Powdered Cellulose, Silicified Microcrystalline Cellulose, Cellulose Acetate, Cellulose Acetate Phthalate, Ceratonia, Cetostearyl Alcohol, Cetrimide, Cetyl Alcohol, Cetylpyridinium Chloride, Chitosan, Chlorhexidine, Chlorobutanol, Chlorocresol, Chlorodifluoroethane (HCFC), Chlorofluorocarbons (CFC), Chloroxylenol, Cholesterol, Citric Acid Monohydrate, Colloidal Silicon Dioxide, Coloring Agents, Copovidone, Corn Oil, Cottonseed Oil, Cresol, Croscarmellose Sodium, Crospovidone, Cyclodextrins, Cyclomethicone, Denatonium Benzoate, Dextrates, Dextrin, Dextrose, Dibutyl Phthalate, Dibutyl Sebacate, Diethanolamine, Diethyl Phthalate, Difluoroethane (HFC), Dimethicone, Dimethyl Ether, Dimethyl Phthalate, Dimethyl Sulfoxide, Dimethylacetamide, Disodium Edetate, Docusate Sodium, Edetic Acid, Erythorbic Acid, Erythritol, Ethyl Acetate, Ethyl Lactate, Ethyl Maltol, Ethyl Oleate, Ethyl Vanillin, Ethylcellulose, Ethylene Glycol Palmitostearate, Ethylene Vinyl Acetate, Ethylparaben, Fructose, Fumaric Acid, Gelatin, Glucose, Liquid, Glycerin, Glyceryl Behenate, Glyceryl Monooleate, Glyceryl Monostearate, Glyceryl Palmitostearate, Glycofurol, Guar Gum, Hectorite, Heptafluoropropane (HFC), Hexetidine, Hydrocarbons (HC), Hydrochloric Acid, Hydroxyethyl Cellulose, Hydroxyethylmethyl Cellulose, Hydroxypropyl Cellulose, Low-substituted Hydroxypropyl Cellulose, Hydroxypropyl Starch, Hypromellose, Hypromellose Acetate Succinate, Hypromellose Phthalate, Imidurea, Inulin, Iron Oxides, Isomalt, Isopropyl Alcohol, Isopropyl Myristate, Isopropyl Palmitate, Kaolin, Lactic Acid, Lactitol, Anhydrous Lactose, Monohydrate Lactose, Spray-Dried Lactose, Lanolin, Lanolin Alcohols, Hydrous Lanolin, Lauric Acid, Lecithin, Leucine, Linoleic Acid, Macrogol, Hydroxystearate Magnesium Aluminum Silicate, Magnesium Carbonate, Magnesium Oxide, Magnesium

Petitioner Merck Ex-1004, 0797

Silicate, Magnesium Stearate, Magnesium Trisilicate, Malic Acid, Maltitol, Maltitol Solution, Maltodextrin, Maltol, Maltose, Mannitol, Medium-chain Triglycerides, Meglumine, Menthol, Methylcellulose, Methylparaben, Mineral Oil, Light Mineral Oil, Mineral Oil and Lanolin Alcohols, Monoethanolamine, Monosodium Glutamate, Monothioglycerol, Myristic Acid, Neohesperidin Dihydrochalcone, Nitrogen, Nitrous Oxide, Octyldodecanol, Oleic Acid, Oleyl Alcohol, Olive Oil, Palmitic Acid, Paraffin, Peanut Oil, Pectin, Petrolatum, Petrolatum and Lanolin Alcohols, Phenol, Phenoxyethanol, Phenylethyl Alcohol, Phenylmercuric Acetate, Phenylmercuric Borate, Phenylmercuric Nitrate, Phosphoric Acid, Polacrilin Potassium, Poloxamer, Polycarbophil, Polydextrose, Polyethylene Glycol, Polyethylene Oxide, Polymethacrylates, Poly(methyl vinyl ether/maleic anhydride), Polyoxyethylene Alkyl Ethers, Polyoxyethylene Castor Oil Derivatives, Polyoxyethylene Sorbitan Fatty Acid Esters, Polyoxyethylene Stearates, Polyvinyl Acetate Phthalate, Polyvinyl Alcohol, Potassium Alginate, Potassium Benzoate, Potassium Bicarbonate, Potassium Chloride, Potassium Citrate, Potassium Hydroxide, Potassium Metabisulfite, Potassium Sorbate, Povidone, Propionic Acid, Propyl Gallate, Propylene Carbonate, Propylene Glycol, Propylene Glycol Alginate, Propylparaben, 2-Pyrrolidone, Raffinose, Saccharin, Saccharin Sodium, Saponite, Sesame Oil, Shellac, Simethicone, Sodium Acetate, Sodium Alginate, Sodium Ascorbate, Sodium Benzoate, Sodium Bicarbonate, Sodium Borate, Sodium Chloride, Sodium Citrate Dihydrate, Sodium Cyclamate, Sodium Hyaluronate, Sodium Hydroxide, Sodium Lactate, Sodium Lauryl Sulfate, Sodium Metabisulfite, Dibasic Sodium Phosphate, Monobasic Sodium Phosphate, Sodium Propionate, Sodium Starch Glycolate, Sodium Stearyl Fumarate, Sodium Sulfite, Sorbic Acid, Sorbitan Esters (Sorbitan Fatty Acid Esters), Sorbitol, Soybean Oil, Starch, Pregelatinized Starch, Sterilizable Maize Starch, Stearic Acid, Stearyl Alcohol, Sucralose, Sucrose, Compressible Sugar, Confectioner's Sugar, Sugar Spheres, Sulfobutylether b-Cyclodextrin, Sulfuric Acid, Sunflower Oil, Hard Fat Suppository Bases, Talc, Tartaric Acid, Tetrafluoroethane (HFC), Thaumatin, Thimerosal, Thymol, Titanium Dioxide, Tragacanth, Trehalose, Triacetin, Tributyl Citrate, Triethanolamine, Triethyl Citrate, Vanillin, Hydrogenated Vegetable Oil, Water, Anionic Emulsifying Wax, Carnauba Wax, Cetyl Esters Wax, Microcrystalline Wax, Nonionic Emulsifying Wax, White Wax, Yellow Wax, Xanthan Gum, Xylitol, Zein, Zinc Acetate, or Zinc Stearate.

[0077] A pharmaceutical composition or vaccine can further comprise a surfactant or emulsifier, or a combination thereof. A surfactant can be polysorbate, polymer glycol, a sorbitan ester, or any combination thereof. In some embodiments, polysorbate can be polysorbate 80. In some embodiments, polymer glycol can be polyethylene glycol. A

Petitioner Merck Ex-1004, 0798

surfactant can be TWEEN. A pharmaceutical composition or vaccine can comprise a specific polyethylene glycol based upon its molecular weight. A pharmaceutical composition or vaccine can comprise a preservative. A pharmaceutical composition or vaccine can comprise a salt. The salt can be an inorganic salt. A pharmaceutical composition or vaccine can further comprise an anti-fungal compound or a salt thereof. An antifungal compound can be thimerosal, phenol, benzethonium chloride, or 2-phenoxyethanol. A pharmaceutical composition or vaccine can further comprise a chelating agent. A chelating agent can be ethylenediaminetetraacetic acid (EDTA). A pharmaceutical composition or vaccine can further comprise a buffering agent. The buffering agent can be succinate buffer agent, tris-acetate- ethylenediaminetetraacetic acid (TAE) buffering agent, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffering agent, phosphate buffering agent, sodium phosphate buffering agent, or a potassium phosphate buffering agent. A pharmaceutical composition or vaccine can further comprise sterile water. When the pharmaceutical composition or vaccine can be administered intramuscularly to a human, the pharmaceutical composition or vaccine can elicit an immune response that lasts from about 1 week to about 30 years. A pharmaceutical composition or vaccine retains at least about 60% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C when the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure. A pharmaceutical composition or vaccine can retain at least about 70% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C when the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure. A pharmaceutical composition or vaccine can retain at least about 80% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C when the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure. A pharmaceutical composition or vaccine can retain at least about 90% of its original biological activity when stored in a sealed container for about 24 months at a temperature of about 2°C–8°C when the sealed container is stored in an atmosphere having about 50% relative humidity and 1 atm of atmospheric pressure.

[0078] The polysaccharide-polypeptide conjugates as described herein can be adjuvanted. Suitable adjuvants can include an aluminum hydroxide gel (alum) or aluminum phosphate, but can also be a salt of calcium, magnesium, iron or zinc, or can be an insoluble suspension of acylated tyrosine, or acylated sugars, cationically or anionically derivatized

Petitioner Merck Ex-1004, 0799

polysaccharides, or polyphophazenes. An adjuvant can be 3M-052. When adjuvanted with aluminum salts, the ratio of aluminum salt to polysaccharide can be less than 10:1 (w/w), less than 8:1(w/w), or more than 2:1(w/w) in a pharmaceutical composition dose or formulation, or a vaccine dose or formulation.

[0079] The amount of polypeptide in a pharmaceutical composition dose or vaccine dose can be selected as an amount which can induces an immunoprotective response without significant adverse effects. A dose or formulation can comprise from 0.1-100 μg of polysaccharide and/or for a polysaccharide polypeptide conjugate from 0.1-50 μg of polysaccharide. A polysaccharide-polypeptide conjugate can comprise from 1-10 μg of polysaccharide, 1-5 μg of polysaccharide, or 2-5 μg of polysaccharide. A polysaccharide-polypeptide conjugate can comprise about 0.5 μg of polysaccharide, about 1 μg of polysaccharide, about 1.5 μg of polysaccharide, about 2 μg of polysaccharide, about 2.5 μg of polysaccharide, about 3 μg of polysaccharide, about 3.5 μg of polysaccharide, about 4 μg of polysaccharide, about 5 μg of polysaccharide, about 6 μg of polysaccharide, about 7 μg of polysaccharide, about 8 μg of polysaccharide, about 9 μg of polysaccharide, about 10 μg of polysaccharide, about 11 μg of polysaccharide, about 12 μg of polysaccharide, about 13 μg of polysaccharide, about 14 μg of polysaccharide, about 15 μg of polysaccharide, about 16 μg of polysaccharide, about 17 μg of polysaccharide, about 18 μg of polysaccharide, about 19 μg of polysaccharide, or about 20 μg of polysaccharide. The content of polypeptide in the vaccine can be in the range from 1-100 μg of polypeptide, 5-50 μg of polypeptide, or 5-25 μg of polypeptide.

[0080] The total amount of capsular polysaccharide, fragment thereof, or combination thereof, individually for each *Streptococcus pneumoniae* serotype in a pharmaceutical composition dose or a vaccine dose in unit dose form, can range from about 0.5 μg to about 5 μg. The total amount of capsular polysaccharide, fragment thereof, or combination thereof, individually for each *Streptococcus pneumoniae* serotype in a pharmaceutical composition dose or a vaccine dose in unit dose form, can range from 0.5 μg to 5 μg. The total amount of capsular polysaccharide, fragment thereof, or combination thereof, individually for each *Streptococcus pneumoniae* serotype in a pharmaceutical composition dose or in a vaccine dose in unit dose form, can range from about 1 μg to about 4 μg. The total amount of capsular polysaccharide, fragment thereof, or combination thereof, individually for each *Streptococcus pneumoniae* serotype in a pharmaceutical composition dose or a vaccine dose in unit dose form, can range from about 1 μg to about 4 μg. The total amount of capsular polysaccharide, fragment thereof, or combination thereof, individually for each *Streptococcus*

Petitioner Merck Ex-1004, 0800

pneumoniae serotype in a pharmaceutical composition dose or in a vaccine dose in unit dose form, can range from about 1 μg to about 8 μg. The total amount of capsular polysaccharide, fragment thereof, or combination thereof, individually for each *Streptococcus pneumoniae* serotype in a pharmaceutical composition dose or a vaccine dose in unit dose form, can range from about 1 μg to about 8 μg. The total amount of capsular polysaccharide, fragment thereof, or combination thereof, individually for each *Streptococcus pneumoniae* serotype in a pharmaceutical composition dose or in a vaccine dose in unit dose form, can range from about 1 μg to about 10 μg. The total amount of capsular polysaccharide, fragment thereof, or combination thereof, individually for each *Streptococcus pneumoniae* serotype in a pharmaceutical composition dose or a vaccine dose in unit dose form, can range from about 1 μg to about 10 μg. The total amount of capsular polysaccharide, fragment thereof, or combination thereof, individually for each *Streptococcus pneumoniae* serotype in a pharmaceutical composition dose or in a vaccine dose in unit dose form, can range from about 1 μg to about 15 μg. The total amount of capsular polysaccharide, fragment thereof, or combination thereof, individually for each *Streptococcus pneumoniae* serotype in a pharmaceutical composition dose or a vaccine dose in unit dose form, can range from about 1 μg to about 15 μg. The total amount of capsular polysaccharide, fragment thereof, or combination thereof, individually for each *Streptococcus pneumoniae* serotype in a pharmaceutical composition dose or in a vaccine dose in unit dose form, can range from about 1 μg to about 20 μg. The total amount of capsular polysaccharide, fragment thereof, or combination thereof, individually for each *Streptococcus pneumoniae* serotype in a pharmaceutical composition dose or a vaccine dose in unit dose form, can range from about 1 μg to about 20 μg. The total amount of capsular polysaccharide, fragment thereof, or combination thereof, individually for each *Streptococcus pneumoniae* serotype in a pharmaceutical composition dose or in a vaccine dose in unit dose form, can range from about 4 μg to about 8 μg. The total amount of capsular polysaccharide, fragment thereof, or combination thereof, individually for each *Streptococcus pneumoniae* serotype in a pharmaceutical composition dose or a vaccine dose in unit dose form, can range from about 4 μg to about 8 μg. The total amount of capsular polysaccharide, fragment thereof, or combination thereof, individually for each *Streptococcus pneumoniae* serotype in a pharmaceutical composition dose or in a vaccine dose in unit dose form, can range from about 2 μg to about 6 μg. The total amount of capsular polysaccharide, fragment thereof, or combination thereof, individually for each *Streptococcus pneumoniae* serotype in a pharmaceutical composition dose or a vaccine dose in unit dose form, can range from about 2

Petitioner Merck Ex-1004, 0801

μg to about 6 μg. The total amount of capsular polysaccharide, fragment thereof, or combination thereof, individually for each *Streptococcus pneumoniae* serotype in a pharmaceutical composition dose or in a vaccine dose in unit dose form, can range from about 3 μg to about 5 μg. The total amount of capsular polysaccharide, fragment thereof, or combination thereof, individually for each *Streptococcus pneumoniae* serotype in a pharmaceutical composition dose or a vaccine dose in unit dose form, can range from about 3 μg to about 5 μg. The total amount of capsular polysaccharide, fragment thereof, or combination thereof, individually for each *Streptococcus pneumoniae* serotype in a pharmaceutical composition dose or a vaccine dose in unit dose form, can be about 0.5 μg, about 1 μg, about 2 μg, about 3 μg, about 4 μg, or about 5 μg. The total amount of capsular polysaccharide, fragment thereof, or combination thereof, individually for each *Streptococcus pneumoniae* serotype in a pharmaceutical composition dose or a vaccine dose in unit dose form, can be 0.5 μg, 1 μg, 2 μg, 3 μg, 4 μg, or 5 μg. In some embodiments, a total amount of unconjugated capsular polysaccharide, fragment thereof, or combination thereof, individually for each *Streptococcus pneumoniae* serotype in a pharmaceutical composition dose or vaccine dose in unit dose form, can range from about 0% to about 30% w/w. In some embodiments, a total amount of unconjugated capsular polysaccharide, fragment thereof, or combination thereof, individually for each *Streptococcus pneumoniae* serotype in a pharmaceutical composition dose or vaccine dose in unit dose form, can range from 0% to 30% w/w. In some embodiments, a total amount of unconjugated capsular polysaccharide, fragment thereof, or combination thereof, individually for each *Streptococcus pneumoniae* serotype in a pharmaceutical composition dose or vaccine dose in unit dose form, can range from about 0% to about 20% w/w. In some embodiments, a total amount of unconjugated capsular polysaccharide, fragment thereof, or combination thereof, individually for each *Streptococcus pneumoniae* serotype in a pharmaceutical composition dose or vaccine dose in unit dose form, can range from 0% to 20% w/w. In some embodiments, a total amount of unconjugated capsular polysaccharide, fragment thereof, or combination thereof, individually for each *Streptococcus pneumoniae* serotype in a pharmaceutical composition dose or vaccine dose in unit dose form, can range from about 0% to about 10% w/w. In some embodiments, a total amount of unconjugated capsular polysaccharide, fragment thereof, or combination thereof, individually for each *Streptococcus pneumoniae* serotype in a pharmaceutical composition dose or vaccine dose in unit dose form, can range from 0% to 10% w/w.

Petitioner Merck Ex-1004, 0802

TABLE 2. Exemplary Total Amount of Polysaccharide in a Pharmaceutical Composition or Vaccine in Unit Dose Form

Polysaccharide Serotype*	Total Amount of Polysaccharide in Polysaccharide-Polypeptide Conjugate**		
1	about 1 μg	about 2 μg	about 4 μg
2	about 1 μg	about 2 μg	about 4 μg
3	about 1 μg	about 2 μg	about 4 μg
4	about 1 μg	about 2 μg	about 4 μg
5	about 1 μg	about 2 μg	about 4 μg
6A	about 1 μg	about 2 μg	about 4 μg
6B	about 1 μg	about 2 μg	about 4 μg
6C	about 1 μg	about 2 μg	about 4 μg
7F	about 1 μg	about 2 μg	about 4 μg
7C	about 1 μg	about 2 μg	about 4 μg
8	about 1 μg	about 2 μg	about 4 μg
9N	about 1 μg	about 2 μg	about 4 μg
9V	about 1 μg	about 2 μg	about 4 μg
10A	about 1 μg	about 2 μg	about 4 μg
11A	about 1 μg	about 2 μg	about 4 μg
12F	about 1 μg	about 2 μg	about 4 μg
14	about 1 μg	about 2 μg	about 4 μg
15A	about 1 μg	about 2 μg	about 4 μg
15B	about 1 μg	about 2 μg	about 4 μg
15C	about 1 μg	about 2 μg	about 4 μg
16F	about 1 μg	about 2 μg	about 4 μg
17F	about 1 μg	about 2 μg	about 4 μg
18C	about 1 μg	about 2 μg	about 4 μg
19F	about 1 μg	about 2 μg	about 4 μg
19A	about 1 μg	about 2 μg	about 4 μg
20A	about 1 μg	about 2 μg	about 4 μg
20B	about 1 μg	about 2 μg	about 4 μg
20 (comprising	about 1 μg	about 2 μg	about 4 μg

Petitioner Merck Ex-1004, 0803

Polysaccharide Serotype*	Total Amount of Polysaccharide in Polysaccharide-Polypeptide Conjugate**		
20A and 20B)			
22F	about 1 μg	about 2 μg	about 4 μg
23F	about 1 μg	about 2 μg	about 4 μg
23A	about 1 μg	about 2 μg	about 4 μg
23B	about 1 μg	about 2 μg	about 4 μg
24F	about 1 μg	about 2 μg	about 4 μg
24B	about 1 μg	about 2 μg	about 4 μg
31	about 1 μg	about 2 μg	about 4 μg
33F	about 1 μg	about 2 μg	about 4 μg
33B	about 1 μg	about 2 μg	about 4 μg
34	about 1 μg	about 2 μg	about 4 μg
35F	about 1 μg	about 2 μg	about 4 μg
35B	about 1 μg	about 2 μg	about 4 μg
38	about 1 μg	about 2 μg	about 4 μg

*Each serotype polysaccharide can be in a pharmaceutical composition or a vaccine in any one of the total amounts listed in the table

**Total amount can refer to the amount of polysaccharide of a serotype in a pharmaceutical composition or a vaccine when the pharmaceutical composition or vaccine is in unit dose form

[0081] A vaccine or pharmaceutical composition can be in unit dose form. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 1, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from about 300 kDa to about 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from about 0.3 to about 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 1, the immunogenic saccharide- CRM_{197} conjugate can have a molecular weight from 300 kDa to 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from 0.3 to 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an

Petitioner Merck Ex-1004, 0804

immunogenic saccharide, fragment, combination thereof of serotype 2, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from about 300kDa to about 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from about 0.3 to about 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 2, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from 300 kDa to 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from 0.3 to 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 3, the immunogenic saccharide- CRM_{197} conjugate can have a molecular weight from about 300 kDa to about 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from about 0.3 to about 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 3, the immunogenic saccharide- CRM_{197} conjugate can have a molecular weight from 300 kDa to 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from 0.3 to 3.0. A pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 4, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from about 300 kDa to about 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from about 0.3 to about 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 4, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from 300 kDa to 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from 0.3 to 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 5, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from about 300 kDa to about 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from about 0.3 to about 3.0. A vaccine or pharmaceutical composition can comprise an

Petitioner Merck Ex-1004, 0805

immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 5, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from 300 kDa to 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from 0.3 to 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 6A, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from about 300 kDa to about 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from about 0.3 to about 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 6A, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from 300 kDa to 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from 0.3 to 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 6B, the immunogenic saccharide- CRM_{197} conjugate can have a molecular weight from about 300 kDa to about 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from about 0.3 to about 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 6B, the immunogenic saccharide- CRM_{197} conjugate can have a molecular weight from 300 kDa to 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from 0.3 to 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 7F, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from about 300 kDa to about 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from about 0.3 to about 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 7F, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from 300kDa to 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from 0.3 to 3.0. A vaccine or pharmaceutical

Petitioner Merck Ex-1004, 0806

composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 7C, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from about 300 kDa to about 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from about 0.3 to about 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 7C, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from 300kDa to 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from 0.3 to 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 9V, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from about 300 kDa to about 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from about 0.3 to about 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 9V, the immunogenic saccharide- CRM_{197} conjugate can have a molecular weight from 300 kDa to 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from 0.3 to 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 14, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from about 300 kDa to about 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from about 0.3 to about 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 14, the immunogenic saccharide- CRM_{197} conjugate can have a molecular weight from 300 kDa to 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from 0.3 to 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 18C, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from about 300 kDa to about 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or

Petitioner Merck Ex-1004, 0807

combination thereof to the CRM_{197} can be from about 0.3 to about 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 18C, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from 300 kDa to 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from 0.3 to 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 19A, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from about 300 kDa to about 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from about 0.3 to about 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 19Athe immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from 300 kDa to 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from 0.3 to 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 19F, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from about 300 kDa to about 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from about 0.3 to about 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 19F, the immunogenic saccharide- CRM_{197} conjugate can have a molecular weight from 300 kDa to 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from 0.3 to 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 23F, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from about 300 kDa to about 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from about 0.3 to about 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 23F, the immunogenic saccharide- CRM_{197} conjugate can have a molecular weight from 300 kDa to

Petitioner Merck Ex-1004, 0808

10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from 0.3 to 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 6C, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from about 300 kDa to about 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from about 0.3 to about 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 6C, the immunogenic saccharide- CRM_{197} conjugate can have a molecular weight from 300 kDa to 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from 0.3 to 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 8, the immunogenic saccharide- CRM_{197} conjugate can have a molecular weight from about 300 kDa to about 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from about 0.3 to about 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 8, the immunogenic saccharide- CRM_{197} conjugate can have a molecular weight from 300 kDa to 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from 0.3 to 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 9N, the immunogenic saccharide- CRM_{197} conjugate can have a molecular weight from about 300 kDa to about 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from about 0.3 to about 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 9N, the immunogenic saccharide- CRM_{197} conjugate can have a molecular weight from 300 kDa to 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from 0.3 to 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 10A, the immunogenic

Petitioner Merck Ex-1004, 0809

saccharide-CRM_{197} conjugate can have a molecular weight from about 300 kDa to about 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from about 0.3 to about 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 10A, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from 300 kDa to 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from 0.3 to 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 11A, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from about 300 kDa to about 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from about 0.3 to about 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 11A, the immunogenic saccharide- CRM_{197} conjugate can have a molecular weight from 300 kDa to 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from 0.3 to 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 12F, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from about 300 kDa to about 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from about 0.3 to about 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 12F, the immunogenic saccharide- CRM_{197} conjugate can have a molecular weight from 300 kDa to 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from 0.3 to 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 15A, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from about 300 kDa to about 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from about 0.3 to about 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate

Petitioner Merck Ex-1004, 0810

comprising an immunogenic saccharide, fragment, combination thereof of serotype 15A, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from 300 kDa to 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from 0.3 to 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 15B, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from about 300 kDa to about 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from about 0.3 to about 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 15B, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from 300 kDa to 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from 0.3 to 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 15C, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from about 300 kDa to about 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from about 0.3 to about 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 15C, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from 300 kDa to 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from 0.3 to 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 16F, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from about 300 kDa to about 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from about 0.3 to about 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 16F, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from 300 kDa to 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from 0.3 to 3.0. A vaccine or pharmaceutical

Petitioner Merck Ex-1004, 0811

composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 17F, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from about 300 kDa to about 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from about 0.3 to about 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 17F, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from 300 kDa to 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from 0.3 to 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 20A, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from about 300 kDa to about 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from about 0.3 to about 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 20A, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from 300 kDa to 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from 0.3 to 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 20B, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from about 300 kDa to about 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from about 0.3 to about 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 20B, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from 300 kDa to 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from 0.3 to 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 22F, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from about 300 kDa to about 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or

Petitioner Merck Ex-1004, 0812

combination thereof to the CRM_{197} can be from about 0.3 to about 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 22F, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from 300 kDa to 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from 0.3 to 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 23A, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from about 300 kDa to about 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from about 0.3 to about 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 23A, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from 300 kDa to 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from 0.3 to 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 23B, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from about 300 kDa to about 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from about 0.3 to about 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 23B, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from 300 kDa to 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from 0.3 to 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 24F the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from about 300 kDa to about 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from about 0.3 to about 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 24F, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from 300 kDa to

Petitioner Merck Ex-1004, 0813

10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from 0.3 to 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 24B, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from about 300 kDa to about 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from about 0.3 to about 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 24B, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from 300 kDa to 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from 0.3 to 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 31, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from about 300 kDa to about 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from about 0.3 to about 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 31, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from 300 kDa to 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from 0.3 to 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 33F, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from about 300 kDa to about 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from about 0.3 to about 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 33F, the immunogenic saccharide- CRM_{197} conjugate can have a molecular weight from 300 kDa to 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from 0.3 to 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 33B, the immunogenic

Petitioner Merck Ex-1004, 0814

saccharide-CRM_{197} conjugate can have a molecular weight from about 300 kDa to about 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from about 0.3 to about 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 33B, the immunogenic saccharide- CRM_{197} conjugate can have a molecular weight from 300 kDa to 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from 0.3 to 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 35F, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from about 300 kDa to about 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from about 0.3 to about 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 35F, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from 300 kDa to 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from 0.3 to 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 34, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from about 300 kDa to about 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from about 0.3 to about 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 34, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from 300 kDa to 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from 0.3 to 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 35B, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from about 300 kDa to about 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from about 0.3 to about 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate

Petitioner Merck Ex-1004, 0815

comprising an immunogenic saccharide, fragment, combination thereof of serotype 35B, the immunogenic saccharide-CRM_{197} conjugate can have a molecular weight from 300 kDa to 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from 0.3 to 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 38, the immunogenic saccharide- CRM_{197} conjugate can have a molecular weight from about 300 kDa to about 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from about 0.3 to about 3.0. A vaccine or pharmaceutical composition can comprise an immunogenic saccharide-CRM_{197} conjugate comprising an immunogenic saccharide, fragment, combination thereof of serotype 38, the immunogenic saccharide- CRM_{197} conjugate can have a molecular weight from 300kDa to 10,000 kDa, and a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} can be from 0.3 to 3.0. The vaccine or pharmaceutical composition can comprise in any combination of serotypes as described above (*e.g.*, see EXAMPLE 6).

[0082] The vaccine dosage can be in the range from 1-5 μg of polysaccharide per polysaccharide polypeptide conjugate delivered intramuscularly as a single 0.5 mL injection. The vaccine dosage can comprise aluminum phosphate (1 mg/mL) and 0.01% polysorbate 80.

Methods of Use

[0083] The saccharide-polypeptide conjugates as described herein can be formulated for use in a vaccine. The pharmaceutical compositions comprising a saccharide-polypeptide conjugate as described herein can be formulated for use in a vaccine. The pharmaceutical compositions comprising a saccharide-polypeptide conjugate as described herein can be used in a vaccine. The vaccine can be administered to a subject. The vaccine can be used as a prophylactic for disease caused by *S. pneumoniae*, such as meningitis, pneumonia, and severe invasive diseases associated with pneumococcal infection. The subject can be a human. The subject or human can be a subject or human in need thereof.

[0084] The saccharide-polypeptide conjugate can be administered by intramuscular injection. The saccharide-polypeptide conjugate can be administered via intramuscular, intraperitoneal, intradermal or subcutaneous routes, or via mucosal administration to the oral/alimentary, respiratory or genitourinary tracts. The saccharide-polypeptide conjugate can be administered via a suppository. The saccharide-polypeptide conjugate can be administered intranasally.

Petitioner Merck Ex-1004, 0816

The saccharide-polypeptide conjugate can elicit an opsonophagocytic response after administration. The saccharide-polypeptide conjugate can elicit the production of antibodies against the serotype of the saccharide-polypeptide conjugate. This antibody response can be measured by an in vitro opsonophagocytic assay after administration of the saccharide-polypeptide conjugate.

[0085] The pharmaceutical composition or vaccine comprising a saccharide-polypeptide conjugate can be administered by intramuscular injection. The pharmaceutical composition or vaccine comprising a saccharide-polypeptide conjugate can be administered via intramuscular, intraperitoneal, intradermal or subcutaneous routes, or via mucosal administration to the oral/alimentary, respiratory or genitourinary tracts. The pharmaceutical composition or vaccine comprising a saccharide-polypeptide conjugate can be administered intranasally. The pharmaceutical composition or vaccine can be administered intramuscularly and can elicit an immune response that lasts from 1 week to 30 years. The saccharide-polypeptide conjugate can elicit an opsonophagocytic response after administration. The pharmaceutical composition or vaccine comprising a saccharide-polypeptide conjugate can elicit the production of antibodies against the serotype of the saccharide-polypeptide conjugate. This antibody response can be measured by an in vitro opsonophagocytic assay after administration of the pharmaceutical composition or vaccine comprising the saccharide-polypeptide conjugate.

[0086] For example, a pharmaceutical composition or vaccine administered to a subject can comprise a saccharide-polypeptide conjugate comprising a capsular polysaccharide or immunogenic fragment thereof from a serotype of *S. pneumoniae* conjugated to a polypeptide, and wherein the serotype is not at least one of, or is not 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, or 23F; the serotype is at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 21, 22, 23, or 24 of 2, 6C, 7C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B); or a combination thereof. A pharmaceutical composition or vaccine administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least nine serotypes selected from the group consisting of 2, 6C, 7C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise at least ten serotypes selected from the group consisting of 2, 6C, 7C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20

Petitioner Merck Ex-1004, 0817

(comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise at least eleven serotypes selected from the group consisting of 2, 6C, 7C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 33B, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise at least twelve serotypes selected from the group consisting of 2, 6C, 7C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 33B, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise at least thirteen serotypes selected from the group consisting of 2, 6C, 7C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 33B, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise at least fourteen serotypes selected from the group consisting of 2, 6C, 7C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 33B, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise at least fifteen serotypes selected from the group consisting of 2, 6C, 7C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 33B, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise at least sixteen serotypes selected from the group consisting of 2, 6C, 7C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 33B, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise at least seventeen serotypes selected from the group consisting of 2, 6C, 7C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 33B, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise at least eighteen serotypes selected from the group consisting of 2, 6C, 7C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 33B, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise at least nineteen serotypes selected from the group consisting of 2, 6C, 7C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 33B, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise at least twenty serotypes selected from the group consisting of 2, 6C, 7C, 7F, 8, 9N,

Petitioner Merck Ex-1004, 0818

10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 33B, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise at least twenty-one serotypes selected from the group consisting of 2, 6C, 7C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 33B, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise at least twenty-two serotypes selected from the group consisting of 2, 6C, 7C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 33B, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). For example, a pharmaceutical composition or vaccine administered to a subject can comprise a saccharide-polypeptide conjugate comprising a capsular polysaccharide or immunogenic fragment thereof from a serotype of *S. pneumoniae* conjugated to a polypeptide, and wherein the serotype is not at least one of, or is not 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, or 23F; the serotype is at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or of 2, 6C, 7C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B); or a combination thereof. A pharmaceutical composition or vaccine administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least nine serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise at least ten serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 33B, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise at least eleven serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 33B, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise at least twelve serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 33B, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise at least thirteen serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 33B, 34, 35B, 35F, 38, and Serogroup 20 (comprising

Petitioner Merck Ex-1004, 0819

serotypes 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise at least fourteen serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 33B, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise at least fifteen serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 33B, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition administered to a subject can comprise at least sixteen serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 33B, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise at least seventeen serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 33B, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise at least eighteen serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 33B, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise at least nineteen serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 33B, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise at least twenty serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 33B, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise at least twenty-one serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 33B, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise at least twenty-two serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 33B, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and

Petitioner Merck Ex-1004, 0820

35B. A pharmaceutical composition or vaccine administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 24F, 33F, and 35B. A pharmaceutical composition or vaccine administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B. A pharmaceutical composition or vaccine administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 20B, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition or vaccine administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, 35B and Serogroup 20 (comprising 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B, and 38. A pharmaceutical composition or vaccine administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition or vaccine administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition or vaccine administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B. A pharmaceutical composition or vaccine administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B and 38. A pharmaceutical composition or vaccine administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition or vaccine administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition or vaccine administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least 13 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 31, 33F,

Petitioner Merck Ex-1004, 0821

33B, 34, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B), or a combination thereof. A pharmaceutical composition or vaccine administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least 14 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 31, 33F, 33B, 34, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B), or a combination thereof. A pharmaceutical composition or vaccine administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least 15 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 31, 33F, 33B, 34, 35B, and 38, and Serogroup 20 (comprising serotypes 20A and 20B), or a combination thereof. A pharmaceutical composition or vaccine administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least 16 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 31, 33F, 33B, 34, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B), or a combination thereof. A pharmaceutical composition or vaccine administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least 17 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 31, 33F, 33B, 34, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B), or a combination thereof. A pharmaceutical composition or vaccine administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least 18 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 33B, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B), or a combination thereof. A pharmaceutical composition or vaccine administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least 1, 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, or 36 additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B), or a combination thereof. A pharmaceutical composition or vaccine administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least 1, 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 or 28 additional serotype comprising 1, 3, 4, 6A, 6B, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 15C,

Petitioner Merck Ex-1004, 0822

16F, 19F, 19A, 22F, 23A, 23B, 24F, 24B, 33F, 33B, 34, 35F, 35B, 38 or Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least 1, 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 or 30 additional serotype 1, 3, 4, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 22F, 23A, 23B, 23F, 24F, 24B, 33F, 33B, 34, 35F, 35B, 38 or Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least 13 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 31, 33F, 33B, 34, 35B, 38, and Serogroup 20 (comprising 20A and 20B), or a combination thereof. A pharmaceutical composition or vaccine administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least 14 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 31, 33F, 33B, 34, 35B, 38, and Serogroup 20 (comprising 20A and 20B), or a combination thereof. A pharmaceutical composition or vaccine administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least 15 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 31, 33F, 33B, 34, 35B, 38, and Serogroup 20 (comprising 20A and 20B), or a combination thereof. A pharmaceutical composition or vaccine administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least 16 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 31, 33F, 33B, 34, 35B, 38, and Serogroup 20 (comprising 20A and 20B), or a combination thereof. A pharmaceutical composition or vaccine administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least 17 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 31, 33F, 33B, 34, 35B, 38, and Serogroup 20 (comprising 20A and 20B) or a combination thereof. A pharmaceutical composition or vaccine administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising at least 18 of the serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 31, 33F, 33B, 34, 35B, 38, and Serogroup 20 (comprising 20A and 20B), or a combination thereof. A pharmaceutical composition or vaccine administered to a

Petitioner Merck Ex-1004, 0823

subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least 1, 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, or 36 additional serotypes comprising 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35F, 35B, 38, and Serogroup 20 (comprising 20A and 20B), or a combination thereof. A pharmaceutical composition or vaccine administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least 1, 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 or 28 additional serotype comprising 1, 3, 4, 6A, 6B, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 22F, 23A, 23B, 24F, 24B, 33F, 33B, 34, 35F, 35B, 38, or Serogroup 20 (comprising 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least 1, 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 or 30 additional serotype 1, 3, 4, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 22F, 23A, 23B, 23F, 24F, 24B, 33F, 33B, 34, 35F, 35B, 38, or Serogroup 20 (comprising 20A and 20B). A pharmaceutical composition or vaccine can comprise administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition or vaccine can comprise administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 additional serotypes selected from 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, 34, and 35B. A pharmaceutical composition or vaccine can comprise administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 20B, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition or vaccine can comprise administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 additional serotypes selected from 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 20B, 22F, 23A, 23B, 33F, 34, and 35B. A pharmaceutical composition or vaccine can comprise administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B,

Petitioner Merck Ex-1004, 0824

33F, 35B, and Serogroup 20 (comprising 20A and 20B). A pharmaceutical composition or vaccine can comprise administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 additional serotypes selected from 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, 35B, and Serogroup 20 (comprising 20A and 20B). A pharmaceutical composition or vaccine can comprise administered to a subject can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 3, 4, 7F, 8, 9N, 11A, 12F, 15A, 15B, 15C, 16F, 19A, 22F, 23A, 23B, and 35B. A pharmaceutical composition or vaccine administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 1, 3, 4, 6A, 7F, 8, 9N, 10A, 11A, 12F, 14, 19A, 19F, 22F, 23A, 23B, 23F, 24F, and 33F. A pharmaceutical composition or vaccine administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 3, 4, 6B, 10A, 15A, 15B, 19A, 19F, 22F, 23F, 24F, and 35B. A pharmaceutical composition or vaccine administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 9N, 15A, 15C, 16F, 23A, 23B, and 33B. A pharmaceutical composition or vaccine administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 9N, 15A, 15C, 16F, 23A, 23B, and 33F. A pharmaceutical composition or vaccine administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 15A, 15C, 16F, 23A, 23B, and 33B. A pharmaceutical composition or vaccine administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 15A, 15C, 16F, 23A, 23B, and 33F. A pharmaceutical composition or vaccine administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B. A pharmaceutical composition or vaccine administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 24F, 31, 33F, 34, 35F, and 35B. A pharmaceutical composition or vaccine administered to a subject can comprise can

Petitioner Merck Ex-1004, 0825

comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B. A pharmaceutical composition or vaccine administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 33F, 34, 35F, and 35B. A pharmaceutical composition or vaccine administered to a subject can comprise can comprise a plurality of saccharide-polypeptide conjugates comprising the serotype 6C and at least one additional serotype selected from 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20A, 22F, 23A, 23B, 33F, and 35B. A pharmaceutical composition or vaccine administered to a subject can comprise a plurality of at least 20 immunogenic saccharide-polypeptide conjugate comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *S. pneumoniae* selected from the group comprising 3, 6C, 7C, 8, 9N, 11A, 12F, 15A, 16F, 19A, 19F, 22F, 23A, 23B, 31, 33F, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise a plurality of at least 20 immunogenic saccharide-polypeptide conjugate comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *S. pneumoniae* selected from the group comprising 3, 6C, 7F, 7C, 8, 9N, 11A, 12F, 15A, 16F, 19A, 19F, 22F, 23A, 23B, 31, 33F, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise a plurality of at least 15 immunogenic saccharide-polypeptide conjugate comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *S. pneumoniae* selected from the group comprising 3, 6C, 7F, 7C, 8, 9N, 11A, 12F, 15A, 16F, 19A, 19F, 22F, 23A, 23B, 31, 33F, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise a plurality of at least 16 immunogenic saccharide-polypeptide conjugate comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *S. pneumoniae* selected from the group comprising 3, 6C, 7F, 7C, 8, 9N, 11A, 12F, 15A, 16F, 19A, 19F, 22F, 23A, 23B, 31, 33F, 35B, 35F, 38, and Serogroup 20

Petitioner Merck Ex-1004, 0826

(comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise a plurality of at least 15 or 16 immunogenic saccharide-polypeptide conjugate comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *S. pneumoniae* selected from the group comprising 3, 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 19A, 19F, 22F, 23A, 23B, 31, 33F, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise a plurality of at least 15 or 16 immunogenic saccharide-polypeptide conjugate comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *S. pneumoniae* selected from the group comprising 3, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 19A, 22F, 23A, 23B, 24F, 33F, 35B, and 38. A pharmaceutical composition or vaccine administered to a subject can comprise a plurality of at least 10 immunogenic saccharide-polypeptide conjugate comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *S. pneumoniae* selected from the group comprising 3, 8, 9N, 12F, 15A, 19A, 22F, 23A, 23B, and 24F. A pharmaceutical composition or vaccine administered to a subject can comprise a plurality of at least 11 immunogenic saccharide-polypeptide conjugate comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *S. pneumoniae* selected from the group comprising 3, 8, 9N, 10A, 12F, 15A, 19A, 22F, 23A, 23B, and 24F. A pharmaceutical composition or vaccine administered to a subject can comprise a plurality of at least 24 immunogenic saccharide-polypeptide conjugate comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *S. pneumoniae* selected from the group comprising 3, 4, 6C, 7C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 19A, 22F, 23A, 23B, 24F, 31, 33F, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise a plurality of at least 20 immunogenic saccharide-polypeptide conjugate comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination

Petitioner Merck Ex-1004, 0827

thereof is from a unique *S. pneumoniae* selected from the group comprising 3, 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 19A, 19F, 22F, 23A, 23B, 31, 33F, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise a plurality of at least 20, 21, 22, 23, 24, 25, 26, or 27 immunogenic saccharide-polypeptide conjugate comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *S. pneumoniae* selected from the group comprising 3, 6C, 7C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 19A, 19F, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise at least 20 serotypes selected from the group consisting of 3, 6C, 7C, 8, 9N, 11A, 12F, 15A, 16F, 19A, 19F, 22F, 23A, 23B, 31, 33F, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise at least 20 serotypes selected from the group consisting of 3, 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 19A, 19F, 22F, 23A, 23B, 31, 33F, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise at least 20 serotypes selected from the group consisting of 3, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 19A, 22F, 23A, 23B, 24F, 33F, 35B, and 38. A pharmaceutical composition or vaccine administered to a subject can comprise at least 10 serotypes selected from the group consisting of 3, 8, 9N, 12F, 15A, 19A, 22F, 23A, 23B, and 24F. A pharmaceutical composition or vaccine administered to a subject can comprise at least 11 serotypes selected from the group consisting of 3, 8, 9N, 10A, 12F, 15A, 19A, 22F, 23A, 23B, and 24F. A pharmaceutical composition or vaccine administered to a subject can comprise at least 24 serotypes selected from the group consisting of 3, 4, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 19A, 22F, 23A, 23B, 24F, 31, 33F, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise at least 20 serotypes selected from the group consisting of 3, 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 19A, 19F, 22F, 23A, 23B, 31, 33F, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). A pharmaceutical composition or vaccine administered to a subject can comprise at least 20, 21, 22, 23, 24, 25, 26, or 27 serotypes selected from the group consisting of 3, 6C, 7C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 19A, 19F, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B).

Petitioner Merck Ex-1004, 0828

[0087] Each dosage can be formulated in 0.5 mL. The formulation can comprise normal saline. The formulation can have a pH of 6.5. The formulation can be a pH from 6.3 to 6.7 or from 6.2 to 6.8. The formulation can have a pH of 5.8. The formulation can be a pH from 5.5 to 6.1 or from 5.6 to 6.0. The formulation can comprise 0.001% to 0.01% polysorbate 80.

[0088] A first composition and second composition can be administered to a subject. The first composition and the second composition can be distinct. The first composition can be a first pharmaceutical composition or vaccine. The second composition can be a second pharmaceutical composition or vaccine. The first pharmaceutical composition or vaccine can be administered to the subject at least about four weeks, six weeks, two months, six months, eight months, one year, two years, or five years before or at least about four weeks, six weeks, two months, six months, eight months, one year, two years, or five years after an administration to the subject a second pharmaceutical composition or vaccine. The first pharmaceutical composition or vaccine can be administered to a subject who is at least 4 weeks old, 6 weeks old, 12 weeks old, 24 weeks old, 6 months old, 8 months old, 10 months old, 12 months old, 14 months old, 15 months old, 16 months old, 17 months old, 18 months old, 19 months old, 20 months old, 21 months old, 22 months old, 23 months old, 2 years old or older. The first pharmaceutical composition or vaccine can be administered to a subject with an age from 4 weeks to 2 years old. The first pharmaceutical composition or vaccine can be administered to a subject with an age from 18 years old to 100 years old. The first pharmaceutical composition can be administered to a subject with an age of 100 years old or older. In some embodiments, the second pharmaceutical composition or vaccine can comprise a saccharide-polypeptide conjugate comprising a polypeptide that has at least about 70%, 80%, 90%, 95%, 99%, or 100% length homology, about 70%, 80%, 90%, 95%, 99%, or 100% sequence homology, or a combination thereof to a polypeptide of a saccharide-polypeptide conjugate of the first pharmaceutical composition or vaccine. For example, the polypeptide in a saccharide-conjugate of the first pharmaceutical composition or vaccine and the polypeptide in a saccharide-conjugate of the second pharmaceutical composition or vaccine can be CRM_{197}. In other embodiments, the second pharmaceutical composition or vaccine can comprise a saccharide-polypeptide conjugate comprising a polypeptide that has at less than 70% length homology, 70% sequence homology, or a combination thereof to a polypeptide of a saccharide-polypeptide conjugate of the first pharmaceutical composition.

[0089] In some embodiments, the first pharmaceutical composition or vaccine can comprise a saccharide-polypeptide conjugate comprising a capsular polysaccharide or immunogenic fragment thereof from a serotype of *S. pneumoniae* conjugated to a polypeptide, and wherein

Petitioner Merck Ex-1004, 0829

the serotype is not at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13, of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, or 23F; the serotype is at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B); or a combination thereof. The first pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 33B, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). The first pharmaceutical composition or vaccine can comprise at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 23, or 24 serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). In some embodiments, the first pharmaceutical composition or vaccine can comprise a saccharide-polypeptide conjugate comprising a capsular polysaccharide or immunogenic fragment thereof from a serotype of *S. pneumoniae* conjugated to a polypeptide, and wherein the serotype is not at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13, of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, or 23F; the serotype is at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B,34, 35F, 35B, 38, and Serogroup 20 (comprising 20A and 20B); or a combination thereof. The first pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 33B, 34, 35B, 35F, 38, and Serogroup 20 (comprising 20A and 20B). The first pharmaceutical composition can comprise at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 23, or 24 serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35B, 35F, 38, and Serogroup 20 (comprising 20A and 20B).The first pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. The first pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 24F, 33F, and 35B. The first pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F,

Petitioner Merck Ex-1004, 0830

33F, and 35B. The first pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B, and 38. The first pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B. The first pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20B, 22F, 23A, 23B, 33F, and 35B. The first pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20A, 22F, 23A, 23B, 33F, and 35B. The first pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 9N, 15A, 15C, 16F, 23A, 23B, and 33B. The first pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 9N, 15A, 15C, 16F, 23A, 23B, and 33F. The first pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 15A, 15C, 16F, 23A, 23B, and 33B. The first pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 15A, 15C, 16F, 23A, 23B, and 33F. The first pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B. . The first pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 24F, 31, 33F, 34, 35F, and 35B. The first pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B. The first pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. The first pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B. The first pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B. The first pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B and 38. The first

Petitioner Merck Ex-1004, 0831

pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B. The first pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B. In other embodiments, the first pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising a serotype that is at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. The first pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. The first pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 3, 6C, 7C, 8, 9N, 11A, 12F, 15A, 16F, 19F, 19A, 22F, 23A, 23B, 31, 33F, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B). The first pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 3, 6C, 7F, 7C, 8, 9N, 11A, 12F, 15A, 16F, 19F, 19A, 22F, 23A, 23B, 31, 33F, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B). The first pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 3, 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 19A, 19F, 22F, 23A, 23B, 31, 33F, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B). The first pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 3, 8, 9N, 12F, 15A, 19A, 22F, 23A, 23B, and 24F. The first pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 3, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 19A, 22F, 23A, 23B, 24F, 33F, 35B, and 38. The first pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 3, 8, 9N, 10A, 12F, 15A, 19A, 22F, 23A, 23B, and 24F. The first pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 3, 4, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 19A, 22F, 23A, 23B, 24F, 31, 33F, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B). The first pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 3, 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 19A, 19F, 22F, 23A, 23B, 31, 33F, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B). When administering any of the first pharmaceutical compositions or vaccines described

Petitioner Merck Ex-1004, 0832

above, the first pharmaceutical composition or vaccine can be distinct from the second pharmaceutical composition or vaccine administered to a subject.

[0090] In some embodiments, the second pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising a serotype that is at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. The second pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. The second pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising a serotype that is at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 33B, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B), wherein the serotype of the first pharmaceutical composition or vaccine and the serotype of the second pharmaceutical composition or vaccine are distinct. The second pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising a serotype that is at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F,33B, 34, 35B, 35F, 38, and Serogroup 20 (comprising 20A and 20B), wherein the serotype of the first pharmaceutical composition or vaccine and the serotype of the second pharmaceutical composition or vaccine are distinct. The second pharmaceutical composition or vaccine can comprise a saccharide-polypeptide conjugate comprising a capsular polysaccharide or immunogenic fragment thereof from a serotype of *S. pneumoniae* conjugated to a polypeptide, and wherein the serotype is not at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13, of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, or 23F; and the serotype is at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B); wherein the serotype of the first pharmaceutical composition or vaccine and the serotype of the second pharmaceutical composition or vaccine are distinct. The second pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 33B, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B), wherein the serotype of the first pharmaceutical composition or vaccine and the serotype of the second pharmaceutical composition or vaccine are distinct. The second pharmaceutical composition or vaccine can comprise at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 serotypes selected from the group

Petitioner Merck Ex-1004, 0833

consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B), wherein the serotype of the first pharmaceutical composition or vaccine and the serotype of the second pharmaceutical composition or vaccine are distinct. The second pharmaceutical composition or vaccine can comprise a saccharide-polypeptide conjugate comprising a capsular polysaccharide or immunogenic fragment thereof from a serotype of *S. pneumoniae* conjugated to a polypeptide, and wherein the serotype is not at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13, of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, or 23F; and the serotype is at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35F, 35B, 38, and Serogroup 20 (comprising 20A and 20B); wherein the serotype of the first pharmaceutical composition or vaccine and the serotype of the second pharmaceutical composition or vaccine are distinct. The second pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24B, 24F, 31, 33F, 33B, 34, 35B, 35F, 38, and Serogroup 20 (comprising 20A and 20B), wherein the serotype of the first pharmaceutical composition or vaccine and the serotype of the second pharmaceutical composition or vaccine are distinct. The second pharmaceutical composition or vaccine can comprise at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 serotypes selected from the group consisting of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35B, 35F, 38, and Serogroup 20 (comprising 20A and 20B), wherein the serotype of the first pharmaceutical composition or vaccine and the serotype of the second pharmaceutical composition or vaccine are distinct. The second pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B, wherein the serotype of the first pharmaceutical composition or vaccine and the serotype of the second pharmaceutical composition or vaccine are distinct. The second pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 24F, 33F, and 35B, wherein the serotype of the first pharmaceutical composition or vaccine and the serotype of the second pharmaceutical composition or vaccine are distinct. The second pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B, wherein the

Petitioner Merck Ex-1004, 0834

serotype of the first pharmaceutical composition or vaccine and the serotype of the second pharmaceutical composition or vaccine are distinct. The second pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B, and 38, wherein the serotype of the first pharmaceutical composition or vaccine and the serotype of the second pharmaceutical composition or vaccine are distinct. The second pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B, wherein the serotype of the first pharmaceutical composition or vaccine and the serotype of the second pharmaceutical composition or vaccine are distinct. The second pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20B, 22F, 23A, 23B, 33F, and 35B, wherein the serotype of the first pharmaceutical composition or vaccine and the serotype of the second pharmaceutical composition or vaccine are distinct. The second pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20A, 22F, 23A, 23B, 33F, and 35B, wherein the serotype of the first pharmaceutical composition or vaccine and the serotype of the second pharmaceutical composition or vaccine are distinct. The second pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 9N, 15A, 15C, 16F, 23A, 23B, and 33B, wherein the serotype of the first pharmaceutical composition or vaccine and the serotype of the second pharmaceutical composition or vaccine are distinct. The second pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 9N, 15A, 15C, 16F, 23A, 23B, and 33F, wherein the serotype of the first pharmaceutical composition or vaccine and the serotype of the second pharmaceutical composition or vaccine are distinct. The second pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 15A, 15C, 16F, 23A, 23B, and 33B, wherein the serotype of the first pharmaceutical composition or vaccine and the serotype of the second pharmaceutical composition or vaccine are distinct. The second pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 15A, 15C, 16F, 23A, 23B, and 33F, wherein the serotype of the first pharmaceutical composition or vaccine and the serotype of the second pharmaceutical composition or vaccine are distinct. The second pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates

Petitioner Merck Ex-1004, 0835

comprising the serotypes 6C, 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B, wherein the serotype of the first pharmaceutical composition or vaccine and the serotype of the second pharmaceutical composition or vaccine are distinct. The second pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 24F, 31, 33F, 34, 35F, and 35B, wherein the serotype of the first pharmaceutical composition or vaccine and the serotype of the second pharmaceutical composition or vaccine are distinct. The second pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B, wherein the serotype of the first pharmaceutical composition or vaccine and the serotype of the second pharmaceutical composition or vaccine are distinct. The second pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 33F, 34, 35F, and 35B, wherein the serotype of the first pharmaceutical composition or vaccine and the serotype of the second pharmaceutical composition or vaccine are distinct. The second pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B, wherein the serotype of the first pharmaceutical composition or vaccine and the serotype of the second pharmaceutical composition or vaccine are distinct. The second pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B, wherein the serotype of the first pharmaceutical composition or vaccine and the serotype of the second pharmaceutical composition or vaccine are distinct. The second pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B, wherein the serotype of the first pharmaceutical composition or vaccine and the serotype of the second pharmaceutical composition or vaccine are distinct. The second pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B, and 38, wherein the serotype of the first pharmaceutical composition or vaccine and the serotype of the second pharmaceutical composition or vaccine are distinct. The second pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B, wherein the serotype of the first pharmaceutical composition or

Petitioner Merck Ex-1004, 0836

vaccine and the serotype of the second pharmaceutical composition or vaccine are distinct. The second pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B, wherein the serotype of the first pharmaceutical composition or vaccine and the serotype of the second pharmaceutical composition or vaccine are distinct. The second pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 3, 6C, 7C, 8, 9N, 11A, 12F, 15A, 16F, 19F, 19A, 22F, 23A, 23B, 31, 33F, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B), wherein the serotype of the first pharmaceutical composition or vaccine and the serotype of the second pharmaceutical composition or vaccine are distinct.. The second pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 3, 6C, 7F, 7C, 8, 9N, 11A, 12F, 15A, 16F, 19F, 19A, 22F, 23A, 23B, 31, 33F, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B), wherein the serotype of the first pharmaceutical composition or vaccine and the serotype of the second pharmaceutical composition or vaccine are distinct. The second pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 3, 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 19A, 19F, 22F, 23A, 23B, 31, 33F, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B), wherein the serotype of the first pharmaceutical composition or vaccine and the serotype of the second pharmaceutical composition or vaccine are distinct. The second pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 3, 8, 9N, 12F, 15A, 19A, 22F, 23A, 23B, and 24F, wherein the serotype of the first pharmaceutical composition or vaccine and the serotype of the second pharmaceutical composition or vaccine are distinct. The second pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 3, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 19A, 22F, 23A, 23B, 24F, 33F, 35B, and 38, wherein the serotype of the first pharmaceutical composition or vaccine and the serotype of the second pharmaceutical composition or vaccine are distinct. The second pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 3, 8, 9N, 10A, 12F, 15A, 19A, 22F, 23A, 23B, and 24F, wherein the serotype of the first pharmaceutical composition or vaccine and the serotype of the second pharmaceutical composition or vaccine are distinct. The second pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 3, 4, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F,

Petitioner Merck Ex-1004, 0837

19A, 22F, 23A, 23B, 24F, 31, 33F, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B), wherein the serotype of the first pharmaceutical composition or vaccine and the serotype of the second pharmaceutical composition or vaccine are distinct. The second pharmaceutical composition or vaccine can comprise a plurality of saccharide-polypeptide conjugates comprising the serotypes 3, 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 19A, 19F, 22F, 23A, 23B, 31, 33F, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B), wherein the serotype of the first pharmaceutical composition or vaccine and the serotype of the second pharmaceutical composition or vaccine are distinct.

[0091] In other embodiments, the first pharmaceutical composition or vaccine and the second pharmaceutical composition or vaccine can be administered at least about 4 weeks apart, at least 6 about weeks apart, at least 2 about months apart, at a least about 3 months apart, at least about 4 months apart, at least about 5 months apart, at least 6 months apart, at least about 7 months apart, at least about 8 months apart, at least about 9 months apart, at least about 10 months apart, at least 11 about months apart, at least about 1 year apart, at least about 1.5 years apart, at least about 2 years apart, at least about 5 years apart, at least about 10 years apart, at least about 15 year, at least about 20 years apart, or at least about 30 years apart.

[0092] In some embodiments, a serotype of the first pharmaceutical composition or vaccine can be from a different bacteria than the serotype of the second pharmaceutical composition or vaccine. For example, the serotype of the first composition or vaccine can be from *Streptococcus pneumoniae*, and the serotype of the second composition or vaccine can be from *Neisseria meningitidis*, *Salmonella enterica*, or *Haemophilus influenzae*. The serotype of the first pharmaceutical composition or vaccine can be from *Neisseria meningitidis*, *Salmonella enterica*, or *Haemophilus influenzae*, and the serotype of the second pharmaceutical composition or vaccine can be from *Streptococcus pneumoniae*.

[0093] In some embodiments, a serotype of the first pharmaceutical composition or vaccine can be from the same bacteria as a serotype for the second pharmaceutical composition or vaccine. For example, the first pharmaceutical composition or vaccine can comprise at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second pharmaceutical composition or vaccine can comprise at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F 38 and Serogroup 20 (comprising serotypes 20A and 20B) from *Streptococcus pneumoniae*. The first pharmaceutical composition or vaccine can comprise at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F,

Petitioner Merck Ex-1004, 0838

24B, 31, 33F,33B, 34, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B), from *Streptococcus pneumoniae*, and the second pharmaceutical composition or vaccine can comprise at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*. The first pharmaceutical composition or vaccine can comprise at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second pharmaceutical composition or vaccine can comprise at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B) from *Streptococcus pneumoniae*. The first pharmaceutical composition or vaccine can comprise at least one of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B) from *Streptococcus pneumoniae*, and the second pharmaceutical composition or vaccine can comprise at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*. The first pharmaceutical composition or vaccine can comprise at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second pharmaceutical composition or vaccine comprises at least one of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B from *Streptococcus pneumoniae*. The first pharmaceutical composition or vaccine can comprise at least one of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B from *Streptococcus pneumoniae*, and the second pharmaceutical composition or vaccine can comprise at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*. The first pharmaceutical composition or vaccine can comprise at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second pharmaceutical composition or vaccine comprises at least one of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, 35B and Serogroup 20 (comprising serotypes 20A and 20B) from *Streptococcus pneumoniae*. The first pharmaceutical composition or vaccine can comprise at least one of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, 35B, and Serogroup 20 (comprising serotypes 20A and 20B) from *Streptococcus pneumoniae*, and the second pharmaceutical composition or vaccine can comprise at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*. The first pharmaceutical composition or vaccine can comprise at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second pharmaceutical composition or vaccine can comprise at least one of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F,

Petitioner Merck Ex-1004, 0839

35B, and Serogroup 20 (comprising serotypes 20A and 20B) from *Streptococcus pneumoniae*. The first pharmaceutical composition or vaccine can comprise at least one of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, 35B and Serogroup 20 (comprising serotypes 20A and 20B) from *Streptococcus pneumoniae*, and the second pharmaceutical composition or vaccine can comprise at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*. The first pharmaceutical composition or vaccine can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second pharmaceutical composition or vaccine can comprise the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B from *Streptococcus pneumoniae*. The first pharmaceutical composition or vaccine can comprise the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B from *Streptococcus pneumoniae*, and the second pharmaceutical composition or vaccine can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*. The first pharmaceutical composition or vaccine can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second pharmaceutical composition or vaccine can comprise the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 20B, 22F, 23A, 23B, 33F, and 35B from *Streptococcus pneumoniae*. The first pharmaceutical composition or vaccine can comprise the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 20B, 22F, 23A, 23B, 33F, and 35B from *Streptococcus pneumoniae*, and the second pharmaceutical composition or vaccine can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*. The first pharmaceutical composition or vaccine can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second pharmaceutical composition or vaccine can comprise the serotypes 6C, 9N, 15A, 15C, 16F, 23A, 23B, and 33B from *Streptococcus pneumoniae*. The first pharmaceutical composition or vaccine can comprise the serotypes 6C, 9N, 15A, 15C, 16F, 23A, 23B, and 33B from *Streptococcus pneumoniae*, and the second pharmaceutical composition or vaccine can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*. The first pharmaceutical composition or vaccine can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second pharmaceutical composition or vaccine can comprise the serotypes 6C, 15A, 15C, 16F, 23A, 23B, and 33B from *Streptococcus pneumoniae*. The first pharmaceutical composition or vaccine can comprise the serotypes 6C, 15A, 15C, 16F, 23A, 23B, and 33B

Petitioner Merck Ex-1004, 0840

from *Streptococcus pneumoniae*, and the second pharmaceutical composition or vaccine can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*. The first pharmaceutical composition or vaccine can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second pharmaceutical composition or vaccine can comprise the serotypes 6C, 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B from *Streptococcus pneumoniae*. The first pharmaceutical composition or vaccine can comprise the serotypes 6C, 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B from *Streptococcus pneumoniae*, and the second pharmaceutical composition or vaccine can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*. The first pharmaceutical composition or vaccine can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second pharmaceutical composition or vaccine can comprise the serotypes 6C, 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B from *Streptococcus pneumoniae*. The first pharmaceutical composition or vaccine can comprise the serotypes 6C, 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 33B, 34, 35F, and 35B from *Streptococcus pneumoniae*, and the second pharmaceutical composition or vaccine can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*. The first pharmaceutical composition or vaccine can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second pharmaceutical composition or vaccine can comprise the serotypes 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20A, 22F, 23A, 23B, 33F, and 35B from *Streptococcus pneumoniae*. The first pharmaceutical composition or vaccine can comprise the serotypes 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20A, 22F, 23A, 23B, 33F, and 35B from *Streptococcus pneumoniae*, and the second pharmaceutical composition or vaccine can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*. The first pharmaceutical composition or vaccine can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second pharmaceutical composition or vaccine can comprise the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, 35B, and Serogroup 20 (comprising serotypes 20A and 20B) from *Streptococcus pneumoniae*. The first pharmaceutical composition or vaccine can comprise the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, 35B, and Serogroup 20 (comprising serotypes 20A and 20B) from *Streptococcus pneumoniae*, and the second

Petitioner Merck Ex-1004, 0841

pharmaceutical composition or vaccine can comprise the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*.

[0094] Different serotypes of *S. pneumoniae* can be more pervasive or endemic in different geographical areas. For example, a certain group of serotypes can commonly cause pneumococcal disease in the United States and Canada. This group of serotypes can be different for Europe, the Middle East, North Africa, Japan and Korea. For example, administration of a pharmaceutical composition or vaccine to a subject comprising a plurality of saccharide-polypeptide conjugates comprising the serotypes 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 19F, 19A, 20A, 22F, 23A, 23B, 33F, 35F, 35B, and 38 can improve protection against *S. pneumoniae* disease for the subject in the United States and/or Canada. Administration of a pharmaceutical composition or vaccine to a subject comprising a plurality of saccharide-polypeptide conjugates comprising the serotypes 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 19F, 19A, 22F, 23A, 23B, 33F, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B) can improve protection against *S. pneumoniae* disease for the subject in the United States and/or Canada. Administration of a pharmaceutical composition or vaccine to a subject comprising a plurality of saccharide-polypeptide conjugates comprising the serotypes 3, 6C, 7C, 8, 9N, 11A, 12F, 15A, 16F, 19F, 19A, 22F, 23A, 23B, 31, 33F, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B) can improve protection against *S. pneumoniae* disease for the subject in the United States. Administration of a pharmaceutical composition or vaccine to a subject comprising a plurality of saccharide-polypeptide conjugates comprising the serotypes 3, 6C, 7F, 7C, 8, 9N, 11A, 12F, 15A, 16F, 19F, 19A, 22F, 23A, 23B, 31, 33F, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B) can improve protection against *S. pneumoniae* disease for the subject in the United States. Administration of a pharmaceutical composition or vaccine to a subject comprising a plurality of saccharide-polypeptide conjugates comprising the serotypes 3, 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 19A, 19F, 22F, 23A, 23B, 31, 33F, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B) can improve protection against *S. pneumoniae* disease for the subject in the United States. Administration of a pharmaceutical composition or vaccine to a subject comprising a plurality of saccharide-polypeptide conjugates comprising the serotypes 3, 8, 9N, 12F, 15A, 19A, 22F, 23A, 23B, and 24F can improve protection against *S. pneumoniae* disease for the subject in the United States. Administration of a pharmaceutical composition or vaccine to a subject comprising a plurality of saccharide-polypeptide conjugates comprising the serotypes 3, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 19A, 22F,

Petitioner Merck Ex-1004, 0842

23A, 23B, 24F, 33F, 35B, and 38 can improve protection against *S. pneumoniae* disease for the subject in the United Kingdom. Administration of a pharmaceutical composition or vaccine to a subject comprising a plurality of saccharide-polypeptide conjugates comprising the serotypes 3, 8, 9N, 10A, 12F, 15A, 19A, 22F, 23A, 23B, and 24F can improve protection against *S. pneumoniae* disease for the subject in the United Kingdom or in Europe. Administration of a pharmaceutical composition or vaccine to a subject comprising a plurality of saccharide-polypeptide conjugates comprising the serotypes 3, 4, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 19A, 22F, 23A, 23B, 24F, 31, 33F, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B) can improve protection against *S. pneumoniae* disease for the subject in Germany. Administration of a pharmaceutical composition or vaccine to a subject comprising a plurality of saccharide-polypeptide conjugates comprising the serotypes 3, 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 19A, 19F, 22F, 23A, 23B, 31, 33F, 35B, 35F, 38 and Serogroup 20 (comprising serotypes 20A and 20B) can improve protection against *S. pneumoniae* disease for the subject in Germany. Administration of a pharmaceutical composition or vaccine to a subject comprising a plurality of saccharide-polypeptide conjugates comprising the serotypes 1, 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 16F, 18C, 19F, 19A, 22F, 23F, 23A, 23B, 24F, 33F, 35F, 35B, and 38 can improve protection against *S. pneumoniae* disease for the subject in the Europe, the Middle East, and/or North Africa. Administration of a pharmaceutical composition or vaccine to a subject comprising a plurality of saccharide-polypeptide conjugates comprising the serotypes 3, 4, 6A, 6B, 6C, 7F, 10A, 11A, 14, 15A, 15B, 15C, 19F, 19A, 22F, 23F, 23A, 24F, 35F, 35B, and 38 can improve protection against *S. pneumoniae* disease for the subject in the Japan and/or Korea. Additionally, invasive clinical disease can be caused by serotype 20B (as opposed to serotype 20A). The subject can be a human who resides in the specific geographical location as indicated above, or can be a human who is visiting the specific geographical location as indicated above.

[0095] A method of administration can comprise administering a first saccharide-polypeptide conjugate composition to a subject; administering a second saccharide-polypeptide conjugate composition to the subject at least four weeks after administering the first saccharide-polypeptide conjugate composition; wherein the first saccharide-polypeptide conjugate composition comprises a first polypeptide that has at least about 70%, 80%, 90%, 95%, 99%, or 100% length homology, about 70%, 80%, 90%, 95%, 99%, or 100% sequence homology, or a combination thereof to a second polypeptide of the second saccharide-polypeptide conjugate composition; and wherein the first saccharide-polypeptide conjugate composition

Petitioner Merck Ex-1004, 0843

comprises a first serotype; wherein the second saccharide-polypeptide conjugate composition comprises a second serotype, and wherein the first serotype and the second serotype are different. In some embodiments, the first serotype is from the same bacteria as the second serotype. For example, the polypeptide in a saccharide-conjugate of the first pharmaceutical composition and the polypeptide in a saccharide-conjugate of the second pharmaceutical composition can be CRM_{197}. In other embodiments, the second pharmaceutical composition comprises a saccharide-polypeptide conjugate comprising a polypeptide that has at less than about 70% length homology, about 70% sequence homology, or a combination thereof to a polypeptide of a saccharide-polypeptide conjugate of the first pharmaceutical composition. The first saccharide-polypeptide conjugate composition can be a vaccine comprising a plurality of unique immunogenic saccharide-polypeptide conjugates that can comprise individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof can be from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 23A and 23B in a vaccine. The first saccharide-polypeptide conjugate composition can be a vaccine comprising at least 2 immunogenic saccharide-polypeptide conjugates that can each comprise individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof can be from a unique *Streptococcus pneumoniae* serotype group, wherein one serotype can be 23A and at least one additional serotype can be selected from the group consisting of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 20B, 22F, 23B, 24F, 24B, 31, 33F, 33B, 34, 35B, 35F, and 38. The first saccharide-polypeptide conjugate composition can be a vaccine comprising at least 2 immunogenic saccharide-polypeptide conjugates that can each comprise individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof can be from a unique *Streptococcus pneumoniae* serotype group, wherein one serotype can be 23B and at least one additional serotype can be selected from the group consisting of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 20B, 22F, 23A, 24F, 24B, 31, 33F, 33B, 34, 35B, 35F, and 38. The second saccharide-polypeptide conjugate composition can be a vaccine comprising a plurality of unique immunogenic saccharide-polypeptide conjugates that can comprise individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or

Petitioner Merck Ex-1004, 0844

combination thereof can be from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 23A and 23B. The second saccharide-polypeptide conjugate composition be a vaccine comprising at least 2 immunogenic saccharide-polypeptide conjugates that can each comprise individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof can be from a unique *Streptococcus pneumoniae* serotype group, wherein one serotype can be 23A and at least one additional serotype can be selected from the group consisting of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 20B, 22F, 23B, 24F, 24B, 31, 33F, 33B, 34, 35B, 35F, and 38, in a vaccine. The second saccharide-polypeptide conjugate composition can be a vaccine comprising at least 2 immunogenic saccharide-polypeptide conjugates that can each comprise individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide, wherein the capsular polysaccharide, fragment thereof, or combination thereof can be from a unique *Streptococcus pneumoniae* serotype group, wherein one serotype can be 23B and at least one additional serotype can be selected from the group consisting of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 20B, 22F, 23A, 24F, 24B, 31, 33F, 33B, 34, 35B, 35F, and 38. The first saccharide-polypeptide conjugate composition and the second saccharide-polypeptide conjugate composition can be distinct.

[0096] In other embodiments, the conducting of any of the methods described herein may not result in more than about: 50%, 40%, 30%, 20%, 10%, 5%, 4%, 3%, 2%, or 1% suppression of an immunity conveyed by a first vaccine containing an immunogenic saccharide-polypeptide conjugate, as measured by antibody response to the saccharide of the immunogenic saccharide-polypeptide conjugate, wherein the antibody response is measured by measuring antibody titers.

Saccharide-polypeptide Conjugate Kits

[0097] The saccharide-polypeptide conjugates, pharmaceutical compositions, and vaccines described herein can be contained in a kit. The kit can further comprise instructions for use. The saccharide-polypeptide conjugates and/or pharmaceutical compositions and/or vaccines can be stored in a container. The container can be a syringe. The container can be a vial. The vial can be a 2 mL borosilicate glass vial. The vial can be 3 mL Vials USP Type I glass, non-siliconized. The top can be an aluminum crimp top. The container can be a vial with a rubber septum or a septum made of flexible, reclose-able material. The container can be a vial with a

Petitioner Merck Ex-1004, 0845

stopper. The stopper can be a non-siliconized stopper. The vial can have a fill volume of 0.6 mL. The extractable volume can be 0.5 mL.

EXAMPLES

[0098] The following examples are included to further describe some aspects of the present disclosure, and should not be used to limit the scope of the embodiments described herein.

EXAMPLE 1

Method of Establishing a Cell Bank

[0099] This example shows the establishment of a cell bank for a *S. pneumoniae* strain for use in producing polysaccharides for a polysaccharide-CRM_{197} conjugate vaccine. Any strain that produces the polysaccharides of **TABLE 1** or of **FIGS. 10A-10F** (e.g., polysaccharides from serotypes 1, 2, 3, 4, 5, 6A, 6B, 7C, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, or 38 and Serogroup 20 (comprising serotypes 20A and 20B)) are established as a cell bank by this method. A *S. pneumoniae* strain is provided by either the Center for Disease Control and Prevention (CDC), United States or the Culture Collection, University of Gothenburg (CCUG). The cell bank process is depicted in **FIG. 2**. A vial of a strain of *S. pneumoniae* is seeded and then is grown overnight on an agar plate. A single colony is picked from this plate and streaked onto a new agar plate for overnight growth. This process is repeated three times to purify the strain before a flask is seeded and is incubated. After allowing the seeded flask sufficient time for growth, the flask contents are harvested by centrifugation. The centrifugation product is resuspended in a medium of about 20% glycerol and then is dispensed into cryogenic vials. The cell bank is contained in the cryogenic vials, which are stored at -70°C until use.

[0100] *S. pneumoniae* strains that produce serotype 6C polysaccharide was acquired by the University of Alabama, Birmingham (UAB) for establishing a 6C cell bank. The cell bank process is depicted in **FIG. 2**. For 6C polysaccharide serotype, a vial of the strain of *S. pneumoniae* is seeded and then is grown overnight on an agar plate. A single colony is picked from this plate and streaked onto a new agar plate for overnight growth. This process is repeated three times to purify the strain before a flask is seeded and is incubated. After allowing the seeded flask sufficient time for growth, the flask contents are harvested by centrifugation. The centrifugation product is resuspended in a medium of about 20% glycerol

Petitioner Merck Ex-1004, 0846

and then is dispensed into cryogenic vials. The cell bank for each strain are contained in the cryogenic vials, which are stored at -70°C until use.

[0101] *S. pneumoniae* strains that produce serotype 8 polysaccharide, serotype 10A polysaccharide, serotype 11A polysaccharide, serotype 12F polysaccharide, serotype 22F polysaccharide, or serotype 33F polysaccharide were acquired by either the Center for Disease Control and Prevention (CDC), United States or the Culture Collection, University of Gothenburg (CCUG) for establishing a cell bank. The cell bank process is depicted in **FIG. 2**. For each polysaccharide serotype, a vial of the strain of *S. pneumoniae* was seeded and then was grown overnight on an agar plate. A single colony was picked from this plate and streaked onto a new agar plate for overnight growth. This process was repeated three times to purify the strain before a flask was seeded and was incubated. After allowing the seeded flask sufficient time for growth, the flask contents were harvested by centrifugation. The centrifugation product was resuspended in a medium of about 20% glycerol and then was dispensed into cryogenic vials. The cell bank for each strain were contained in the cryogenic vials, which were stored at -70°C until use.

EXAMPLE 2

Method of Isolating and Purifying Polysaccharides

[0102] This examples shows how polysaccharides are isolated and purified from cell banks for use in a polysaccharide-CRM_{197} conjugate vaccine. Any of the polysaccharides of **TABLE 1** or of **FIGS. 10A-10F** (e.g., polysaccharides from serotypes 1, 2, 3, 4, 5, 6A, 6B, 7C, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33B, 33F, 34, 35F, 35B, or 38 and Serogroup 20 (comprising serotypes 20A and 20B)) are isolated and purified by this method. This process for the isolation and purification of polysaccharides is shown in **FIG. 4**. A vial of a cell bank produced as described in **EXAMPLE 1** is used to inoculate a flask containing an appropriate culture medium and incubated overnight. This culture is used to seed a new culture for fermentation in a fermenter. The culture is allowed to grow under the appropriate conditions for that strain. After fermentation, the cells are inactivated and then are treated with deoxycholate to break open the cells and release the capsular polysaccharide. Subsequently, debris is removed by centrifugation, which is followed by a depth filter step through a 0.45 µm filter and the cell free broth is collected. The product is then purified and the polysaccharide size is determined. Quality control tests are performed on the product, such as a purity assay and confirmation of strain inactivation (no growth after plating).

Petitioner Merck Ex-1004, 0847

[0103] A cell bank of an *S. pneumoniae* strain that produces serotype 8, serotype 10A, serotype 11A, serotype 12F, serotype 22F, or serotype 33F was used to inoculate a flask containing the appropriate culture medium and incubated overnight. This culture was used to seed a new culture for fermentation in a fermenter. The culture was allowed to grow under the appropriate conditions for the specific strain. After fermentation, the cells were inactivated and then were treated with deoxycholate to break open the cells and release the capsular polysaccharide. Subsequently, debris was removed by centrifugation, which was followed by a depth filter step through a 0.45 μm filter and the cell free broth was collected. The product was then purified and the polysaccharide size was determined. Quality control tests were performed on the product, such as a purity assay and confirmation of strain inactivation (no growth after plating).

EXAMPLE 3

Method of CRM_{197} production

[0104] This example shows the production of CRM_{197} (SEQ ID NO: 1) for use in saccharide polypeptide vaccines, which is depicted in **FIG. 3.** A vial from a working cell bank of CRM_{197} was seeded into a flask containing the appropriate culture medium and incubated overnight. The fermentation culture was centrifuged and the contents were harvested, were clarified, and then were filtered through a 0.2 μm filter. The filtrate was concentrated about ten-fold, and then was diafiltered to exchange the buffer and remove contaminants. The protein was further purified by microfiltration using an appropriate pore size membrane. Then the filtrate was further purified by anion exchange chromatography, followed by ammonium sulfate precipitation, and filtration (0.45 μm). A final purification using hydrophobic chromatography was conducted, and the highly purified CRM_{197} protein was concentrated and diafiltered, and then filtered through a 0.2 μm filter. The product was stored at -70°C until use in conjugation with a polysaccharide, such as any polysaccharide listed in **TABLE 1** or of **FIGS. 10A-10F** (e.g., polysaccharides from serotypes 1, 2, 3, 4, 5, 6A, 6B, 7C, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B)). Quality control tests were performed on the product to assess concentration (BCA for Lowry NLT 15 mg/mL), purity (SDS-PAGE gel with a target of ≥ 95% pure), confirming CRM_{197} identification (using a monoclonal antibody), assessing size (SDS-Page with a target of ~58 kDa), assessing endotoxin level (LAL kit with target of <1 IU/μg of protein), assessing lack of toxicity (Vero Cytotoxicity or DT enzyme test; non-lethal

Petitioner Merck Ex-1004, 0848

in Guinea Pigs), assessing pH (meter, target of 6.5 ± 0.2), and assessing appearance (visually, target for clear liquid).

EXAMPLE 4

Method of conjugation

[0105] This example shows the conjugation of a capsular polysaccharide to a polypeptide via CDAP conjugation chemistry. The polysaccharide are isolated as described in **EXAMPLE 2** and are thawed. The polypeptide is chosen from any of the following: diphtheria and tetanus toxoids, and variants thereof (*e.g.*, DT, CRM_{197},TT), cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, non-typable *H. influenzae* P4 protein, non-typable *H. influenzae* P6 protein, *M catarrhalis* uspA, keyhole limpet haemocyanin (KLH), OMPC from *N. meningitidis*, the purified protein derivative of tuberculin(PPD), protein D from *H. influenzae*, or any fragment thereof. The polysaccharide is activated via CDAP chemistry in a CDAP solution. The polypeptide is added to the activated polysaccharides, and then the conjugation reaction is quenched. The reaction mixture is filtered, is diafiltrated, and is concentrated. The polysaccharide polypeptide conjugates are then separated using gel filtration chromatography. These conjugates are then concentrated for the final polysaccharide polypeptide conjugate.

EXAMPLE 5

Method of Polysaccharide-CRM_{197} Conjugation

[0106] This example shows the conjugation of a capsular polysaccharide to CRM_{197} via CDAP conjugation chemistry. The isolated polysaccharide is any polysaccharide as described in **TABLE 1** or of **FIGS. 10A-10F** (e.g., polysaccharides from serotypes 1, 2, 3, 4, 5, 6A, 6B, 7C, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B)), or in **EXAMPLE 2**, and CRM_{197} is as described in **EXAMPLE 3**. The conjugation process is depicted in **FIG. 5**. The polysaccharide and CRM_{197} are thawed. The polysaccharide is mixed with CDAP solution, which is then incubated at the appropriate temperature for polysaccharide activation. A calculated amount of CRM_{197} is added to the activated polysaccharides. The reaction is then quenched using glycine or any other suitable quenching reagent. The conjugation reaction mixture is filtered, is diafiltered, and is

Petitioner Merck Ex-1004, 0849

concentrated for the polysaccharide-CRM_{197} conjugates. The polysaccharide-CRM_{197} conjugates are then purified by gel filtration chromatography. The fractions containing the polysaccharide-CRM_{197} conjugates are pooled, are concentrated, and then are filtered through a 0.2 μm filter. The product is stored at 2-8°C.

[0107] The isolated polysaccharide from serotype 8, serotype 10A, serotype 11A, serotype 12F, serotype 22F, or serotype 33F as described in **TABLE 1** and **EXAMPLE 2**, and CRM_{197} as described in **EXAMPLE 3** were thawed. The polysaccharide from serotype 8, serotype 10A, serotype 11A, serotype 12F, serotype 22F, or serotype 33F were mixed with CDAP solution, which was then incubated at the appropriate temperature for polysaccharide activation. A calculated amount of CRM_{197} was added to the activated polysaccharides. The reaction was then quenched using glycine or any other suitable quenching reagent. The conjugation reaction mixture was filtered, was diafiltered, and was concentrated for the polysaccharide-CRM_{197} conjugates. The polysaccharide-CRM_{197} conjugates were then purified by gel filtration chromatography. The fractions containing the polysaccharide-CRM_{197} conjugates were pooled, were concentrated, and then were filtered through a 0.2 μm filter. The product is stored at 2-8°C.

EXAMPLE 6

Combinations of Polysaccharide-CRM_{197} Conjugates in a Vaccine Composition

[0108] This example shows combinations of polysaccharides that are used in a polysaccharide-CRM_{197} conjugate vaccine. Each polysaccharide serotype is individually conjugated to a CRM_{197} to produce the polysaccharide CRM_{197} conjugate. The polysaccharide-CRM_{197} conjugates for each serotype are combined into vaccine composition. See the below **TABLE 3** for exemplary polysaccharide-CRM_{197} conjugate serotype combinations.

TABLE 3. Combinations of Polysaccharides in Polysaccharide-CRM_{197} Conjugate Vaccines

List of Polysaccharide Serotype Combinations
1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F
6C, 8, 9N, 10A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B
6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B
6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 24F, 33F, and 35B
6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B

Petitioner Merck Ex-1004, 0850

List of Polysaccharide Serotype Combinations
6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B
6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B
6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B
6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B
6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B and 38
6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B
1, 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 20A, 22F, 23A, 23B, 24F, 33F, 35F, 35B, and 38
3, 4, 6C, 7F, 8, 9N, 11A, 12F, 15A, 15B, 15C, 16F, 19A, 22F, 23A, 23B, and 35B
1, 3, 4, 6A, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 19A, 19F, 22F, 23A, 23B, 23F, 24F, and 33F
3, 4, 6B, 6C, 10A, 15A, 15B, 19A, 19F, 22F, 23F, 24F, and 35B
6C, 9N, 15A, 15C, 16F, 20A, 23A, 23B, 24F, 31, 34, 35F, and 38
6C, 15A, 15C, 16F, 23A, 23B, 24F, 31, 34, 35F, and 38
6C, 15A, 15C, 16F, 23A, 23B, and 35B
6C, 9N, 15A, 15C, 16F, 23A, 23B, and 35B
6C, 9N, 15A, 15C, 16F, 23A, 23B, 33F, and 35B
6C, 9N, 15A, 15C, 16F, 17F, 20A, 23A, 23B, 33F, 34, 35F, 35B, 24F, and 31
6C, 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 33F, 34, 35F, 35B, 24F, and 31
6C, 9N, 15A, 15C, 16F, 23A, 23B, 33F, 35F, and 35B
1, 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 20A, 22F, 23A, 23B, 24F, 33F, 35F, 35B, and 38
1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 19F, 19A, 20A, 22F, 23F, 23A, 23B, 24F, 31, 33F, 35F, 35B, and 38
1, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 20A, 22F, 23F, 23A, 23B, 24F, 31, 33F, 35F, 35B, and 38
6C, 15A, 15C, 16F, 23A, 23B, 31, 33F, 34, 35B, and 38
1, 4, 6A, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15C, 16F, 17F, 19F, 19A, 20A, 22F, 23A, 23B, 31, 33F, 34, 35B, 35F, 38
1, 3, 4, 6A, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15C, 16F, 17F, 19F, 19A, 20A, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, 35F, and 38
1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 20B, and 23F

Petitioner Merck Ex-1004, 0851

List of Polysaccharide Serotype Combinations
6C, 8, 9N, 10A, 12F, 15A, 15B, 15C, 16F, 20B, 22F, 23A, 23B, 33F, and 35B
6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 20B, 22F, 23A, 23B, 33F, and 35B
6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 20B, 22F, 23A, 23B, 24F, 33F, and 35B
6C, 8, 9N, 10A, 11A, 12F, 15A, 20B, 22F, 23A, 23B, 24F, 33F, and 35B
6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 20B, 22F, 23A, 23B, 33F, and 35B
6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 20B, 22F, 23A, 23B, 33F, and 35B
6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 20B, 22F, 23A, 23B, 33F, and 35B
6C, 8, 9N, 10A, 11A, 12F, 15A, 20B, 22F, 23A, 23B, 24F, 33F, and 35B
6C, 10A, 11A, 15A, 15B, 15C, 20B, 22F, 23A, 24B, 33F, 35B and 38
6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 20B, 22F, 23A, 23B, 33F, and 35B
1, 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 24F, 33F, 35F, 35B, and 38
3, 4, 6C, 7F, 8, 9N, 11A, 12F, 15A, 15B, 15C, 16F, 19A, 20B, 22F, 23A, 23B, and 35B
1, 3, 4, 6A, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, and 33F
3, 4, 6B, 6C, 10A, 15A, 15B, 19A, 19F, 20B, 22F, 23F, 24F, and 35B
6C, 9N, 15A, 15C, 16F, 20A, 20B, 23A, 23B, 24F, 31, 34, 35F, and 38
6C, 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 34, 35F, and 38
6C, 15A, 15C, 16F, 20B, 23A, 23B, and 35B
6C, 9N, 15A, 15C, 16F, 20B, 23A, 23B, and 35B
6C, 9N, 15A, 15C, 16F, 20B, 23A, 23B, 33F, and 35B
6C, 9N, 15A, 15C, 16F, 17F, 20A, 20B, 23A, 23B, 33F, 34, 35F, 35B, 24F, and 31
6C, 9N, 15A, 15C, 16F, 20B, 23A, 23B, 33F, 35F, and 35B
1, 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 24F, 33F, 35F, 35B, and 38
1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 19F, 19A, 20A, 20B, 22F, 23F, 23A, 23B, 24F, 31, 33F, 35F, 35B, and 38
1, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 20A, 20B, 22F, 23F, 23A, 23B, 24F, 31, 33F, 35F, 35B, and 38
6C, 15A, 15C, 16F, 20B, 23A, 23B, 31, 35B, 34, 35F, and 38

Petitioner Merck Ex-1004, 0852

List of Polysaccharide Serotype Combinations
1, 4, 6A, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15C, 16F, 17F, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 31, 33F, 34, 35B, 35F, 38
1, 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 20B, 22F, 23A, 23B, 24F, 33F, 35F, 35B, and 38
6C, 9N, 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 34, 35F, and 38
1, 2, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 19F, 19A, 20B, 22F, 23F, 23A, 23B, 24F, 31, 33F, 35F, 35B, and 38
1, 3, 4, 5, 6A, 6B, 6C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 20B, 22F, 23F, 23A, 23B, 24F, 31, 33F, 35F, 35B, and 38
1, 4, 6A, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15C, 16F, 17F, 19F, 19A, 20B, 22F, 23A, 23B, 31, 33F, 34, 35B, 35F, 38
1, 3, 4, 6A, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15C, 16F, 17F, 19F, 19A, 20B, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, 35F, and 38
6C, 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 33F, 34, 35F, and 35B
6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20A, 22F, 23A, 23B, 33F, and 35B
6C, 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 33F, 34, 35F, and 35B
6C, 9N, 15A, 15C, 16F, 23A, 23B, and 33F
6C, 15A, 15C, 16F, 23A, 23B, and 33F
6C, 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 24F, 31, 33F, 34, 35F, and 35B
3, 6C, 7C, 8, 9N, 11A, 12F, 15A, 16F, 19A, 19F, 20 (comprising 20A and 20B), 22F, 23A, 23B, 31, 33F, 35B, 35F, and 38
3, 6C, 7C, 7F, 8, 9N, 11A, 12F, 15A, 16F, 19A, 19F, 20 (comprising 20A and 20B), 22F, 23A, 23B, 31, 33F, 35B, 35F, and 38
3, 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 19A, 19F, (comprising 20A and 20B), 22F, 23A, 23B, 31, 33F, 35B, 35F, 38
3, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 19A, 22F, 23A, 23B, 24F, 33F, 35B, and 38
3, 8, 9N, 12F, 15A, 19A, 22F, 23A, 23B, and 24F
3, 8, 9N, 10A, 12F, 15A, 19A, 22F, 23A, 23B, and 24F
3, 4, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 19A, (comprising 20A and 20B), 22F, 23A, 23B, 24F, 31, 33F, 35B, 35F, and 38
3, 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 19A, 19F, (comprising 20A and 20B),

Petitioner Merck Ex-1004, 0853

List of Polysaccharide Serotype Combinations
22F, 23A, 23B, 31, 33F, 35B, 35F, and 38

EXAMPLE 7

Formulation of Polysaccharide-CRM_{197} Conjugate Vaccine

[0109] This example shows a formulation for a polysaccharide-CRM_{197} conjugate vaccine. Any combination of polysaccharide-CRM_{197} conjugates from **EXAMPLE 5** or the combinations listed in **EXAMPLE 6** are used in the vaccine composition. In addition to the polysaccharide-CRM_{197} conjugates, the vaccine is composed of normal saline and 0.01% Polysorbate 80, with a pH of 6.5 ± 0.2. This formulation of the polysaccharide CRM_{197} vaccine composition is stored in 2 mL glass vials with an aluminum crimp top and stopper at 2-8°C. The vial is filled to a volume of 0.60 mL to allow for an extractable volume of 0.50 mL.

EXAMPLE 8

Process for Formulating a Polysaccharide-CRM_{197} Conjugate Vaccine

[0110] This example shows the formulating process for a polysaccharide CRM_{197} conjugate vaccine. The polysaccharide CRM_{197} conjugates are prepared as described in the above Examples. The formulation process is depicted in **FIG. 6**. 0.9% w/v sterile saline is added to a formulation container. Each type of conjugate is separately sterilized and the appropriate volume of each monovalent bulk polysaccharide-CRM_{197} conjugate is added (volume is based on the polysaccharide concentration) to the formulation container one at a time. The pH is adjusted to 6.5 ± 0.2 using 1 N HCl or 1 N NaOH. Optionally, an adjuvant is added to the polysaccharide-CRM_{197} conjugate mixture, such as aluminum phosphate to 1 mg/mL final concentration. A calculated volume of sterile saline is added to reach the appropriate final concentration, and then the mixture is blended for approximately two hours. The formulate in the bulk container is then attached to the filling machine, which fills, stoppers, and seals the vials of the final vaccine product. These vial then are inspected, labeled, and packed for distribution. During this process, the pH, appearance of the vaccine, and the vaccine sterility is monitored. At the filling, stoppering, and sealing step, the appearance, pH, identity of polysaccharides, identity of CRM_{197}, sterility, saccharide content, total protein content, endotoxin content, pyrogen content, general safety, osmolality, and extractable volume are assessed. 1-5 microgram of polysaccharide per each serotype CRM_{197} conjugate is contained in a 0.5 mL of vaccine in a vial.

Petitioner Merck Ex-1004, 0854

EXAMPLE 9

Rabbit Toxicology Safety Study

[0111] This example shows the rabbit toxicology studies that are used to assess vaccine safety. The polysaccharide polypeptide conjugate is produced according to the above **EXAMPLES 1**, **2**, **3**, and **5**, and comprises the *S. pneumoniae* polysaccharides from the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B, which are conjugated to CRM_{197}. Alternatively, the polysaccharide polypeptide conjugate is produced according to the above Examples 1, 2, 3, and 5, and comprises the *S. pneumoniae* polysaccharides from the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 20B, 22F, 23A, 23B, 33F, and 35B, which are conjugated to CRM_{197}. These polysaccharide-CRM_{197} conjugates are used in a vaccine formulated according to **EXAMPLES 6** and **7**. This polysaccharide CRM_{197} conjugate vaccine is administered intramuscularly on days 1, 22, 43, 64, and 85 to rabbits, 5 months of age, (10/gender) at a dose of 0.5 ml/injection into the right thigh muscle. 0.5 ml 0.9% sodium chloride is injected in the Control groups (10/gender). 0.125 mg aluminum phosphate as an adjuvant is injected in the AlP04 group (10/gender). A 4 week dose free recovery period is undertaken in five (5) animals/gender/group after the last injection. Treatment-related mortality and any other toxicologically relevant changes in relative food consumption, ophthalmoscopic parameters, heart rate, respiratory rate, gross anatomy or organ weight is assessed to support lack of systemic toxicity. Injection site are analyzed for reactions and any chronic inflammation. Specific serum antibodies to each of the polysaccharides in the polysaccharide CRM_{197} conjugate vaccine is found in the test group.

[0112] The polysaccharide polypeptide conjugate is produced according to the above Examples 1, 2, 3, and 5, and comprises the *S. pneumoniae* polysaccharides from the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B, which are conjugated to CRM_{197}. Alternatively, the polysaccharide polypeptide conjugate is produced according to the above Examples 1, 2, 3, and 5, and comprises the *S. pneumoniae* polysaccharides from the serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 20B, 22F, 23A, 23B, 33F, and 35B, which are conjugated to CRM_{197}. These polysaccharide CRM_{197} conjugates are used in a vaccine formulated according to **EXAMPLES 6** and **7**. This polysaccharide CRM_{197} conjugate vaccine is administered intramuscularly with and without an aluminum phosphate adjuvant as a single 0.5 mL injection into the left thigh muscle of 3 male rabbits/group, 5 months of age. 0.5 ml 0.9% sodium chloride is administered to the control group (3 males). Treatment related mortality, changes in body weight, relative food consumption, or treatment related macroscopic or microscopic lesions is assessed.

Petitioner Merck Ex-1004, 0855

EXAMPLE 10

Composition and Method of Administration of a Vaccine

[0113] This example shows a vaccine composition and a method of administration of that vaccine composition. An aqueous vaccine composition is composed of an immunogenic polysaccharide polypeptide conjugate comprising capsular polysaccharides from any serotype from **TABLE 1** conjugated to any one of diphtheria and tetanus toxoids, and variants thereof (*e.g.*, DT, CRM_{197},TT), cholera toxoid, pertussis toxoid, inactivated or mutant pneumococcal pneumolysin, pneumococcal surface protein A, pneumococcal adhesion protein A, pneumococcal lipoprotein PsaA, C5a peptidase group A or group B streptococcus, non-typable *H. influenzae* P4 protein, non-typable *H. influenzae* P6 protein, *M catarrhalis* uspA, keyhole limpet haemocyanin (KLH), OMPC from *N. meningitidis*, the purified protein derivative of tuberculin(PPD), protein D from *H. influenzae,* or any fragment thereof; or any combination of polysaccharide polypeptide conjugates as described in Example 6. The aqueous vaccine is formulated to contain from 1-4 microgram of each serotype of polysaccharide per polysaccharide polypeptide conjugate, aluminum phosphate (1 mg/mL), and 0.01% polysorbate 80 in a single 0.5 mL injection delivered intramuscularly to a subject. This vaccine is administered to subject, wherein the subject is a child of 4 weeks to 2 years old. This vaccine is administered to subject, wherein the subject is a child of 2 to 18 years old. This vaccine is administered to a subject, wherein the subject is an adult of at least 50 years of age.

EXAMPLE 11

Vaccination for Protection Against Endemic *S. pneumoniae* Serotypes in the United States and Canada

[0114] This example shows a vaccination of a subject that provides protection against *S. pneumoniae* for a subject residing in or visiting the United States and/or Canada. The aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate comprising capsular polysaccharides from the *S. pneumoniae* serotypes 3, 4, 6A, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 19F, 19A, 22F, 23A, 23B, 33F, 35F, 35B, 38 and Serogroup 20 (comprising serotypes 20A and 20B), such as serotypes 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant. A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to the subject.

Petitioner Merck Ex-1004, 0856

After administration, immunity of the subject against *S. pneumoniae* disease endemic in the United States and Canada is improved.

EXAMPLE 12

Vaccination for Protection against Endemic *S. pneumoniae* Serotypes in Europe, the Middle East, and North Africa

[0115] This example shows a vaccine composition that provides protection against *S. pneumoniae* for subjects residing in or visiting Europe, the Middle East, and/or North Africa. The aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate comprising capsular polysaccharides from the *S. pneumoniae* serotypes 1, 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 16F, 18C, 19F, 19A, 22F, 23F, 23A, 23B, 24F, 33F, 35F, 35B, and 38, such as serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35F. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant. A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to the subject. After administration, immunity of the subject against *S. pneumoniae* disease endemic in Europe, the Middle East, and North Africa is improved.

EXAMPLE 13

Vaccination for Protection Against Endemic *S. pneumoniae* Serotypes in Japan and Korea

[0116] This example shows a vaccine composition that provides protection against *S. pneumoniae* for subjects residing in or visiting Japan and/or Korea. The aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate comprising capsular polysaccharides from the *S. pneumoniae* serotypes 3, 4, 6A, 6B, 6C, 7F, 10A, 11A, 14, 15A, 15B, 19F, 19A, 22F, 23F, 23A, 24F, 35F, 35B, and 38, such as serotypes 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B, and 38. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant. A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to the subject. After administration, immunity of the subject against *S. pneumoniae* disease endemic in Japan and Korea is improved.

EXAMPLE 14

Method of Making a 6C-CRM_{197} Conjugate Pharmaceutical Composition

Petitioner Merck Ex-1004, 0857

[0117] This example shows a method of making a saccharide-polypeptide conjugate pharmaceutical composition. A 6C serotype capsular polysaccharide is isolated from *S. pneumoniae*. The CRM_{197} is produced and purified. The 6C polysaccharide is mixed with CDAP solution, which is then incubated at the appropriate temperature for 6C polysaccharide activation. A calculated amount of CRM_{197} is added to the activated 6C polysaccharides. The reaction is then quenched using glycine or any other suitable quenching reagent. The conjugation reaction mixture is filtered, is diafiltered, and is concentrated for the 6C-CRM_{197} conjugates. The 6C-CRM_{197} conjugates are then purified by gel filtration chromatography. The fractions containing the 6C-CRM_{197} conjugates are pooled, are concentrated, and then are filtered through a 0.2 μm filter. The 6C-CRM_{197} conjugate is formulated for use in a vaccine and is contacted with an excipient.

EXAMPLE 15

Method of Making a 20B-CRM_{197} Conjugate Pharmaceutical Composition

[0118] This example shows a method of making a saccharide-polypeptide conjugate pharmaceutical composition. A 20B serotype capsular polysaccharide is isolated from *S. pneumoniae*. The CRM_{197} is produced and purified. The 20B polysaccharide is mixed with CDAP solution, which is then incubated at the appropriate temperature for 20B polysaccharide activation. A calculated amount of CRM_{197} is added to the activated 20B polysaccharides. The reaction is then quenched using glycine or any other suitable quenching reagent. The conjugation reaction mixture is filtered, is diafiltered, and is concentrated for the 20B-CRM_{197} conjugates. The 20B-CRM_{197} conjugates are then purified by gel filtration chromatography. The fractions containing the 20B-CRM_{197} conjugates are pooled, are concentrated, and then are filtered through a 0.2 μm filter. The 20B-CRM_{197} conjugate is formulated for use in a vaccine and is contacted with an excipient.

EXAMPLE 16

Composition of a Kit with a Saccharide-Polypeptide Conjugate

[0119] This example shows the composition of a kit with a saccharide-polypeptide conjugate. A saccharide-polypeptide conjugate in a vial or a pre-filled syringe is placed in a container. The container is further associated with instructions of use.

Petitioner Merck Ex-1004, 0858

EXAMPLE 17

Vaccination Against *S. pneumoniae*

[0120] This example shows a vaccine composition and administration of the vaccine composition to a human subject. An aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 22F, 23A, 23B, 33F, and 35B. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0121] A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to a human subject. Twenty-eight days later blood is drawn from the human subject and tested for antibodies against each serotype of polysaccharide of the immunogenic saccharide-polypeptide conjugate in the vaccine by in vitro opsonophagocytic assays. After administration of the vaccine, the human subject is found to have antibodies against each serotype of polysaccharide of the immunogenic saccharide-polypeptide conjugate in the vaccine, and therefore, human subject's immunity against these *S. pneumonaie* serotypes is improved.

EXAMPLE 18

Vaccination Against *S. pneumoniae*

[0122] This example shows a vaccine composition and administration of the vaccine composition to a human subject. An aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 20B, 22F, 23A, 23B, 33F, and 35B. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0123] A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to a human subject. Twenty-eight days later blood is drawn from the human subject and tested for antibodies against each serotype of polysaccharide of the immunogenic saccharide-polypeptide conjugate in the vaccine by in vitro opsonophagocytic assays. After administration of the vaccine, the human subject is found to have antibodies against each serotype of polysaccharide of the immunogenic saccharide-polypeptide conjugate in the vaccine, and therefore, human subject's immunity against these *S. pneumonaie* serotypes is improved.

Petitioner Merck Ex-1004, 0859

EXAMPLE 19

Vaccination Against *S. pneumoniae*

[0124] This example shows a vaccine composition and administration of the vaccine composition to a human subject. An aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes of any of the combinations listed in **TABLE 3**. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0125] A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to a human subject. Twenty-eight days later blood is drawn from the human subject and tested for antibodies against each serotype of polysaccharide of the immunogenic saccharide-polypeptide conjugate in the vaccine by in vitro opsonophagocytic assays. After administration of the vaccine, the human subject is found to have antibodies against each serotype of polysaccharide of the immunogenic saccharide-polypeptide conjugate in the vaccine, and therefore, human subject's immunity against these *S. pneumonaie* serotypes is improved.

EXAMPLE 20

Method of Vaccination

[0126] This example shows vaccine compositions and a dosing schedule for the vaccine compositions for a human subject. A first aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. A second aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 22F, 23A, 23B, 33F, and 35B. Each aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0127] A single 0.5 mL dose of the first aqueous vaccine is given by intramuscular injection to a human subject. Four weeks later a single 0.5 mL dose of the second aqueous vaccine is given by intramuscular injection to the human subject. Alternatively to four weeks later, one year later a single 0.5 mL of the second aqueous vaccine is given by intramuscular injection to the human subject.

Petitioner Merck Ex-1004, 0860

EXAMPLE 21

Method of Vaccination

[0128] This example shows vaccine compositions and a dosing schedule for the vaccine compositions for a human subject. A first aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. A second aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 20B, 22F, 23A, 23B, 33F, and 35B. Each aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0129] A single 0.5 mL dose of the first aqueous vaccine is given by intramuscular injection to a human subject. Four weeks later a single 0.5 mL dose of the second aqueous vaccine is given by intramuscular injection to the human subject. Alternatively to four weeks later, one year later a single 0.5 mL of the second aqueous vaccine is given by intramuscular injection to the human subject.

EXAMPLE 22

Method of Vaccination for an Adult Aged 50 Years Old or Older

[0130] This example shows vaccine compositions and a dosing schedule for the vaccine compositions for an adult age 50 years old or older. A first aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. A second aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 22F, 23A, 23B, 33F, and 35B. Alternatively, a second aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 20B, 22F, 23A, 23B, 33F, and 35B. Each aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0131] A single 0.5 mL dose of the first aqueous vaccine is given by intramuscular injection to an adult age 50 years old or older. Four weeks later a single 0.5 mL dose of the second aqueous vaccine is given by intramuscular injection to the adult aged 50 years old or older.

Petitioner Merck Ex-1004, 0861

Alternatively to four weeks later, one year later a single 0.5 mL of the second aqueous vaccine is given by intramuscular injection to the adult aged 50 years old or older.

EXAMPLE 23

Method of Vaccination for a Child Aged 2 to 18 Years Old

[0132] This example shows vaccine compositions and a dosing schedule for the vaccine compositions for a child aged 2 to 18 years old. A first aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. A second aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 22F, 23A, 23B, 33F, and 35B. Alternatively, a second aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 20B, 22F, 23A, 23B, 33F, and 35B. Each aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0133] A single 0.5 mL dose of the first aqueous vaccine is given by intramuscular injection to a child aged 2 to 18 years old. Four weeks later a single 0.5 mL dose of the second aqueous vaccine is given by intramuscular injection to the child aged 2 to 18 years old. Alternatively to four weeks later, one year later a single 0.5 mL of the second aqueous vaccine is given by intramuscular injection to the child aged 2 to 18 years old.

EXAMPLE 24

Method of Vaccination for a Child Aged 4 Weeks to 2 Years Old

[0134] This example shows vaccine compositions and a dosing schedule for the vaccine compositions for a child aged 4 weeks to 2 years old. A first aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. A second aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 22F, 23A, 23B, 33F, and 35B. Alternatively, a second aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S.*

Petitioner Merck Ex-1004, 0862

pneumoniae serotypes 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 20B, 22F, 23A, 23B, 33F, and 35B. Each aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0135] A single 0.5 mL dose of the first aqueous vaccine is given by intramuscular injection to a child aged 4 weeks to 2 years old. Four weeks later a single 0.5 mL dose of the second aqueous vaccine is given by intramuscular injection to the child who is 2-18 years old. Alternatively to four weeks later, one year later a single 0.5 of the second aqueous vaccine is given by intramuscular injection to the child aged 4 weeks to 2 years old.

EXAMPLE 25

Method of Vaccination for *S. pneumoniae* and *N. meningitidis*

[0136] This example shows vaccine compositions and a dosing schedule that provides protection against *S. pneumoniae* and *N. meningitidis*. A first aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 22F, 23A, 23B, 33F, and 35B conjugated to CRM_{197}. A second aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing *N. meningitidis* group C capsular saccharide conjugated to CRM_{197}. Each vaccine is formulated for intramuscular injection.

[0137] A single 0.5 mL dose of the first aqueous vaccine is given by intramuscular injection to a subject. One month later a single dose of the second aqueous vaccine is given by intramuscular injection to a subject. Alternatively, the first aqueous vaccine is given by intramuscular injection one month after the second aqueous vaccine is given by intramuscular injection.

EXAMPLE 26

Method of Vaccination for *S. pneumoniae* and *S. enterica*

[0138] This example shows vaccine compositions and a dosing schedule that provides protection against *S. pneumoniae* and *S. enterica*. A first aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 22F, 23A, 23B, 33F, and 35B conjugated to CRM_{197}. A second aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing *S. enterica*

Petitioner Merck Ex-1004, 0863

subspecies enterica serovar Typhi Vi-capsular saccharide conjugated to CRM_{197}. Each aqueous vaccine is formulated for intramuscular injection.

[0139] A single 0.5 mL dose of the first aqueous vaccine is given by intramuscular injection to a subject. One month later a single dose of the second aqueous vaccine is given by intramuscular injection to a subject. Alternatively, the first aqueous vaccine is given by intramuscular injection one month after the second aqueous vaccine by intramuscular injection.

EXAMPLE 27

Method of Vaccination for *S. pneumoniae*, *N. meningitidis*, and *H. influenzae*

[0140] This example shows vaccine compositions and a dosing schedule that provides protection against *S. pneumoniae*, *N. meningitidis*, and *H. influenzae*. A first aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing capsular polysaccharides from the *S. pneumoniae* 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 22F, 23A, 23B, 33F, and 35B conjugated to a tetanus toxoid carrier protein. A second aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate containing *N. meningitidis* group C and group Y saccharide and *H. influenzae* type b saccharide individually conjugated a tetanus toxoid carrier protein. Each aqueous vaccine is formulated for intramuscular injection.

[0141] A single 0.5 mL dose of the first aqueous vaccine is given by intramuscular injection to a subject. One month later a single dose of the second aqueous vaccine is given by intramuscular injection to a subject. Alternatively, the first aqueous vaccine is given by intramuscular injection one month after the second aqueous vaccine by intramuscular injection.

EXAMPLE 28

Serotype 23A Vaccination Against *S. pneumoniae*

[0142] This example shows a vaccine composition and administration of the vaccine composition to a human subject. An aqueous vaccine composition is comprised of immunogenic saccharide-polypeptide conjugates comprising a capsular polysaccharide from the *S. pneumoniae* serotypes 23A. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0143] A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to a human subject. Twenty-eight days later blood is drawn from the human subject and tested for

Petitioner Merck Ex-1004, 0864

antibodies against each serotype of polysaccharide of the immunogenic saccharide-polypeptide conjugate in the vaccine by in vitro opsonophagocytic assays. After administration of the vaccine, the human subject is found to have antibodies against each serotype of polysaccharide of the immunogenic saccharide-polypeptide conjugate in the vaccine, and therefore, human subject's immunity against these *S. pneumonaie* serotypes is improved.

EXAMPLE 29

Serotype 23B Vaccination Against *S. pneumoniae*

[0144] This example shows a vaccine composition and administration of the vaccine composition to a human subject. An aqueous vaccine composition is comprised of immunogenic saccharide-polypeptide conjugates comprising a capsular polysaccharide from the *S. pneumoniae* serotypes 23B. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant.

[0145] A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to a human subject. Twenty-eight days later blood is drawn from the human subject and tested for antibodies against each serotype of polysaccharide of the immunogenic saccharide-polypeptide conjugate in the vaccine by in vitro opsonophagocytic assays. After administration of the vaccine, the human subject is found to have antibodies against each serotype of polysaccharide of the immunogenic saccharide-polypeptide conjugate in the vaccine, and therefore, human subject's immunity against these *S. pneumonaie* serotypes is improved.

EXAMPLE 30

Vaccination for Protection Against Endemic *S. pneumoniae* Serotypes in the United States

[0146] This example shows a vaccination of a subject that provides protection against *S. pneumoniae* for a subject residing in or visiting the United States. The aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate comprising capsular polysaccharides from the *S. pneumoniae* serotypes 3, 6C, 7C, 8, 9N, 11A, 12F, 15A, 16F, 19A, 19F, 22F, 23A, 23B, 31, 33F, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). The immunogenic saccharide-polypeptide conjugates are made using the methods as described herein. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant. A single 0.5 mL dose

Petitioner Merck Ex-1004, 0865

of the aqueous vaccine is given by intramuscular injection to the subject. After administration, immunity of the subject against *S. pneumoniae* disease endemic in the United States is improved.

EXAMPLE 31

Vaccination for Protection Against Endemic *S. pneumoniae* Serotypes in the United States

[0147] This example shows a vaccination of a subject that provides protection against *S. pneumoniae* for a subject residing in or visiting the United States. The aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate comprising capsular polysaccharides from the *S. pneumoniae* serotypes 3, 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 19A, 19F, 22F, 23A, 23B, 31, 33F, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). The immunogenic saccharide-polypeptide conjugates are made using the methods as described herein. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant. A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to the subject. After administration, immunity of the subject against *S. pneumoniae* disease endemic in the United States is improved.

EXAMPLE 32

Vaccination for Protection Against Endemic *S. pneumoniae* Serotypes in the United Kingdom

[0148] This example shows a vaccination of a subject that provides protection against *S. pneumoniae* for a subject residing in or visiting the United Kingdom. The aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate comprising capsular polysaccharides from the *S. pneumoniae* serotypes 3, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 19A, 22F, 23A, 23B, 24F, 33F, 35B, and 38. The immunogenic saccharide-polypeptide conjugates are made using the methods as described herein. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant. A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to the subject. After administration, immunity of the subject against *S. pneumoniae* disease endemic in the United Kingdom is improved.

Petitioner Merck Ex-1004, 0866

EXAMPLE 33

Vaccination for Protection Against Endemic *S. pneumoniae* Serotypes in Germany

[0149] This example shows a vaccination of a subject that provides protection against *S. pneumoniae* for a subject residing in or visiting Germany. The aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate comprising capsular polysaccharides from the *S. pneumoniae* serotypes 3, 4, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 19A, 22F, 23A, 23B, 24F, 31, 33F, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). The immunogenic saccharide-polypeptide conjugates are made using the methods as described herein. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant. A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to the subject. After administration, immunity of the subject against *S. pneumoniae* disease endemic in Germany is improved.

EXAMPLE 34

Vaccination for Protection Against Endemic *S. pneumoniae* Serotypes in Germany

[0150] This example shows a vaccination of a subject that provides protection against *S. pneumoniae* for a subject residing in or visiting Germany. The aqueous vaccine composition is composed of an immunogenic saccharide-polypeptide conjugate comprising capsular polysaccharides from the *S. pneumoniae* serotypes 3, 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 19A, 22F, 23A, 23B, 31, 33F, 35B, 35F, 38, and Serogroup 20 (comprising serotypes 20A and 20B). The immunogenic saccharide-polypeptide conjugates are made using the methods as described herein. The aqueous vaccine is formulated to contain from 1-5 microgram of each serotype and aluminum phosphate as an adjuvant. A single 0.5 mL dose of the aqueous vaccine is given by intramuscular injection to the subject. After administration, immunity of the subject against *S. pneumoniae* disease endemic in Germany is improved.

[0151] While preferred embodiments of the present invention have been shown and described herein, it will be apparent to those skilled in the art that such embodiments are provided by way of example only. It is not intended that the invention be limited by the specific examples provided within the specification. While the invention has been described with reference to the aforementioned specification, the descriptions and illustrations of the embodiments herein are not meant to be construed in a limiting sense. Numerous variations,

Petitioner Merck Ex-1004, 0867

changes, and substitutions will now occur to those skilled in the art without departing from the invention. Furthermore, it shall be understood that all aspects of the invention are not limited to the specific depictions, configurations or relative proportions set forth herein which depend upon a variety of conditions and variables. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is therefore contemplated that the invention shall also cover any such alternatives, modifications, variations or equivalents. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

Petitioner Merck Ex-1004, 0868