CLAIMS

WHAT IS CLAIMED IS:

1. A pharmaceutical composition in unit dose form comprising 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, 7F, 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.
2. The pharmaceutical composition of claim 1, comprising a plurality of at least 9 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, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 35B, 35F, and 38.
3. The pharmaceutical composition of claim 1, comprising a plurality of at least 10 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, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 35B, 35F, and 38.
4. The pharmaceutical composition of claim 1, comprising a plurality of at least 13 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 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.
5. The pharmaceutical composition of claim 1, comprising a plurality of at least 14 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular

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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 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 24F, 33F and 35B.

6. The pharmaceutical composition of claim 1, comprising a plurality of at least 13 unique immunogenic saccharide-polypeptide 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 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B.
7. The pharmaceutical composition of claim 1, comprising a plurality of at least 12 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 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B, and 38.
8. The pharmaceutical composition of claim 1, comprising a plurality of at least 13 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 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.
9. The pharmaceutical composition of claim 1, comprising a plurality of at least 13 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 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B.
10. The pharmaceutical composition of claim 1, comprising a plurality of at least 13 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 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B.

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11. The pharmaceutical composition of claim 1, comprising a plurality of at least 12 unique immunogenic saccharide-polypeptide conjugates each comprising a unique serotype selected from the group consisting of 6C, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B and 38.
12. The pharmaceutical composition of claim 1, comprising a plurality of at least 11 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 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.
13. The pharmaceutical composition of claim 1, comprising a plurality of at least 11 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 6C, 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B.
14. The pharmaceutical composition of claim 1, comprising a plurality of at least 15 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 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B.
15. The pharmaceutical composition of claim 1, comprising a plurality of at least 16 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 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 20B, 22F, 23A, 23B, 33F, and 35B.
16. The pharmaceutical composition of claim 1, comprising a plurality of at least 8 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 6C, 9N, 15A, 15C, 16F, 23A, 23B, and 35B.

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17. The pharmaceutical composition of claim 1, comprising a plurality of at least 7 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 6C, 15A, 15C, 16F, 23A, 23B, and 35B.
18. The pharmaceutical composition of claim 1, comprising a plurality of at least 15 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 6C, 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 24F, 31, 33F, 34, 35F, and 35B.
19. The pharmaceutical composition of claim 1, comprising a plurality of at least 13 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 6C, 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 33F, 34, 35F, and 35B.
20. The pharmaceutical composition of claim 1, comprising a plurality of at least 15 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 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20A, 22F, 23A, 23B, 33F, and 35B.
21. The pharmaceutical composition of claim 1, comprising a plurality of at least 15 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 6C, 9N, 15A, 15C, 16F, 17F, 20A, 23A, 23B, 24F, 31, 34, 35F, 35B, and 38.
22. The pharmaceutical composition of claim 1, comprising a plurality of at least 15 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

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unique *Streptococcus pneumoniae* serotype selected from a group consisting of 6C, 9N, 15A, 15C, 16F, 17F, 20A, 23A, 23B, 24F, 31, 34, 35F, 35B, and 38.

23. The pharmaceutical composition of claim 1, comprising a plurality of at least 15, 16, or 20 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 3, 6C, 7C, 8, 9N, 11A, 12F, 15A, 16F, 19A, 19F, 20A, 20B, 22F, 23A, 23B, 31, 33F, 35B, 35F, and 38.
24. The pharmaceutical composition of claim 1, comprising a plurality of at least 15, 16, or 20 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 3, 6C, 8, 9N, 11A, 12F, 15A, 15C, 16F, 19A, 19F, 20A, 20B, 22F, 23A, 23B, 31, 33F, 35B, 35F, and 38.
25. The pharmaceutical composition of claim 1, comprising a plurality of at least 15, 16, or 20 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 3, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 19A, 22F, 23A, 23B, 24F, 33F, 35B, and 38.
26. The pharmaceutical composition of claim 1, comprising a plurality of at least 15, 16, or 20 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 3, 4, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 19A, 20A, 20B, 22F, 23A, 23B, 24F, 31, 33F, 35B, 35F, and 38.
27. The pharmaceutical composition of claim 1, comprising a plurality of at least 15, 16, or 20 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

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thereof is from a unique Streptococcus pneumoniae serotype selected from a group consisting of 6C, 9N, 15A, 15C, 16F, 17F, 20A, 23A, 23B, 24F, 31, 34, 35F, 35B, and 38.

28. The pharmaceutical composition of any one of claims 1-27, wherein the unique *Streptococcus pneumoniae* serotype is not any of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F.

29. A pharmaceutical composition in unit dose form comprising 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, 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.

30. The pharmaceutical composition of claim 29, comprising a plurality of at least 15 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, 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 33F, 35F, 35B, and 38.

31. The pharmaceutical composition of claim 29, comprising a plurality of at least 25 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 3, 4, 6A, 6C, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 24B, 33F, 35F, 35F, 35B and 38.

32. The pharmaceutical composition of claim 29, comprising a plurality of at least 27 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide,

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wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique *Streptococcus pneumoniae* serotype selected from a group consisting of 1, 3, 4, 6A, 6B, 6C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 15B, 16F, 18C, 19F, 19A, 20A, 20B, 22F, 23F, 23A, 23B, 24F, 33F, 35F, and 35B.

33. The pharmaceutical composition of claim 29, comprising a plurality of at least 19 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 3, 4, 6A, 6B, 6C, 7F, 10A, 11A, 14, 15A, 15B, 19F, 19A, 20A, 20B, 22F, 23F, 23A, 24F, 35B and 38.

34. A pharmaceutical composition in unit dose form comprising 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, 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.

35. The pharmaceutical composition of claim 34, wherein the at least one additional serotype is selected from the group consisting of 1, 3, 4, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 23F, 24F, 33F, 35F, 35B, and 38.

36. The pharmaceutical composition of claim 34, wherein the at least one additional serotype is selected from the group consisting of 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.

37. The pharmaceutical composition of claim 34, 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.

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38. The pharmaceutical composition of claim 34, 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.
39. The pharmaceutical composition of claim 34, 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.
40. The pharmaceutical composition of claim 34, wherein the at least one additional serotype is selected from the group consisting of 8, 9N, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 23B, 33F, and 35B.
41. The pharmaceutical composition of claim 34, wherein the at least one additional serotype is selected from the group consisting of 8, 9N, 10A, 11A, 15A, 15B, 15C, 20B, 22F, 23A, 23B, 33F, and 35B.
42. The pharmaceutical composition of claim 34, wherein the at least one additional serotype is selected from the group consisting of 9N, 15A, 15C, 16F, 23A, 23B, and 35B.
43. The pharmaceutical composition of claim 34, wherein the at least one additional serotype is selected from the group consisting of 15A, 15C, 16F, 23A, 23B, and 35B.
44. The pharmaceutical composition of claim 34, wherein the at least one additional serotype is selected from the group consisting of 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 24F, 31, 33F, 34, 35F, and 35B.
45. The pharmaceutical composition of claim 34, wherein the at least one additional serotype is selected from the group consisting of 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 33F, 34, 35F, and 35B.
46. The pharmaceutical composition of claim 34, wherein the at least one additional serotype is selected from the group consisting of 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20A, 22F, 23A, 23B, 33F, and 35B.
47. The pharmaceutical composition of claim 4, wherein the at least one additional serotype consists of 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.
48. The pharmaceutical composition of claim 34, wherein the at least one additional serotype consists of 8, 9N, 11A, 12F, 15A, 15B, 16F, 22F, 23A, 23B, 33F, and 35B.
49. The pharmaceutical composition of claim 34, wherein the at least one additional serotype consists of 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B.
50. The pharmaceutical composition of claim 34, wherein the at least one additional serotype consists of 10A, 11A, 15A, 15B, 15C, 22F, 23A, 24B, 33F, 35B and 38.

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51. The pharmaceutical composition of claim 4, wherein the at least one additional serotype consists of 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B.
52. The pharmaceutical composition of claim 34, 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.
53. The pharmaceutical composition of claim 34, wherein the at least one additional serotype consists of 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B.
54. The pharmaceutical composition of claim 34, 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.
55. The pharmaceutical composition of claim 34, wherein the at least one additional serotype consists of 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 20B, 23A, 23B, 33F, and 35B.
56. The pharmaceutical composition of claim 34, wherein the at least one additional serotype consists of 3, 4, 6B, 10A, 15A, 15B, 19A, 19F, 22F, 23F, 24F, and 35B.
57. The pharmaceutical composition of claim 34, wherein the at least one additional serotype consists of 15A, 15C, 16F, 23A, 23B, and 35B.
58. The pharmaceutical composition of claim 34, wherein the at least one additional serotype consists of 9N, 15A, 15C, 16F, 23A, 23B, 35B.
59. The pharmaceutical composition of claim 34, wherein the at least one additional serotype consists of 9N, 15A, 15C, 16F, 23A, 23B, 33F, and 35B.
60. The pharmaceutical composition of claim 34, wherein the at least one additional serotype consists of 9N, 15A, 15C, 16F, 17F, 20A, 23A, 23B, 33F, 34, 35F, 35B, 24F, and 31.
61. The pharmaceutical composition of claim 34, wherein the at least one additional serotype consists of 9N, 15A, 15C, 16F, 23A, 23B, 33F, 35F, and 35B.
62. The pharmaceutical composition of claim 34, wherein the at least one additional serotype consists of 9N, 15A, 15C, 16F, 23A, 23B, and 35B.
63. The pharmaceutical composition of claim 34, wherein the at least one additional serotype consists of the group consisting of 15A, 15C, 16F, 23A, 23B, and 35B.
64. The pharmaceutical composition of claim 34, wherein the at least one additional serotype consists of 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 24F, 31, 33F, 34, 35F, and 35B.
65. The pharmaceutical composition of claim 34, wherein the at least one additional serotype consists of 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 33F, 34, 35F, and 35B.
66. The pharmaceutical composition of claim 34, wherein the at least one additional serotype consists of 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20A, 22F, 23A, 23B, 33F, and 35B.

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67. The pharmaceutical composition of claim 34, wherein the at least one additional serotype consists of 3, 7C, 8, 9N, 10A, 11A, 12F, 15A, 16F, 19A, 19F, 20, 22F, 23A, 23B, 31, 33F, 35B, 35F, and 38.
68. The pharmaceutical composition of claim 34, wherein the at least one additional serotype consists of 3, 8, 9N, 11A, 12F, 15A, 15B, 16F, 19A, 19F, 20, 22F, 23A, 23B, 31, 33F, 35B, 35F, and 38.
69. The pharmaceutical composition of claim 34, wherein the at least one additional serotype consists of 3, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 19A, 22F, 23A, 23B, 24F, 33F, 35B, and 38.
70. The pharmaceutical composition of claim 34, wherein the at least one additional serotype consists of 3, 4, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 19A, 20, 22F, 23A, 23B, 24F, 31, 33F, 35B, 35F, and 38.
71. The pharmaceutical composition of claim 34, wherein the at least one additional serotype consists of 3, 8, 9N, 11A, 12F, 15A, 15B, 16F, 19A, 19F, 20, 22F, 23A, 23B, 31, 33F, 35B, 35F, and 38.
72. A pharmaceutical composition in unit dose form comprising 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, 7C, 7F, 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.
73. The pharmaceutical composition of claim 72, wherein the at least one additional serotype consists of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, 34, and 35B.
74. The pharmaceutical composition of claim 72, wherein the at least one additional serotype is selected from the group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 22F, 23A, 23B, 33F, and 35B.

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75. The pharmaceutical composition of claim 72, wherein the at least one additional serotype is selected from the group consisting of 6C, 15A, 15C, 16F, 23A, 23B, 24F, 31, 33F, 34, 35F, and 35B.
76. The pharmaceutical composition of claim 72, wherein the at least one additional serotype is selected from the group consisting of 6C, 15A, 15C, 16F, 23A, 23B, 24F, 31, 33F, 35F, and 35B.
77. A pharmaceutical composition comprising 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, 7C, 7F, 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.
78. The pharmaceutical composition of claim 77, 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 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 20B, 22F, 23A, 23B, 33F, and 35B.
79. The pharmaceutical composition of claim 77, 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 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 23B, 33F, and 35B.
80. The pharmaceutical composition of claim 77, further comprising at least one unique immunogenic saccharide-polypeptide conjugate 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, 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, 35B, and 38.
81. The pharmaceutical composition of any one of claims 1-80, wherein the 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 0.5 μg to 5 μg.
82. The pharmaceutical composition of any one of claims 1-81, wherein the total amount of capsular polysaccharide, fragment thereof, or combination thereof, individually for each

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Streptococcus pneumoniae serotype in the pharmaceutical composition in unit dose form, ranges from about 1 μg to about 4 μg.

83. The pharmaceutical composition of any one of claims 1-82, wherein the 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 1 μg to 4 μg.
84. The pharmaceutical composition of any one of claims 1-83, wherein the total amount of capsular polysaccharide, fragment thereof, or combination thereof, individually for each *Streptococcus pneumoniae* serotype in the pharmaceutical composition in unit dose form, is selected from the group consisting of about 1 μg, about 2 μg, and about 4 μg.
85. The pharmaceutical composition of any one of claims 1-84, wherein the total amount of capsular polysaccharide, fragment thereof, or combination thereof, individually for each *Streptococcus pneumoniae* serotype in the pharmaceutical composition in unit dose form, is selected from the group consisting of 1 μg, 2 μg, and 4 μg.
86. The pharmaceutical composition of any one of claims 1-85, wherein a total amount of unconjugated 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% to about 30% w/w.
87. The pharmaceutical composition of any one of claims 1-86, wherein a total amount of unconjugated 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% to about 20% w/w.
88. The pharmaceutical composition of any one of claims 1-87, wherein a total amount of unconjugated 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% to about 10% w/w.
89. The pharmaceutical composition of any one of claims 29, 30, 32, 34-66, and 77-88, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 1, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

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90. The pharmaceutical composition of any one of claims 1-3, 28, 29, 34-66, and 72-89, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 2, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.
91. The pharmaceutical composition of any one of claims 29-90, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 3, the immunogenic saccharide- CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.
92. The pharmaceutical composition of any one of claims 26, 29-66, 70, and 72-91, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 4, the immunogenic saccharide- CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.
93. The pharmaceutical composition of any one of claims 29, 34-66 and 72-92, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide- CRM_{197} conjugate comprises serotype 5, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.
94. The pharmaceutical composition of any one of claims 29-66 and 72-93, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 6A, the immunogenic saccharide- CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

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95. The pharmaceutical composition of any one of claims 29, 30, 32-66 and 72-94, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide- CRM_{197} conjugate comprises serotype 6B, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.
96. The pharmaceutical composition of any one of claims 25, 26, 29-66, 69, 70, and 72-95, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 7F, the immunogenic saccharide- CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.
97. The pharmaceutical composition of any one of claims 23, 29, 34, 67, and 80-95, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 7C, the immunogenic saccharide- CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.
98. The pharmaceutical composition of any one of claims 29, 34-66, and 72-96, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide- CRM_{197} conjugate comprises serotype 9V, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.
99. The pharmaceutical composition of any one of claims 29, 30, 32-66, and 72-98, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide- CRM_{197} conjugate comprises serotype 14, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the

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capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

100. The pharmaceutical composition of any one of claims 29, 30, 34-66, and 72-99, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide- CRM_{197} conjugate comprises serotype 18C, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

101. The pharmaceutical composition of any one of claims 29-100, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 19A, the immunogenic saccharide- CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

102. The pharmaceutical composition of any one of claims 23, 24, 27, 29-66, 67, 68, and 71-101, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 19F, the immunogenic saccharide- CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

103. The pharmaceutical composition of any one of claims 29, 32-66, and 72-102, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide- CRM_{197} conjugate comprises serotype 23F, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

104. The pharmaceutical composition of any one of claims 1-103, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 6C, the immunogenic saccharide- CRM_{197} conjugate has a molecular

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weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

105. The pharmaceutical composition of any one of claims 1-6, 8-10, 12-15, 20, 23-28-32, and 34-104, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 8, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

106. The pharmaceutical composition of any one of claims 1-6, 8-10, 12-15, 20, 23-32, and 34-105, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 9N, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

107. The pharmaceutical composition of any one of claims 1-4, 6-8, 10-12, 14, 15, 20, 25, 26, 28-66, 69, 70, and 72-106, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 10A, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

108. The pharmaceutical composition of any one of claims 1-15, 20, and 23-107, wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 11A, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

109. The pharmaceutical composition of any one of claims 1-6, 8-10, 12, 14, 15, 23-32, and 34-108, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 12F, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio

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(w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

110. The pharmaceutical composition of any one of claims 1-109, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 15A, the immunogenic saccharide- CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

111. The pharmaceutical composition of any one of claims 1-5, 7-9, 11-15, 20, 2428-66, and 68-110, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 15B, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

112. The pharmaceutical composition of any one of claims 1-3, 7, 11, 14-22, 25, 26, 28-31, 34-66, 69, 70 and 72-111, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 15C, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

113. The pharmaceutical composition of any one of claims 1-3, 5, 9, 13-32, and 34-112, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 16F, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

114. The pharmaceutical composition of any one of claims 1-3, 18, 21, 22, 28, 29, 34-66, and 72-102, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 17F, the immunogenic saccharide-CRM_{197}

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conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

115. The pharmaceutical composition of any one of claims 1-3, 20-24, 26-68, and 70-114, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 20A, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

116. The pharmaceutical composition of any one of claims 1-3, 15, 18-19, and 23. 24, 26-68, and 70-115, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 20B, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

117. The pharmaceutical composition of any one of claims 1-15, 17, 20, and 23-116, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 22F, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

118. The pharmaceutical composition of any one of claims 1-117, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 23A, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

119. The pharmaceutical composition of any one of claims 1-32, and 34-118, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate

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comprises serotype 23B, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

120. The pharmaceutical composition of any one of claims 1-3, 5, 6, 10, 18, 19, 21, 22, 25, 26, 28-30, 32-66, 69, 70, and 72-119, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 24F, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

121. The pharmaceutical composition of any one of claims 1-3, 7, 28-31, 34-66, and 72-120, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 24B, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

122. The pharmaceutical composition of any one of claims 1-3, 18, 19, 21, 22-24, 26-29, 34-68, and 70-121, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 31, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

123. The pharmaceutical composition of any one of claims 1-15, 20, 23-32, and 34-122, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 33F, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

124. The pharmaceutical composition of any one of claims 1, 16-19, 23-29, 34-66, and 72-123, wherein the immunogenic saccharide-polypeptide conjugate comprises an

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immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 33B, the immunogenic saccharide- CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

125. The pharmaceutical composition of any one of claims 1, 2, 18, 19, 21-24, 26-32, 34-68, and 70-124, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide- CRM_{197} conjugate comprises serotype 35F, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

126. The pharmaceutical composition of any one of claims 1, 18, 19, 21, 22, 28, 29, 34-66, and 72-125, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 34, the immunogenic saccharide- CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

127. The pharmaceutical composition of any one of claims 1-15 and 18-126, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide- CRM_{197} conjugate comprises serotype 35B, the immunogenic saccharide- CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

128. The pharmaceutical composition of any one of claims 1-3, 7, 11, 21-31, and 33-127, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide- CRM_{197} conjugate comprises serotype 38, the immunogenic saccharide- CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

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129. The pharmaceutical composition of any one of claims 1-128, wherein at least one of the unique immunogenic saccharide-polypeptide conjugate comprises the capsular polysaccharide.

130. The pharmaceutical composition of any one of claims 1-129, wherein at least one of the unique immunogenic saccharide-polypeptide conjugate comprises the fragment of the capsular polysaccharide.

131. The pharmaceutical composition of claim 130, wherein the fragment of the capsular polysaccharide comprises a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, or a hexasaccharide.

132. The pharmaceutical composition of any one of claims 1-131, wherein at least one polypeptide of the plurality is an oligopeptide.

133. The pharmaceutical composition of any one of claims 1-132, wherein at least one polypeptide of the plurality 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*, PspA, any fragment thereof, or any combination thereof.

134. The pharmaceutical composition of any one of claims 1-133, wherein the polypeptide comprises a mixture of polypeptides.

135. The pharmaceutical composition of any one of claims 1-134, wherein a toxin activity of at least one of the polypeptides of the plurality is at least partly mitigated.

136. The pharmaceutical composition of any one of claims 1-135, further comprising an adjuvant.

137. The pharmaceutical composition in unit dose form of claim 136, wherein the adjuvant is an aluminum-cation comprising adjuvant.

138. The pharmaceutical composition of claim 136, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide.

139. The pharmaceutical composition of any one of claims 1-138, wherein the pharmaceutical composition is in the form of an intramuscularly injectable composition, intradermally injectable composition, subcutaneously injectable composition, or an intranasally administrable composition.

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140. The pharmaceutical composition of any one of claims 1-139, further comprising a chelating agent.

141. The pharmaceutical composition of claim 140, wherein the chelating agent comprises ethylenediaminetetraacetic acid (EDTA).

142. The pharmaceutical composition of any one of claims 1-141, which is in a form of a suspension.

143. The pharmaceutical composition of any one of claims 1-142, which is in the form of a homogenous mixture or a heterogeneous mixture.

144. The pharmaceutical composition of claim 143, wherein the pharmaceutical composition is in a form of a homogenous mixture.

145. The pharmaceutical composition of any one of claims 1-144, which is contained in a pre-filled syringe.

146. The pharmaceutical composition of any one of claims 1-145, further comprising a surfactant , an emulsifier, or a combination thereof.

147. The pharmaceutical composition of claim 146, comprising the surfactant, wherein the surfactant is a polysorbate, a polymer glycol, a sorbitan ester, or any combination thereof.

148. The pharmaceutical composition of claim 147, comprising the polysorbate, wherein the polysorbate is polysorbate 80.

149. The pharmaceutical composition of claim 148, comprising the polymer glycol, wherein the polymer glycol is polyethylene glycol.

150. The pharmaceutical composition of any one of claims 1-149, further comprising a buffering agent.

151. The pharmaceutical composition of claim 150, wherein the buffering agent is a succinate buffer agent, a tris-acetate- ethylenediaminetetraacetic 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.

152. The pharmaceutical composition of any one of claims 1-151, further comprising sterile water.

153. The pharmaceutical composition of any one of claims 1-152, 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.

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154. The pharmaceutical composition of any one of claims 1-153, 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.

155. The pharmaceutical composition of any one of claims 1-154, wherein the unique immunogenic saccharide-polypeptide conjugate elicits an opsonophagocytic response.

156. The pharmaceutical composition of any one of claims 1-155, 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.

157. The pharmaceutical composition of any one of claims 1-156, wherein the pharmaceutical composition is in unit dose form.

158. The pharmaceutical composition of any one of claims 1-157, further comprising a preservative.

159. The pharmaceutical composition of any one of claims 1-158, further comprising an anti-fungal compound or a salt thereof.

160. The pharmaceutical composition of any one of claims 1-159, further comprising a salt.

161. The pharmaceutical composition of any one of claims 1-160, wherein the salt is an inorganic salt.

162. The pharmaceutical composition of any one of claims 1-161, wherein the immunogenic saccharide-polypeptide conjugate is isolated and purified.

163. The pharmaceutical composition of any one of claims 1-162, which is a vaccine.

164. The pharmaceutical composition of any one of claims 1-163, 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.

165. The pharmaceutical composition of any one of claims 1-164, wherein the immunogenic saccharide-polypeptide conjugate is produced using bacteria.

166. The pharmaceutical composition of any one of claims 1-165, wherein the immunogenic saccharide-polypeptide conjugate is produced using *E. coli*.

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167. The pharmaceutical composition of any one of claims 1-166, wherein the immunogenic saccharide-polypeptide comprises the capsular polysaccharide at least partially embedded in the polypeptide.

168. The pharmaceutical composition of any one of claims 1-167, wherein the immunogenic saccharide-polypeptide comprises the capsular polysaccharide chemically cross-linked to the polypeptide.

169. The pharmaceutical composition of any one of claims 1-168, wherein the immunogenic saccharide-polypeptide comprises the capsular polysaccharide at least partially chemically cross-linked to the polypeptide.

170. A vaccine in unit dose form comprising at 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.

171. The vaccine of claim 170, comprising at least 2 immunogenic saccharide-polypeptide conjugates each 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 group, wherein one serotype is 23A 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, 20B, 22F, 23B, 24F, 24B, 31, 33F, 33B, 34, 35B, 35F, and 38, or a combination thereof.

172. The vaccine of claim 170, comprising at least 2 immunogenic saccharide-polypeptide conjugates each 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 group, wherein one serotype is 23B 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, 20B, 22F, 23A, 24F, 24B, 31, 33F, 33B, 34, 35B, 35F, and 38.

173. The vaccine of any one of claims 170-172, wherein the total amount of capsular polysaccharide, fragment thereof, or combination thereof, individually for each *Streptococcus pneumoniae* serotype in the vaccine in unit dose form, ranges from 0.5 μg to 5 μg.

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174. The vaccine of any one of claims 170-173, wherein the 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 1 μg to about 4 μg.

175. The vaccine of any one of claims 170-174, wherein the total amount of capsular polysaccharide, fragment thereof, or combination thereof, individually for each *Streptococcus pneumoniae* serotype in the vaccine in unit dose form, ranges from 1 μg to 4 μg.

176. The vaccine of any one of claims 170-175, wherein the total amount of capsular polysaccharide, fragment thereof, or combination thereof, individually for each *Streptococcus pneumoniae* serotype in the vaccine in unit dose form, is selected from the group consisting of about 1 μg, about 2 μg, and about 4 μg.

177. The vaccine of any one of claims 170-176, wherein the total amount of capsular polysaccharide, fragment thereof, or combination thereof, individually for each *Streptococcus pneumoniae* serotype in vaccine in unit dose form, is selected from the group consisting of 1 μg, 2 μg, and 4 μg.

178. The vaccine of any one of claims 170-177, wherein a total amount of unconjugated capsular polysaccharide, fragment thereof, or combination thereof, individually for each *Streptococcus pneumoniae* serotype in the vaccine in unit dose form, ranges from about 0% to about 30% w/w.

179. The vaccine of any one of claims 170-178, wherein a total amount of unconjugated capsular polysaccharide, fragment thereof, or combination thereof, individually for each *Streptococcus pneumoniae* serotype in the vaccine in unit dose form, ranges from about 0% to about 20% w/w.

180. The vaccine of any one of claims 170-179, wherein a total amount of unconjugated capsular polysaccharide, fragment thereof, or combination thereof, individually for each *Streptococcus pneumoniae* serotype in the vaccine in unit dose form, ranges from about 0% to about 10% w/w.

181. The vaccine of any one of claims 170-180, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 23A, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

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182. The vaccine of any one of claims 170-181, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 23B, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

183. The vaccine of any one of claims 171-182, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 1, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

184. The vaccine of any one of claims 171-183, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 2, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

185. The vaccine of any one of claims 171-184, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 3, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

186. The vaccine of any one of claims 171-185, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 4, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

187. The vaccine of any one of claims 171-186, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide- CRM_{197} conjugate comprises serotype 5, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and

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10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

188. The vaccine of any one of claims 171-187, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 6A, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

189. The vaccine of any one of claims 171-188, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 6B, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

190. The vaccine of any one of claims 171-189, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 7F, the immunogenic saccharide- CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

191. The vaccine of any one of claims 171-190, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 7C, the immunogenic saccharide- CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

192. The vaccine of any one of claims 171-191, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide- CRM_{197} conjugate comprises serotype 9V, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

193. The vaccine of any one of claims 171-192, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and

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wherein the immunogenic saccharide- CRM_{197} conjugate comprises serotype 14, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

194. The vaccine of any one of claims 171-193, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide- CRM_{197} conjugate comprises serotype 18C, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

195. The vaccine of any one of claims 171-194, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 19A, the immunogenic saccharide- CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

196. The vaccine of any one of claims 171-195, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 19F, the immunogenic saccharide- CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

197. The vaccine of any one of claims 171-196, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide- CRM_{197} conjugate comprises serotype 23F, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

198. The vaccine of any one of claims 171-197, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 6C, the immunogenic saccharide- CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

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199. The vaccine of any one of claims 171-198, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide- CRM_{197} conjugate comprises serotype 8, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

200. The vaccine of any one of claims 171-199, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide- CRM_{197} conjugate comprises serotype 9N, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

201. The vaccine of any one of claims 171-200, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 10A, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

202. The vaccine of any one of claims 171-201, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 11A, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

203. The vaccine of any one of claims 171-202, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 12F, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

204. The vaccine of any one of claims 171-203, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 15A, the immunogenic saccharide- CRM_{197} conjugate has a molecular weight of from 300 kDa and

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10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

205. The vaccine of any one of claims 171-204, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 15B, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

206. The vaccine of any one of claims 171-205, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 15C, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

207. The vaccine of any one of claims 171-206, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 16F, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

208. The vaccine of any one of claims 171-207, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 17F, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

209. The vaccine of any one of claims 171-208, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 20A, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

210. The vaccine of any one of claims 171-209, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and

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wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 20B, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

211. The vaccine of any one of claims 171-210, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 22F, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

212. The vaccine of any one of claims 171-211, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 24F, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

213. The vaccine of any one of claims 171-212, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 24B, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

214. The vaccine of any one of claims 171-213, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 31, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

215. The vaccine of any one of claims 171-214, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide-CRM_{197} conjugate comprises serotype 33F, the immunogenic saccharide-CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

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216. The vaccine of any one of claims 171-215, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide- CRM_{197} conjugate comprises serotype 33B, the immunogenic saccharide- CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

217. The vaccine of any one of claims 171-216, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide- CRM_{197} conjugate comprises serotype 35F, the immunogenic saccharide- CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

218. The vaccine of any one of claims 171-217, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide- CRM_{197} conjugate comprises serotype 34, the immunogenic saccharide- CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

219. The vaccine of any one of claims 171-218, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide- CRM_{197} conjugate comprises serotype 35B, the immunogenic saccharide- CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

220. The vaccine of any one of claims 171-219, wherein the immunogenic saccharide-polypeptide conjugate comprises an immunogenic saccharide-CRM_{197} conjugate, and wherein the immunogenic saccharide- CRM_{197} conjugate comprises serotype 38, the immunogenic saccharide- CRM_{197} conjugate has a molecular weight of from 300 kDa and 10,000 kDa, and wherein a ratio (w/w) of the capsular polysaccharide, fragment thereof, or combination thereof to the CRM_{197} is from 0.3 to 3.0.

221. The vaccine of any one of claims 170-220, wherein at least one of the immunogenic saccharide-polypeptide conjugate comprises the capsular polysaccharide.

222. The vaccine of any one of claims 170-221, wherein at least one of the immunogenic saccharide-polypeptide conjugates comprises the fragment of the capsular polysaccharide.

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223. The vaccine of claim 222, wherein the fragment of the capsular polysaccharide comprises a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, or a hexasaccharide.

224. The vaccine of any one of claims 170-223, wherein at least one polypeptide is an oligopeptide.

225. The vaccine of any one of claims 170-224, wherein at least one 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, PspA, any fragment thereof, or any combination thereof.

226. The vaccine of any one of claims 170-225, wherein the polypeptide comprises a mixture of polypeptides.

227. The vaccine of any one of claims 1-226, wherein a toxin activity of at least one of the polypeptides is at least partly mitigated.

228. The vaccine of any one of claims 1-227, further comprising an adjuvant.

229. The vaccine in unit dose form of claim 228, wherein the adjuvant is an aluminum-cation comprising adjuvant.

230. The vaccine of claim 228, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide.

231. The vaccine or pharmaceutical composition of any one of claims 1-230, wherein the vaccine is in the form of an intramuscularly injectable composition, intradermally injectable composition, subcutaneously injectable composition, or an intranasally administrable composition.

232. The vaccine or pharmaceutical composition of any one of claims 1-231, further comprising a chelating agent.

233. The vaccine or pharmaceutical composition of claim 232, wherein the chelating agent comprises ethylenediaminetetraacetic acid (EDTA).

234. The vaccine or pharmaceutical composition of any one of claims 1-233, which is in a form of a suspension.

235. The vaccine or pharmaceutical composition of any one of claims 1-234, which is in the form of a homogenous mixture or a heterogeneous mixture.

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236. The vaccine or pharmaceutical composition of claim 235, wherein the vaccine is in a form of a homogenous mixture.

237. The vaccine or pharmaceutical composition of any one of claims 1-236, which is contained in a pre-filled syringe.

238. The vaccine or pharmaceutical composition of any one of claims 1-237, further comprising a surfactant, an emulsifier, or a combination thereof.

239. The vaccine or pharmaceutical composition of claim 238, comprising the surfactant, wherein the surfactant is a polysorbate, a polymer glycol, a sorbitan ester, or any combination thereof.

240. The vaccine or pharmaceutical composition of claim 239, comprising the polysorbate, wherein the polysorbate is polysorbate 80.

241. The vaccine or pharmaceutical composition of claim 240, comprising the polymer glycol, wherein the polymer glycol is polyethylene glycol.

242. The vaccine or pharmaceutical composition of any one of claims 1-241, further comprising a buffering agent.

243. The vaccine or pharmaceutical composition of claim 242, wherein the buffering agent is a succinate buffer agent, a tris-acetate- ethylenediaminetetraacetic 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.

244. The vaccine or pharmaceutical composition of any one of claims 1-243, further comprising sterile water.

245. The vaccine or pharmaceutical composition of any one of claims 1-244, wherein when the vaccine 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.

246. The vaccine or pharmaceutical composition of any one of claims 1-245, wherein when the vaccine is administered intramuscularly to a human, the pharmaceutical composition elicits an immune response that lasts from about 1 week to about 30 years.

247. The vaccine or pharmaceutical composition of any one of claims 1-246, wherein the unique immunogenic saccharide-polypeptide conjugate elicits an opsonophagocytic response.

248. The vaccine or pharmaceutical composition of any one of claims 1-247, wherein the vaccine retains at least about 80% of its original biological activity when stored in a

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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.

249. The vaccine or pharmaceutical composition of any one of claims 1-248, wherein the vaccine is in unit dose form.

250. The vaccine or pharmaceutical composition of any one of claims 1-249, further comprising a preservative.

251. The vaccine or pharmaceutical composition of any one of claims 1-250, further comprising an anti-fungal compound or a salt thereof.

252. The vaccine or pharmaceutical composition of any one of claims 1-251, further comprising a salt.

253. The vaccine or pharmaceutical composition of any one of claims 1-252, wherein the salt is an inorganic salt.

254. The vaccine or pharmaceutical composition of any one of claims 1-253, wherein the immunogenic saccharide-polypeptide conjugate is isolated and purified.

255. The vaccine or pharmaceutical composition of any one of claims 1-254, which is a vaccine.

256. The vaccine or pharmaceutical composition of any one of claims 1-255, 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.

257. The vaccine or pharmaceutical composition of any one of claims 1-256, wherein the immunogenic saccharide-polypeptide conjugate is produced using bacteria.

258. The vaccine or pharmaceutical composition of any one of claims 1-257, wherein the immunogenic saccharide-polypeptide conjugate is produced using E. coli.

259. The vaccine or pharmaceutical composition of any one of claims 1-258, wherein the immunogenic saccharide-polypeptide comprises the capsular polysaccharide at least partially embedded in the polypeptide.

260. The vaccine or pharmaceutical composition of any one of claims 1-259, wherein the immunogenic saccharide-polypeptide comprises the capsular polysaccharide chemically cross-linked to the polypeptide.

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261. The vaccine or pharmaceutical composition of any one of claims 1-260, wherein the immunogenic saccharide-polypeptide comprises the capsular polysaccharide at least partially chemically cross-linked to the polypeptide

262. A method comprising administering to a subject a first composition, wherein the first composition is the pharmaceutical composition of any one of claims 1-169 or the vaccine of any one of 170-262.

263. The method of claim 262, 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 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.

264. The method of claim 263, wherein the second composition comprises an immunogenic saccharide-polypeptide conjugate comprising a capsular polysaccharide or fragment of the capsular polysaccharide from a serotype of *Streptococcus pneumoniae* conjugated to the second polypeptide.

265. The method of any one of claims 263-264, wherein the second composition comprises the pharmaceutical composition of any one of claims 1-169 or the vaccine of any one of claims 170-262, wherein the serotype of the first composition and the serotype of the second composition are distinct.

266. The method of any one of claims 263-265, wherein the second composition comprises a plurality of at least 13 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, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F.

267. The method of any one of claims 263-266, wherein the second composition comprises at least 1 unique immunogenic saccharide-polypeptide conjugate comprising 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, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F, and 33F.

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268. The method of any one claims 263-267, wherein the second composition comprising at least 1 unique immunogenic saccharide-polypeptide conjugate comprising 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, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 35F, 35B, and 38, wherein the serotype of the first composition and the serotype of the second composition are distinct.

269. The method of claim 268, wherein the immunogenic saccharide polypeptide conjugate of the second composition comprises a capsular polysaccharide.

270. The method of claim 269, wherein the immunogenic saccharide polypeptide conjugate of the second composition comprises a fragment of the capsular polysaccharide.

271. The method of claim 270, wherein the fragment of the capsular polysaccharide of the second composition comprises a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, or a hexasaccharide.

272. The method of any one of claims 263-271, wherein the second polypeptide is an oligopeptide.

273. The method of any one of claims 263-272, 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*, PspA, any fragment thereof, or any combination thereof.

274. The method of any one of claims 263-273, wherein a toxin activity of the second polypeptide is at least partly mitigated.

275. The method of any one of claims 263-274, wherein the second composition further comprises an adjuvant.

276. The method of claim 275, wherein the adjuvant is an aluminum-cation comprising adjuvant.

277. The method of claim 276, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide.

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278. The method any one of claims 263-277, wherein the second composition is in the form of an intramuscularly injectable composition, intradermally injectable composition, subcutaneously injectable composition, or an intranasally administrable composition.

279. The method of any one of claims 263-278, wherein the second composition is in unit dose form.

280. The method of any one of claims 263-279, wherein the second composition further comprises a chelating agent.

281. The method of claim 280, wherein the chelating agent is ethylenediaminetetraacetic acid (EDTA).

282. The method any one of claims 263-281, wherein the second composition is in a form of a suspension.

283. The method any one of claims 263-282, wherein the second composition is in a form of a homogenous mixture or a heterogeneous mixture.

284. The method any one of claims 263-283, wherein the second composition is in the form of a homogenous mixture.

285. The method any one of claims 263-284, wherein the second composition is contained in a pre-filled syringe.

286. The method any one of claims 263-285, wherein the second composition further comprises a surfactant, an emulsifier, or a combination thereof.

287. The method claim 286, wherein the second composition further comprises the surfactant, wherein the surfactant is polysorbate, polymer glycol, a sorbitan ester, or any combination thereof.

288. The method of claim 287, wherein the second composition comprises the polysorbate, wherein the polysorbate is polysorbate 80.

289. The method of claim 288, wherein the second composition comprises the polymer glycol wherein the polymer glycol is polyethylene glycol.

290. The method any one of claims 263-289, wherein the second composition further comprises a buffering agent.

291. The method of claim 290, wherein the buffering agent is a succinate buffer agent, a tris-acetate- ethylenediaminetetraacetic 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.

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292. The method any one of claims 263-291, wherein the second composition further comprises sterile water.

293. The method any one of claims 263-292, wherein when the second 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.

294. The method any one of claims 263-293, wherein when the second composition is administered intramuscularly to a human, the pharmaceutical composition elicits an immune response that lasts from about 1 week to about 30 years.

295. The method any one of claims 263-294, wherein when the unique immunogenic saccharide-polypeptide conjugate of the second composition elicits an opsonophagocytic response.

296. The method of any one of claims 263-295, wherein when the second composition is administered intramuscularly, the second composition elicits an immune response that lasts from about 1 week to about 30 years.

297. The method of any one of claims 263-296, wherein second 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.

298. The method of any one of claims 263-297, wherein the second composition further comprises a preservative.

299. The method of any one of claims 263-298, wherein the second composition further comprises an anti-fungal compound or a salt thereof.

300. The method of any one of claims 263-299, wherein the second composition further comprises a salt.

301. The method of claim 300, wherein the salt is an inorganic salt.

302. The method of any one of claims 263-301, wherein the immunogenic saccharide-polypeptide conjugate of the second composition is isolated and purified.

303. The method of any one of claims 263-302, wherein the second composition is a vaccine.

304. The method of any one of claims 263-303, which is a method of vaccinating a subject.

305. The method of claim 304, wherein the subject is a human.

306. The method of any one of claims 304-305, wherein the subject or human is a subject or human in need thereof.

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307. The method of any one of claims 263-306, wherein the immunogenic saccharide-polypeptide conjugates of the second composition 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.

308. The method of any one of claims 263-307, 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 of the second composition, wherein the antibody response is measured by measuring antibody titers.

309. The method of any one of claims 263-308, wherein the immunogenic saccharide-polypeptide conjugate of the second composition is produced using bacteria.

310. The method of any one of claims 263-309, wherein the immunogenic saccharide-polypeptide conjugate of the second composition is produced using *E. coli*.

311. The method of any one of claims 263-310, wherein the immunogenic saccharide-polypeptide of the second composition comprises the capsular polysaccharide at least partially embedded in the polypeptide.

312. The method of any one of claims 263-311, wherein the immunogenic saccharide-polypeptide of the second composition comprises the capsular polysaccharide chemically cross-linked to the polypeptide.

313. The method of any one of claims 263-312, wherein the immunogenic saccharide-polypeptide of the second composition comprises the capsular polysaccharide at least partially chemically cross-linked to the polypeptide.

314. 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;

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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 170-261.

315. The method of claim 314, wherein the first immunogenic saccharide-polypeptide conjugate composition is the pharmaceutical composition of any one of claims 1-158 wherein the first immunogenic saccharide-polypeptide conjugate composition and the second immunogenic saccharide-polypeptide conjugate composition are distinct.

316. The method of any one of claims 314-315, wherein the second immunogenic saccharide-polypeptide conjugate composition is the pharmaceutical composition of any one of claims 1-169 wherein the first immunogenic saccharide-polypeptide conjugate composition and the second immunogenic saccharide-polypeptide conjugate composition are distinct.

317. The method of any one of claims 314-316, wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a capsular polysaccharide.

318. The method of any one of claims 314-317, wherein the first immunogenic saccharide-polypeptide conjugate composition comprises a fragment of the capsular polysaccharide.

319. The method of any one of claims 314-318, wherein the fragment of the capsular polysaccharide comprises a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, or a hexasaccharide.

320. The method of any one of claims 314-319, wherein the first polypeptide is an oligopeptide.

321. The method of any one of claims 314-320, wherein the second polypeptide is an oligopeptide.

322. The method of any one of claims 314-321, wherein the first 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.

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meningitidis, a purified protein derivative of tuberculin(PPD), protein D from *H. influenzae*, any fragment thereof, or any combination thereof .

323. The method of any one of claims 314-322, wherein a toxin activity of the first polypeptide is at least partly mitigated.

324. The method of any one of claims 314-323, 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*, PspA, any fragment thereof, or any combination thereof.

325. The method of any one of claims 314-324, wherein a toxin activity of the second polypeptide is at least partly mitigated.

326. The method of any one of claims 314-325, wherein the first serotype is from a same bacteria as the second serotype.

327. The method of any one of claims 314-326, wherein the first serotype is from a different bacteria than the second serotype.

328. The method of any one of claims 314-327, wherein the first serotype is from *Streptococcus pneumoniae*.

329. The method of any one of claims 314-328, wherein the first serotype is from *Neisseria meningitidis*, *Salmonella enterica*, or *Haemophilus influenzae*.

330. The method of any one of claims 314-329, wherein the second serotype is from *Streptococcus pneumoniae*.

331. The method of any one of claims 314-330, wherein the second bacteria is from *Neisseria meningitidis*, *Salmonella enterica*, or *Haemophilus influenzae*.

332. The method of any one of claims 314-331, wherein the first serotype comprises at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, or 23F from *Streptococcus pneumoniae*, and the second serotype comprises at least eight of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, or 38 from *Streptococcus pneumoniae*.

333. The method of any one of claims 314-331, wherein the first serotype comprises at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, or 23F from *Streptococcus pneumoniae*, and the second serotype comprises at least eight of 2, 6C, 8, 9N, 10A, 11A,

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12F, 15A, 15B, 15C, 16F, 17F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, or 38 from *Streptococcus pneumoniae*.

334. The method of any one of claims 314-331, wherein the first serotype comprises at least eight of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, and 38 from *Streptococcus pneumoniae*, and the second serotype comprises at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*.

335. The method of any one of claims 314-331, wherein the first serotype comprises at least eight of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, and 38 from *Streptococcus pneumoniae*, and the second serotype comprises at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*.

336. The method of any one of claims 314-331, wherein the first serotype comprises 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second serotype comprises 6C, 9N, 15A, 15C, 16F, 23A, 23B, and 35B from *Streptococcus pneumoniae*.

337. The method of any one of claims 314-331, wherein the first serotype comprises 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second serotype comprises 6C, 15A, 16F, 23A, 23B, and 35B from *Streptococcus pneumoniae*.

338. The method of any one of claims 314-331, wherein the first serotype comprises 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second serotype comprises 6C, 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 24F, 31, 33F, 34, 35F, and 35B from *Streptococcus pneumoniae*.

339. The method of any one of claims 314-331, wherein the first serotype comprises 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second serotype comprises 6C, 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 33F, 34, 35F, and 35B from *Streptococcus pneumoniae*.

340. The method of any one of claims 314-331, wherein the first serotype comprises 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*, and the second serotype comprises 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20A, 22F, 23A, 23B, 33F, and 35B from *Streptococcus pneumoniae*.

341. The method of any one of claims 314-331, wherein the first serotype comprises 6C, 9N, 15A, 15C, 16F, 23A, 23B, and 35B from *Streptococcus pneumoniae*, and the second

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serotype comprises 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*.

342. The method of any one of claims 314-331, wherein the first serotype comprises 6C, 15A, 16F, 23A, 23B, and 35B from *Streptococcus pneumoniae*, and the second serotype comprises 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*.

343. The method of any one of claims 314-331, wherein the first serotype comprises 6C, 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 24F, 31, 33F, 34, 35F, and 35B from *Streptococcus pneumoniae*, and the second serotype comprises 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*.

344. The method of any one of claims 314-331, wherein the first serotype comprises 6C, 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 33F, 34, 35F, and 35B from *Streptococcus pneumoniae*, and the second serotype comprises 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*.

345. The method of any one of claims 314-331, wherein the first serotype comprises 6C, 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20A, 22F, 23A, 23B, 33F, and 35B from *Streptococcus pneumoniae*, and the second serotype comprises 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*.

346. The method of any one of claims 314-331, wherein the first serotype comprises at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, or 23F from *Streptococcus pneumoniae*, and the second serotype comprises at least eight of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, 38, and Serogroup 20 (comprising serotypes 20A and 20B) from *Streptococcus pneumoniae*.

347. The method of any one of claims 314-331, wherein the first serotype comprises at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, or 23F from *Streptococcus pneumoniae*, and the second serotype comprises at least eight of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, 38, and Serogroup 20 (comprising serotypes 20A and 20B) from *Streptococcus pneumoniae*.

348. The method of any one of claims 314-331, wherein the first serotype comprises at least eight of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, 38, and Serogroup 20 (comprising serotypes 20A and 20B) from *Streptococcus pneumoniae*, and the second serotype comprises at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*.

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349. The method of any one of claims 314-331, wherein the first serotype comprises at least eight of 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, 38, and Serogroup 20 (comprising serotypes 20A and 20B) from *Streptococcus pneumoniae*, and the second serotype comprises at least one of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F from *Streptococcus pneumoniae*.

350. The method of any one of claims 314-349, wherein the first immunogenic saccharide-polypeptide conjugate composition further comprises an adjuvant.

351. The method of claim 350, wherein the adjuvant is an aluminum-cation comprising adjuvant.

352. The method of claim 351, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide.

353. The method of any one of claims 314-352, wherein the first immunogenic saccharide-polypeptide conjugate composition is in the form of an intramuscularly injectable composition, intradermally injectable composition, subcutaneously injectable composition, or an intranasally administrable composition.

354. The method of any one of claims 314-353, wherein the first immunogenic saccharide-polypeptide conjugate composition further comprises a chelating agent.

355. The method of claim 354, wherein the chelating agent is ethylenediaminetetraacetic acid (EDTA).

356. The method of any one of claims 314-355, wherein the first immunogenic saccharide-polypeptide conjugate composition is in a form of a suspension.

357. The method of any one of claims 302-356, wherein the first immunogenic saccharide-polypeptide conjugate composition is in a form of a homogenous mixture or a heterogeneous mixture.

358. The method of any one of claims 314-357, wherein the first immunogenic saccharide-polypeptide conjugate composition is in a form of a homogenous mixture.

359. The method of any one of claims 314-358, wherein the first immunogenic saccharide-polypeptide conjugate composition is contained in a pre-filled syringe.

360. The method of any one of claims 314-359, wherein the first immunogenic saccharide-polypeptide conjugate composition further comprises a surfactant or emulsifier, or a combination thereof.

361. The method of claim 360, wherein the first immunogenic saccharide-polypeptide composition comprises the surfactant wherein the surfactant is a polysorbate, a polymer glycol, a sorbitan ester, or any combination thereof.

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362. The method of claim 361, wherein the first immunogenic saccharide-polypeptide composition comprises the polysorbate wherein the polysorbate is polysorbate 80.

363. The method claim 361, wherein the first immunogenic saccharide-polypeptide composition comprises the polymer glycol wherein the polymer glycol is polyethylene glycol.

364. The method of any one of claims 314-363, wherein the first immunogenic saccharide-polypeptide conjugate composition further comprises a buffering agent.

365. The method of claim 364, wherein the buffering agent is a succinate buffer agent, a tris-acetate- ethylenediaminetetraacetic 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.

366. The method of any one of claims 314-365, wherein the first immunogenic saccharide-polypeptide conjugate composition further comprises sterile water.

367. The method of any one of claims 314-366, wherein when the first immunogenic saccharide-polypeptide conjugate 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.

368. The method of any one of claims 314-367, wherein when first immunogenic saccharide-polypeptide conjugate composition is administered intramuscularly to a human, the first immunogenic saccharide-polypeptide conjugate composition elicits an immune response that lasts from about 1 week to about 30 years.

369. The method any one of claims 314-368, wherein when first immunogenic saccharide-polypeptide conjugate composition elicits an opsonophagocytic response.

370. The method of any one of claims 314-369, wherein the first immunogenic saccharide-polypeptide conjugate 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.

371. The method of any one of claims 314-370, wherein the second immunogenic saccharide-polypeptide conjugate composition further comprises an adjuvant.

372. The method of claim 371, wherein the adjuvant is an aluminum-cation comprising adjuvant.

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373. The method of claim 372, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide.

374. The method of any one of claims 314-373, wherein the second immunogenic saccharide-polypeptide conjugate composition is in a form of an intramuscularly injectable composition, intradermally injectable composition, subcutaneously injectable composition, or an intranasally administrable composition.

375. The method of any one of claims 314-374, wherein the second immunogenic saccharide-polypeptide conjugate composition further comprises a chelating agent.

376. The method of claim 375, wherein the chelating agent is ethylenediaminetetraacetic acid (EDTA).

377. The method of any one of claims 314-376, wherein the second immunogenic saccharide-polypeptide conjugate composition is in a form of a suspension.

378. The method of any one of claims 314-377, wherein the second immunogenic saccharide-polypeptide conjugate composition is in a form of a homogenous mixture or a heterogeneous mixture.

379. The method of claim 378, wherein the second immunogenic saccharide-polypeptide conjugate composition is in a form of a homogenous mixture.

380. The method of any one of claims 314-379, wherein the second immunogenic saccharide-polypeptide conjugate composition is contained in a pre-filled syringe.

381. The method of any one of claims 314-380, wherein the second immunogenic saccharide-polypeptide conjugate composition further comprises a surfactant, an emulsifier, or a combination thereof.

382. The method of claim 381, wherein the second immunogenic saccharide-polypeptide conjugate composition comprises the surfactant wherein the surfactant is polysorbate, polymer glycol, a sorbitan ester, or any combination thereof.

383. The method of claim 382, wherein the second immunogenic saccharide-polypeptide conjugate composition comprises the polysorbate wherein the polysorbate is polysorbate 80.

384. The method of claim 383, wherein the second immunogenic saccharide-polypeptide conjugate composition comprises the polymer glycol wherein the polymer glycol is polyethylene glycol.

385. The method of any one of claims 314-384, wherein the second immunogenic saccharide-polypeptide conjugate composition further comprises a buffering agent.

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386. The method of claim 385, wherein the buffering agent is a succinate buffer agent, a tris-acetate- ethylenediaminetetraacetic 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.

387. The method of any one of claims 314-386, wherein the second immunogenic saccharide-polypeptide conjugate composition further comprises sterile water.

388. The method of any one of claims 314-387, wherein when the second immunogenic saccharide-polypeptide conjugate 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.

389. The method of any one of claims 314-388, wherein when the second immunogenic saccharide-polypeptide conjugate composition is administered intramuscularly to a human, the second immunogenic saccharide-polypeptide conjugate composition elicits an immune response that lasts from about 1 week to about 30 years.

390. The method of any one of claims 314-389, wherein when the second immunogenic saccharide-polypeptide conjugate composition elicits an opsonophagocytic response.

391. The method of any one of claims 314-390, wherein the second immunogenic saccharide-polypeptide conjugate 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.

392. The method of any one of claims 314-391, wherein the first immunogenic saccharide-polypeptide conjugate composition and/or the second immunogenic saccharide polypeptide composition is in unit dose form.

393. The method of any one of claims 314-392, wherein the first immunogenic saccharide-polypeptide conjugate composition and/or the second immunogenic saccharide polypeptide composition further comprises a preservative.

394. The method of any one of claims 314-393, wherein the first immunogenic saccharide-polypeptide conjugate composition and/or the second immunogenic saccharide polypeptide composition further comprises an anti-fungal compound or a salt thereof.

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395. The method of any one of claims 314-394, wherein the first immunogenic saccharide-polypeptide conjugate composition and/or the second immunogenic saccharide polypeptide composition further comprise a salt.

396. The method of claim 395, wherein the salt is an inorganic salt.

397. The method of any one of claims 314-396, wherein the first immunogenic saccharide-polypeptide conjugate composition and/or the second immunogenic saccharide polypeptide composition is isolated and purified.

398. The method of any one of claims 314-397, wherein the first immunogenic saccharide-polypeptide conjugate composition and/or the second immunogenic saccharide polypeptide composition which is a vaccine.

399. The method of any one of claims 314-398, which is a method of vaccinating a subject.

400. The method of any claim 399, wherein the subject is a human.

401. The method of any one of claims 399-400, wherein the subject or human is a subject or human in need thereof.

402. The method of any one of claims 314-401, wherein the first immunogenic saccharide-polypeptide conjugate composition and/or the second immunogenic saccharide polypeptide composition comprise immunogenic saccharide-polypeptide conjugate(s) 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.

403. The method of any one of claims 314-402, wherein administration of the second immunogenic saccharide-polypeptide conjugate composition does not result in more than about: 50%, 40%, 30%, 20%, 10%, 5%, 4%, 3%, 2%, or 1% suppression of an immunity as measured by antibody response to the saccharide of the immunogenic saccharide-polypeptide conjugate, wherein the antibody response is measured by measuring antibody titers.

404. The method of any one of claims 314-403, wherein the first immunogenic saccharide-polypeptide conjugate composition and/or the second immunogenic saccharide polypeptide composition is produced using a bacteria.

405. The method of any one of claims 314-404, wherein the first immunogenic saccharide-polypeptide conjugate composition and/or the second immunogenic saccharide polypeptide composition is produced using *E. coli*.

406. The method of any one of claims 314-405, wherein the first immunogenic saccharide-polypeptide conjugate composition and/or the second immunogenic saccharide

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polypeptide composition comprises the capsular polysaccharide at least partially embedded in the polypeptide.

407. The method of any one of claims 314-406, wherein the first immunogenic saccharide-polypeptide conjugate composition and/or the second immunogenic saccharide polypeptide composition comprises the capsular polysaccharide chemically cross-linked to the polypeptide.

408. The method of any one of claims 314-407, wherein the first immunogenic saccharide-polypeptide conjugate composition and/or the second immunogenic saccharide polypeptide composition comprises the capsular polysaccharide at least partially chemically cross-linked to the polypeptide.

409. A method of making a composition comprising:

contacting the immunogenic saccharide-polypeptide conjugate of any one of claims 1-261 with an excipient, an adjuvant, or any combination thereof.

410. The method of claim 409, wherein the composition is injectable or intranasally administrable.

411. The method claim 410, comprising contacting with the adjuvant, wherein the adjuvant is aluminum phosphate, aluminum sulfate, or aluminum hydroxide.

412. The method of any one of claims 409-411, wherein the composition is in the form of an intramuscularly injectable composition.

413. The method of any one of claims 409-412, further comprising contacting the composition with a chelating agent.

414. The method of claim 413, further comprising contacting the composition with a chelating agent, wherein the chelating agent is ethylenediaminetetraacetic acid (EDTA).

415. The method of any one of claims 409-414, wherein the composition is in a form of a suspension.

416. The method of any one of claims 409-415, wherein the composition is in a form of a homogenous mixture or a heterogeneous mixture.

417. The method of claim 416, wherein the composition is in a form of a homogenous mixture.

418. The method of any one of claims 409-417, wherein the composition is contained in a pre-filled syringe.

419. The method of any one of claims 409-418, further comprising contacting the composition with a surfactant, an emulsifier, or a combination thereof.

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420. The method of claim 419, comprising contacting the composition with the surfactant, wherein the surfactant is a polysorbate, a polymer glycol, a sorbitan ester, or any combination thereof.

421. The method of claim 420, comprising contacting the composition with the polysorbate, wherein the polysorbate is polysorbate 80.

422. The method of claim 421, comprising contacting the composition with the polymer glycol, wherein the polymer glycol is polyethylene glycol.

423. The method of any one of claims 409-422, further comprising contacting the composition with a buffering agent.

424. The method of claim 423, wherein the buffering agent is a succinate buffer agent, a tris-acetate- ethylenediaminetetraacetic 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.

425. The method of any one of claims 409-424, further comprising contacting the composition with sterile water.

426. The method of any one of claims 409-425, wherein when the 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.

427. The method of any one of claims 409-426, wherein when the composition is administered intramuscularly to a human, the composition elicits an immune response that lasts from about 1 week to about 30 years.

428. The method of any one of claims 409-427, wherein the immunogenic saccharide-polypeptide conjugate elicits an opsonophagocytic response.

429. The method of any one of claims 409-428, wherein the 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.

430. The method of any one of claims 409-429, wherein the composition further comprises a preservative.

431. The method of any one of claims 409-430, wherein the composition further comprises an anti-fungal compound or a salt thereof.

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432. The method of any one of claims 409-431, wherein the composition further comprises a salt.

433. The method claim 432, wherein the salt is an inorganic salt.

434. The method of any one of claims 409-433, wherein the immunogenic saccharide-polypeptide conjugate of the composition is isolated and purified.

435. The method of any one of claims 409-434, wherein the composition is used as a vaccine.

436. The method of any one of claims 409-435, wherein the composition is used for vaccinating a subject.

437. The method of claim 436, wherein the subject is a human.

438. The method of any one of claims 185-437, wherein the subject or human is a subject or human in need thereof.

439. The method of any one of claims 409-438, wherein the immunogenic saccharide-polypeptide conjugate(s) of the composition 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.

440. The method of any one of claims 409-439, wherein the immunogenic saccharide-polypeptide conjugate of the composition is produced using bacteria.

441. The method of any one of claims 409-440, wherein the immunogenic saccharide-polypeptide conjugate of the composition is produced using E. coli.

442. The method of any one of claims 409-441, wherein the immunogenic saccharide-polypeptide conjugate of the composition comprises the capsular polysaccharide at least partially embedded in the polypeptide.

443. The method of any one of claims 409-442, wherein the immunogenic saccharide-polypeptide conjugate of the composition comprises the capsular polysaccharide chemically cross-linked to the polypeptide.

444. The method of any one of claims 409-443, wherein the immunogenic saccharide-polypeptide conjugate of the composition comprises the capsular polysaccharide at least partially chemically cross-linked to the polypeptide.

445. A kit comprising a pharmaceutical composition of any of claims 1-169 or the vaccine of any one of claims 170-261 contained in a container.

446. The kit of claim 445, further comprising instructions for use.

447. The kit of any one of claims 445-446, wherein the container is a syringe.

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448. The kit of any one of claims 445-446, wherein the container is a vial comprising a cap, wherein the cap comprises a rubber septum.

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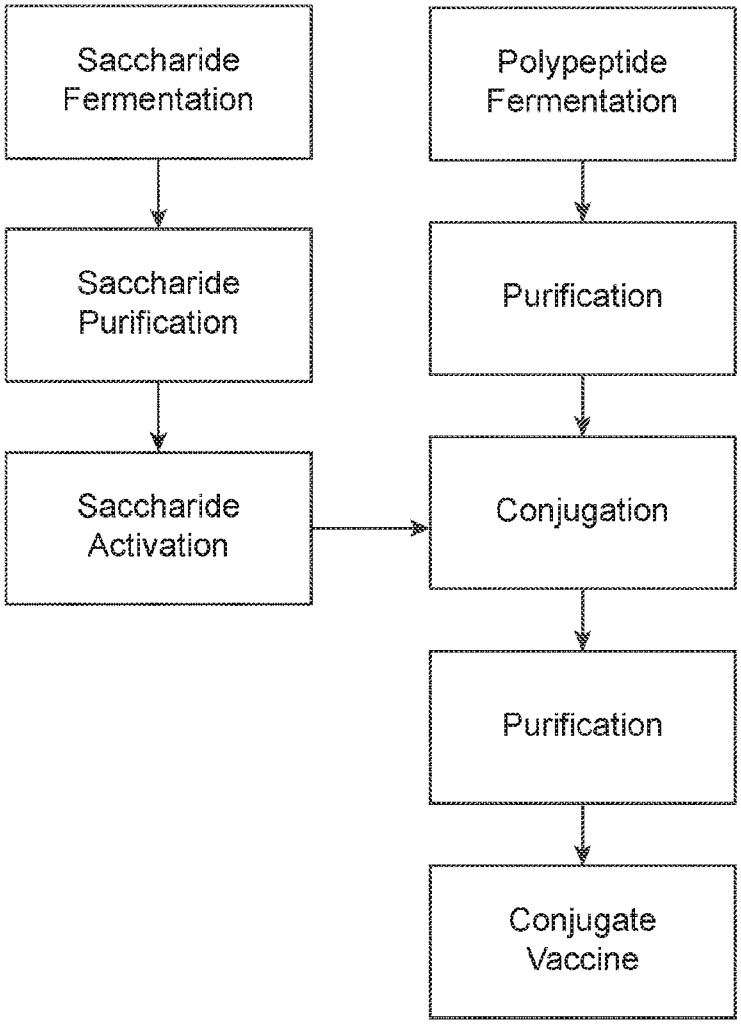

Saccharide Fermentation
Saccharide Purification
Saccharide Activation
Polypeptide Fermentation
Purification
Conjugation
Purification
Conjugate Vaccine

FIG. 1

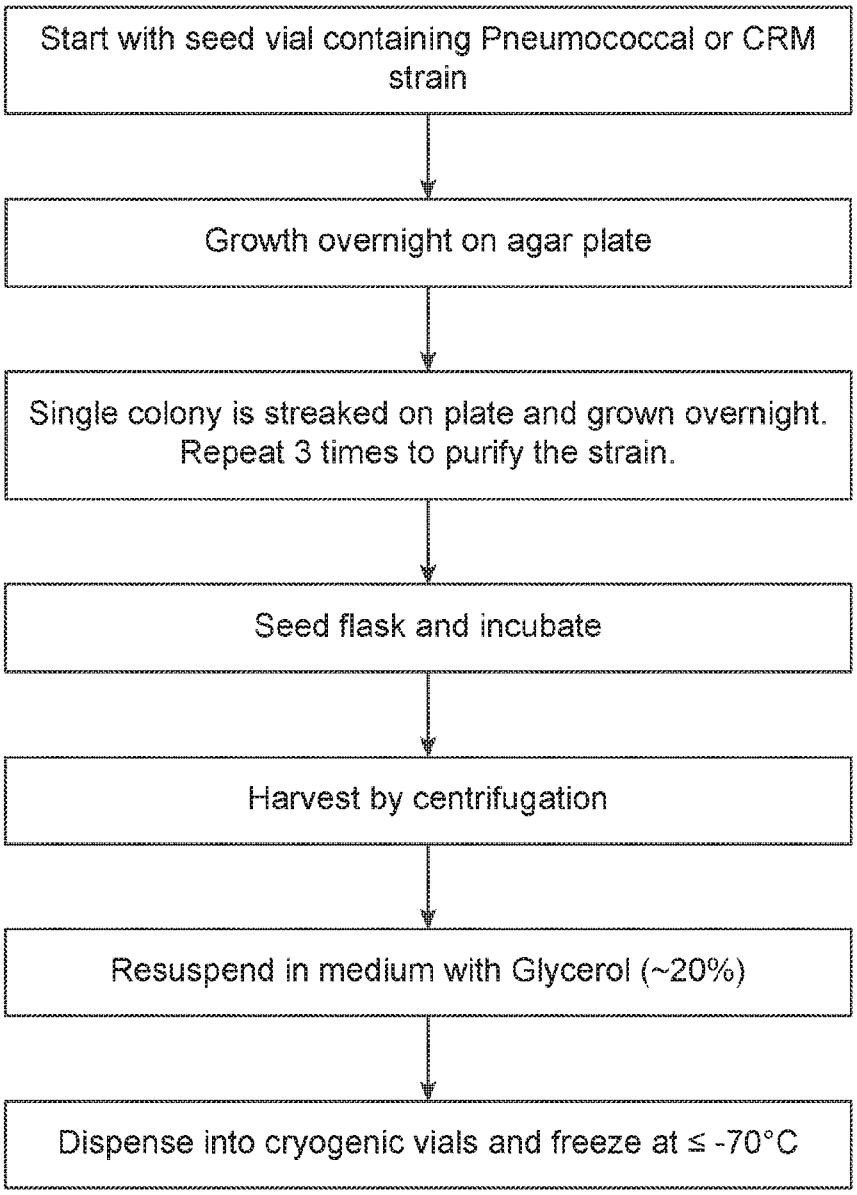

Start with seed vial containing Pneumococcal or CRM strain
Growth overnight on agar plate
Single colony is streaked on plate and grown overnight. Repeat 3 times to purify the strain.
Seed flask and incubate
Harvest by centrifugation
Resuspend in medium with Glycerol (~20%)
Dispense into cryogenic vials and freeze at ≤ -70°C

FIG. 2

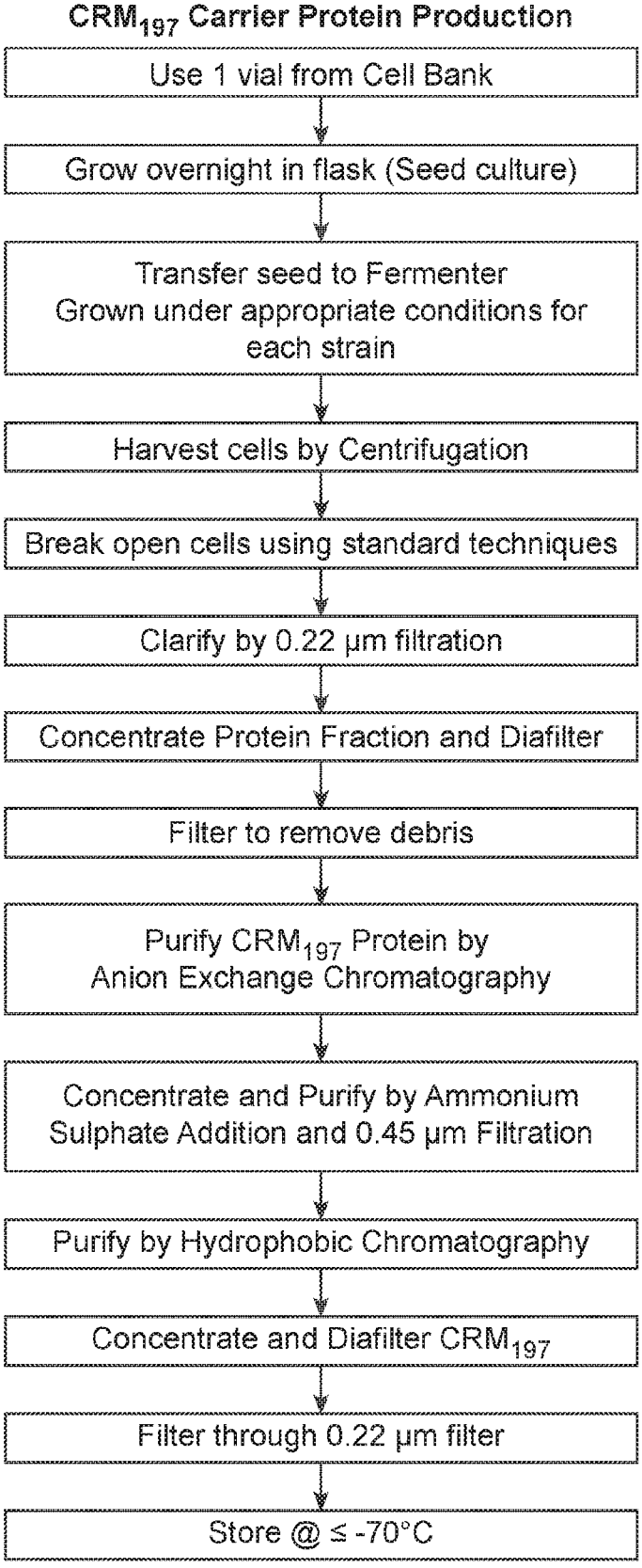


FIG. 3

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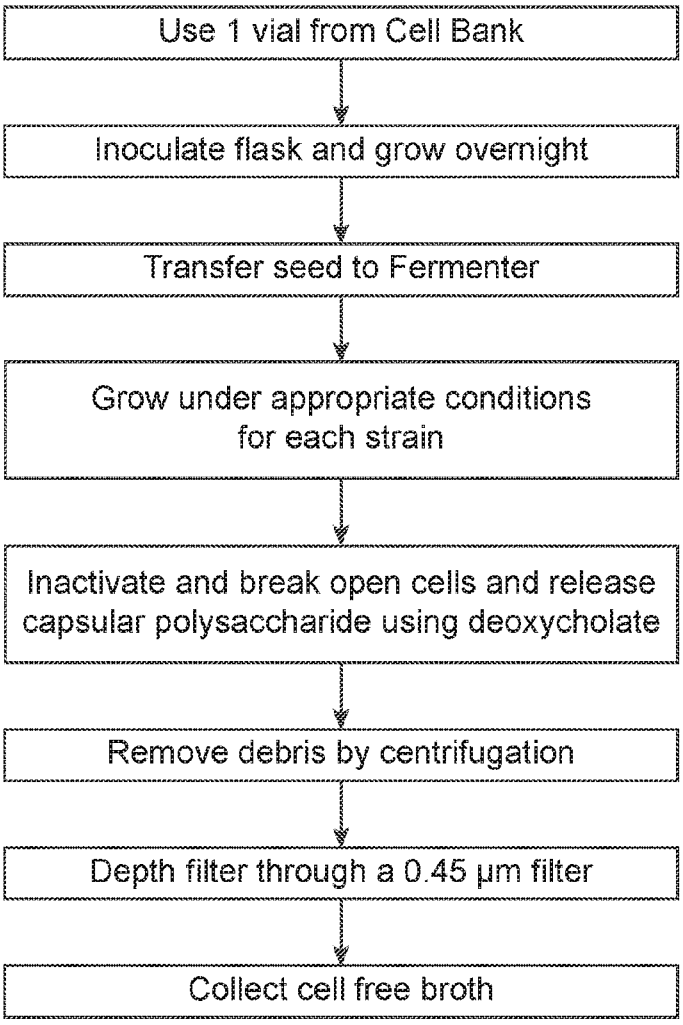

Use 1 vial from Cell Bank
Inoculate flask and grow overnight
Transfer seed to Fermenter
Grow under appropriate conditions for each strain
Inactivate and break open cells and release capsular polysaccharide using deoxycholate
Remove debris by centrifugation
Depth filter through a 0.45 µm filter
Collect cell free broth

FIG. 4

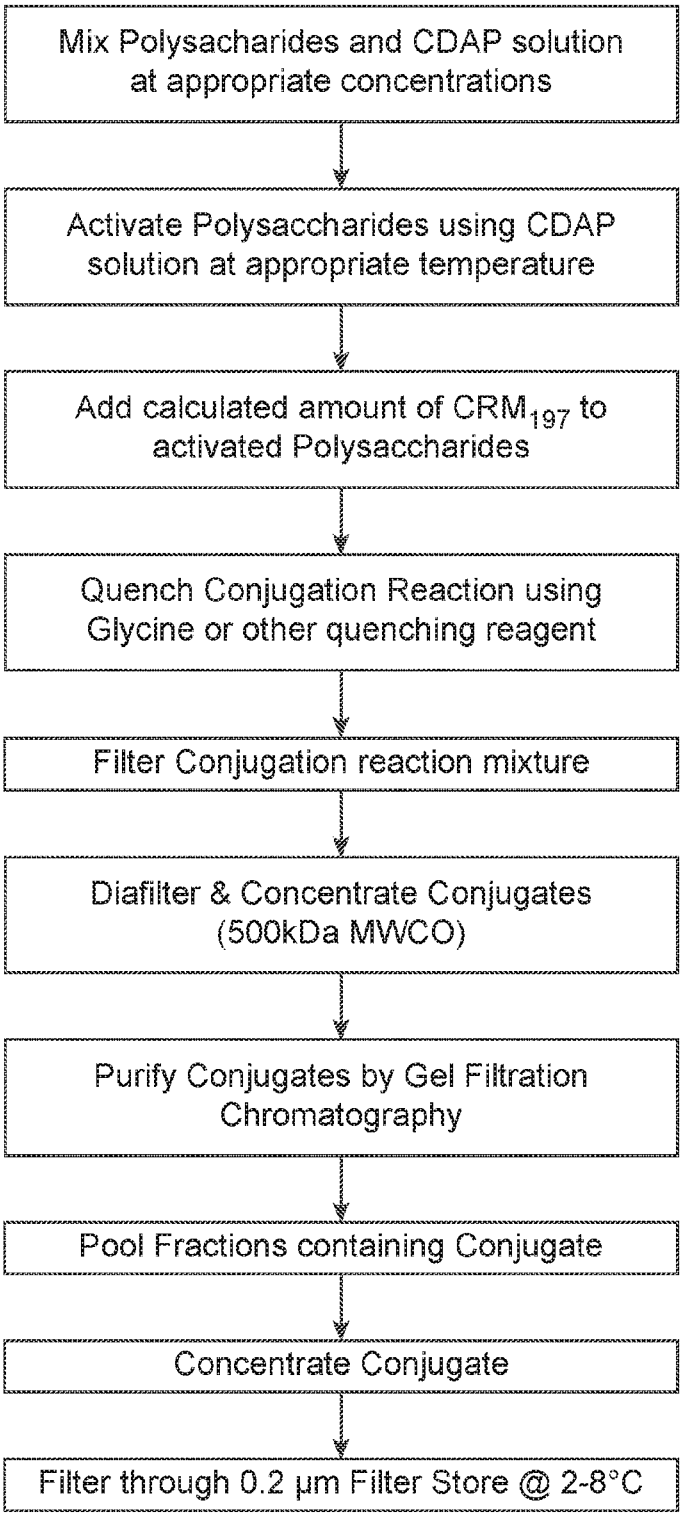

Mix Polysacharides and CDAP solution at appropriate concentrations
Activate Polysaccharides using CDAP solution at appropriate temperature
Add calculated amount of CRM_{197} to activated Polysaccharides
Quench Conjugation Reaction using Glycine or other quenching reagent
Filter Conjugation reaction mixture
Diafilter & Concentrate Conjugates (500kDa MWCO)
Purify Conjugates by Gel Filtration Chromatography
Pool Fractions containing Conjugate
Concentrate Conjugate
Filter through 0.2 µm Filter Store @ 2-8°C

FIG. 5

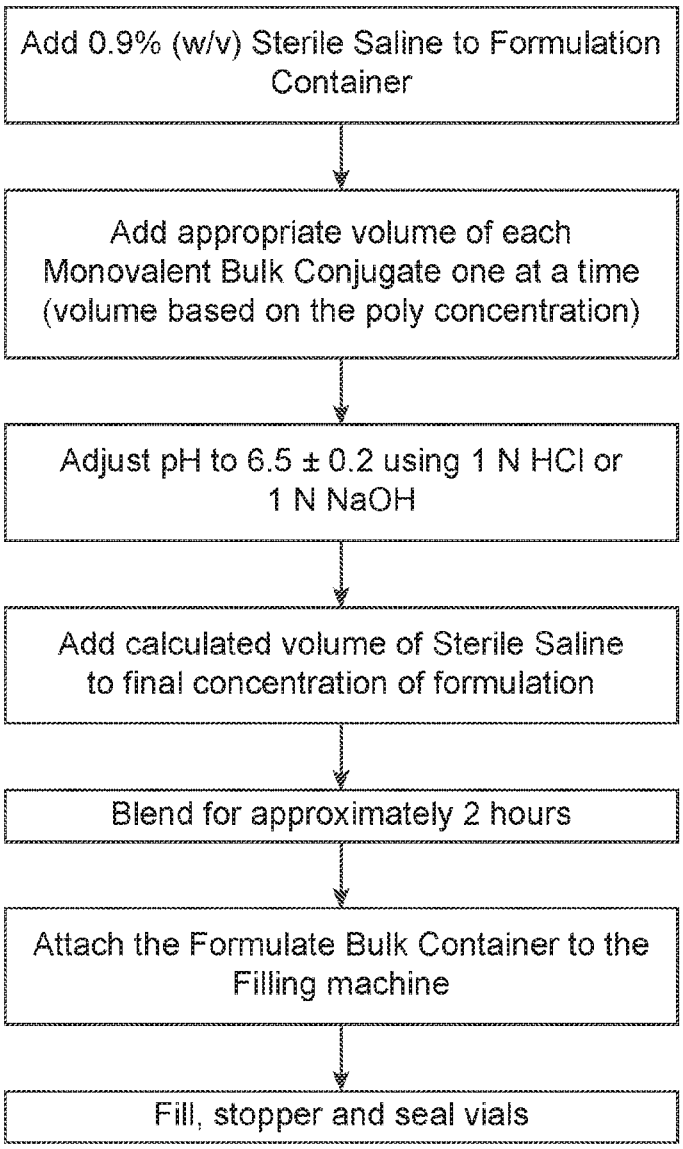

Add 0.9% (w/v) Sterile Saline to Formulation Container
Add appropriate volume of each Monovalent Bulk Conjugate one at a time (volume based on the poly concentration)
Adjust pH to 6.5 ± 0.2 using 1 N HCl or 1 N NaOH
Add calculated volume of Sterile Saline to final concentration of formulation
Blend for approximately 2 hours
Attach the Formulate Bulk Container to the Filling machine
Fill, stopper and seal vials

FIG. 6

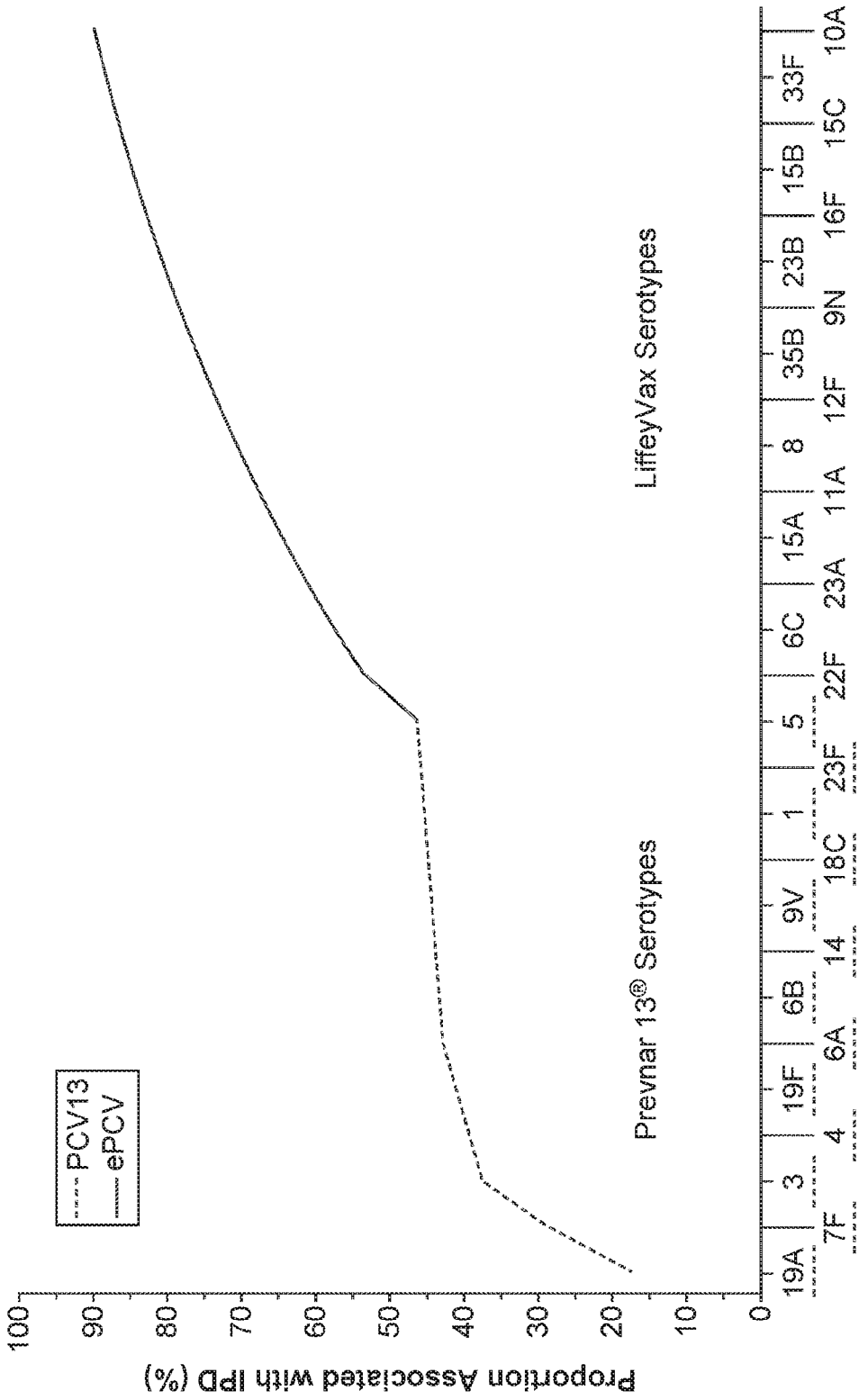

PCV13
ePCV
Proportion Associated with IPD (%)
100
90
80
70
60
50
40
30
20
10
0
Prevnar 13® Serotypes
LiffeyVax Serotypes
19A
7F
3
4
19F
6A
6B
14
9V
18C
1
23F
5
22F
6C
23A
15A
11A
8
12F
35B
9N
23B
16F
15B
15C
33F
10A
S. pneumoniae Serotypes

FIG. 7

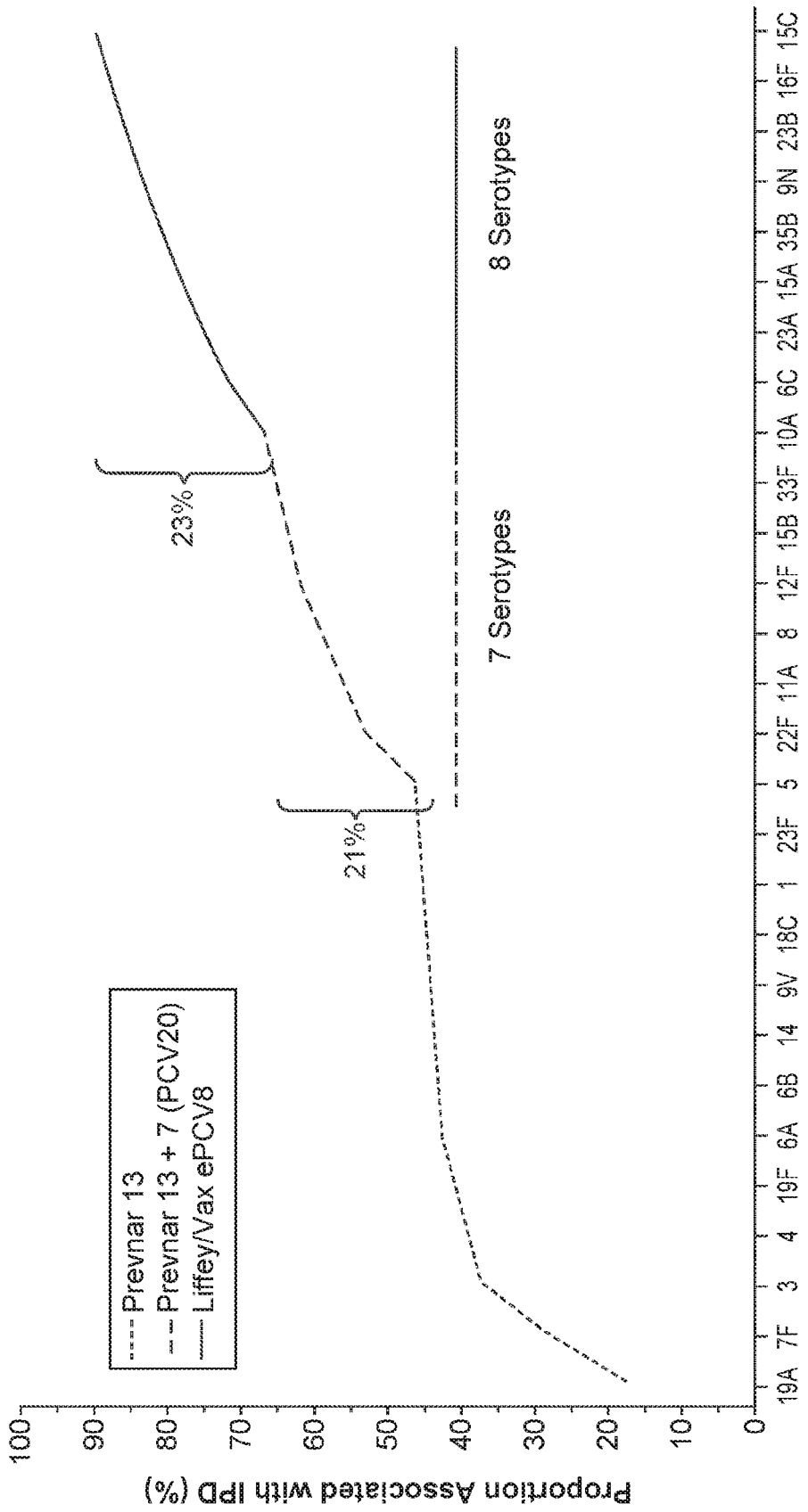

Proportion Associated with IPD (%)
100
90
80
70
60
50
40
30
20
10
0
Prevnar 13
Prevnar 13 + 7 (PCV20)
Liffey/Vax ePCV8
23%
21%
7 Serotypes
8 Serotypes
19A 7F 3 4 19F 6A 6B 14 9V 18C 1 23F 5 22F 11A 8 12F 15B 33F 10A 6C 23A 15A 35B 9N 23B 16F 15C
S. pneumoniae Serotypes

FIG. 8

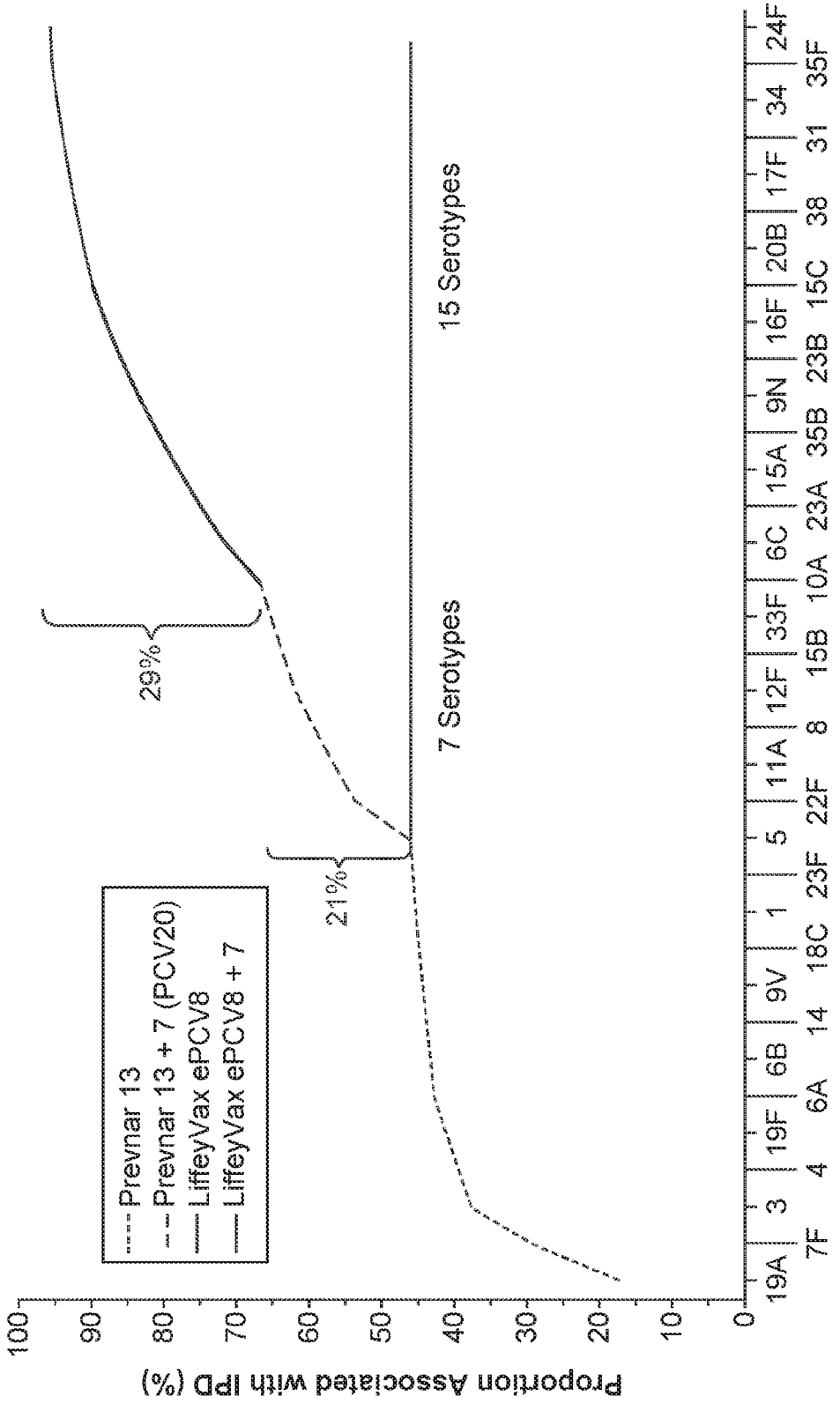

Proportion Associated with IPD (%)
100
90
80
70
60
50
40
30
20
10
0
Prevnar 13
Prevnar 13 + 7 (PCV20)
LiffeyVax ePCV8
LiffeyVax ePCV8 + 7
29%
21%
7 Serotypes
15 Serotypes
19A 7F 3 4 19F 6A 6B 14 9V 18C 1 23F 5 22F 11A 8 12F 15B 33F 10A 6C 23A 15A 35B 9N 23B 16F 15C 20B 38 17F 31 34 35F 24F
S. pneumoniae Serotypes

FIG. 9

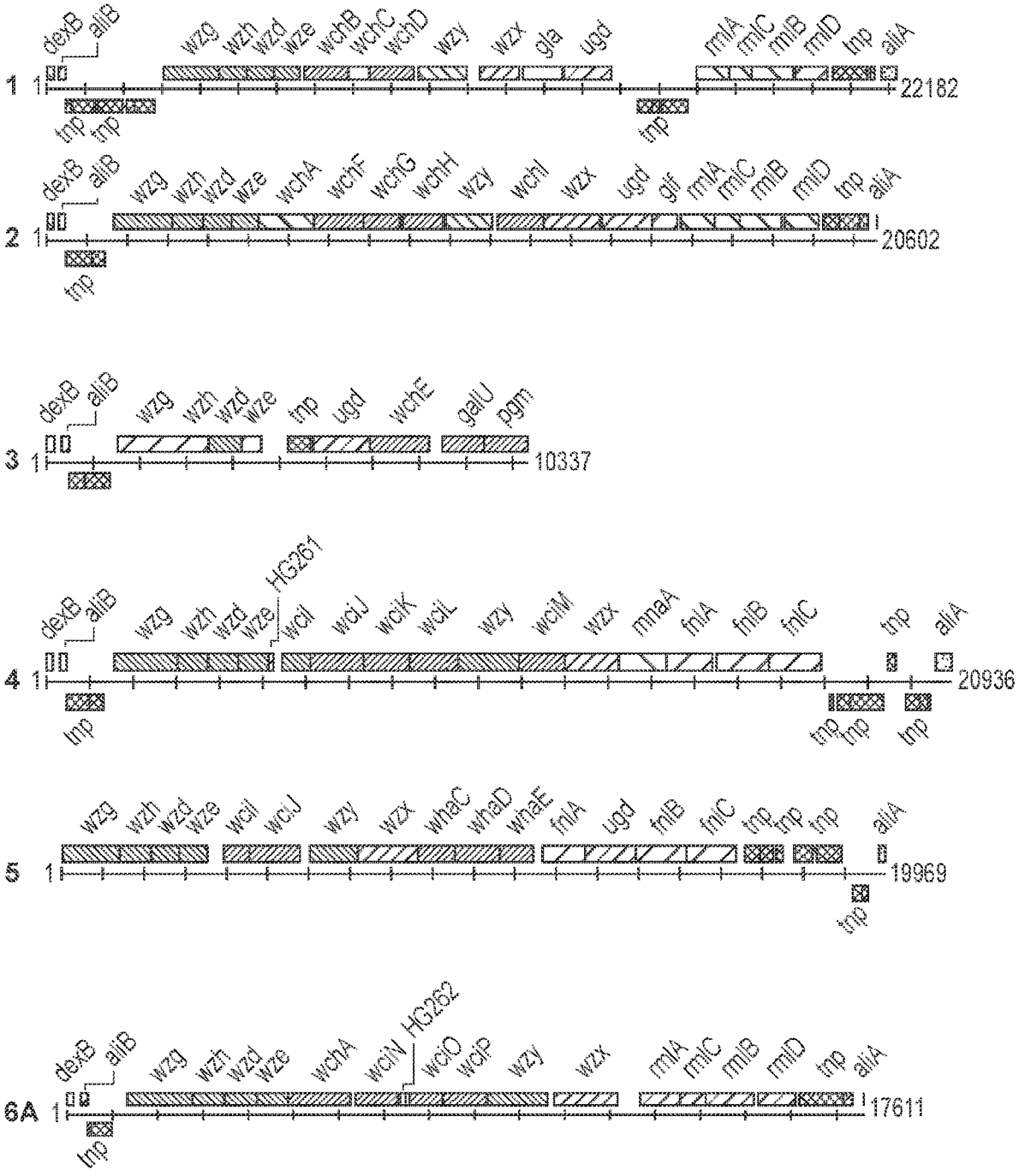

1
dexB
aliB
wzg
wzh
wzd
wze
wchB
wchC
wchD
wzy
wzx
gla
ugd
rmlA
rmlC
rmlB
rmlD
tnp
aliA
22182
2
wchA
wchF
wchG
wchH
wchI
ugd
glf
20602
3
galU
pgm
10337
4
HG261
wciI
wciJ
wciK
wciL
wciM
mnaA
fnlA
fnlB
fnlC
20936
5
whaC
whaD
whaE
19969
6A
HG262
wciN
wciO
wciP
17611

FIG. 10A

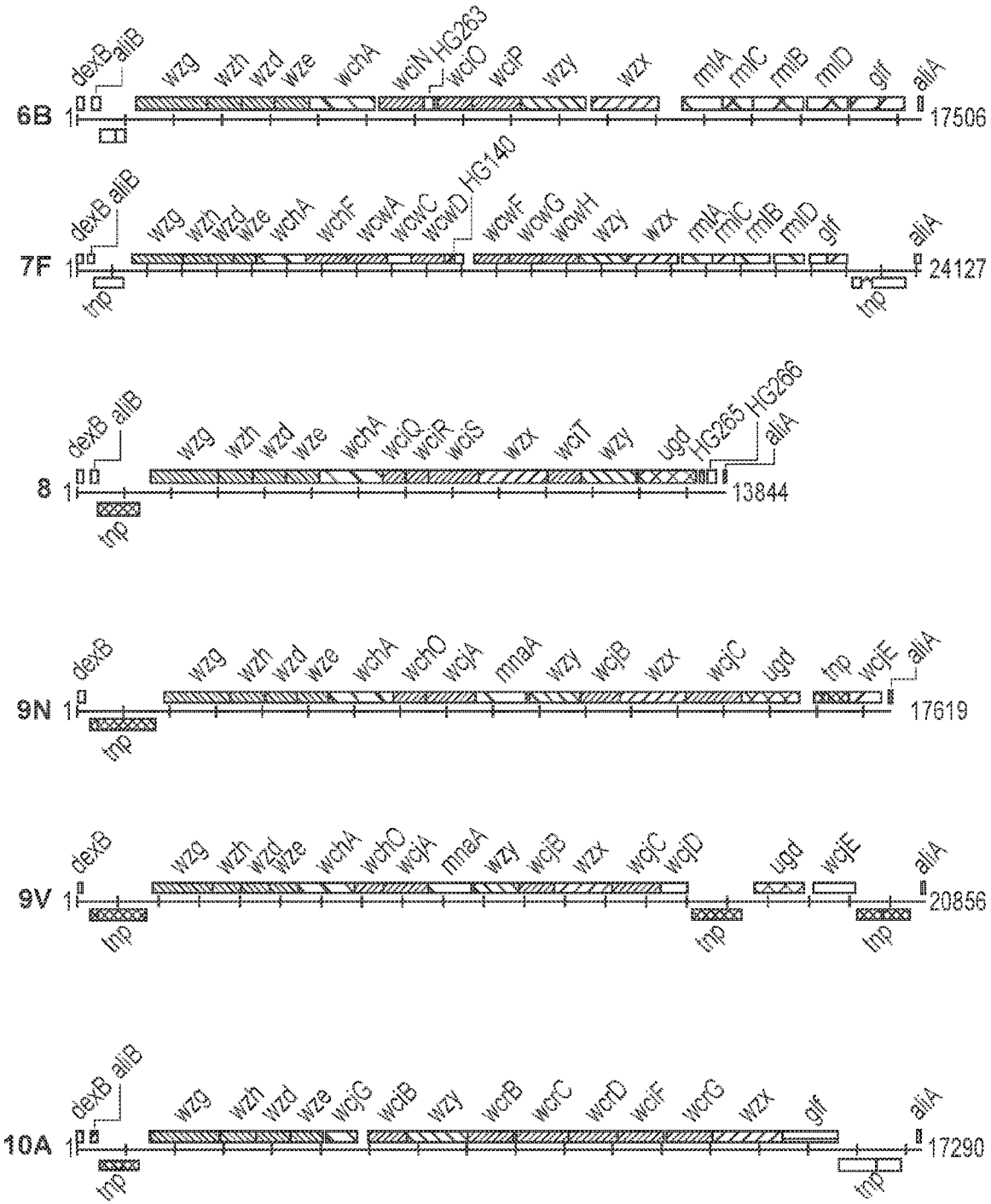

6B
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aliB
wzg
wzh
wzd
wze
wchA
wciN
HG263
wciO
wciP
wzy
wzx
rmlA
rmlC
rmlB
rmlD
glf
aliA
1
17506
7F
dexB
aliB
wzg
wzh
wzd
wze
wchA
wciF
wcwA
wcwC
wcwD
HG140
wcwF
wcwG
wcwH
wzy
wzx
rmlA
rmlC
rmlB
rmlD
glf
aliA
tnp
tnp
1
24127
8
dexB
aliB
wzg
wzh
wzd
wze
wchA
wciQ
wciR
wciS
wzx
wciT
wzy
ugd
HG265
HG266
aliA
tnp
1
13844
9N
dexB
wzg
wzh
wzd
wze
wchA
wchO
wcjA
rmaA
wzy
wcjB
wzx
wcjC
ugd
tnp
wcjE
aliA
tnp
1
17619
9V
dexB
wzg
wzh
wzd
wze
wchA
wchO
wcjA
rmaA
wzy
wcjB
wzx
wcjC
wcjD
ugd
wcjE
aliA
tnp
tnp
tnp
1
20856
10A
dexB
aliB
wzg
wzh
wzd
wze
wciG
wciB
wzy
wcrB
wcrC
wcrD
wciF
wcrG
wzx
glf
aliA
tnp
tnp
1
17290

FIG. 10B

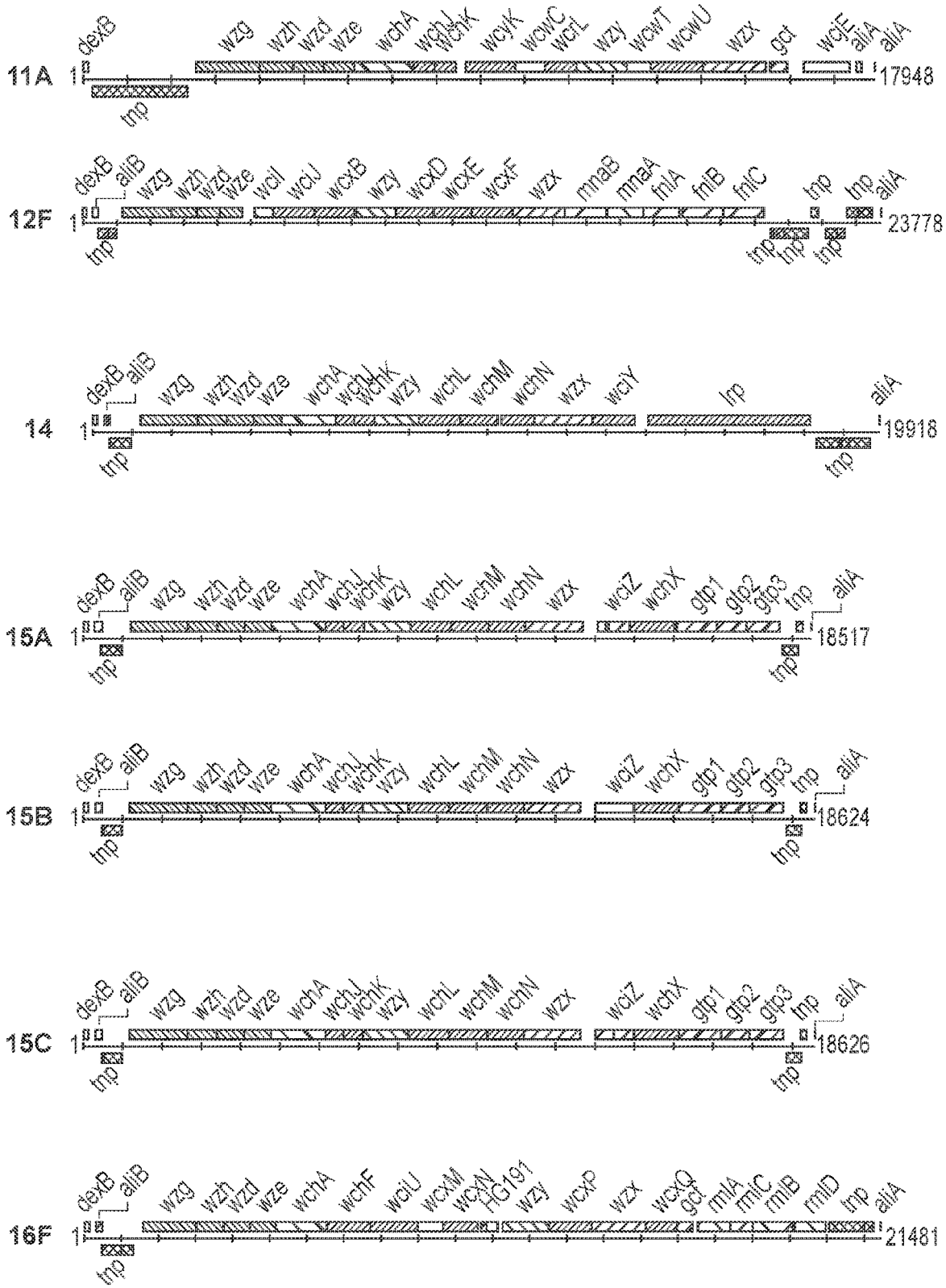


FIG. 10C

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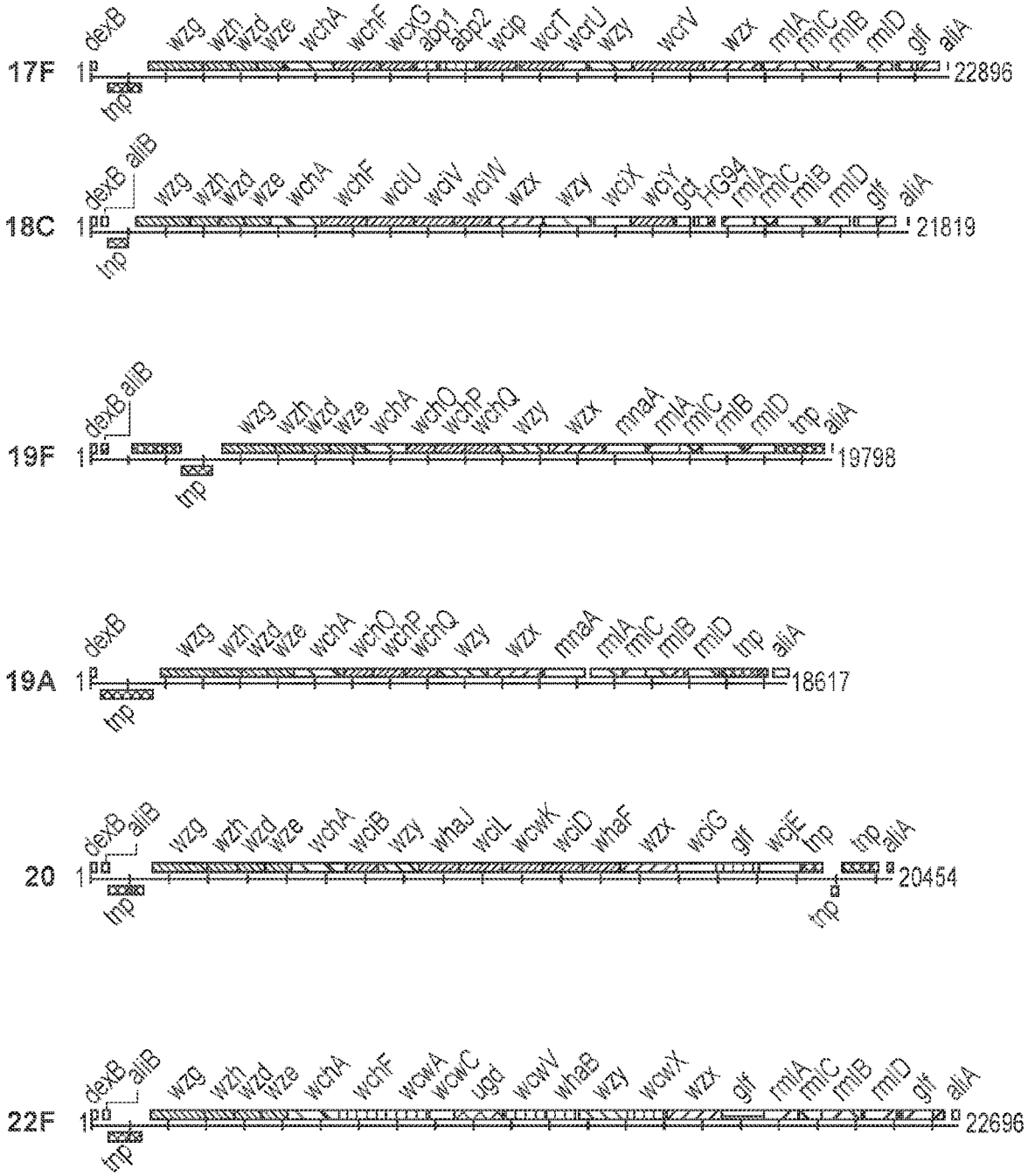

17F
dexB
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tnp
22896
18C
dexB aliB
wzg wzh wzd wze wchA wchF wciU wciV wciW wzx wzy wciX wciY gct rfbG94 rmlA rmlC rmlB rmlD glf aliA
tnp
21819
19F
dexB aliB
wzg wzh wzd wze wchA wchO wchP wchQ wzy wzx rmaA rmlA rmlC rmlB rmlD tnp aliA
tnp
19798
19A
dexB
wzg wzh wzd wze wchA wchO wchP wchQ wzy wzx rmaA rmlA rmlC rmlB rmlD tnp aliA
tnp
18617
20
dexB aliB
wzg wzh wzd wze wchA wciB wzy whaJ wciL wcwK wciD whaF wzx wciG glf wciE tnp tnp aliA
tnp
tnp
20454
22F
dexB aliB
wzg wzh wzd wze wchA wchF wcwA wcwC ugd wcwV whaB wzy wcwX wzx glf rmlA rmlC rmlB rmlD glf aliA
tnp
22696

FIG. 10D

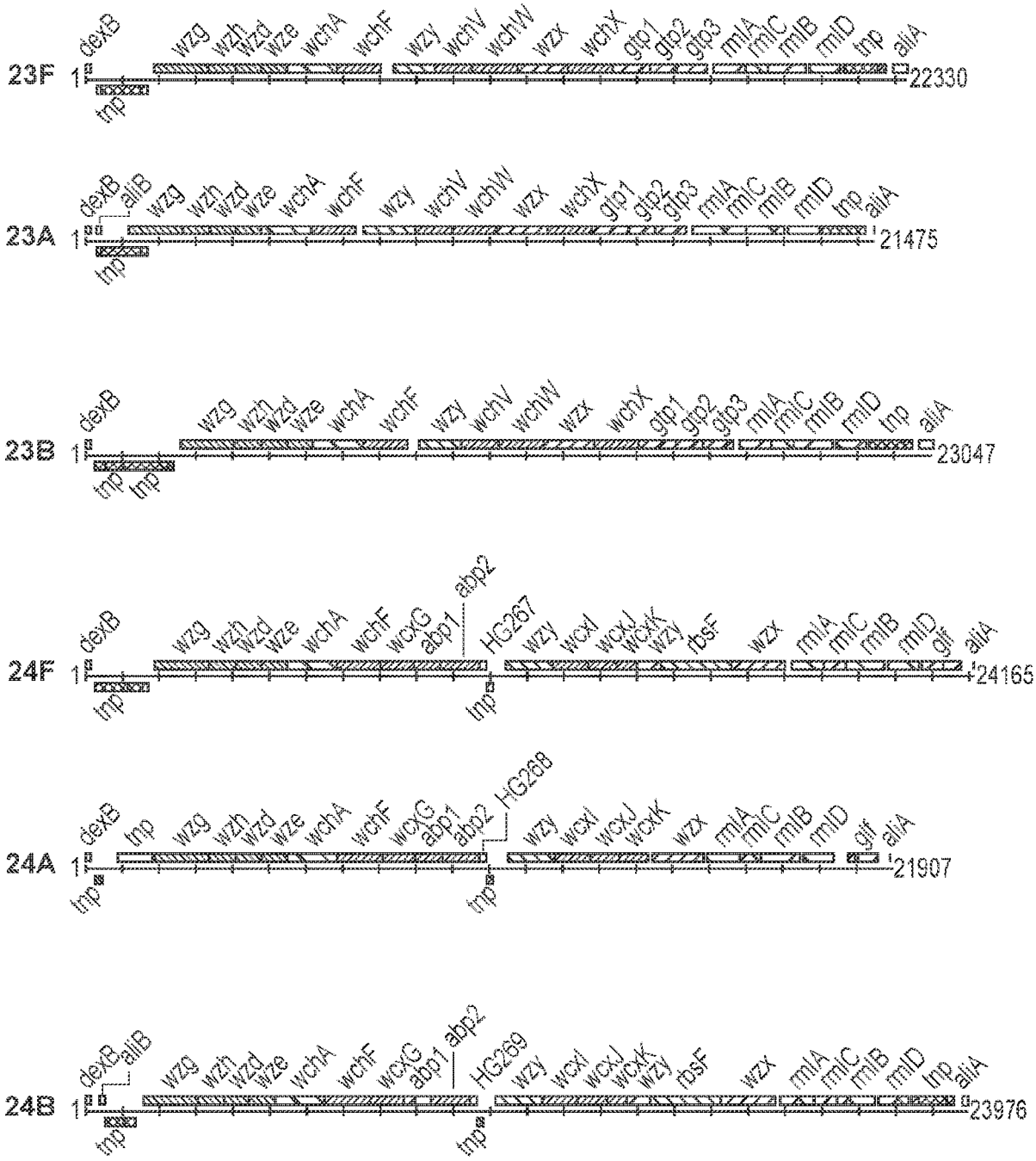

23F
dexB
wzg
wzh
wzd
wze
wchA
wchF
wzy
wchV
wchW
wzx
wchX
glp1
glp2
glp3
rmlA
rmlC
rmlB
rmlD
tnp
aliA
tnp
1
22330
23A
dexB
aliB
21475
23B
tnp
23047
24F
abp2
HG267
wcxI
wcxJ
wcxK
wzy
rbsF
gtf
24165
24A
HG268
21907
24B
HG269
23976

FIG. 10E

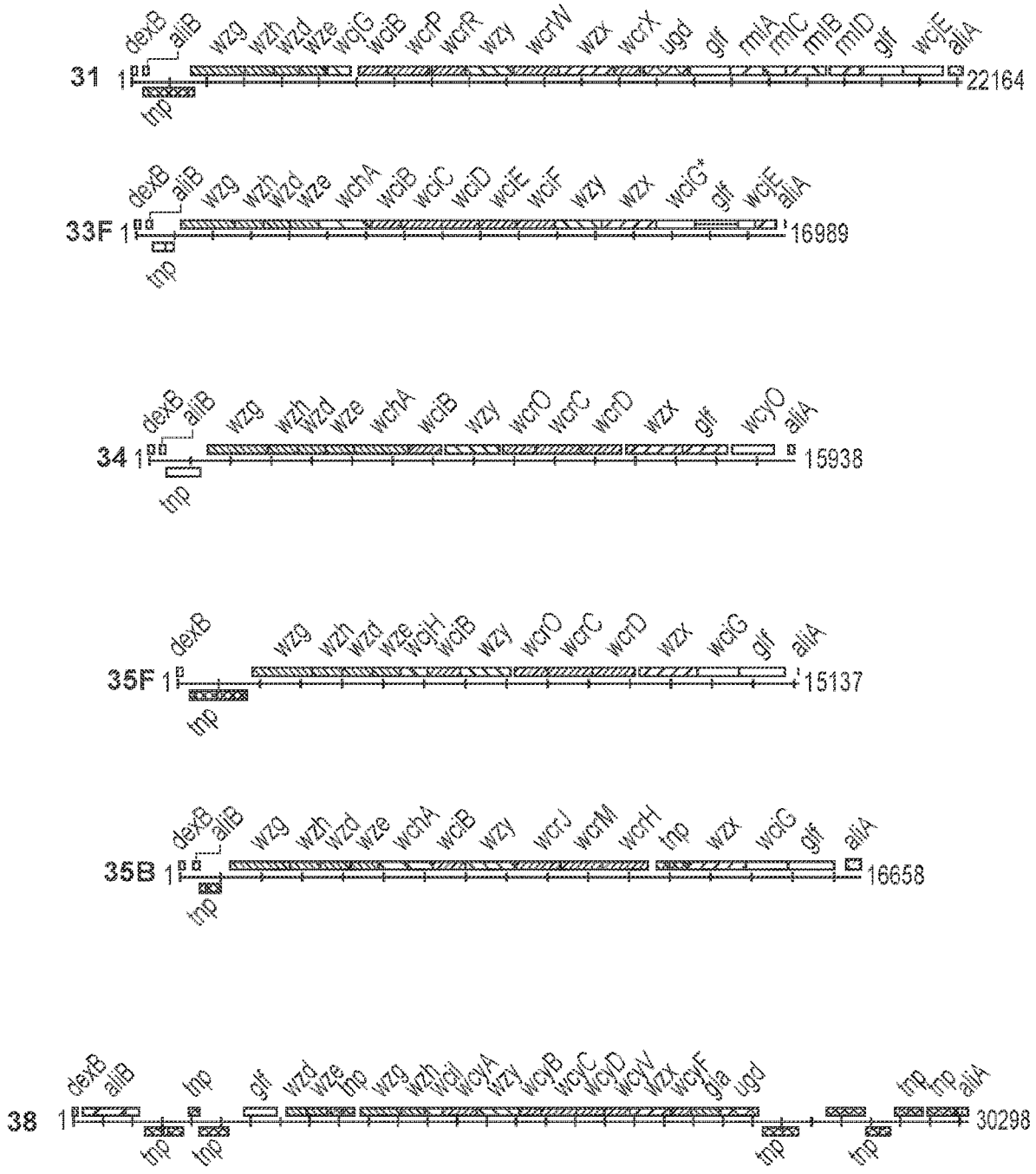

31
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aliB
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wzh
wzd
wze
wciG
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wzx
wcrX
ugd
glf
rmlA
rmlC
rmlB
rmlD
glf
wciE
aliA
tnp
33F
1
16989
dexB
aliB
wzg
wzh
wzd
wze
wchA
wciB
wciC
wciD
wciE
wciF
wzy
wzx
wciG*
glf
wciE
aliA
tnp
34
1
15938
dexB
aliB
wzg
wzh
wzd
wze
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wzy
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aliA
tnp
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wzx
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tnp
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wzd
wze
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wciB
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wcrM
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wciG
glf
aliA
tnp
38
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aliB
tnp
tnp
glf
wzd
wze
tnp
wzg
wzh
wcl
wcyA
wzy
wcyB
wcyC
wcyD
wcyV
wzx
wcyF
gla
ugd
tnp
tnp
tnp
tnp
aliA
tnp
tnp

FIG. 10F

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/54391

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/09, A61K 39/00 (2018.01)
CPC - A61K 39/092, A61K 47/646, A61K 2039/6087, A61K 2039/62, A61K 2039/70

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	WO 2017/067962 A1 (GLAXOSMITHKLINE BIOLOGICALS S.A.) 27 April 2017 (27.04.2017) pg 9, ln 10-15, pg 9, ln 23-26, pg 11, ln 14-20, pg 28, ln 28-30, pg 43, ln 1-2, pg 43, ln 15-20	1, 77-80 ----- 28/1, 34-46
Y	US 2017/0021006 A1 (PFIZER Inc.) 26 January 2017 (26.01.2017) Claim 1	28/1
Y	WO 2016/149771 A1 (GAMMA VACCINES PTY LIMITED) 29 September 2016 (29.09.2016) pg 18, ln 6-12	34-46

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

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 January 2019	11 FEB 2019

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Lee W. Young 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/US 18/54391

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out 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).

2. ☐ 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.

3. Additional comments:

Petitioner Merck Ex-1004, 0938

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/54391

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.: 81-169, 174-448
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:

- see extra sheet for Box No. III Observations where unity of invention is lacking -

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.:
1, 28/1, 34-46, 77-80 limited to serotypes 2, 6C, 7F, 7C, 8, 9N, 10A, 11A

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, 0939

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/54391

Continuation of:
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 searched, the appropriate additional search fees must be paid.

Group I+: Claims 1-80, 170-173, drawn to a pharmaceutical composition or vaccine in unit dose form comprising a plurality of unique immunogenic saccharide-polypeptide conjugates. The plurality of unique immunogenic saccharide-polypeptide conjugates will be searched to the extent that the capsular polysaccharides encompasses eight Streptococcus pneumoniae serotypes 2, 6C, 7F, 7C, 8, 9N, 10A, 11A. It is believed that claims 1, 28(in part), 34-46, 77-80 encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass capsular polysaccharide serotypes 2, 6C, 7F, 7C, 8, 9N, 10A, 11A. Additional capsular polysaccharide serotypes will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected capsular polysaccharide serotypes. 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. An exemplary election would be a pharmaceutical composition or vaccine encompasses 9 capsular polysaccharide serotypes of 6C, 15A, 15B, 15C, 17F, 20A, 20B, 23A, 23B (Claims 2, 28(in part), 34-46, 72, 74-80, 170-173).

The inventions listed as Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

The inventions of Groups I+ each include the special technical feature of a specific set of capsular polysaccharide selected from a group of S. pneumoniae serotypes, not required by any of the other inventions of Groups I+.

Common Technical Features

The inventions of Groups I+ share the technical feature of Claims 1, 29, 34, 72, 77, 170.

However, these shared technical features do not represent a contribution over prior art in view of WO 2017/067962 A1 to Glaxosmithkline Biologicals S.A. (hereinafter 'GSK'); WO 2016/149771 A1 to Gamma Vaccines PTY Limited (hereinafter 'GVPL'); and the article "Biochemical, Genetic, and Serological Characterization of Two Capsule Subtypes among Streptococcus pneumoniae Serotype 20 Strains. DISCOVERY OF A NEW PNEUMOCOCCAL SEROTYPE" by Calix et al. (hereinafter 'Calix') (The Journal Of Biological Chemistry VOL. 287, NO. 33, pp. 27885-27894, August 10, 2012).

GSK teaches (instant claim 1) a pharmaceutical composition in unit dose form (pg 43, ln 1-2, the vaccine of the invention may be administered as a single dose.; pg 43, ln 15-20, a vaccine containing the immunogenic compositions of the invention and a pharmaceutically acceptable excipient or carrier), comprising 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 (pg 9, ln 10-15, the invention will comprise capsular polysaccharide antigens (suitably conjugated), wherein the polysaccharides are derived from at least ten serotypes of S. pneumoniae. The number of S. pneumoniae capsular polysaccharides can range from 10 different serotypes (or valences "v") to 23 different serotypes (23v, a 23 valent composition).; pg 11, ln 14-20, Examples of carrier proteins which may be used in the present invention are DT (Diphtheria toxoid), TT (tetanus toxoid) or fragment C of TT, DT, CRM197 other DT point mutants, such as CRM176, CRM228, CRM 45.), wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique Streptococcus pneumoniae serotype selected from a group consisting of 2, 6C, 7F, 7C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, I 7F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35B, 35F, and 38 (pg 9, ln 23-26, In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.), 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 (pg 43, ln 1-2), ranges from about 0.5 ug to about 5 ug (pg 28, ln 28-30, In the immunogenic composition of the invention contains each S. pneumoniae capsular saccharide at a dose of between 0.1-20 ug; 0.5-1.0 ug; 0,5-5 ug or 1-3 ug of saccharide.).

GSK teaches (instant claim 29) a pharmaceutical composition in unit dose form (pg 43, ln 1-2, pg 43, ln 15-20) comprising a plurality of at least 23 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide (pg 9, ln 10-15, pg 11, ln 14-20), 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, 7C, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, I 7F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 35F, 34, 35B, and 38 (pg 9, ln 23-26), 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 (pg 43, ln 1-2), ranges from about 0.5 ug to about 5 ug (pg 28, ln 28-30). GSK does not specifically teaches a composition comprising 29 unique immunogenic saccharide-polypeptide conjugates. GVPL teaches a vaccine composition comprising any one or more of S. pneumoniae serotypes (more than 29 serotypes) (pg 18, ln 6-12, the vaccines may comprise any one of more of S. pneumoniae serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, IOC, 10F, 11A, 11B, 11C, 11D, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20, 21, 22A, 22F, 23A, 23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F, 29, 31, 32A, 32F, 33A, 33B, 33C, 33D, 33F, 34, 35A, 35B, 35C, 35F, 36, 37, 38, 39, 40, 41A, 41F, 42, 43, 44, 45, 46, 47A, 47F, and/or 48.). It would have been obvious to one of ordinary skill in the art to have prepared a vaccine comprising 29 unique immunogenic saccharide-polypeptide conjugates, as taught by GVPL.

----continued on next sheet----

Petitioner Merck Ex-1004, 0940

Continuation of:
Box No. III. Observations where unity of invention is lacking

GSK teaches (instant claim 34) a pharmaceutical composition in unit dose form (pg 43, ln 1-2, pg 43, ln 15-20) comprising at least 2 immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide (pg 9, ln 10-15, pg 11, ln 14-20), wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique Streptococcus pneumoniae serotype group, wherein 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, l 7F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35B, 35F, and 38 (pg 9, ln 23-26), 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 (pg 43, ln 1-2), ranges from about 0.5 ug to about 5 ug (pg 28, ln 28-30). GSK does not specifically teaches a composition comprising serotype 6C. GVPL teaches a vaccine composition comprising any one or more of S. pneumoniae serotypes comprising 6C (pg 18, ln 6-12, the vaccines may comprise any one of more of S. pneumoniae serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D,.....). It would have been obvious to one of ordinary skill in the art to have prepared a vaccine comprising serotype 6C, as taught by GVPL.

GSK teaches (instant claim 72) a pharmaceutical composition in unit dose form (pg 43, ln 1-2, pg 43, ln 15-20) comprising at least 2 immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide (pg 9, ln 10-15, pg 11, ln 14-20), wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique Streptococcus pneumoniae serotype group, wherein at least one additional serotype is 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, l7F, 20A, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35B, and 38 (pg 9, ln 23-26), 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 (pg 43, ln 1-2), ranges from about 0.5 ug to about 5 ug (pg 28, ln 28-30). GSK does not specifically teaches a composition comprising serotype 20B. Calix teaches serotype 20B (abstract, We examined the capsule structures of two serologically dissimilar serotype 20 strains, 20alpha and 20beta, by extensive biochemical analysis. 20alpha was composed of the previously described serotype 20 hexasaccharide repeat unit, whereas the 20beta was composed of a novel heptasaccharide repeat unit containing an extra branching alpha-glucose residue.). It would have been obvious to one of ordinary skill in the art to have prepared a vaccine comprising serotype 20B, which was first identified by Calix.

GSK teaches (instant claim 77) a pharmaceutical composition comprising 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, 7C, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, l 7F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35F, 35B, and 38 (pg 9, ln 10-15, pg 9, ln 23-26, pg 11, ln 14-20).

GSK teaches (instant claim 170) a vaccine in unit dose form (pg 43, ln 1-2, pg 43, ln 15-20) comprising at 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 (pg 9, ln 10-15, pg 9, ln 23-26, pg 11, ln 14-20). GSK does not specifically teaches serotypes 23A or 23B, or a combination thereof. GVPL teaches a vaccine composition comprising any one or more of S. pneumoniae serotypes comprising 23A or 23B (pg 18, ln 6-12, the vaccines may comprise any one of more of S. pneumoniae serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D,...23A, 23B....). It would have been obvious to one of ordinary skill in the art to have prepared a vaccine comprising serotype 23A or 23B, as taught by GVPL.

As said technical features were known in the art at the time of the invention, these cannot be considered special technical features that would otherwise unify the groups.

Groups I+ therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Petitioner Merck Ex-1004, 0941

Electronic Acknowledgement Receipt

EFS ID:	38826976
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:	23628
Filer:	Chelsea E. Witte-Garcia/Kristy Alaura
Filer Authorized By:	Chelsea E. Witte-Garcia
Attorney Docket Number:	P1143.70000US04
Receipt Date:	10-MAR-2020
Filing Date:	27-SEP-2018
Time Stamp:	19:09:15
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-CEW.pdf	26190 d99803cfe3b5ab30f171b1654cd2499b34ab1dd0	no	1

Warnings:

Information:					
2	Transmittal Letter	P114370000US04-IDSBODY-CEW.pdf	31928 fe70356112900e3d2b8db5130a0901e6c8a83dc5	no	3
Warnings:					
Information:					
3	Information Disclosure Statement (IDS) Form (SB08)	P114370000US04-IDS1449-CEW.pdf	29450 05b61b9adf1a57ab5c19306d6cf02a203c069f9d	no	2
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
4	Other Reference-Patent/App/Search documents	P114370000EP00_EESR_mailed_11-04-2019.pdf	990833 0d411f634dc88d42570a3a3295d18035dfedd8ec	no	10
Warnings:					
Information:					
5	Other Reference-Patent/App/Search documents	P114370000WO00_ISR_mailed_09-13-2017.pdf	2007552 560ab03c99668b10c4c2b9f19fd71c6a752d5f1c	no	12
Warnings:					
Information:					
6	Other Reference-Patent/App/Search documents	P114370000WO00_IPRP_maile d_10-11-2018.pdf	1449928 abac10f92a9c09cb6693bfb1b75ce878d0373108	no	10
Warnings:					
Information:					
7	Foreign Reference	CN104069488A_Eng_Abst.pdf	1866836 becbd31e4c868812d2056c29ffa79c5145afae3a	no	11
Warnings:					
Information:					
8	Foreign Reference	EP2865392A1.pdf	3605501 c17c156345e89a06ae3db5139850121d904214e1	no	28
Warnings:					
Information:					

9	Foreign Reference	WO2008143709A2.pdf	6238467 aeec22f91514a6ee45e130df5c702c3b645fc5db	no	59
Warnings:					
Information:					
10	Foreign Reference	WO2011100151A1.pdf	4670735 d60819c97ef3ac1695e35db39828d3ac5bd4b35d	no	37
Warnings:					
Information:					
11	Foreign Reference	WO2016207905A2.pdf	3167581 bf8ea041955ea441890ad1e85d6e0c0893c1f0f2	no	24
Warnings:					
Information:					
12	Foreign Reference	WO2018064444A1.pdf	8884731 bdf3bf677badb8a6cd6e0e0d6abbc744fb5ff35a	no	75
Warnings:					
Information:					
13	Foreign Reference	WO2019070994A1.pdf	25439884 7fb62cbceef79096252e6367f70a87d2c4910732	no	195
Warnings:					
Information:					
14	Non Patent Literature	Bentley_et_al_PLoS_Genet_2006_v2_pe31.pdf	13946128 ab8b6af83e765ab9e5c2fae07927e05d3ccb0b1c	no	8
Warnings:					
Information:					
15	Non Patent Literature	Durando_et_al_Clin_Mircrobiol_Infect_2013_v19_p1.pdf	249627 14229e68fd85377391898dcb7a4b357e61dcb0f4	no	9
Warnings:					
Information:					

16	Non Patent Literature	Hu_et_al_Chem_Soc_Rev_2016_v45_p1969.pdf	4096266 15508251bc606109bf7ba9df58f47c39c8425e1a	no	29
Warnings:					
Information:					
17	Non Patent Literature	Jefferies_et_al_Hum_Vaccin_2011_v7_p1012.pdf	1789475 9d458301086c296eda7b2d1c81e0eba45f5e0fb5	no	8
Warnings:					
Information:					
18	Non Patent Literature	Kolkman_et_al_J_Biol_Chem_1997_v272_p19502.pdf	3943190 7b119c218dd65bc42d09434e5439c24cb9600218	no	8
Warnings:					
Information:					
19	Non Patent Literature	Skinner_et_al_Vaccine_2011_v29_p8870.pdf	1540072 be7268865d0f6e17d89110ce10278d6dfcc316dc	no	7
Warnings:					
Information:					
		Total Files Size (in bytes):	83974374		

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.

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	1645
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
- [x] 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
- [ ] 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):

Form PTO-1449

Copies of cited references

Remarks

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	WOLF, GREENFIELD & SACKS, P.C.		
Signature	/Chelsea E. Witte-Garcia/		
Printed name	Chelsea E. Witte-Garcia, Ph.D.		
Date	March 10, 2020	Reg. No.	75,713

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: March 10, 2020 Electronic Signature for Kristy J. Alaura: /Kristy J. Alaura/

7915411.1

DOCKET NO.: P1143.70000US04

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor:	Bruce D. Forrest
Application No.:	16/089,040
Confirmation No.:	8702
Filed:	September 27, 2018
For:	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF
Examiner:	Not Yet Assigned
Art Unit:	1645

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: March 10, 2020 Electronic Signature for Kristy J. Alaura: /Kristy J. Alaura/

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

STATEMENT FILED PURSUANT TO THE DUTY OF DISCLOSURE UNDER 37 C.F.R. §§ 1.56, 1.97 AND 1.98

Commissioner:

Pursuant to the duty of disclosure under 37 C.F.R. §§ 1.56, 1.97 and 1.98, the undersigned requests consideration of this Information Disclosure Statement.

PART I: Compliance with 37 C.F.R. § 1.97

This Information Disclosure Statement has been filed before the mailing of a first Office Action on the merits in the above-referenced Application. Therefore, no fee or certification is required.

PART II: Information Cited

The undersigned hereby makes of record in the present Application the information listed on the attached Form PTO-1449 (modified PTO/SB/08). The order of presentation of the references should not be construed as an indication of the importance of the references.

Petitioner Merck Ex-1004, 0947

The undersigned would also like to bring to the Examiner's attention the enclosed search reports and other communication from corresponding and related international and foreign patent applications:

Serial No.	Date of Mailing	Type of Communication	Docket No.
EP 17776881.9	11-04-2019	Extended European Search Report	P1143.70000EP00
PCT/US2017/025621	09-03-2017	International Search Report and Written Opinion	P1143.70000WO00
PCT/US2017/025621	10-11-2018	International Preliminary Report on Patentability	P1143.70000WO00

PART III: Remarks

Documents cited anywhere in the Information Disclosure Statement are enclosed unless otherwise indicated. It is respectfully requested that:

1. The Examiner consider completely the cited information, along with any other information, in reaching a determination concerning the patentability of the present claims;

2. The enclosed Form PTO-1449 (modified PTO/SB/08) be signed by the Examiner to evidence that the cited information has been fully considered by the United States Patent and Trademark Office during the examination of the present Application; and

3. The citations for the information be printed on any patent which issues from the present Application.

By submitting this Information Disclosure Statement, the undersigned makes no representation that a search has been performed, of the extent of any search performed, or that more relevant information does not exist.

By submitting this Information Disclosure Statement, the undersigned makes no representation that the information cited in the Statement is, or is considered to be, material to patentability as defined in 37 C.F.R. § 1.56(b).

By submitting this Information Disclosure Statement, the undersigned makes no representation that the information cited in the Statement is, or is considered to be, in fact, prior art as defined by 35 U.S.C. § 102.

Notwithstanding any statements by the undersigned, the Examiner is urged to form his or her own conclusion regarding the relevance of the cited information.

An early and favorable action is hereby requested.

The Director is hereby authorized to charge any deficiency in the fees due, or credit any overpayment, by the filing of this Information Disclosure Statement to our Deposit Account No. 23/2825, under Docket No. P1143.70000US04, from which the undersigned is authorized to draw.

Respectfully submitted,

Electronic signature: /Chelsea E. Witte-Garcia/
Chelsea E. Witte-Garcia, Ph.D.
Registration No.: 75,713
WOLF, GREENFIELD & SACKS, P.C.
600 Atlantic Avenue
Boston, Massachusetts 02210-2206
617.646.8000

Docket No.: P1143.70000US04
Date: March 10, 2020

Docket No.: P1143.70000US04
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor:	Bruce D. Forrest
Application No.:	16/089,040
Confirmation No.:	8702
Filed:	September 27, 2018
For:	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF
Examiner:	Not Yet Assigned
Art Unit:	1645

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: March 10, 2020 Electronic Signature for Katelyn R. Farmer: /Katelyn R. Farmer/

FIRST PRELIMINARY AMENDMENT UNDER 37 C.F.R. § 1.115

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

INTRODUCTORY COMMENTS

Prior to examination on the merits, please amend the above-identified U.S. patent application as follows.

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

Remarks begin on page 8 of this paper.

Petitioner Merck Ex-1004, 0950

AMENDMENTS TO THE CLAIMS

Please amend the claims as follows. Insertions are indicated by underlining, and deletions are indicated by strikeouts and/or double bracketing.

1. (Original) A pharmaceutical composition comprising 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, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, and 38.

2. - 28. (Canceled)

29. (Original) 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, 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, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 20B, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, and 38.

30. - 61. (Canceled)

62. (Original) 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, 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,

Petitioner Merck Ex-1004, 0951

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, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, and 38.

63. - 66. (Canceled)

67. (Original) A pharmaceutical composition comprising 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, 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, 34, 35F, 35B, and 38.

68. - 70. (Canceled)

71. (Currently amended) The pharmaceutical composition of claim 1 ~~any one of claims 1-70~~, wherein at least one of the unique immunogenic saccharide-polypeptide conjugates comprises the capsular polysaccharide or the fragment of the capsular polysaccharide.

72. (Canceled)

73. (Currently amended) The pharmaceutical composition of claim 71 [[72]], wherein the fragment of the capsular polysaccharide comprises a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, or a hexasaccharide.

74. (Currently amended) The pharmaceutical composition of claim 1 ~~any one of claims 1-73~~, wherein at least one polypeptide of the plurality is an oligopeptide and/or at least one polypeptide comprises a mixture of polypeptides.

Petitioner Merck Ex-1004, 0952

75. (Currently amended) The pharmaceutical composition of claim 1 ~~any one of claims 1-74~~, wherein at least one polypeptide of the plurality 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*, PspA, any fragment thereof, or any combination thereof.

76. - 77. (Original)

78. (Currently amended) The pharmaceutical composition of claim 1 ~~any one of claims 1-77~~, further comprising an adjuvant; a chelating agent; a surfactant , an emulsifier, or a combination thereof; a buffering agent; a preservative; a salt; and/or an anti-fungal compound or a salt thereof.

79. - 80. (Canceled)

81. (Currently amended) The pharmaceutical composition of claim 1 ~~any one of claims 1-80~~, wherein the pharmaceutical composition is in the form of an intramuscularly injectable composition, intradermally injectable composition, subcutaneously injectable composition, or an intranasally administrable composition.

82. - 96. (Canceled)

97. (Currently amended) The pharmaceutical composition of claim 1 ~~any one of claims 1-96~~, wherein the unique immunogenic saccharide-polypeptide conjugate elicits an opsonophagocytic response.

98. - 105. (Canceled)

Petitioner Merck Ex-1004, 0953

106. (Currently amended) The pharmaceutical composition of claim 1 ~~any one of claims 1-105~~, wherein the immunogenic saccharide-polypeptide conjugate(s) are individually, respectively, or collectively present in an amount of at least 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.

107. - 108. (Canceled)

109. (Currently amended) The pharmaceutical composition of claim 1 ~~any one of claims 1-108~~, wherein the immunogenic saccharide-polypeptide comprises:

(i) the capsular polysaccharide at least partially embedded in the polypeptide,

(ii) the capsular polysaccharide chemically cross-linked to the polypeptide, and/or

(iii) the capsular polysaccharide at least partially chemically cross-linked to the polypeptide.

110. - 111. (Canceled)

112. (Currently amended) A method comprising administering to a subject a first composition, wherein the first composition is the pharmaceutical composition of claim 1 ~~any one of claims 1-111~~.

113. (Original) The method of claim 112, 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 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.

114. (Original) The method of claim 113, wherein the second composition comprises an immunogenic saccharide-polypeptide conjugate comprising a capsular polysaccharide or fragment of the capsular polysaccharide from a serotype of *Streptococcus pneumoniae* conjugated to the second polypeptide.

Petitioner Merck Ex-1004, 0954

115. - 153. (Canceled)

154. (Currently amended) The method of claim 113 [[153]], wherein the subject is a human.

155. - 156. (Canceled)

157. (Currently amended) The method of claim 113 ~~any one of claims 113-156~~, 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 of the second composition, wherein the antibody response is measured by measuring antibody titers.

158. - 162. (Canceled)

163. (Original) 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.

Petitioner Merck Ex-1004, 0955

164. - 255. (Canceled)

256. (Currently amended) A method of making a composition comprising:

contacting the immunogenic saccharide-polypeptide conjugate of claim 1 ~~any one of claims 1-70~~ with an excipient, an adjuvant, or any combination thereof.

257. - 295. (Canceled)

Petitioner Merck Ex-1004, 0956

REMARKS

Claims 1-295 were previously pending in this application. Claims 2-28, 30-61, 63-66, 68-70, 72, 76, 77, 79, 80, 82-96, 98-105, 107, 108, 110, 111, 115-153, 155, 156, 158-162, 164, 164-255, and 257-295 are canceled without prejudice or disclaimer. Claims 71, 73-75, 78, 81, 97, 106, 109, 112, 154, 157, and 256 are amended to remove multiple dependencies and reduce total claim number. Support for the amendment to claims may be found throughout the specification and claims as originally filed. No new matter is added to the application.

Upon entry of this amendment, claims 1, 29, 62, 67, 71, 73-75, 78, 81, 97, 106, 109, 112-114, 154, 157, 163, and 256 will be pending for examination, with claims 1, 29, 62, 67, and 163 being independent claims.

Applicant believes no fee is due with this Amendment, other than the enclosed fee. However, if a fee is due with this Amendment, please charge our Deposit Account No. 23/2825, under Docket No. P1142.70000US04 from which the undersigned is authorized to draw.

Dated: March 10, 2020

Respectfully submitted,

By /Chelsea E. Witte-Garcia/
Chelsea E. Witte-Garcia, Ph.D.
Registration No.: 75,713
WOLF, GREENFIELD & SACKS, P.C.
600 Atlantic Avenue
Boston, Massachusetts 02210-2206
617.646.8000

Petitioner Merck Ex-1004, 0957

FEE TRANSMITTAL

Complete if known	
Application Number	16/089,040-Conf. #8702
Filing Date	September 27, 2018
First Named Inventor	Bruce D. Forrest
Examiner Name	Not Yet Assigned
Art Unit	1645
Practitioner Docket No.	P1143.70000US04

[x] Applicant asserts small entity status. See 37 CFR 1.27.

[ ] Applicant certifies micro entity status. See 37 CFR 1.29. Form PTO/SB/15A or B or equivalent must either be enclosed or have been submitted previously.

TOTAL AMOUNT OF PAYMENT ($)460.00

METHOD OF PAYMENT (check all that apply)

[ ] Check [x] Credit Card [ ] Money Order [ ] None [ ] Other (please identify): _____

[x] Deposit Account Deposit Account Number: 23/2825 Deposit Account Name: Wolf, Greenfield & Sacks, P.C.

For the above-identified deposit account, the Director is hereby authorized to (check all that apply):

[ ] Charge fee(s) indicated below

[ ] Charge fee(s) indicated below, **except for the filing fee**

[ ] Charge any additional fee(s) or underpayment of fee(s) under 37 CFR 1.16 and 1.17, **except for any excess claims fees or multiple dependent claim fee**

[x] Charge any additional fee(s) or underpayment of fee(s) under 37 CFR 1.16 and 1.17

[x] Credit any overpayment of fee(s)

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

FEE CALCULATION

1. BASIC FILING, SEARCH, AND EXAMINATION FEES (U = undiscounted fee; S = small entity fee; M = micro entity fee)

	FILING FEES			SEARCH FEES			EXAMINATION FEES			
Application Type	**U ($)**	**S ($)**	**M ($)**	**U ($)**	**S ($)**	**M ($)**	**U ($)**	**S ($)**	**M ($)**	**Fees Paid ($)**
Utility	300	150*	75	660	330	165	760	380	190	
Design	200	100	50	160	80	40	600	300	150	
Plant	200	100	50	420	210	105	620	310	155	
Reissue	300	150	75	660	330	165	2,200	1,100	550	
Provisional	280	140	70	0	0	0	0	0	0	

* The $150 small entity status filing fee for a utility application is further reduced to $75 for a small entity status applicant who files the application via EFS-Web.

2. EXCESS CLAIM FEES

Fee Description	**Undiscounted Fee ($)**	**Small Entity Fee ($)**	**Micro Entity Fee ($)**
Each claim over 20 (including Reissues)	100	50	25
Each independent claim over 3 (including Reissues)	460	230	115
Multiple dependent claims	820	410	205

Total Claims		**Extra Claims**		**Fee ($)**		**Fee Paid ($)**
20	- 20 or HP =		x		=	

HP = highest number of total claims paid for, if greater than 20.

Multiple Dependent Claims	
Fee ($)	**Fee Paid ($)**

Indep. Claims		**Extra Claims**		**Fee ($)**		**Fee Paid ($)**
5	- 3 or HP =	2	x	230.00	=	460.00

HP = highest number of independent claims paid for, if greater than 3.

3. APPLICATION SIZE FEE

If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due 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).

Total Sheets		**Extra Sheets**		**Number of each additional 50 or fraction thereof**		**Fee ($)**		**Fee Paid ($)**
99	- 100 =		/50 =		(round **up** to a whole number) x		=	

4. OTHER FEE(S)

	Fees Paid ($)
Non-English specification, $130 fee (no small or micro entity discount)	
Non-electronic filing fee under 37 CFR 1.16(t) for a utility application, $400 fee ($200 small or micro entity)	
Other (e.g., late filing surcharge):	

SUBMITTED BY

Signature	/Chelsea E. Witte-Garcia/	Registration No. (Attorney/Agent)	75,713	Telephone	617.646.8000
Name (Print/Type)	Chelsea E. Witte-Garcia, Ph.D.			Date	March 10, 2020

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: March 10, 2020 Electronic Signature for Katelyn R. Farmer: /Katelyn R. Farmer/

8023998.1

Electronic Patent Application Fee Transmittal

Application Number:	16089040
Filing Date:	27-Sep-2018
Title of Invention:	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF
First Named Inventor/Applicant Name:	Bruce D. FORREST
Filer:	Chelsea E. Witte-Garcia/Katelyn Farmer
Attorney Docket Number:	P1143.70000US04

Filed as Small Entity

Filing Fees for U.S. National Stage under 35 USC 371

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
INDEPENDENT CLAIMS IN EXCESS OF 3	2614	2	230	460
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	**460**

Electronic Acknowledgement Receipt

EFS ID:	38823378
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:	23628
Filer:	Chelsea E. Witte-Garcia/Katelyn Farmer
Filer Authorized By:	Chelsea E. Witte-Garcia
Attorney Docket Number:	P1143.70000US04
Receipt Date:	10-MAR-2020
Filing Date:	27-SEP-2018
Time Stamp:	15:49:43
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$460
RAM confirmation Number	E202030F50117530
Deposit Account	232825
Authorized User	Katelyn Farmer

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

37 CFR 1.17 (Patent application and reexamination processing fees)

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-CEW.pdf	26241 918232219da2cf0b01e000430692fd7e720e0020	no	1
Warnings:					
Information:					
2	Applicant Response to Pre-Exam Formalities Notice	P114370000US04-NTFMP-CEW.pdf	25049 96d50b270c7a6dfde4af05f00fc27fe28e18c6bf	no	1
Warnings:					
Information:					
3		P114370000US04-PAMN-CEW.pdf	45688 5b7c98d28b461aba381026f40833a9afda31c8e0	yes	8

Multipart Description/PDF files in .zip description

Document Description	Start	End
Preliminary Amendment	1	1
Claims	2	7
Applicant Arguments/Remarks Made in an Amendment	8	8

Warnings:					
Information:					
4	Fee Worksheet (SB06)	P114370000US04-FEE-CEW.pdf	32651 adb293b14edc7e56db18e5030a145b5c579f839a	no	1
Warnings:					
Information:					
5	Fee Worksheet (SB06)	fee-info.pdf	30796 dabf5ff83cb3a36646bd48ccc79e4c9eaba6728f	no	2

Warnings:	
Information:	
Total Files Size (in bytes):	160425

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.

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	1645
Examiner Name	Not Yet Assigned
Attorney Docket Number	P1143.70000US04

ENCLOSURES (*Check all that apply*)

- [x] 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
- [ ] 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):

-First Preliminary Amendment
-Response to Notification of Insufficiency

Remarks

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	WOLF, GREENFIELD & SACKS, P.C.		
Signature	/Chelsea E. Witte-Garcia/		
Printed name	Chelsea E. Witte-Garcia, Ph.D.		
Date	March 10, 2020	Reg. No.	75,713

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: March 10, 2020 Electronic Signature for Katelyn R. Farmer: /Katelyn R. Farmer/

8024000.1

Docket No.: P1143.70000US04
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor:	Bruce D. Forrest
Application No.:	16/089,040
Confirmation No.:	8702
Filed:	September 27, 2018
For:	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF
Examiner:	Not Yet Assigned
Art Unit:	1645

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: March 10, 2020 Electronic Signature for Katelyn R. Farmer: /Katelyn R. Farmer/

RESPONSE TO NOTICE TO FILE MISSING PARTS OF APPLICATION

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

In response to the Notification of Insufficiency mailed January 10, 2020, Applicant respectfully submits a Preliminary Amendment and the required fees.

Please charge our Credit Card in the amount of $460.00 covering the fees set forth in 37 C.F.R. § 1.16(h). The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed with this response to our Deposit Account No. 23/2825, under Docket No. P1143.70000US04.

Dated: March 10, 2020

Respectfully submitted,

By /Chelsea E. Witte-Garcia/
Chelsea E. Witte-Garcia, Ph.D.
Registration No.: 75,713
WOLF, GREENFIELD & SACKS, P.C.
600 Atlantic Avenue
Boston, Massachusetts 02210-2206
617.646.8000

8024001.1

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		Indep	Depend	Indep	Depend	Indep	Depend
1			1				51						
2			1				52						
3			1				53						
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5				1			55						
6				1			56						
7				1			57						
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45							95						
46							96						
47							97						
48							98						
49							99						
50							100						
Total Indep	0		5		0								
Total Depend	0	↵	15	↵	0	↵							
Total Claims	0		20		0								

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))	20 minus 20 =	*	x 50 =	0.00	OR		
INDEPENDENT CLAIMS (37 CFR 1.16(h))	5 minus 3 =	* 2	x 230 =	460			
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))				0.00			
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	1060		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.

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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 ACCEPTANCE LETTER

OC000000115596211

Date Mailed: 03/23/2020

NOTICE OF ACCEPTANCE OF APPLICATION UNDER 35 U.S.C 371 AND 37 CFR 1.495

The applicant is hereby advised that the United States Patent and Trademark Office, in its capacity as a Designated / Elected Office (37 CFR 1.495), has ACCEPTED the above identified international application for national patentability examination in the United States Patent and Trademark Office.

The United States Application Number assigned to the application is shown above. A Filing Receipt will be issued for the present application in due course. **THE DATE APPEARING ON THE FILING RECEIPT AS THE "FILING DATE or 371(c) DATE" IS THE DATE ON WHICH THE LAST OF THE 35 U.S.C. 371 (c)(1) and (c)(2) REQUIREMENTS HAS BEEN RECEIVED IN THE OFFICE. THIS DATE IS SHOWN BELOW.** The filing date of the above identified application is the international filing date of the international application (Article 11(3) and 35 U.S.C. 363)

09/27/2018
DATE OF RECEIPT OF 35 U.S.C.
371(c)(1) and (c)(2) REQUIREMENTS

The following items have been received:

- Indication of Small Entity Status
- Copy of the International Application filed on 09/27/2018
- Copy of the International Search Report filed on 09/27/2018
- Preliminary Amendments filed on 03/10/2020
- Information Disclosure Statements filed on 03/10/2020
- Inventor's Oath or Declaration filed on 09/27/2018
- U.S. Basic National Fees filed on 09/27/2018
- Assignment filed on 09/27/2018
- Authorize Access to Search Results filed on 09/27/2018
- Priority Documents 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

Petitioner Merck Ex-1004, 0968

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)

TONI M HOOD

Telephone: (571) 272-3654

FORM PCT/DO/EO/903 (371 Acceptance Notice)

Petitioner Merck Ex-1004, 0969

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

23628
WOLF GREENFIELD & SACKS, P.C.
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INTERNATIONAL APPLICATION NO.	
PCT/US17/25621	
I.A. FILING DATE	PRIORITY DATE
03/31/2017	03/31/2016

CONFIRMATION NO. 8702
371 WITHDRAWAL NOTICE

OC00000011559572O

Date Mailed: 03/23/2020

Letter Regarding a New Notice and/or the Status of the Application

If a new notice or Filing Receipt is enclosed, applicant may disregard the previous notice mailed on 01/10/2020. The time period for reply runs from the mail date of the new notice. Within the time period for reply, applicant is required to file a reply in compliance with the requirements set forth in the new notice to avoid abandonment of the application.

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 the reply is not filed electronically via EFS-Web, the reply must be accompanied by a copy of the new notice.

If the Office previously granted a petition to withdraw the holding of abandonment or a petition to revive under 37 CFR 1.137, the status of the application has been returned to pending status.

TONI M HOOD

Telephone: (571) 272-3654

FORM PCT/DO/EO/WITH (371 Withdrawal Notice)

Petitioner Merck Ex-1004, 0970

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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	1645	1060	P1143.70000US04	20	5

CONFIRMATION NO. 8702

FILING RECEIPT

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

OC000000115596210

Date Mailed: 03/23/2020

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, 0971

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

424

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.

Petitioner Merck Ex-1004, 0972

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

Petitioner Merck Ex-1004, 0973

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, 0974

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
16/089,040	09/27/2018	Bruce D. FORREST	P1143.70000US04

CONFIRMATION NO. 8702

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

PUBLICATION NOTICE

OC000000116284602

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

Publication No.US-2020-0113993-A1
Publication Date:04/16/2020

NOTICE OF PUBLICATION OF APPLICATION

The above-identified application will be electronically published as a patent application publication pursuant to 37 CFR 1.211, et seq. The patent application publication number and publication date are set forth above.

The publication may be accessed through the USPTO's publically available Searchable Databases via the Internet at www.uspto.gov. The direct link to access the publication is currently http://www.uspto.gov/patft/.

The publication process established by the Office does not provide for mailing a copy of the publication to applicant. A copy of the publication may be obtained from the Office upon payment of the appropriate fee set forth in 37 CFR 1.19(a)(1). Orders for copies of patent application publications are handled by the USPTO's Public Records Division. The Public Records Division can be reached by telephone at (571) 272-3150 or (800) 972-6382, by facsimile at (571) 273-3250, by mail addressed to the United States Patent and Trademark Office, Public Records Division, Alexandria, VA 22313-1450 or via the Internet.

In addition, information on the status of the application, including the mailing date of Office actions and the dates of receipt of correspondence filed in the Office, may also be accessed via the Internet through the Patent Electronic Business Center at www.uspto.gov using the public side of the Patent Application Information and Retrieval (PAIR) system. The direct link to access this status information is currently https://portal.uspto.gov/pair/PublicPair. Prior to publication, such status information is confidential and may only be obtained by applicant using the private side of PAIR.

Further assistance in electronically accessing the publication, or about PAIR, is available by calling the Patent Electronic Business Center at 1-866-217-9197.

Office of Data Managment, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

Petitioner Merck Ex-1004, 0975

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/089,040	09/27/2018	Bruce D. FORREST	P1143.70000US04	8702

23628 7590 06/29/2020

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

EXAMINER
BASKAR, PADMAVATHI

ART UNIT	PAPER NUMBER
1645	

NOTIFICATION DATE	DELIVERY MODE
06/29/2020	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

Patents_eOfficeAction@WolfGreenfield.com
WGS_eOfficeAction@WolfGreenfield.com

Petitioner Merck Ex-1004, 0976

Office Action Summary	Application No. 16/089,040	Applicant(s) FORREST et al.	
	Examiner Padmavathi Baskar	Art Unit 1645	AIA (FITF) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 2 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☑ Responsive to communication(s) filed on 03/10/2020.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL.** 2b) ☐ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☑ Claim(s) 1,29,62,67,71,73-75,78,81,97,106,109,112-114,154,157,163 and 256 is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☐ Claim(s) _____ is/are rejected.

8) ☐ Claim(s) _____ is/are objected to.

9) ☑ Claim(s) 1,29,62,67,71,73-75,78,81,97,106,109,112-114,154,157,163 and 256 are subject to restriction and/or election requirement

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov.**

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date _____.

4) ☐ Other: _____.

Petitioner Merck Ex-1004, 0977

Notice of Pre-AIA or AIA Status

The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

DETAILED ACTION

1. The preliminary amendment filed on 03/10/2020 is entered. Claims 1, 29, 62, 67, 71, 73-75, 78, 81, 97, 106, 109, 112-114, 154, 157, 163 and 256 are pending.

REQUIREMENT FOR UNITY OF INVENTION

2. As provided in 37 CFR 1.475(a), a national stage application shall relate to one invention only or to a group of inventions so linked as to form a single general inventive concept ("requirement of unity of invention"). Where a group of inventions is claimed in a national stage application, the requirement of unity of invention shall be fulfilled only when there is a technical relationship among those inventions involving one or more of the same or corresponding special technical features. The expression "special technical features" shall mean those technical features that define a contribution which each of the claimed inventions, considered as a whole, makes over the prior art.

The determination whether a group of inventions is so linked as to form a single general inventive concept shall be made without regard to whether the inventions are claimed in separate claims or as alternatives within a single claim. See 37 CFR 1.475(e).

When Claims Are Directed to Multiple Categories of Inventions:

As provided in 37 CFR 1.475(b), a national stage application containing claims to different categories of invention will be considered to have unity of invention if the claims are drawn only to one of the following combinations of categories:

(1)A product and a process specially adapted for the manufacture of said product; or
(2)A product and process of use of said product; or
(3)A product, a process specially adapted for the manufacture of the said product, and a use of the said product; or
(4)A process and an apparatus or means specifically designed for carrying out the said process; or
(5)A product, a process specially adapted for the manufacture of the said product, and an apparatus or means specifically designed for carrying out the said process.

Otherwise, unity of invention might not be present. See 37 CFR 1.475(c).

3. Restriction is required under 35 U.S.C. 121 and 372.

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 accordance with 37 CFR 1.499, applicant is required, in reply to this action, to elect a single invention to which the claims must be restricted.

Group I, claims 1 (in part), 71, 73, 74, 75, 78, 81, 97,106, 109 and 256 drawn to a pharmaceutical composition and a method of making composition,

(See paragraph # 5).

Group 2-4, Claims 29 or 62 or 67 drawn to a pharmaceutical composition comprising at least two immunogenic polypeptide conjugates

(See paragraph # 5).

Group 5 or 6 claims 112-114, 154,157 or 163 drawn to a method comprising administering a pharmaceutical composition.

(See paragraph # 5).

4. The groups of inventions1-6 listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature of linking groups appears to be that they are all related to polysaccharides. However, US 2015/0374811 A1 teaches 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). Therefore, the technical feature of linking groups I-5 does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art and hence unity of invention is lacking.

5. This application contains claims directed to more than one invention. These inventions are deemed to lack unity of invention because they are not so linked as to form a single general inventive concept under PCT Rule 13.1 as indicated above.

The inventions are as follows:

Claim 1 Or claim 112 , please select at least eight or more or any one combination of eight or more capsular polysaccharide conjugated to a polypeptide from serotype 2, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35B, 35F, and 38.

Claim 2, please select at least two or more or any one combination of two or more capsular polysaccharide conjugated to a polypeptide from serotype 6C and at least one additional serotype is selected from the group consisting of 1, 2, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 17F, 20A, 20B, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, and 38.

Claim 3, please select at least two or more or any one combination of two or more capsular polysaccharide conjugated to a polypeptide from serotype 20B and at least one additional serotype is 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, 22F, 23A, 23B, 24F, 31, 33F, 34, 35B, and 38.

Claim 67, please select at least two or more or any one combination of two or more capsular polysaccharide conjugated to a polypeptide from serotype 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, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, and 38.

Applicant is required, in reply to this action, to elect a single combination of serotype and indicate clearly what are the serotypes selected for prosecution to which the claims shall be restricted. The reply must also identify the claims readable on the elected invention, including any claims subsequently added. An argument that a claim is allowable is considered non-responsive unless accompanied by an election.

6. Applicant is advised that the reply to this requirement to be complete must include (i) an election of invention to be examined even though the requirement may be traversed (37 CFR 1.143) and (ii) identification of the claims encompassing the elected invention.

The election of an invention may be made with or without traverse. To preserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the restriction requirement, the election shall be treated as an election without traverse. Traversal must be presented at the time of election in order to be considered timely. Failure to timely traverse the requirement will result in the loss of right to petition under 37 CFR 1.144. If claims are added after the election, applicant must indicate which of these claims are readable on the elected invention.

Should applicant traverse on the ground that the inventions have unity of invention (37 CFR 1.475(a)), applicant must provide reasons in support thereof. Applicant may submit evidence or identify such evidence now of record showing the inventions to be obvious variants or clearly admit on the record that this is the case. Where such evidence or admission is provided by applicant, if the examiner finds one of the inventions unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. 103(a) of the other invention.

7. Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a request under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(i).

8. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Padmavathi Baskar whose telephone number is (571)272-0853. The examiner can normally be reached on Mon-Fri 9-5:30pm.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Nickol can be reached on 571-272-0835. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Respectfully,

/Padmavathi Baskar/
Primary Examiner, Art Unit 1645
Padmavathi Baskar, Ph.D. (Microbiology and Immunology)

Docket No.: P1143.70000US04
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor:	Bruce D. Forrest
Application No.:	16/089,040
Confirmation No.:	8702
Filed:	September 27, 2018
For:	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF
Examiner:	Padmavathi Baskar
Art Unit:	1645

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 28, 2020 Signature: /Chelsea E. Dowling/ (Chelsea E. Dowling)

AMENDMENT AND RESPONSE TO RESTRICTION/ ELECTION OF SPECIES REQUIREMENT

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Commissioner:

In response to the Restriction/Election of Species Requirement, dated June 29, 2020, Applicant respectfully requests entrance of the following Amendment and consideration of the following Remarks. The deadline for responding to the Office Action was August 29, 2020. A four-month extension of time from August 29, 2020, up to and including December 29, 2020, is hereby respectfully requested, and the extension fee is being paid using the credit card payment option of the USPTO's Electronic Filing System. Applicant thus submits that the present Response is timely submitted on Monday, December 28, 2020.

Please amend the above-identified U.S. patent application as follows:

Amendments to the Claims are reflected in the Listing of Claims, which begins on page 2 of this paper.

Remarks begin on page 7 of this paper.

Petitioner Merck Ex-1004, 0982

AMENDMENTS OT THE CLAIMS

This Listing of Claims will replace all prior versions, and listings, of claims in the Application:

Listing of Claims

1.-66. (Canceled)

67. (Currently Amended) A pharmaceutical composition comprising an immunogenic saccharide-polypeptide conjugate comprising, individually, at least two unique capsular polysaccharides, fragments thereof, or combinations thereof, conjugated to a polypeptide, wherein each of the at least two unique capsular polysaccharides, or fragments thereof, ~~or combinations thereof~~ is [[are]] from a *Streptococcus pneumoniae* serotype selected from a 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, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 34, 35F, 35B, and 38.

68.-70. (Canceled)

71. (Currently Amended) The pharmaceutical composition of claim 67, [[1]] wherein at least one of the ~~unique~~ immunogenic saccharide-polypeptide conjugates comprises the capsular polysaccharide or the fragment of the capsular polysaccharide.

72. (Canceled)

73. (Previously Presented) The pharmaceutical composition of claim 71, wherein the fragment of the capsular polysaccharide comprises a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, or a hexasaccharide.

74. (Currently Amended) The pharmaceutical composition of claim 67 [[1]], wherein at least one polypeptide of the plurality is an oligopeptide and/or at least one polypeptide comprises a mixture of polypeptides.

75. (Currently Amended) The pharmaceutical composition of claim 67 [[1]], wherein at least one polypeptide of the plurality 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*, PspA, any fragment thereof, or any combination thereof.

76. - 77. (Canceled)

78. (Currently Amended) The pharmaceutical composition of claim 67 [[1]], further comprising an adjuvant; a chelating agent; a surfactant, an emulsifier, or a combination thereof; a buffering agent; a preservative; a salt; and/or an anti-fungal compound or a salt thereof.

79. - 80. (Canceled)

81. (Currently Amended) The pharmaceutical composition of claim 67 [[1]], wherein the pharmaceutical composition is in the form of an intramuscularly injectable composition, intradermally injectable composition, subcutaneously injectable composition, or an intranasally administrable composition.

82. - 96. (Canceled)

97. (Currently Amended) The pharmaceutical composition of claim 67 [[1]], wherein the unique immunogenic saccharide-polypeptide conjugate elicits an opsonophagocytic response.

98. - 105. (Canceled)

106. (Currently Amended) The pharmaceutical composition of claim 67 [[1]], wherein the immunogenic saccharide-polypeptide conjugate(s) are individually, respectively, or collectively present in an amount of at least about 0.001 %, by weight, based on the weight of the composition.

107. - 108. (Canceled)

109. (Currently Amended) The pharmaceutical composition of claim 67 [[1]], wherein the immunogenic saccharide-polypeptide comprises:

(i) the capsular polysaccharide at least partially embedded in the polypeptide,

(ii) the capsular polysaccharide chemically cross-linked to the polypeptide, and/or

(iii) the capsular polysaccharide at least partially chemically cross-linked to the polypeptide.

110. - 111. (Canceled)

112. (Currently Amended) A method comprising administering to a subject a first composition, wherein the first composition is the pharmaceutical composition of claim 67 [[1]].

113. (Currently Amended) The method of claim 112, 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 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~~.

114. (Original) The method of claim 113, wherein the second composition comprises an immunogenic saccharide-polypeptide conjugate comprising a capsular polysaccharide or fragment

of the capsular polysaccharide from a serotype of *Streptococcus pneumoniae* conjugated to the second polypeptide.

115. - 153. (Canceled)

154. (Previously Presented) The method of claim 113, wherein the subject is a human.

155. - 255. (Canceled)

256. (Currently Amended) A method of making a composition comprising:

contacting the immunogenic saccharide-polypeptide conjugate of claim 67 [[1]] with an excipient, an adjuvant, or any combination thereof.

257 - 295. (Canceled)

296. (New) The pharmaceutical composition of claim 67, wherein at least one of the unique immunogenic saccharide-polypeptide conjugates comprises the capsular polysaccharides or the fragments of the capsular polysaccharide.

297. (New) The pharmaceutical composition of claim 67, wherein a toxin activity of at least one of the polypeptides of the plurality is at least partly mitigated.

298. (New) The pharmaceutical composition of claim 67, further comprising at least one additional immunogenic saccharide-polypeptide conjugate comprising a capsular polysaccharide, or a fragment thereof, from a unique *Streptococcus pneumoniae* 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, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 35F, 35B, and 38.

299. (New) The pharmaceutical composition of claim 67, wherein each of the immunogenic saccharide-polypeptide conjugates is present in an amount of about: 0.001% by weight based on the weight of the pharmaceutical composition.

300. (New) The method of claim 112, wherein the pharmaceutical composition is administered intramuscularly.

REMARKS

Applicant requests entrance of this Preliminary Amendment to amend the claims of the Application. After entrance of the present Amendment, one (1) independent claim, 20 total claims, and no multiply dependent claims will be pending in this case. Claims 1, 29, 62, 157, 163, and 256 have been canceled without prejudice or disclaimer. Claims 71, 74, 75, 68, 81, 97, 106, 109, 112, 113, and 256 have been amended. New claims 296-300 have been added. Support for the claim amendments and new claims can be found throughout the specification and claims of the originally filed Application. No new matter is added to the Application by the present Amendment.

Restriction Requirement

In response to the Restriction/Election of Species Requirement set forth in the Office Action, mailed June 29, 2020, Applicant hereby elects Group 4 (claim 67), drawn to a pharmaceutical composition comprising at least two immunogenic polypeptide conjugates. Applicant submits that claims 67, as well as claims 71-75, 78, 81, 97, 106, 109, and 296-299, encompass the elected invention.

Election of Species Requirement

Furthermore, Applicant hereby elects, for search purposes only, the following species: a combination comprising capsular polysaccharides from serotypes 35B and 23A and/or 23B conjugated to a polypeptide. Applicant submits that claims 67, 71-75, 78, 81, 97, 106, 109, and 296-299 encompass the elected species.

Having made the above elections, Applicant reserves the right to pursue the subject matter of the non-elected claims in one or more future applications claiming priority to the present Application.

Applicant believes all fees due with this Response are being paid by credit card. However, if any additional fees are due, please charge them to our Deposit Account No. 23/2825, under Docket No. P1143.70000US04, from which the undersigned is authorized to draw.

Dated: December 28, 2020

Respectfully submitted,

By /Chelsea E. Witte-Garcia/
Chelsea E. Witte-Garcia, Ph.D.
Registration Number: 75,713
WOLF, GREENFIELD & SACKS, P.C.
600 Atlantic Avenue
Boston, Massachusetts 02210-2206
617.646.8000

Electronic Patent Application Fee Transmittal

Application Number:	16089040
Filing Date:	27-Sep-2018
Title of Invention:	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF
First Named Inventor/Applicant Name:	Bruce D. FORREST
Filer:	Chelsea E. Witte-Garcia/Chelsea Dowling
Attorney Docket Number:	P1143.70000US04

Filed as Small Entity

Filing Fees for U.S. National Stage under 35 USC 371

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension - 4 months with $0 paid	2254	1	1160	1160
Miscellaneous:				
			Total in USD ($)	**1160**

Electronic Acknowledgement Receipt

EFS ID:	41502978
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:	23628
Filer:	Chelsea E. Witte-Garcia/Chelsea Dowling
Filer Authorized By:	Chelsea E. Witte-Garcia
Attorney Docket Number:	P1143.70000US04
Receipt Date:	28-DEC-2020
Filing Date:	27-SEP-2018
Time Stamp:	18:18:44
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$1160
RAM confirmation Number	E2020BRI19394420
Deposit Account	232825
Authorized User	Chelsea Dowling

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

37 CFR 1.17 (Patent application and reexamination processing fees)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal Letter	P114370000US04-TRN-CEW.pdf	26277 d678190cbd8dd8e07d3d73c5985ebb832a20e5f9	no	1
Warnings:					
Information:					
2	Fee Worksheet (SB06)	P114370000US04-FEE-CEW.pdf	32638 8cba415bdaf77126e611cc7f005b16367614b8ee	no	1
Warnings:					
Information:					
3	Extension of Time	P114370000US04-EXT-CEW.pdf	24706 bda27a7ac7d5fb5f54f7d9d915d0e8e14a991523	no	1
Warnings:					
Information:					
4		P114370000US04-ERRRAMN-CEW.pdf	44330 b3fbe5ec75e49825bd315f9719dcd369eefb47ea	yes	8

Multipart Description/PDF files in .zip description

Document Description	Start	End
Response to Election / Restriction Filed	1	1
Claims	2	6
Applicant Arguments/Remarks Made in an Amendment	7	8

Warnings:					
Information:					
5	Fee Worksheet (SB06)	fee-info.pdf	31247 a174da1738fa93e5f0f8cc9468eae4adf0bb85ab	no	2

Warnings:	
Information:	
Total Files Size (in bytes):	159198

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.

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	1645
Examiner Name	Padmavathi Baskar
Attorney Docket Number	P1143.70000US04

ENCLOSURES (*Check all that apply*)

- [X] Fee Transmittal Form
  - [ ] Fee Attached
- [ ] Amendment/Reply
  - [ ] After Final
  - [ ] Affidavits/declaration(s)
- [X] 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
- [ ] 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):

-Second Preliminary Amendment and Response to Restriction and Species Election Requirement

Remarks

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	WOLF, GREENFIELD & SACKS, P.C.		
Signature	/Chelsea E. Witte-Garcia/		
Printed name	Chelsea E. Witte-Garcia, Ph.D.		
Date	December 28, 2020	Reg. No.	75,713

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 28, 2020 Signature: /Chelsea E. Dowling/ (Chelsea E. Dowling)

8740933.1

FEE TRANSMITTAL

Complete if known	
Application Number	16/089,040-Conf. #8702
Filing Date	September 27, 2018
First Named Inventor	Bruce D. Forrest
Examiner Name	Padmavathi Baskar
Art Unit	1645
Practitioner Docket No.	P1143.70000US04

[x] Applicant asserts small entity status. See 37 CFR 1.27.

[ ] Applicant certifies micro entity status. See 37 CFR 1.29. Form PTO/SB/15A or B or equivalent must either be enclosed or have been submitted previously.

TOTAL AMOUNT OF PAYMENT ($)1,160.00

METHOD OF PAYMENT (check all that apply)

[ ] Check [x] Credit Card [ ] Money Order [ ] None [ ] Other (please identify): _____

[x] Deposit Account Deposit Account Number: 23/2825 Deposit Account Name: Wolf, Greenfield & Sacks, P.C.

For the above-identified deposit account, the Director is hereby authorized to (check all that apply):

[ ] Charge fee(s) indicated below

[ ] Charge fee(s) indicated below, **except for the filing fee**

[x] Charge any additional fee(s) or underpayment of fee(s) under 37 CFR 1.16 and 1.17, **except for any excess claims fees or multiple dependent claim fee**

[ ] Charge any additional fee(s) or underpayment of fee(s) under 37 CFR 1.16 and 1.17

[x] Credit any overpayment of fee(s)

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

FEE CALCULATION

1. BASIC FILING, SEARCH, AND EXAMINATION FEES (U = undiscounted fee; S = small entity fee; M = micro entity fee)

	FILING FEES			SEARCH FEES			EXAMINATION FEES			
Application Type	**U ($)**	**S ($)**	**M ($)**	**U ($)**	**S ($)**	**M ($)**	**U ($)**	**S ($)**	**M ($)**	**Fees Paid ($)**
Utility	320	160*	80	700	350	175	800	400	200	
Design	220	110	55	160	80	40	640	320	160	
Plant	220	110	55	440	220	110	660	330	165	
Reissue	320	160	80	700	350	175	2,320	1,160	580	
Provisional	300	150	75	0	0	0	0	0	0	

* The $160 small entity status filing fee for a utility application is further reduced to $80 for a small entity status applicant who files the application via EFS-Web.

2. EXCESS CLAIM FEES

Fee Description	**Undiscounted Fee ($)**	**Small Entity Fee ($)**	**Micro Entity Fee ($)**
Each claim over 20 (including Reissues)	100	50	25
Each independent claim over 3 (including Reissues)	480	240	120
Multiple dependent claims	860	430	215

Total Claims	**Extra Claims**	**Fee ($)**	**Fee Paid ($)**
20 - or HP =	_____	x _____	= _____

HP = highest number of total claims paid for, if greater than 20.

Indep. Claims	**Extra Claims**	**Fee ($)**	**Fee Paid ($)**
1 - or HP =	_____	x _____	= _____

HP = highest number of independent claims paid for, if greater than 3.

Multiple Dependent Claims	
Fee ($)	**Fee Paid ($)**

3. APPLICATION SIZE FEE

If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is $420 ($210 for small entity) ($105 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).

Total Sheets	**Extra Sheets**	**Number of each additional 50 or fraction thereof**	**Fee ($)**	**Fee Paid ($)**
_____ - 100 =	_____ /50 =	_____ (round **up** to a whole number) x	_____	= _____

4. OTHER FEE(S)

	Fees Paid ($)
Non-English specification, $130 fee (no small or micro entity discount)	
Non-electronic filing fee under 37 CFR 1.16(t) for a utility application, $400 fee ($200 small or micro entity)	
Other (e.g., late filing surcharge): 2254 Extension for response within fourth month	1,160.00

SUBMITTED BY

Signature	/Chelsea E. Witte-Garcia/	Registration No. (Attorney/Agent)	75,713	Telephone	617.646.8000
Name (Print/Type)	Chelsea E. Witte-Garcia, Ph.D.			Date	December 28, 2020

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 28, 2020 Signature: /Chelsea E. Dowling/ (Chelsea E. Dowling)

8740930.1

PTO/AIA/22 (10-20)
Approved for use through 11/30/2020. 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.

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a)	Docket Number (Optional) P1143.70000US04
Application Number 16/089,040-Conf. #8702	Filed September 27, 2018
For SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF	
Art Unit 1645	Examiner Padmavathi Baskar

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above-identified application.

The requested extension and fee are as follows (check time period desired and enter the appropriate fee below):

		Fee	Small Entity Fee	Micro Entity Fee	
☐	One month (37 CFR 1.17(a)(1))	$220	$110	$55	$
☐	Two months (37 CFR 1.17(a)(2))	$640	$320	$160	$
☐	Three months (37 CFR 1.17(a)(3))	$1,480	$740	$370	$
☒	Four months (37 CFR 1.17(a)(4))	$2,320	$1,160	$580	$ 1,160.00
☐	Five months (37 CFR 1.17(a)(5))	$3,160	$1,580	$790	$

☒ Applicant asserts small entity status. See 37 CFR 1.27.

☐ Applicant certifies micro entity status. See 37 CFR 1.29.
Form PTO/SB/15A or B or equivalent must either be enclosed or have been submitted previously.

☐ A check in the amount of the fee is enclosed.

☒ Payment by credit card. ~~Form PTO-2038 is attached.~~

☐ The Director has already been authorized to charge fees in this application to a Deposit Account.

☒ The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number 23/2825 .

☒ Payment made via EFS-Web.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

I am the

☐ applicant.

☐ attorney or agent of record. Registration number _____ .

☒ attorney or agent acting under 37 CFR 1.34. Registration number 75,713 .

/Chelsea E. Witte-Garcia/	December 28, 2020
Signature	Date
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		2017-0021006	A1	Watson et al.	01-26-2017

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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)
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(54) Title: STREPTOCOCCAL VACCINE

(57) Abstract: The present invention relates to photon-irradiated streptococcal vaccine preparations and methods for their use.

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STREPTOCOCCAL VACCINE

Incorporation by Cross-Reference

This Application claims priority from Australian Provisional Patent Application Number 2015901098 entitled *"Streptococcal Vaccine"* filed on 26 March 2015, the entire content of which is incorporated herein by cross reference.

Technical Field

The present invention relates generally to the field of vaccines. More specifically, the present invention relates to streptococcal vaccine preparations and methods for their use.

Background

Streptococci are a genus of spheroidal bacteria belonging to the family *Streptococcaceae.* There are many different species of streptococci, some of which cause disease in humans and animals. Others are important in the manufacture of various fermented products.

Individual streptococcal species are classified into two key groups based on their haemolytic properties (alpha- and beta-haemolytic). Alpha-haemolytic streptococci include *Streptococcus pneumoniae* and *Viridans* streptococci. The beta-haemolytic group is made up of Group A and Group B streptococci. Group B streptococci usually inhabit the digestive system and the vagina of women without adverse effect. Most people quickly develop a natural immunity to Group B streptococci although they can cause more serious types of infection in newborn infants. Group A streptococci commonly inhabit the throat and skin surface, and are a common cause of infection in adults and children. Although most Group A infections do not usually pose a serious threat to health (e.g. throat infections, cellulitis, impetigo, sinusitis, middle ear infections) Group A Streptococci can establish a more serious invasive infection by penetrating deeper into the tissues and organs of the body (e.g. pneumonia, sepsis, meningitis, necrotising fasciitis) and can trigger serious sequelae including acute post-streptococcal glomerulonephritis and acute rheumatic fever.

In addition, Enterococcal (faecal) streptococcal species occur in significant numbers in the bowel and can cause endocarditis and urinary tract infections.

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Streptococcus pneumoniae (also called pneumococcus) is an important human pathogen accounting for significant morbidity and mortality in human and animal populations. It causes serious conditions including pneumonia, meningitis, sinusitis, and otitis media. An estimated 1.6 million people die globally from invasive pneumococcal disease each year and approximately one million of those are children. There are many different serotypes of *S. pneumoniae* (>90) distinguishable on the basis of capsule chemical structure and immunogenicity. The capsular polysaccharide is considered to be an essential virulence factor of *S. pneumoniae* as non-encapsulated strains are virtually absent among *S. pneumoniae* that are responsible for non-invasive pneumococcal disease. Capsular polysaccharides are thus used as vaccine antigens in current pneumococcal vaccines.

Current pneumococcal conjugate vaccines cover only a selected set of serotypes, (e.g. PCV7 (7 serotypes), PCV10 (10 serotypes) and PCV13 (13 serotypes)). In many populations the introduction of the PCV7 vaccine targeting serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F significantly reduced the burden of pneumococcal disease. However, despite their efficacy against disease caused by targeted vaccine serotypes, serotype replacement often reduces the net effect of vaccination. For example, in a number of locations including the USA, England, Germany, Wales, and The Netherlands, serotype 19A was reported to be a common emerging non-vaccine serotype after PCV7 introduction. The emergence of non-vaccine serotypes upon the implementation of pneumococcal conjugate vaccines thus raises a problem.

In consideration of the emergence of non-vaccine serotypes associated with pneumococcal conjugate vaccines, a need exists for new streptococcal vaccines capable of inducing immunity against a broader range of serotypes.

Colonisation of the upper respiratory tract is the obligatory first step in the pathogenesis of pneumococcal disease, and therefore considered the most important risk factor for invasive pneumococcal disease. It also provides the basis for horizontal spread of pneumococci in the community, making it an important target for preventive measures.

Accordingly, a need exists for vaccines capable of inducing immunity against a broad range of pneumococcal serotypes and which are suited to upper respiratory tract administration.

Summary of the Invention

The invention relates to an improved streptococcal vaccine that reduces or alleviates at least one deficiency of existing streptococcal vaccines.

Accordingly, the invention relates to at least the following embodiments:

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Embodiment 1. A method for preventing or treating an infection by streptococcal bacteria in a subject, the method comprising administering a therapeutically effective amount of photon-irradiated streptococcal bacteria to the subject to thereby prevent or treat the infection.

Embodiment 2. The method according to embodiment 1, wherein the method prevents or treats infection by a plurality of different streptococcal species and/or serotypes.

Embodiment 3. The method according to embodiment 2, wherein the photon-irradiated streptococcal bacteria comprise different: *Streptococcus* species, streptococcal serotypes, and/or streptococcal derivatives.

Embodiment 4. The method according to any one of embodiments 1 to 3, wherein the streptococcal infection comprises infection by one or more serotypes of *Streptococcus pneumoniae.*

Embodiment 5. The method according to any one of embodiments 1 to 4, wherein the infection by streptococcal bacteria is any one or more of a respiratory tract infection, pneumonia, an ear infection, an ear-ache, a middle ear infection, otitis media, sinusitis, meningitis, conjunctivitis, bacteraemia, septicaemia, a joint infection, a bone infection, septic arthritis, sepsis, osteomyelitis, a soft tissue infection, cellulitis, myositis, periorbital cellulitis, an abscess, necrotising fasciitis, impetigo, peritonitis, a cardiac infection, endocarditis, and/or pericarditis.

Embodiment 6. A method of inducing an immune response against streptococcal bacteria in a subject, the method comprising administering a therapeutically effective amount of photon-irradiated streptococcal bacteria to the subject to thereby induce the immune response.

Embodiment 7. The method according to embodiment 6, wherein the immune response comprises a heterotypic immune response against a different streptococcal species and/or serotype to that administered to the subject.

Embodiment 8. The method according to embodiment 6 or embodiment 7, wherein the therapeutically effective amount of photon-irradiated streptococcal bacteria comprise different: *Streptococcus* species, streptococcal serotypes, and/or streptococcal derivatives.

Embodiment 9. The method according to embodiment 7 or embodiment 8, wherein the different streptococcal species and/or serotype is a *Streptococcus pneumoniae* serotype.

Embodiment 10. The method according to any one of embodiments 6 to 9, wherein the immune response comprises any one or more of:

(i) a B-lymphocyte response;

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(ii) an innate immune response induced at least in part by interaction of double-stranded RNA from the photon-irradiated streptococcal bacteria with Toll-like receptors;

(iii) a T-lymphocyte response.

Embodiment 11. The method according to any one of embodiments 1 to 10, wherein the photon-irradiated streptococcal bacteria comprise at least one *Streptococcus pneumoniae* serotype, or at least one unencapsulated derivative of a *Streptococcus pneumoniae* serotype.

Embodiment 12. The method according to any one of embodiments 1 to 11, wherein the photon-irradiated streptococcal bacteria comprise mutant streptococcal bacteria comprising:

(i) one or more defective DNA repair proteins; and/or

(ii) a genetic alteration causing defective DNA repair capacity.

Embodiment 13. The method according to embodiment 12, wherein the photon-irradiated mutant streptococcal bacteria comprise a defect in at least one gene that encodes a DNA repair protein.

Embodiment 14. The method according to embodiment 13, wherein the photon-irradiated mutant streptococcal bacteria are *Streptococcus pneumoniae* mutants comprising a defect in one or more genes selected from a gene encoding a DNA alkylation repair protein, a gene encoding DNA polymerase 4, *hexA, hexB, mutS, radC, recA, recF, recN, recO, uvrA, uvrB, uvrC* or *uvrD*.

Embodiment 15. The method according to any one of embodiments 12 to 14, wherein the defective DNA repair protein is mutated, truncated or absent in the photon-irradiated mutant streptococcal bacteria.

Embodiment 16. The method according to any one of embodiments 12 to 15, wherein the photon-irradiated mutant streptococcal bacteria comprise streptococcal Rxl strain derivatives in which:

(i) any one or more of autolysin, hemolysin, pneumolysin, and/or PsaA protein is/are defective; and/or

(ii) any one or more of autolysin, hemolysin, and/or a PsaA protein is/are absent.

Embodiment 17. The method according to embodiment 16, wherein the photon-irradiated streptococcal Rx1 strain derivatives:

(i) comprise any one or more of a gene encoding a defective autolysin protein, a gene encoding defective hemolysin, a gene encoding defective pneumolysin, and/or a gene encoding defective PsaA protein; or

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(ii) are derivatives in which any one or more of a gene encoding autolysin, a gene encoding hemolysin, and/or a gene encoding PsaA protein, is/are absent.

Embodiment 18. The method according to embodiment 16 or embodiment 17, wherein the autolysin is LytA.

Embodiment 19. The method according to any one of embodiments 16 to 18, wherein the photon-irradiated streptococcal Rx1 strain derivatives:

(i) comprise a gene encoding defective LytA protein, and a gene encoding defective pneumolysin; or

(ii) do not contain the *lytA* gene, and comprise a gene encoding a defective pneumolysin.

Embodiment 20. The method according to any one of embodiments 12 to 19, wherein the photon-irradiated mutant streptococcal bacteria require a reduced dosage of photon-irradiation for inactivation compared to that required by corresponding non-mutant streptococcal bacteria.

Embodiment 21. The method according to any one of embodiments 1 to 20, wherein some or all of the photon-irradiated streptococcal bacteria comprise modified streptococcal bacteria comprising a sequence of DNA encoding an RNA transcript, wherein the RNA transcript comprises a region of self-complementarity capable of forming a double-stranded portion after transcription.

Embodiment 22. The method according to embodiment 21, wherein the portion of the double-stranded RNA transcript is at least: 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65 or 70 base pairs in length.

Embodiment 23. The method according to embodiment 21 or embodiment 22, wherein the double-stranded RNA transcript initiates an innate immune response in the subject comprising Toll-like receptor activation.

Embodiment 24. The method according to embodiment 23, wherein the Toll-like receptor is Toll-like receptor-3 (TLR-3).

Embodiment 25. The method according to any one of embodiments 1 to 24, wherein the photon-irradiated streptococcal bacteria comprise auxotrophic mutant streptococcal bacteria.

Embodiment 26. The method according to any one of embodiments 1 to 24, wherein the photon-irradiated streptococcal bacteria comprise at least one recombinant DNA portion encoding an antigen or a component thereof that:

(i) inactivates or attenuates the bacteria; and/or

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(ii) induces or enhances an immune response in the subject.

Embodiment 27. The method according to embodiment 26, wherein the recombinant DNA portion replaces or disrupts an endogenous gene necessary for pathogenicity, infection, multiplication, growth *in vivo,* or any combination thereof.

Embodiment 28. The method according to any one of embodiments 1 to 27, wherein the photon-irradiated streptococcal bacteria comprise whole killed streptococcal bacteria.

Embodiment 29. The method according to any one of embodiments 1 to 28, wherein the photon-irradiated streptococcal bacteria are administered to the subject mucosally or intranasally.

Embodiment 30. The method according to any one of embodiments 1 to 29, wherein the photon-irradiated streptococcal bacteria are administered to the subject in combination with an adjuvant.

Embodiment 31. The method according to any one of embodiments 1 to 30, wherein one, two or three separate doses of the photon-irradiated streptococcal bacteria are administered to the subject.

Embodiment 32. The method according to any one of embodiments 1 to 31, wherein the subject is a human.

Embodiment 33. The method according to any one of embodiments 1 to 32, wherein the photon-irradiated streptococcal bacteria are administered a total dosage of at least: 8 kilogray (kGy), 9kGy, 10kGy, 11kGy, 12kGy, 15kGy, 20kGy, 25kGy, 30 kGy, 40kGy, or 50 kGy of photon-irradiation.

Embodiment 34. The method according to any one of embodiments 1 to 33, wherein the photon-irradiated streptococcal bacteria comprise gamma-irradiated streptococcal bacteria, X-irradiated streptococcal bacteria, or a combination thereof.

Embodiment 35. The method according to any one of embodiments 1 to 33, wherein the photon-irradiated streptococcal bacteria comprise X-irradiated streptococcal bacteria.

Embodiment 36. The method according to any one of embodiments 1 to 33, wherein the photon-irradiated streptococcal bacteria comprise a combination of gamma-irradiated streptococcal bacteria and X-irradiated streptococcal bacteria.

Embodiment 37. The method according to embodiment 20 or embodiment 33, wherein the photon-irradiation is gamma-irradiation.

Embodiment 38. The method according to embodiment 20 or embodiment 33, wherein the photon-irradiation is X-irradiation.

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Embodiment 39. The method according to embodiment 20 or embodiment 33, wherein the photon-irradiation is a combination of gamma-irradiation and X-irradiation.

Embodiment 40. The method according to any one of embodiments 1 to 39, wherein the photon-irradiated streptococcal bacteria comprise streptococcal Rxl strain derivatives:

(i) comprise a gene encoding a defective LytA protein, a gene encoding defective pneumolysin, and a gene encoding defective PsaA protein;

(ii) comprise a gene encoding a defective LytA protein, a gene encoding defective pneumolysin, and in which a gene encoding PsaA protein is absent;

(iii) comprise a gene encoding a defective PsaA protein, a gene encoding defective pneumolysin, and in which a gene encoding LytA protein is absent; or

(iv) comprise a gene encoding defective pneumolysin, in which a gene encoding LytA protein is absent, and in which a gene encoding PsaA protein is absent.

Embodiment 41. A vaccine composition comprising photon-irradiated streptococcal bacteria and a pharmaceutically-acceptable excipient, diluent and/or carrier, wherein:

(i) the photon-irradiated streptococcal bacteria comprise. mutant streptococcal bacteria comprising one or more defective DNA repair proteins, and/or mutant streptococcal bacteria in which at least one type of DNA repair protein is absent; and/or

(ii) the photon-irradiated streptococcal bacteria comprise modified streptococcal bacteria comprising a sequence of DNA encoding an RNA transcript, wherein the RNA transcript comprises a region of self-complementarity capable of forming a double-stranded portion after transcription.

Embodiment 42. The vaccine composition according to embodiment 41, wherein the mutant streptococcal bacteria are *Streptococcus pneumoniae* mutants comprising a defect in one or more genes selected from. a gene encoding a DNA alkylation repair protein, a gene encoding DNA polymerase 4, *hexA, hexB, mutS, radC, recA, recF, recN, recO, uvrA, uvrB, uvrC* or *uvrD.*

Embodiment 43. The vaccine composition according to embodiment 4 1 or embodiment 42, wherein the modified streptococcal bacteria are modified *Streptococcus pneumoniae* bacteria and the portion of the double-stranded RNA transcript is at least. 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65 or 70 base pairs in length.

Embodiment 44. The vaccine composition according to any one of embodiments 4 1 to 43, wherein the photon-irradiated streptococcal bacteria comprise at least one recombinant DNA portion encoding an antigen or a component thereof that:

(i) inactivates or attenuates the bacteria; or

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(ii) induces or enhances an immune response in the subject.

Embodiment 45. The vaccine composition according to embodiment 44, wherein the recombinant DNA portion replaces or disrupts an endogenous gene necessary for pathogenicity, infection, reproduction or any combination thereof.

Embodiment 46. The vaccine according to any one or more of embodiments 41 to 45, wherein the photon-irradiated streptococcal bacteria comprise streptococcal Rxl strain derivative strains in which:

(i) any one or more of autolysin, hemolysin, pneumolysin, and/or PsaA protein is/are defective; and/or

(ii) any one or more of autolysin, hemolysin, and/or a PsaA protein is/are absent.

Embodiment 47. The vaccine according to embodiment 46, wherein the photon-irradiated streptococcal Rx1 strain derivatives:

(i) comprise any one or more of a gene encoding a defective autolysin protein, a gene encoding defective hemolysin, a gene encoding defective pneumolysin, and/or a gene encoding defective PsaA protein; or

(ii) are derivatives in which any one or more of a gene encoding autolysin, a gene encoding hemolysin, and/or a gene encoding PsaA protein, is/are absent.

Embodiment 48. The vaccine according to any one of embodiments 41 to 47, wherein the photon-irradiated streptococcal Rxl strain derivatives:

(i) comprise a gene encoding defective LytA protein, and a gene encoding defective pneumolysin; or

(ii) do not contain the *lytA* gene, and comprise a gene encoding a defective pneumolysin.

Embodiment 49. The vaccine according to any one of embodiments 41 to 48, wherein the photon-irradiated mutant streptococcal bacteria comprise streptococcal Rxl strain derivatives:

(i) comprise a gene encoding a defective LytA protein, a gene encoding defective pneumolysin, and a gene encoding defective PsaA protein;

(ii) comprise a gene encoding a defective LytA protein, a gene encoding defective pneumolysin, and in which a gene encoding PsaA protein is absent;

(iii) comprise a gene encoding a defective PsaA protein, a gene encoding defective pneumolysin, and in which a gene encoding LytA protein is absent; or

(iv) comprise a gene encoding defective pneumolysin, in which a gene encoding LytA protein is absent, and in which a gene encoding PsaA protein is absent.

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Embodiment 50. The vaccine composition according to any one of embodiments 4 1 to 49, wherein the photon-irradiated streptococcal bacteria comprise whole-attenuated or whole-killed streptococcal bacteria.

Embodiment 51. The vaccine composition according to any one of embodiments 4 1 to 50, further comprising an adjuvant.

Embodiment 52. The vaccine composition according to any one of embodiments 4 1 to 51, wherein the vaccine composition is formulated for mucosal or intranasal administration or formulated for injection intramuscularly, subcutaneously or intradermally.

Embodiment 53. The vaccine composition according to any one of embodiments 4 1 to 52, wherein the photon-irradiated streptococcal bacteria comprise gamma-irradiated mutant streptococcal bacteria and/or gamma-irradiated modified streptococcal bacteria.

Embodiment 54. The vaccine composition according to any one of embodiments 4 1 to 52, wherein the photon-irradiated streptococcal bacteria comprise X-irradiated mutant streptococcal bacteria and/or X-irradiated modified streptococcal bacteria.

Embodiment 55. The vaccine composition according to any one of embodiments 4 1 to 52, wherein the photon-irradiated streptococcal bacteria comprise:

(i) a combination of gamma-irradiated and X-irradiated mutant streptococcal bacteria; and/or

(ii) a combination of gamma-irradiated and X-irradiated modified streptococcal bacteria.

Embodiment 56. A method for preparing the vaccine composition according to any one of embodiments 4 1 to 55, the method comprising:

(i) photon-irradiating a preparation of streptococcal bacteria to thereby attenuate or kill the bacteria; and

(ii) combining the photon-irradiated streptococcal bacteria with a pharmaceutically-acceptable excipient, diluent and/or carrier.

Embodiment 57. The method according to embodiment 56, wherein said photon-irradiating the preparation of streptococcal bacteria comprises exposing the bacteria to gamma-radiation.

Embodiment 58. The method according to embodiment 56, wherein said photon-irradiating the preparation of streptococcal bacteria comprises exposing the bacteria to X-radiation.

Embodiment 59. The method according to embodiment 56, wherein said photon-irradiating the preparation of streptococcal bacteria comprises exposing the bacteria to gamma-radiation and X-radiation.

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Embodiment 60. A vaccine composition prepared by the method of any one of embodiments 56 to 59.

Embodiment 61. A vaccine composition according to any one of embodiments 41 to 55 or 60 for use in preventing or treating an infection by streptococcal bacteria.

Embodiment 62. Use of photon-irradiated streptococcal bacteria in the preparation of a medicament for preventing or treating an infection by streptococcal bacteria, wherein the medicament is the vaccine composition according to any one of embodiments 41 to 55 or 60.

Embodiment 63. The vaccine composition according to embodiment 61 or the use according to embodiment 62, wherein the infection by streptococcal bacteria is any one or more of a respiratory tract infection, pneumonia, ear infection, earache, middle ear infection, otitis media, sinusitis, meningitis, conjunctivitis, bacteraemia, septicaemia, a joint infection, a bone infection, septic arthritis, osteomyelitis, a soft tissue infection, cellulitis, myositis, periorbital cellulitis, an abscess, peritonitis, a cardiac infection, endocarditis, and pericarditis.

Brief Description of the Figures

Preferred embodiments of the present invention will now be described, by way of non-limiting example only, with reference to the accompanying figures wherein:

Figure 1 shows the results of Western blot analysis detecting PdT and LytA in *S. pneumoniae* D39 and various derivatives using anti-LytA and anti-PdT antisera. This confirms the successful deletion of the *lytA* gene and the substitution of *ply* with the mutant gene coding for PdT, leading to the generation of a *lytA* null mutant (autolysin-deficient), pneumolysin mutant derivative (PdT) Rxl strain, designated *S. pneumoniae* Rxl (ALytA, PdT). Lanes: 1. Purified Ply and LytA (5 ng each) as a control; 2. *S. pneumoniae* D39; 3, *S. pneumoniae* Rxl; 4, Rxl (ALytA, PdT) vaccine strain (pre-irradiation). The recombinant purified Ply and LytA proteins are larger in size due to the presence of a His6-tag;

Figure 2 shows the results of a haemolytic assay performed to confirm the lack of haemolytic activity in the derivative Rxl strain *S. pneumoniae* Rxl (ALytA, PdT) carrying the PdT mutant form of pneumolysin;

Figure 3 shows the predicted secondary structure of the mRNA transcript encoded by the *AlytA* gene;

Figure 4 provides graphs depicting *S. pneumoniae* Rxl (ALytA, PdT) viability following exposure to various dosages of gamma-irradiation on ice **(Figure 4A)** or dry ice **(Figure 4B);**

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Figure 5 provides images of gram-stained *S. pneumoniae* Rxl (ALytA, PdT) indicative of the effect of gamma-irradiation on physical morphology. DI: dry ice; KGY: kilogray;

Figure 6 provides scanning electron microscopy images of *S. pneumoniae* Rxl (ALytA, PdT) indicative of the effect of gamma-irradiation on physical morphology;

Figure 7 is a graph showing (A) duration of median survival and (B) percentage survival in mice vaccinated with gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT) against intranasal challenge with live *Streptococcus pneumoniae* strain D39 (serotype *2*). * P = <0.05; γ-PN = gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT);

Figure 8 provides a series of graphs indicative of immunity in mice vaccinated with gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT) against heterotypic challenge with EF3030 (serotype 19F) **(Figure 8A & Figure 8B)** or P9 (serotype 6A) **(Figure 8C).** EF3030 bacterial counts were determined 96 hours post challenge in the lungs **(Figure 8A)** and nasopharynx **(Figure 8B). Figure 8C** shows the duration of survival in mice 21 days post challenge with P9. γ-PN = gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT);

Figure 9 provides a series of graphs showing the results of ELISAs measuring *S. pneumoniae-specific* antibody responses in mice vaccinated intranasally with gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT). Individual graphs show specific IgG responses in serum for antigens Ply, CbpA, GlpO and NanA; and IgG and IgA antibody responses to PspA and to un-irradiated whole Rxl (ALytA, PdT) bacterial cells. Mice were vaccinated with γ-PN (gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT)) or were unvaccinated (PBS control);

Figure 10 provides a series of graphs showing the results of ELISAs measuring *S. pneumoniae-specific* antibody responses in mice vaccinated intraperitoneally with gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT). Individual graphs show specific IgG titres in serum against a whole cell lysate of *S. pneumoniae* Rxl (ALytA, PdT) cells (WC) **(Figure 10A),** and purified antigens Ply **(Figure 10B)** and CbpA **(Figure IOC).** γ-PN = gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT);

Figure 11 provides a graph showing the percentage survival of wild-type C57/BL6 and B-cell deficient C57/BL6 (uMT) mice vaccinated intranasally with gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT) and challenged with a lethal dose of *Streptococcus pneumoniae* strain D39. ** P = <0.01; γ-PN = gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT);

Figure 12 shows the results of cytokine analyses conducted on supernatants from cultured splenocytes derived from mice vaccinated intranasally with *S. pneumoniae* Rxl

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(ALytA, PdT). Interleukin-17A (IL-17A) **(Figure 12A)** and interferon-gamma (IFN-γ) levels **(Figure 12B)** in supernatants from the splenocytes following stimulation with *S. pneumoniae* antigen MalX, ConA, or the whole gamma-irradiated Rxl (ALytA, PdT) vaccine are indicated. **Figures 12C** and **12D** show the results of intracellular cytokine staining performed on cultured splenocytes in the presence of MalX or the vaccine to gauge the proportion of Thl cells (using IFN-γ +), Th2 cells (IL-4+), Thl7 cells (IL-17+) and T-reg cells (Foxp3+) induced after 72h of stimulation. γ-PN = gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT);

Figure 13 shows percentage change in body weight following challenge with influenza virus strain A/PR8 of (i) mice given PBS as a control **(Figure 13A)** and (ii) mice intranasally vaccinated *S. pneumoniae* Rxl (ALytA, PdT) **(Figure 13B)** three weeks prior to A/PR8 challenge. γ-PN = gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT);

Figure 14 shows bacterial counts in the lungs **(Figure 14A)** and nasopharynx **(Figure 14B)** after challenge with *S. pneumoniae* strain EF3030 (serotype 19F) of mice that had been intranasally vaccinated with cholera toxin (CT) only (control) or *S. pneumoniae* Rxl (ALytA, PdT) plus cholera toxin. **Figure 14C** and **Figure 14D** shows the duration of survival of mice challenged with *S. pneumoniae* strain D39 (serotype 2) or *S. pneumoniae* strain P9 (serotype 6A), respectively, that had been intranasally vaccinated with CT only (control) or *S. pneumoniae* Rxl (ALytA, PdT) plus CT. * $p = <0.05$; ** $p = <0.01$; γ-PN = gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT);

Figure 15 shows the results of intracellular cytokine staining performed to gauge the proportion of Thl cells (using **IFN-7+),** Th2 cells (IL-4+), Thl7 cells (IL-17+) & T-reg cells (Foxp3+) induced after 72h of stimulation of splenocytes with the gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT) vaccine or MalX antigen from mice vaccinated intranasally with cholera toxin (CT) only (control) or *S. pneumoniae* Rxl (ALytA, PdT) plus CT **(Figure 15A** and **Figure 15B).** Cytokine analyses were also conducted on supernatants from cultured splenocytes. Interleukin-17A (IL-17A) and interferon-gamma (IFN-γ) levels **(Figure 15C)** in supernatants from splenocytes following stimulation with MalX or the gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT) vaccine, are indicated. γ-PN = gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT);

Figure 16 shows the results of ELISAs measuring *S. pneumoniae-specific* antibody responses to un-irradiated whole Rxl (ALytA, PdT) bacterial cells in serum from mice vaccinated intranasally with gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT) in the

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presence or absence of cholera toxin adjuvant. γ-PN = gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT);

Figure 17 provides a graph showing the percentage survival of wild-type C57/BL6 mice vaccinated intranasally with gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT) and challenged with a lethal dose of *S. pneumoniae* strain D39. Mice were injected with neutralising antibodies to IFN-γ or IL-17, or relevant isotype control antibodies at 24 h before challenge, 6 h post challenge and 48 h post challenge. * P = <0.05, ** p = <0.01; γ-PN = gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT);

Figure 18 shows the total number of T effector cells (Thl and Thl7), γδ T cells (γδTI and γδT17) and phagocytic cells (macrophages and neutrophils) in the lungs induced 24 and 48 hours after live challenge with *S. pneumoniae* D39 in mice vaccinated with gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT) compared to un-vaccinated mice;

Figure 19 shows the results of PCR and Western blot analyses of strains D39, Rxl, Rxl (ALytA, PdT) and Rxl (ALytA, PdT, APsaA). It also shows growth of Rxl, Rxl (ALytA, PdT) and Rxl (ALytA, PdT, APsaA). For PCR, the genetic loci for *ply, lytA* and *psaA* genes were amplified **(Figure 19A).** For the Western blot, antisera against Ply, LytA and PsaA were used **(Figure 19B).** For growth, bacteria were inoculated at $A_{600}$of 0.05 into SILAC RPMI 1640 Flex Media (supplemented with glucose) and statically cultured at 37°C in 5% CO_2 **(Figure 19C).** This confirms the successful deletion of the *psaA* gene from Rxl (ALytA, PdT) to generate Rxl (ALytA, PdT, APsaA) and demonstrates the growth defect of the Rxl (ALytA, PdT, APsaA) strain in media not supplemented with Mn^{2+};

Figure 20 shows the predicted secondary structure of the mRNA transcript encoded by the *ApsaA* gene; and

Figure 21 provides a graph depicting *S. pneumoniae* Rxl (ALytA, PdT, APsaA) viability following exposure to various dosages of gamma-irradiation on dry ice **(Figure 21A).** The scanning electron microscopy images of the physical morphology of *S. pneumoniae* Rxl (ALytA, PdT, APsaA) that had either not been irradiated **(Figure 21B)** or irradiated at 25 kGy **(Figure 21C)** are shown.

Definitions

As used in this application, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the phrase "protein" also includes a plurality of proteins.

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As used herein, the term "comprising" means "including." Variations of the word "comprising", such as "comprise" and "comprises," have correspondingly varied meanings. Thus, for example, a vaccine "comprising" gamma-irradiated streptococcal strain A may consist exclusively of gamma-irradiated streptococcal strain A or may include one or more additional components (e.g. gamma-irradiated streptococcal strain B).

As used herein the term "plurality" means more than one. In certain specific aspects or embodiments, a plurality may mean 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, or more, and any integer derivable therein, and any range derivable therein.

The term "therapeutically effective amount" as used herein, includes within its meaning a non-toxic but sufficient amount of an agent or composition for use in the present invention to provide the desired therapeutic effect. The exact amount required will vary from subject to subject depending on factors such as the species being treated, the age and general condition of the subject, the severity of the condition being treated, the particular agent being administered, the mode of administration and so forth. Thus, it is not possible to specify an exact "effective amount" applicable to all embodiments. However, for any given case, an appropriate "effective amount" may be determined by one of ordinary skill in the art using only routine experimentation.

As used herein the term "photon-radiation" will be understood to encompass both gamma-radiation (i.e. gamma-rays) and X-radiation (i.e. X-rays). Accordingly, a "photon-irradiated" material may be one that has been exposed to gamma-radiation and which has consequently become "gamma-irradiated", one that has been exposed to X-radiation and which has consequently become "X-irradiated", or both. By way of non-limiting example only, to become photon-irradiated a material may be subjected to photon-radiation at energies of at least O.OlMeV, at least O.lMeV, at least 0.5MeV, between O.OlMeV and 0.5MeV, between O.OlMeV and IMeV, between 0.01 MeV and lOMeV, between 0.5MeV and 20MeV, between 0.5MeV and 15MeV, between 0.5MeV and lOMeV, between 0.5MeV and 5MeV, between 0.5MeV and 2MeV, or between IMeV and 2MeV (e.g. 1.25MeV).

As used herein, the term "attenuated" in the context of bacteria will be understood to mean that the bacteria are capable of establishing only non-pathogenic infection in a host to which they are administered, for a time period sufficient to induce an immune response in the host. The bacteria are not however capable of establishing long-term infection or establishing

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pathogenic infection that is detrimental to a non-immunocompromised host to which the attenuated bacteria are administered.

As used herein the terms "induce", "inducing", "enhance" and "enhancing" in the context of immunity or an immune response refer to an increase in immunity or an immune response above existing levels which may be absent or measurable.

As used herein, the term "subject" includes any animal of economic, social or research importance including bovine, equine, ovine, primate, avian and rodent species. Hence, a "subject" may be a mammal such as, for example, a human or a non-human mammal (e.g. a pig, cat, dog, cow, horse, or sheep). Also included within the scope of this term are laboratory animals (e.g. rodents, rabbits, and the like), birds (e.g. poultry), fish and crustaceans.

As used herein the terms "prevent", "prevention" and "preventing" in the context of a given infection and/or a disease or condition arising from the infection will be understood to mean that a subject has a reduced propensity to develop the infection, and/or disease or condition upon exposure to a pathogenic organism causative of the infection, disease or condition. The reduced propensity to develop the infection and/or disease or condition will be understood to include both a diminished propensity and a lack of any propensity.

As used herein the terms "treat" and "treating" in the context of a given infection and/or a disease or condition arising from the infection will be understood to encompass reducing the number of pathogenic organisms infecting a subject and/or reducing any symptoms of the infection and/or symptoms of a disease or condition arising from the infection.

It will be understood that use of the term "about" herein in reference to a recited numerical value includes the recited numerical value and numerical values within plus or minus ten percent of the recited value.

It will be understood that use of the term "between" herein when referring to a range of numerical values encompasses the numerical values at each endpoint of the range. For example, a polypeptide of between 10 residues and 20 residues in length is inclusive of a polypeptide of 10 residues in length and a polypeptide of 20 residues in length.

Any description of prior art documents herein, or statements herein derived from or based on those documents, is not an admission that the documents or derived statements are part of the common general knowledge of the relevant art.

For the purposes of description all documents referred to herein are hereby incorporated by reference in their entirety unless otherwise stated.

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Detailed Description

The following detailed description conveys exemplary embodiments of the present invention in sufficient detail to enable those of ordinary skill in the art to practice the present invention. Features or limitations of the various embodiments described do not necessarily limit other embodiments of the present invention or the present invention as a whole. Hence, the following detailed description does not limit the scope of the present invention, which is defined only by the claims.

Currently used vaccines against streptococcal infection are generally polysaccharide vaccines containing purified capsular polysaccharides from multiple serotypes (most recently the PCV23 vaccine containing polysaccharides from 23 serotypes), or conjugated vaccines containing capsular polysaccharides conjugated to diphtheria toxoid or other protein antigens of non-streptococcal origin. Serotype replacement is a significant problem associated with polysaccharide vaccines, and conjugate vaccines induce immunity against only a subset of the serotypes covered by the polysaccharide vaccines. For example, there are more than 90 recognised serotypes of *Streptococcus pneumoniae* and most have been shown to cause disease. The immunity induced by currently available streptococcal vaccines is thus inadequate to establish broad immunity against the majority of streptococcal species and/or serotypes within certain pathogenic species. In contrast to viruses, the creation of safe live-attenuated bacterial vaccines is difficult. Comparatively, bacteria have a far larger number of genes and it is thus more difficult to preclude the reversion of mutant/attenuated bacteria back to a pathogenic form after administration to a vaccine recipient. Live bacterial vaccines are also unsuitable for administration to immunocompromised individuals such as those undergoing chemotherapy for malignancies, HIV patients, and young or elderly subjects. These individuals are effectively precluded from receiving any form of live attenuated streptococcal vaccine, despite being more susceptible to various forms of streptococcal infection.

The present invention provides vaccines capable of inducing heterotypic immunity against different streptococcal species and/or different streptococcal serotypes (also referred to herein as "vaccines of the invention" or "a vaccine of the invention"). The vaccines contain killed streptococcal bacteria thus alleviating potential issues arising from live attenuated vaccines. The vaccines are also capable of inducing immunity against a broad range of streptococcal species and/or serotypes, thus reducing the potential impact of serotype replacement.

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Also provided herein are methods for manufacturing vaccines of the invention, as well as medicaments and pharmaceutical compositions comprising the vaccines.

The present invention also relates to methods of preventing or treating streptococcal infection in a subject. The methods involve administration of a vaccine of the invention to a subject. The vaccine may be administered for prophylactic or therapeutic purposes. The methods may induce heterotypic immunity in the subject against a plurality of different streptococcal species and/or subtypes.

Although not a required limitation, vaccines of the present invention are preferably formulated for and administered via mucosal surfaces. For example, the vaccines may be administered intranasally which in certain embodiments may stimulate more effective immune responses depending on the particular target disease or condition.

Streptococcal Vaccine Preparations

- Streptococcal strains

Vaccines of the invention are based on attenuated or whole killed streptococcal bacteria and derivatives thereof attenuated or killed by exposure to gamma-irradiation. The streptococcal bacteria may be pathogenic bacteria capable of establishing a detrimental infection in a host organism. Vaccines of the invention may comprise combinations of different streptococcal bacteria attenuated or killed by exposure to photon-radiation (e.g. gamma-radiation and/or X-radiation) including, for example, combinations of different streptococcal species, and/or combinations of different streptococcal serotypes within the same streptococcal species.

The streptococcal bacteria may, for example, be alpha-, beta-, or gamma-haemolytic streptococci, as classified according to well-characterised haemolytic properties or lack thereof in the case of gamma-haemolytic streptococcal bacteria.

Non-limiting examples of suitable alpha-haemolytic streptococcal bacteria include *Streptococcus pneumoniae* and viridans streptococci (e.g. *S. mutans, S. sanguinis, S. mitis, S. oralis, S. sobrinus, S. milleri).* Also within the scope of the present invention are individual serotypes of these streptococcal species.

Non-limiting examples of suitable beta-haemolytic streptococcal bacteria include those classified under the Lancefield grouping (Groups A-H, L, N and R/S) based on the carbohydrate composition of cell wall bacterial antigens (polysaccharides). For example, the beta-haemolytic bacteria may include any one or more of *S. pyogenes* (Group A), *S. agalactiae* (Group B), *S. equisimilis* (Group C), *S. equi* (Group C), *S. zooepidemicus* (Group

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C), *S. dysgalactiae* (Group C), *Enterococcus faecalis* (Group D), *S. bovis* (Group D), *S. milleri* (Group E), *S. mutans* (Group E), *S. anginosus* (Group F), *S. canis* (Group G), *S. dysgalactiae* (Group G), *S. sanguis* (Group H), *S. dysgalactiae* (Group L), *Lactococcus lactis* (Group N), and *S. suis* (Group R/S). Also within the scope of the present invention are individual serotypes of these streptococcal species.

In some embodiments, vaccines of the invention comprise one or more serotypes of *Streptococcus pneumoniae*. Accordingly, the vaccines may comprise any one of more of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, IOC, 10F, 11A, 11B, 11C, 11D, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20, 21, 22A, 22F, 23A, 23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F, 29, 31, 32A, 32F, 33A, 33B, 33C, 33D, 33F, 34, 35A, 35B, 35C, 35F, 36, 37, 38, 39, 40, 41A, 41F, 42, 43, 44, 45, 46, 47A, 47F, and/or 48.

In some embodiments, the vaccines comprise any one or more of *S. pneumoniae* serotypes 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.

- Streptococcal derivatives

Vaccines of the present invention may comprise photon-irradiated (e.g. gamma-irradiated and/or X-irradiated) streptococcal derivatives. The streptococcal derivatives may be recombinant forms of streptococcal bacteria arising from artificial genetic manipulation, or naturally-occurring mutant forms of streptococcal bacteria. Without any particular limitation, the streptococcal derivatives may comprise one or more genetic modifications that reduce pathogenicity.

By way of non-limiting example only, the streptococcal derivatives may comprise a genetic alteration which disrupts or removes the capsule locus *(cps)*. For example, any one or more of the *S. pneumoniae cpsA, cpsB, cpsC, cpsD* and/or *cpsE* genes, or homologous genes in other streptococcal species, may be modified in order to prevent, disrupt or modify capsule production (e.g. by recombination and the like). Alternatively, the streptococcal derivatives may have spontaneous mutations in these or other genes resulting in naturally-occurring non-encapsulated streptococcal bacteria. The streptococcal derivatives may lack all or at least a portion of the capsule locus. In some embodiments, the streptococcal derivatives lacking a capsule are *Streptococcus pneumoniae* strain Rxl, or Rxl derivatives.

Additionally or alternatively, the streptococcal derivatives may comprise a genetic alteration which reduces or prevents the production or activity of other target proteins. By

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way of non-limiting example only, the genetic alteration may exist in: one or more genes encoding a choline-binding protein; one or more genes encoding an autolysin (e.g. *S. pneumoniae lytA, lytB, lytC* or homologous genes in other streptococcal bacteria); one or more genes that confer a nutrient/cofactor (e.g. a metal ion) requirement for growth (e.g. *S. pneumoniae psaA* or homologous genes in other streptococcal bacteria); one or more genes encoding a protective antigen (e.g. *S. pneumoniae pspA* or homologous genes in other streptococcal bacteria); and/or one or more genes encoding virulence determinants or regulators (e.g. *S. pneumoniae codY, comC, comD, cps2A, csp4A, glpO, mgrA, nanA, nanB, pavA, pcpA, phtA, phtB, phtD, phtE, piuA, piaA, ply, prtA, psaA, psrP, rrgA, rrgB, spxB,* and homologs of these genes in other streptococcal bacteria).

Additionally or alternatively, the streptococcal derivatives may comprise a genetic alteration resulting in an auxotroph with reduced pathogenicity and/or growth *in vivo*. By way of non-limiting example only, the genetic alteration may exist in one or more genes encoding a thymidylate synthase.

Additionally or alternatively, the streptococcal derivatives may comprise one or more (external) genes from: a streptococcal bacterium that is of the same species but a different serotype; a streptococcal bacterium that is from a different species; a non-streptococcal bacterium; or a human or a non-human mammal (e.g. a pig, cat, dog, cow, horse, or sheep); a laboratory animal (e.g. a rodent or rabbit); a bird; and/or a subject to which the recombinant streptococcal bacteria are to be administered. In some embodiments, the external gene or genes disrupt or otherwise inactivate one or more endogenous gene or genes (e.g. any one or more genes as set out in the paragraph directly above). In other embodiments, the external gene or genes do not disrupt or inactivate any endogenous gene. By way of non-limiting example only, the external gene or genes encode proteins that induce or enhance an immune response in a subject to which the streptococcal derivatives are administered. The immune response may be innate, adaptive, or both. In some embodiments, the external gene or genes encode an immunomodulator (e.g. a cytokine, chemokine, antibody, fusion protein, peptide, protein, and/or hormone). In other embodiments, the external gene or genes may comprise an antigen from another different family of bacteria (e.g. a *Mycoplasma pneumoniae* antigen, a *Haemophilus influenzae* antigen, a *Chlamydophila pneumoniae* antigen, a *Moraxella catarrhalis* antigen, a *Staphylococcus aureus* antigen), a viral antigen (e.g. an adenovirus antigen, a coronavirus antigen, an influenza virus antigen, a parainfluenza virus antigen, a metapneumovirus antigen, a rhinovirus antigen, a respiratory syncitial virus antigen, an HIV antigen, a hepatitis virus antigen, or a herpes virus antigen, a measles virus antigen, a mumps

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virus antigen, a papillomavirus virus antigen, a rubella virus antigen, a Varicella Zoster virus antigen), a fungal/yeast antigen, a helminthic antigen, and/or a protozoan antigen.

Additionally or alternatively, the streptococcal derivatives may comprise a genetic alteration causing the bacteria to overexpress one or more target genes. In this context, "overexpression" will be understood to mean a level of expression that is increased compared to expression of the same gene without the genetic modification in a corresponding streptococcal bacterium, under the same biological conditions. The overexpression of a given target gene may, for example, induce or enhance an immune response in a subject against streptococcal strains that are parental to the streptococcal derivatives administered and/or against the streptococcal derivatives themselves. By way of non-limiting example only, the genetic alteration may increase the production of one or more genes in the streptococcal derivatives encoding a protein capable of activating the complement system (e.g. *S. pneumoniae cbpA, pspA, ply,* or homologs of these genes in other streptococcal bacteria).

Additionally or alternatively, the streptococcal derivatives may comprise a genetic alteration causing defective DNA repair capacity. The use of vaccines comprising photon-irradiated (e.g. gamma-irradiated and/or X-irradiated) streptococcal derivatives with decreased capacity to repair DNA lesions arising from photon-irradiation may be advantageous insofar as the dose of photon-irradiation needed for attenuation or inactivation can be reduced, while vaccine efficacy and safety can conversely be increased. In some embodiments, the streptococcal derivatives comprise a genetic alteration that disrupts or inactivates expression of one or more genes encoding a protein in a mismatch repair system (e.g. *S. pneumoniae hex* locus or homologs of this locus in other streptococcal bacteria). In other embodiments, the streptococcal derivatives comprise a genetic alteration that disrupts or inactivates expression of one or more genes encoding a DNA alkylation repair protein (e.g. *S. pneumoniae* DNA polymerase 4, *hexA, hexB, mutS, radC, recA, recF, recN, recO, uvrA, uvrB, uvrC, uvrD* or homologs of these genes in other streptococcal bacteria).

Additionally or alternatively, the streptococcal derivatives may comprise a genetic alteration that facilitates production of a double-stranded RNA (dsRNA). The dsRNA may be mRNA or tRNA. Without limitation, the length of the dsRNA may be more than 10, more than 15, more than 20, more than 25, more than 30, more than 35, more than 40, more than 45, more than 50, more than 55, more than, more than 65 or more than 70 base pairs in length. Additionally or alternatively, the length of the dsRNA may be: between about 10 and about 70 base pairs (bp); between about 10 and about 50 base pairs (bp); between about 10 and about 30 base pairs (bp); between about 20 and about 70 base pairs (bp); between about 20

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and about 60 base pairs (bp); between about 20 and about 50 base pairs (bp); between about 20 and about 40 base pairs (bp); between about 20 and about 30 base pairs (bp); between about 30 and about 70 base pairs (bp); between about 40 and about 70 base pairs (bp); between about 50 and about 70 base pairs (bp); between about 60 and about 70 base pairs (bp); between about 30 and about 60 base pairs (bp); between about 30 and about 50 base pairs (bp); or between about 30 and about 40 base pairs (bp); in length. In some embodiments, the dsRNA is a component of a larger RNA molecule that is otherwise single-stranded. The larger RNA molecule may comprise multiple dsRNA components. The dsRNA may be an internal component or an end component of the larger RNA molecule. In some embodiments, the dsRNA may comprise a termination stem-loop sequence. The dsRNA may arise from a region of self-complementarity within the larger RNA molecule. Coding region(s)/exon(s) within a given gene of a streptococcal derivative can be engineered to include one or more region(s) of self-complementarity and thereby produce a dsRNA portion when transcribed.

The dsRNA may be capable of recognition by Toll-like receptor (TLR) proteins expressed by cells in a subject to which the streptococcal derivatives are administered. The TLR proteins may be located in the endoplasmic reticulum and/or endosomal compartment of the cells. The TLR proteins may be Toll-like receptor 3 (TLR3) proteins. Without limitation, the cells may be any one or more of B lymphocytes, T lymphocytes, natural killer cells and/or dendritic cells. Recognition of the dsRNA by the TLR3 protein may induce an immune response in the subject. The immune response may be an innate immune response. The immune response may be an interferon type-1 response and/or comprise the release of inflammatory cytokines.

In general, streptococcal derivatives used in vaccines of the invention will have a significant degree of genetic similarity to the parent strain from which they derive. By way of non-limiting example, a "streptococcal derivative" as referred to herein may have more than 70%, more than 75%, more than 80%, more than 85%, more than 90%, more than 92%, more than 94%, more than 95%, more than 96%, more than 97%, more than 98%, or more than 99% sequence homology to the parent streptococcal strain from which it is derived. By way of further non-limiting example, a "streptococcal derivative" as referred to herein may comprise a genetic alteration in one, two, three, four, five or more than five genes or regulatory sequences necessary for expression of those genes, when compared to corresponding of its parent strain. The genetic alteration may increase, decrease, or prevent expression of the gene or genes in question.

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In one non-limiting embodiment, the streptococcal bacteria derivative may be an Rxl strain. The autolysin gene *(lytA)* gene may be deleted or rendered non-functional in the Rxl derivative strain. Additionally or alternatively, the pneumolysin gene *(ply)* may be deleted or rendered non-functional in the Rxl derivative strain. For example, the *ply* gene may be replaced with another gene such as a toxoid version of *ply*.

Additionally or alternatively, the pneumococcal surface antigen A gene *(psaA)* gene may be deleted or otherwise rendered non-functional. The *psaA* gene of *S. pneumoniae* encodes the pneumococcal surface antigen A (PsaA) involved in Mn^{2+} transport and resistance to oxidative stress. A *psa*A-deletion mutant as described herein may be defective in growth in low Mn^{2+} environments and/or defective in pneumococcal competence. In non-limiting embodiments, a *psa*A -deletion mutant of Rxl (ALytA, PdT) may be used to create a vaccine candidate strain Rxl (ALytA, PdT, APsaA) with reduced virulence, reduced competence, and/or reduced growth in low Mn^{2+} environments. These features may add additional biosecurity and biosafety to Rxl (ALytA, PdT, APsaA) making it a suitable vaccine candidate for preparing a gamma-irradiated *S. pneumoniae* vaccine. Furthermore, fermentor growth of Rxl (ALytA, PdT, APsaA) under Mn^{2+} stress conditions may induce changes in gene expression that increases the level of production of protective antigens. An improved level of protection may thus be afforded by use of vaccine product with higher level of expression of protective antigens.

Techniques for the genetic manipulation of bacteria are well known to those of ordinary skill in the art (see, for example, Vennison *"Laboratory Manual for Genetic Engineering"*, PHI Learning Pvt. Ltd., 2010; Zyskind and Bernstein, *"Recombinant DNA Laboratory Manual"*, Elsevier, 2014; Bose, *"Genetic Manipulation of Staphylococci"* in "Methods in Molecular Biology", Springer Protocols, volume 1106, pages 101-111, 2014; Hakenbeck and Chhatwal, *"Molecular Biology of Streptococci"*, Horizon Scientific Press, 2007; Morona *et al., "The effect that mutations in the conserved capsular polysaccharide biosynthesis genes cpsA, cpsB and cpsD have on virulence of Streptococcus pneumoniae"*, J. Infect. Dis. 189: 1905-1913, 2004; Morona *et al., "Mutational analysis of the carboxy-terminal [YGX]4 repeat domain of CpsD, an autophosphorylating tyrosine kinase required for capsule biosynthesis in Streptococcus pneumonia"*, J. Bacteriol. 185: 3009-3019, 2003; McAllister *et al, "Molecular analysis of the psa permease complex of Streptococcus pneumoniae"*, Mol. Microbiol. 53:889-901, 2004; Mahdi *et al., "Identification of a novel pneumococcal vaccine antigen preferentially expressed during meningitis in mice"*, J. Clin. Invest. 122:2208-2220, 2012.

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Photon-radiation

Streptococcal bacteria and derivatives thereof in vaccines of the invention may be exposed to photon-radiation. As noted above, the term "photon-radiation" will be understood to encompass both gamma-radiation (i.e. gamma-rays) and X-radiation (i.e. X-rays). Accordingly, "photon-irradiated" streptococcal bacteria or their derivatives may be "gamma-irradiated" by way of exposure to gamma-radiation (i.e. gamma-rays), "X-irradiated" by way of exposure to X-radiation (i.e. X-rays), or both. As known to those of ordinary skill in the art, X-rays are identical to gamma-rays except they are emitted by the passage of electrons through an electric field of a nucleus rather than the nucleus itself upon radioactive decay. By way of non-limiting example only, to become photon-irradiated a material may be subjected to photon-radiation at energies of at least O.OlMeV, at least O.lMeV, at least 0.5MeV, between O.OlMeV and 0.5MeV, between O.OlMeV and lMeV, between 0.01 MeV and lOMeV, between 0.5MeV and 20MeV, between 0.5MeV and 15MeV, between 0.5MeV and lOMeV, between 0.5MeV and 5MeV, between 0.5MeV and 2MeV, or between lMeV and 2MeV (e.g. 1.25MeV).

Streptococcal bacteria and derivatives thereof in vaccines of the invention may be gamma-irradiated. Any suitable source of gamma-radiation may be used. Suitable gamma emitters include, but are not limited to Ba^{137}, Co^{60}, Cs^{137}, Ir^{192}, U^{235}, Se^{75} and Yb^{169}.

Gamma-irradiation of the streptococcal bacteria and their derivatives may be performed using commercially available devices, for example, a Gammacell irradiator manufactured by Atomic Energy of Canada Ltd., Canada (e.g. Gammacell 40 Irradiator, Gammacell 220 Irradiator, Gammacell 1000 irradiator, Gammacell 3000 irradiator), a gamma-irradiator manufactured by J. L. Shepherd and Associates (San Fernando, California, USA), or a Nordion Gamma Cell-1000 irradiator manufactured by Nordion Inc. (Kanata, Ontario, Canada). Other suitable devices are described, for example, in United States Patent No. 3,557,370 and United States Patent No. 3,567,938.

Additionally or alternatively, streptococcal bacteria and derivatives thereof in vaccines of the invention may be X-irradiated. Any suitable source of X-radiation may be used. Suitable sources of X-radiation include, but are not limited to, the eXelis® sterilization X-ray machine manufactured by IBA Industrial (Louvain-la-Neuve, Belgium). Other suitable devices include for example, the RS2400® and RS3400® manufactured by Rad Source Technologies Inc. (Suwanee, Georgia, USA).

In general, the streptococcal bacteria and their derivatives are exposed to a dose of photon-radiation (e.g. gamma-radiation and/or X-radiation) sufficient to attenuate or

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inactivate the bacteria/derivatives. Preferably, the dose of photon-radiation is sufficient to attenuate or inactivate the bacteria and their derivatives without substantially disrupting the structure of antigens (e.g. surface antigens). The immunogenicity of antigenic determinants may therefore be retained by the photon-irradiated bacteria and their derivatives. Preferably, the dose of photon-radiation is administered to the bacteria or their derivatives over a period of time and at a level sufficient to ensure that all bacteria/derivatives under treatment are exposed without adversely affecting the structural integrity of antigenic determinants.

As known to those of ordinary skill in the art, a measure for an absorbed dose of radiation is the gray (Gy), which is defined as 1 joule of energy deposited in 1 kilogram of mass. An old unit of measure for this is the rad, which stands for "radiation absorbed dose", where 1 Gy = 100 rad.

Streptococcal bacteria and derivatives thereof for use in accordance with the present invention may be exposed to a total dose of photon-radiation (e.g. gamma-radiation and/or X-radiation) in the range of about 1 X 10^3 rad and about 2 X 10^9 rad (or about 10 Gy to about 2 X 10^4 kGy). In certain embodiments of the invention, the streptococcal bacteria or derivatives are exposed to a total dose of X-radiation and/or gamma-radiation of between about 1 X 10^3 rad and about 2 X 10^9 rad, between about 1 X 10^3 rad and about 1 X 10^9 rad, between about 1 X 10^3 rad and about 1 X 10^8 rad, between about 1 X 10^3 rad and about 1 X 10^7 rad, between about 1 X 10^3 rad and about 1 X 10^6 rad, between about 1 X 10^3 rad and about 1 X 10^5 rad, between about 1 X 10^3 rad and about 1 X 10^4 rad, between about 1 X 10^3 rad and about 2 X 10^9 rad, between about 1 X 10^4 rad and about 2 X 10^9 rad, between about 1 X 10^5 rad and about 2 X 10^9 rad, between about 1 X 10^6 rad and about 2 X 10^9 rad, between about 1 X 10^7 rad and about 2 X 10^9 rad, between about 1 X 10^8 rad and about 2 X 10^9 rad or between about 1 X 10^9 rad and about 2 X 10^9 rad.

In some embodiments of the invention, the streptococcal bacteria or derivatives are exposed to a total dose of photon-radiation (e.g. X-radiation and/or gamma-radiation) of between about 6.5 X 10^4 rad and about 2 X 10^7 rad (about 0.65 KGy to about 200 kGy). In other embodiments of the invention, the streptococcal bacteria or their derivatives are exposed to a total photon-radiation dose of about 10 kGy to about 12 kGy, about 12 kGy to about 14 kGy, about 14 kGy to about 16 kGy, about 10 kGy to about 20 kGy, about 14 kGy to about 20 kGy, more than 10 about 12 kGy to about 14 kGy, more than 1 2kGy, more than 14 kGy, more than 16 kGy, more than 18 kGy, more than 20 kGy, 1.26 X 10^6 rad (12.6 kGy), a total photon-radiation dose of about 1 X 10^6 rad (about 10 kGy) photon-rays, or a total photon-radiation dose of about 1 X 10^5 rad (1 KGy).

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The optimal dose of photon-radiation (e.g. gamma-radiation and/or X-radiation) may be influenced by factors such as the medium in which the streptococcal bacteria or their derivatives are present, the amount of bacteria/derivatives to be treated, and/or the subtype or strain under treatment. Accordingly, the total dose of photon-radiation, the exposure time and/or the level of photon-radiation applied over the period of exposure may be optimised to enhance the effectiveness of the treatment.

The total dose of photon-radiation (e.g. X-radiation and/or gamma-radiation) may be administered to the streptococcal bacteria or their derivatives cumulatively over a period of time. For example, photon-radiation on may be administered to the bacteria/derivatives at a level lower than that of the total dose, over a time period sufficient to achieve the total dose of photon-radiation required.

In one embodiment, preparations of streptococcal bacteria or their derivatives are maintained in a frozen and/or lyophilised state while being exposed to photon-radiation (e.g. gamma-radiation and/or X-radiation). This may facilitate the preservation of biological integrity and avoid unnecessary damage of antigens thereby enhancing the immunogenicity of photon-irradiated bacterial preparations, and in particular, their ability to elicit cross-reactive/cross-protective immunity against, for example, heterologous subtypes. In general, a photon-radiation dose of 10-20 kGy (e.g. more than 10, more than 12, more than 14, more than 16, or more than 18 kGy) may be effective for treating preparations of frozen and/or lyophilised streptococcal bacteria or their derivatives.

As mentioned above, it is preferable that treatment with photon-radiation is sufficient to inactivate the bacteria/derivatives without substantially disrupting the structure of bacterial antigens. Attenuation and/or inactivation of the streptococcal bacteria or their derivatives may be assessed using methods generally known in the art.

For example, bacterial attenuation and/or inactivation can be assessed by determining the number of viable bacteria that form colonies on agar media (i.e. the colony forming units) after being treated with photon-radiation (e.g. gamma-radiation and/or X-radiation).

The integrity of antigenic determinants can be assessed, for example, by reactivity with panels of monospecific antisera raised against purified native antigenic components using Western blotting, FACs analysis, or enzymatic assays of surface components.

Prophylactic and Therapeutic Methods

The invention provides prophylactic methods for the prevention of streptococcal infection in a subject. Also provided are therapeutic methods for treating streptococcal

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infection in a subject. The methods comprise administering photon-irradiated streptococcal bacteria and/or photon-irradiated derivatives thereof to the subject, for example, in the form of a vaccine of the invention. The photon-irradiated streptococcal bacteria and/or derivatives thereof may be gamma-irradiated, X-irradiated, or both.

The methods induce or enhance the immune response against streptococcal bacteria in the subject. The immune response may be cross-protective/heterologous insofar as it may induce or enhance the immune response against multiple serotypes of streptococcal bacteria. The methods may also comprise administering multiple different photon-irradiated (e.g. gamma-irradiated, X-irradiated, or both) streptococcal species or derivatives thereof, to thereby generate immunity against multiple species of streptococcal bacteria and various serotypes thereof.

The methods may induce or enhance an immune response against any one or more of the following streptococcal bacterial species: *Streptococcus agalactiae, Streptococcus bovis, Streptococcus canis, Streptococcus dysgalactiae, Streptococcus equi, Streptococcus equinus, Streptococus equisimilis, Enterococcus faecalis, Enterococcus faecium, Streptococcus iniae, S. milleri, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus salivarius, Streptococcus sanguinis, Streptococcus suis* and *Streptococcus uberis.*

In some embodiments, the methods comprise preventing or treating a streptococcal infection caused by a given streptococcal species by administering to the subject a photon-irradiated (e.g. gamma-irradiated and/or X-irradiated) preparation of the same streptococcal species. The streptococcal species serotype causative of the infection may be different to the photon-irradiated streptococcal species serotype administered.

By way of non-limiting example only, the methods may be used to prevent or treat:

(i) an infection, disease or condition caused by any one or more serotypes of *Streptococcus agalactiae* by administering one or more photon-irradiated (e.g. gamma-irradiated and/or X-irradiated) serotype(s) of *Streptococcus agalactiae.* The photon-irradiated *Streptococcus agalactiae* serotype(s) administered may differ from the serotype(s) causative of the infection, disease or condition;

(ii) an infection, disease or condition caused by any one or more serotypes of *Streptococcus bovis* by administering one or more photon-irradiated (e.g. gamma-irradiated and/or X-irradiated) serotype(s) of *Streptococcus bovis.* The photon-irradiated *Streptococcus bovis* serotype(s) administered may differ from the serotype(s) causative of the infection, disease or condition;

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(iii) an infection, disease or condition caused by any one or more serotypes of *Streptococcus canis* by administering one or more photon-irradiated (e.g. gamma-irradiated and/or X-irradiated) serotype(s) of *Streptococcus canis*. The photon-irradiated *Streptococcus canis* serotype(s) administered may differ from the serotype(s) causative of the infection, disease or condition;

(iv) an infection, disease or condition caused by any one or more serotypes of *Streptococcus dysgalactiae* by administering one or more photon-irradiated (e.g. gamma-irradiated and/or X-irradiated) serotype(s) of *Streptococcus dysgalactiae*. The photon-irradiated *Streptococcus dysgalactiae* serotype(s) administered may differ from the serotype(s) causative of the infection, disease or condition;

(v) an infection, disease or condition caused by any one or more serotypes of *Streptococcus equi* by administering one or more photon-irradiated (e.g. gamma-irradiated and/or X-irradiated) serotype(s) of *Streptococcus equi*. The photon-irradiated *Streptococcus equi* serotype(s) administered may differ from the serotype(s) causative of the infection, disease or condition;

(vi) an infection, disease or condition caused by any one or more serotypes of *Streptococcus equinus* by administering one or more photon-irradiated (e.g. gamma-irradiated and/or X-irradiated) serotype(s) of *Streptococcus equinus*. The photon-irradiated *Streptococcus equinus* serotype(s) administered may differ from the serotype(s) causative of the infection, disease or condition;

(vii) an infection, disease or condition caused by any one or more serotypes of *Streptococcus agalactiae* by administering one or more photon-irradiated (e.g. gamma-irradiated and/or X-irradiated) serotype(s) of *Streptococcus agalactiae*. The photon-irradiated *Streptococcus agalactiae* serotype(s) administered may differ from the serotype(s) causative of the infection, disease or condition;

(viii) an infection, disease or condition caused by any one or more serotypes of *Streptococcus equisimilis* by administering one or more photon-irradiated (e.g. gamma-irradiated and/or X-irradiated) serotype(s) of *Streptococcus equisimilis*. The photon-irradiated *Streptococcus equisimilis* serotype(s) administered may differ from the serotype(s) causative of the infection, disease or condition;

(ix) an infection, disease or condition caused by any one or more serotypes of *Enterococcus faecalis* by administering one or more photon-irradiated (e.g. gamma-irradiated and/or X-irradiated) serotype(s) of *Enterococcus faecalis*. The photon-irradiated

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Enterococcus faecalis serotype(s) administered may differ from the serotype(s) causative of the infection, disease or condition;

(x) an infection, disease or condition caused by any one or more serotypes of *Enterococcus faecium* by administering one or more photon-irradiated (e.g. gamma-irradiated and/or X-irradiated) serotype(s) of *Enterococcus faecium.* The photon-irradiated *Enterococcus faecium* serotype(s) administered may differ from the serotype(s) causative of the infection, disease or condition;

(xi) an infection, disease or condition caused by any one or more serotypes of *Streptococcus iniae* by administering one or more photon-irradiated (e.g. gamma-irradiated and/or X-irradiated) serotype(s) of *Streptococcus iniae.* The photon-irradiated *Streptococcus iniae* serotype(s) administered may differ from the serotype(s) causative of the infection, disease or condition;

(xii) an infection, disease or condition caused by any one or more serotypes of *Streptococcus milleri* by administering one or more photon-irradiated (e.g. gamma-irradiated and/or X-irradiated) serotype(s) of *Streptococcus milleri.* The photon-irradiated *Streptococcus milleri* serotype(s) administered may differ from the serotype(s) causative of the infection, disease or condition;

(xiii) an infection, disease or condition caused by any one or more serotypes of *Streptococcus mutans* by administering one or more photon-irradiated (e.g. gamma-irradiated and/or X-irradiated) serotype(s) of *Streptococcus mutans.* The photon-irradiated *Streptococcus mutans* serotype(s) administered may differ from the serotype(s) causative of the infection, disease or condition;

(xiv) an infection, disease or condition caused by any one or more serotypes of *Streptococcus pneumoniae* by administering one or more photon-irradiated (e.g. gamma-irradiated and/or X-irradiated) serotype(s) of *Streptococcus pneumoniae.* The photon-irradiated *Streptococcus pneumoniae* serotype(s) administered may differ from the serotype(s) causative of the infection, disease or condition;

(xv) an infection, disease or condition caused by any one or more serotypes of *Streptococcus pyogenes* by administering one or more photon-irradiated (e.g. gamma-irradiated and/or X-irradiated) serotype(s) of *Streptococcus pyogenes.* The photon-irradiated *Streptococcus pyogenes* serotype(s) administered may differ from the serotype(s) causative of the infection, disease or condition;

(xvi) an infection, disease or condition caused by any one or more serotypes of *Streptococcus salivarius* by administering one or more photon-irradiated (e.g. gamma-

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irradiated and/or X-irradiated) serotype(s) of *Streptococcus salivarius*. The photon-irradiated *Streptococcus salivarius* serotype(s) administered may differ from the serotype(s) causative of the infection, disease or condition;

(xvii) an infection, disease or condition caused by any one or more serotypes of *Streptococcus sanguinis* by administering one or more photon-irradiated (e.g. gamma-irradiated and/or X-irradiated) serotype(s) of *Streptococcus sanguinis*. The photon-irradiated *Streptococcus sanguinis* serotype(s) administered may differ from the serotype(s) causative of the infection, disease or condition;

(xviii) an infection, disease or condition caused by any one or more serotypes of *Streptococcus suis* by administering one or more photon-irradiated (e.g. gamma-irradiated and/or X-irradiated) serotype(s) of *Streptococcus suis*. The photon-irradiated *Streptococcus suis* serotype(s) administered may differ from the serotype(s) causative of the infection, disease or condition; and/or

(xix) an infection, disease or condition caused by any one or more serotypes of *Streptococcus uberis* by administering one or more photon-irradiated (e.g. gamma-irradiated and/or X-irradiated) serotype(s) of *Streptococcus uberis*. The photon-irradiated *Streptococcus uberis* serotype(s) administered may differ from the serotype(s) causative of the infection, disease or condition.

In some embodiments, the methods are utilised to prevent or treat an infection, disease or condition caused by one or more serotypes of *Streptococcus pneumoniae*. The methods may comprise inducing an immune response in a subject against a plurality of different *S. pneumoniae* serotypes, by administering one or more serotypes of photon-irradiated (e.g. gamma-irradiated and/or X-irradiated) *S. pneumoniae* to the subject. In some embodiments the methods comprise administering a single photon-irradiated (e.g. gamma-irradiated and/or X-irradiated) serotype of *S. pneumoniae*.

In some embodiments, the methods comprise preventing or treating an infection, disease or condition caused by one or more serotypes of *Streptococcus pneumoniae*. The methods comprise administering to a subject at least one serotype of photon-irradiated (e.g. gamma-irradiated and/or X-irradiated) *S. pneumoniae*, and may induce an immune response in the subject against any one or more of *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, IOC, 10F, 11A, 11B, 11C, 11D, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20, 21, 22A, 22F, 23A, 23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F, 29, 31, 32A, 32F, 33A, 33B, 33C, 33D, 33F, 34, 35A, 35B, 35C, 35F, 36, 37, 38, 39, 40, 41A, 41F,

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42, 43, 44, 45, 46, 47A, 47F, and/or 48. In some embodiments, the methods may induce an immune response in the subject against any one or more of *S. pneumoniae* serotypes 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. The photon-irradiated *S. pneumoniae* serotype(s) administered may differ from the serotype(s) causative of the infection, disease or condition.

The disease or condition may be any that is caused by infection of the particular species or serotype of streptococcal bacteria. By way of non-limiting example only, the disease or condition may be any one or more of: pneumonia, ear infection, earache, middle ear infection, otitis media, sinusitis, meningitis, conjunctivitis, bacteraemia, septicaemia, a joint infection, a bone infection, septic arthritis, osteomyelitis, a soft tissue infection, cellulitis, myositis, periorbital cellulitis, an abscess, peritonitis, a cardiac infection, endocarditis, and pericarditis.

The subject may be any animal of economic, social or research importance including bovine, equine, ovine, primate, avian and rodent species. Accordingly, the subject may be a mammal such as, for example, a human or a non-human mammal (e.g. a pig, cat, dog, cow, horse, or sheep). The subject may be a laboratory animal (e.g. a rodent such as a mouse, rat, or guinea pig; a rabbit, and the like), a bird (e.g. poultry), a fish or a crustacean.

The photon-irradiated (e.g. gamma-irradiated and/or X-irradiated) streptococcal bacteria and/or photon-irradiated derivatives thereof may be administered to the subject by any suitable route including, for example, parenteral (e.g. intradermal, intravenous, intraspinal, intraperitoneal, subcutaneous or intramuscular), oral, topical, or mucosal routes (e.g. intranasal). In some embodiments, administration is by the mucosal route. For example, the administration may be intranasal.

Without being limited to specific mechanism(s) of action, the methods may induce an immune response in the subject comprising one or more of the following:

(i) production of antibodies that bind specifically to antigen(s) of the streptococcal bacteria causative of the infection, disease or condition;

(ii) $CD4^+$ T lymphocyte responses specific for antigen(s) of the streptococcal bacteria causative of the infection, disease or condition;

(iii) $CD8^+$ T lymphocyte responses specific for antigen(s) of the streptococcal bacteria causative of the infection, disease or condition.

In some embodiments, the methods may induce an immune response in the subject that may be Interleukin-17A (IL-17A) dependent, IL-17A independent, and/or that comprises activation of the innate immune system including the production of cytokines (e.g. IFN-γ) and/or activation of Toll-like receptors (e.g. TLR-3). This may assist in reducing the

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activation threshold for B cells and/or enhancing the quality or quantity of antibody responses against antigens of interest.

By way of non-limiting example only, an immune response induced or enhanced in a subject by the method may be increased by at least about 10%, at least about 20%, at least about 25%, at least about 50%, at least about 75%, at least about 90%, at least about two-fold, at least about five-fold, at least about ten-fold, at least about twenty-fold, at least about fifty-fold, or at least about 100-fold, when compared to a suitable control. The suitable control may, for example, be a measurement of the same immune response prior to performing the method under otherwise similar, substantially identical, or identical conditions.

Methods for detecting and quantifying immune response are well known to those of ordinary skill in the field and include, for example, solid-phase heterogeneous assays (e.g. enzyme-linked immunosorbent assay), solution phase assays (e.g. electrochemiluminescence assay), amplified luminescent proximity homogeneous assays, flow cytometry, intracellular cytokine staining, functional T-cell assays, functional B-cell assays, functional monocyte-macrophage assays, dendritic and reticular endothelial cell assays, measurement of NK cell responses, oxidative burst assays, cytotoxic specific cell lysis assays, pentamer binding assays, and phagocytosis and apoptosis evaluation.

Vaccine Formulations

The streptococcal bacteria and their derivatives described herein may be incorporated into pharmaceutical compositions. The compositions can stimulate an immune response against pathogenic organisms capable of establishing infection in a host that may culminate in a disease or condition. Accordingly, the compositions may be vaccines, including preventative vaccines (i.e. vaccines administered for the purpose of preventing infections and/or diseases/conditions) and therapeutic vaccines (i.e. vaccines administered for the purpose of treating infections and/or diseases/conditions). A vaccine of the present invention may therefore be administered to a recipient for prophylactic, ameliorative, palliative, or therapeutic purposes. It will be understood that all such vaccines are collectively encompassed by reference herein to "vaccines of the invention" or a "vaccine of the invention". Non-limiting examples of suitable streptococcal bacteria and their derivatives suitable for incorporation into vaccines of the invention are described above in the subsections entitled *"Streptococcal strains"* and *"Streptococcal derivatives"*. The streptococcal bacteria and their derivatives of the vaccines are attenuated or inactivated by photon-radiation (e.g. gamma-radiation and/or X-radiation). The photon-radiation may be

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applied to the bacteria/derivatives before, during or after combining them with other reagent(s) to provide a vaccine formulation.

- Formulations

Vaccines of the invention may be prepared using methods known to those of ordinary skill in the art. Non-limiting examples of suitable methods are described in Gennaro *et al.* (Eds), (1990), *"Remington's Pharmaceutical Sciences"*, Mack Publishing Co., Easton, Pennsylvania, USA, and methods for vaccine preparation are generally described in Voller *et al.*, (1978), "*New Trends and Developments in Vaccines*", University Park Press, Baltimore, Maryland, USA.

The vaccines may comprise a pharmaceutically acceptable carrier, excipient, diluent and/or adjuvant. "Pharmaceutically acceptable" carriers, excipients, diluents and/or adjuvants as contemplated herein are substances which do not produce adverse reaction(s) when administered to a particular recipient such as a human or non-human animal. Pharmaceutically acceptable carriers, excipients, diluents and adjuvants are generally also compatible with other ingredients of the vaccine. Non-limiting examples of suitable excipients, diluents, and carriers can be found in the *"Handbook of Pharmaceutical Excipients"* 4th Edition, (2003) Rowe *et al.* (Eds), The Pharmaceutical Press, London, American Pharmaceutical Association, Washington.

Non-limiting examples of pharmaceutically acceptable carriers, excipients or diluents include demineralised or distilled water; saline solution; vegetable based oils such as peanut oil, safflower oil, olive oil, cottonseed oil, maize oil, sesame oils, arachis oil or coconut oil; silicone oils, including polysiloxanes, such as methyl polysiloxane, phenyl polysiloxane and methylphenyl polysolpoxane; volatile silicones; mineral oils such as liquid paraffin, soft paraffin or squalane; cellulose derivatives such as methyl cellulose, ethyl cellulose, carboxymethylcellulose, sodium carboxymethylcellulose or hydroxypropylmethylcellulose; lower alkanols, for example ethanol or isopropanol; lower aralkanols; lower polyalkylene glycols or lower alkylene glycols, for example polyethylene glycol, polypropylene glycol, ethylene glycol, propylene glycol, 1,3-butylene glycol or glycerin; fatty acid esters such as isopropyl palmitate, isopropyl myristate or ethyl oleate; polyvinylpyrridone; agar; carrageenan; gum tragacanth or gum acacia, and petroleum jelly. Typically, the carrier or carriers will form from 10% to 99.9% by weight of the compositions.

Vaccines of the present invention may be in a form suitable for administration by injection, in the form of a formulation suitable for oral ingestion (such as capsules, tablets,

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caplets, elixirs, for example), in the form of an ointment, cream or lotion suitable for topical administration, in a form suitable for delivery as an eye drop, in an aerosol form suitable for administration by inhalation, such as by intranasal inhalation or oral inhalation, or in a form suitable for parenteral administration, that is, intradermal, subcutaneous, intramuscular or intravenous injection.

Solid forms of the vaccines for oral administration may contain binders acceptable in human and veterinary pharmaceutical practice, sweeteners, disintegrating agents, diluents, flavourings, coating agents, preservatives, lubricants and/or time delay agents. Suitable binders include gum acacia, gelatine, corn starch, gum tragacanth, sodium alginate, carboxymethylcellulose or polyethylene glycol. Suitable sweeteners include sucrose, lactose, glucose, aspartame or saccharine. Suitable disintegrating agents include corn starch, methylcellulose, polyvinylpyrrolidone, guar gum, xanthan gum, bentonite, alginic acid or agar. Suitable diluents include lactose, sorbitol, mannitol, dextrose, kaolin, cellulose, calcium carbonate, calcium silicate or dicalcium phosphate. Suitable flavouring agents include peppermint oil, oil of wintergreen, cherry, orange or raspberry flavouring. Suitable coating agents include polymers or copolymers of acrylic acid and/or methacrylic acid and/or their esters, waxes, fatty alcohols, zein, shellac or gluten. Suitable preservatives include sodium benzoate, vitamin E, alpha-tocopherol, ascorbic acid, methyl paraben, propyl paraben or sodium bisulphite. Suitable lubricants include magnesium stearate, stearic acid, sodium oleate, sodium chloride or talc. Suitable time delay agents include glyceryl monostearate or glyceryl distearate.

Liquid forms of the vaccines for oral administration may contain, in addition to the above agents, a liquid carrier. Suitable liquid carriers include water, oils such as olive oil, peanut oil, sesame oil, sunflower oil, safflower oil, arachis oil, coconut oil, liquid paraffin, ethylene glycol, propylene glycol, polyethylene glycol, ethanol, propanol, isopropanol, glycerol, fatty alcohols, triglycerides or mixtures thereof.

Suspensions comprising the vaccines for oral administration may further comprise dispersing agents and/or suspending agents. Suitable suspending agents include sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethyl-cellulose, poly-vinyl-pyrrolidone, sodium alginate or acetyl alcohol. Suitable dispersing agents include lecithin, polyoxyethylene esters of fatty acids such as stearic acid, polyoxyethylene sorbitol mono- or di-oleate, -stearate or -laurate, polyoxyethylene sorbitan mono- or di-oleate, -stearate or -laurate and the like.

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For preparation of the vaccines as injectable solutions or suspensions, non-toxic parenterally acceptable diluents or carriers may be used such as Ringer's solution, isotonic saline, phosphate buffered saline, ethanol and 1,2 propylene glycol.

Vaccine emulsions for oral administration may further comprise one or more emulsifying agents. Suitable emulsifying agents include dispersing agents as exemplified above or natural gums such as guar gum, gum acacia or gum tragacanth.

Topical formulations of the vaccines comprise an active ingredient(s) (e.g. photon-irradiated streptococcal bacteria and/or derivatives thereof) together with one or more acceptable carriers, and optionally any other therapeutic ingredients. Formulations suitable for topical administration include liquid or semi-liquid preparations suitable for penetration through the skin to the site of where treatment is required, such as liniments, lotions, creams, ointments or pastes, and drops suitable for administration to the eye, ear or nose.

When formulated as drops, the vaccines may comprise sterile aqueous or oily solutions or suspensions. These may be prepared by dissolving the active ingredient in an aqueous solution of a bactericidal and/or fungicidal agent and/or any other suitable preservative, and optionally including a surface active agent. The resulting solution may then be clarified by filtration, transferred to a suitable container and sterilised. For example, sterilisation may be achieved by filtration followed by transfer to a container by an aseptic technique. Examples of bactericidal and fungicidal agents suitable for inclusion in the drops are phenylmercuric nitrate or acetate (0.002%), benzalkonium chloride (0.01%) and chlorhexidine acetate (0.01%). Suitable solvents for the preparation of an oily solution include glycerol, diluted alcohol and propylene glycol.

When formulated as lotions, the vaccines include those suitable for application to the skin or eye. An eye lotion may comprise a sterile aqueous solution optionally containing a bactericide and may be prepared by methods similar to those described above in relation to the preparation of drops. Lotions or liniments for application to the skin may also include an agent to hasten drying and to cool the skin, such as an alcohol or acetone, and/or a moisturiser such as glycerol, or oil such as castor oil or arachis oil.

When formulated as creams, ointments or pastes, the vaccines may be semi-solid formulations of the active ingredient for external application. They may be made by mixing the active ingredient in finely-divided or powdered form, alone or in solution or suspension in an aqueous or non-aqueous fluid, with a greasy or non-greasy basis. The basis may comprise hydrocarbons such as hard, soft or liquid paraffin, glycerol, beeswax, a metallic soap; a mucilage; an oil of natural origin such as almond, corn, arachis, castor or olive oil; wool fat

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or its derivatives, or a fatty acid such as stearic or oleic acid together with an alcohol such as propylene glycol or macrogols.

The vaccines may include any suitable surfactant such as an anionic, cationic or non-ionic surfactant such as sorbitan esters or polyoxyethylene derivatives thereof. Suspending agents such as natural gums, cellulose derivatives or inorganic materials such as silicaceous silicas, and other ingredients such as lanolin, may also be included.

The vaccines may be administered in the form of liposomes. Liposomes are generally derived from phospholipids or other lipid substances, and are formed by mono- or multi-lamellar hydrated liquid crystals that are dispersed in an aqueous medium. Any non-toxic, physiologically acceptable and metabolisable lipid capable of forming liposomes can be used. The vaccines in liposome form may contain stabilisers, preservatives, excipients and the like. The preferred lipids are the phospholipids and the phosphatidyl cholines (lecithins), both natural and synthetic. Methods to form liposomes are known in the art, and in relation to this specific reference is made to: Prescott, Ed., Methods in Cell Biology, Volume XIV, Academic Press, New York, N.Y. (1976), p. 33 *et seq.*

-Adjuvants

Adjuvant(s) may be included in vaccines of the invention, although experimental data provided herein demonstrates that photon-irradiated streptococcal bacteria and their derivatives can induce immunity without requiring such adjuvants. Accordingly, vaccines of the invention may or may not comprise an adjuvant.

In general, adjuvant activity in the context of a vaccine composition includes, but is not limited to, the ability to enhance the immune response (quantitatively or qualitatively) induced by immunogenic components in the vaccine (e.g. photon-irradiated streptococcal bacteria and/or derivatives thereof). This may reduce the dose or level of the immunogenic components required to produce an immune response and/or reduce the number or the frequency of immunisations required to produce the desired immune response.

Preferably, an adjuvant will enhance the immune response induced and/or enhanced by component(s) of the vaccine thereby improving protective efficacy. Preferably, the adjuvant will enable the induction of protective immunity utilising a lower dose of other active component(s) (e.g. photon-irradiated streptococcal bacteria and/or derivatives thereof).

Non-limiting examples of adjuvants suitable for inclusion in vaccines of the invention and methods for their preparation are described in *"Vaccine Adjuvants: Preparation Methods*

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and Research Protocols (Methods in Molecular Medicine)", (2000), Ohagan (Ed), Humana Press Inc. Any suitable adjuvant may be included in a vaccine of the invention.

Specific examples of such adjuvants include, but are not limited to, aluminium hydroxide; polypeptide adjuvants including interferons, interleukins, and other cytokines; AMPHIGEN, oil-in-water and water-in-oil emulsions; and saponins such as QuilA.

For example, an aluminium-based adjuvant may be utilised. Suitable aluminium-based adjuvants include, but are not limited to, aluminium hydroxide, aluminium phosphate and combinations thereof. Other specific examples of aluminium-based adjuvants that may be utilised are described in European Patent No. 1216053 and United States Patent No. 6,372,223.

Oil in water emulsions may be utilised as adjuvants in vaccines of the invention. Oil in water emulsions are well known in the art. In general, the oil in water emulsion will comprise a metabolisable oil, for example, a fish oil, a vegetable oil, or a synthetic oil. Examples of suitable oil in water emulsions include those described in European Patent No. 0399843, United States Patent No. 7,029,678 and PCT Publication No. WO 2007/006939. The oil in water emulsion may be utilised in combination with other adjuvants and/or immunostimulants.

Non-limiting examples of other suitable adjuvants include immunostimulants such as granulocyte-macrophage colony-stimulating factor (GM-CSF), monophosphoryl lipid A (MPL), cholera toxin (CT) or its constituent subunit, heat labile enterotoxin (LT) or its constituent subunit, toll-like receptor ligand adjuvants such as lipopolysaccharide (LPS) and derivatives thereof (e.g. monophosphoryl lipid A and 3-Deacylated monophosphoryl lipid A), muramyl dipeptide (MDP), Toll-like receptor (TLR) agonists (e.g. TLR-2, TLR-3 agonists) and F protein of Respiratory Syncytial Virus (RSV).

Adjuvants in vaccines of the invention may typically include emollients, emulsifiers, thickening agents, preservatives, bactericides and buffering agents. Another type of "self adjuvant" is provided by the conjugation of immunogenic peptides to lipids such as the water soluble lipopeptides Pam3Cys or its dipalmitoyl derivative Pam2Cys. Such adjuvants have the advantage of accompanying and immunogenic component into the antigen presenting cell (such as dendritic cells) and thus producing enhanced antigen presentation and activation of the cell at the same time (see, for example, Brown and lackson, (2005), *"Lipid based self adjuvanting vaccines",* Current Drug Delivery, 23:83).

Suitable adjuvants are commercially available such as, for example, Freund's Incomplete Adjuvant and Complete Adjuvant (Difco Laboratories, Detroit, Mich.); Merck

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Adjuvant 65 (Merck and Company, Inc., Rahway, N.J.); AS-2 (SmithKline Beecham, Philadelphia, Pa.); aluminium salts such as aluminium hydroxide gel (alum) or aluminium phosphate; salts of calcium, iron or zinc; an insoluble suspension of acylated tyrosine; acylated sugars; cationically or anionically derivatized polysaccharides; polyphosphazenes; biodegradable microspheres; monophosphoryl lipid A and quil A. Cytokines, such as GM-CSF or interleukin-2, -7, or -12, may also be used as adjuvants.

In certain embodiments, an adjuvant included in a vaccine of the invention may induce an immune response predominantly of the TH1 type. Suitable adjuvants for use in eliciting a predominantly THl-type response include, for example, a combination of monophosphoryl lipid A, preferably 3-de-O-acylated monophosphoryl lipid A (3D-MPL) together with an aluminium salt. For example, the composition or vaccine may be formulated with adjuvant AS04 containing aluminium hydroxide (alum) and 3-O-deacylated monophosphorylated lipid A (MPL) such as described in Thoelen *et al.* (2001), "A *prophylactic hepatitis B vaccine with a novel adjuvant system",* Vaccine, 19:2400-2403. Other known adjuvants, which preferentially induce a TH1 type immune response, include CpG containing oligonucleotides. The oligonucleotides are characterised in that the CpG dinucleotide is unmethylated. Such oligonucleotides are known to those of ordinary skill in the field and are described, for example, in PCT Publication No. WO 1996/02555. Immunostimulatory DNA sequences are also described, for example, in Sato *et al.,* (1996), *"Immunostimulatory DNA sequences necessaryfor effective intradermal gene immunization",* Science, 273:352-354.

Another example of an adjuvant is a saponin, preferably QS21 (Aquila Biopharmaceuticals Inc., Framingham, Mass.), which may be used alone or in combination with other adjuvants. For example, an enhanced adjuvant system may be utilised involving the combination of a monophosphoryl lipid A and saponin derivative, such as the combination of QS21 and 3D-MPL as described in PCT Publication No. WO 1994/00153, or a less reactogenic composition where the QS21 is quenched with cholesterol, as described in PCT publication No. WO 1996/33739. Other alternative formulations comprise an oil-in-water emulsion and tocopherol. An adjuvant formulation involving QS21, 3D-MPL and tocopherol in an oil-in-water emulsion is described in PCT Publication No. WO 1995/17210. An adjuvant included in a composition of the invention may include a formulation involving QS21, 3D-MPL and tocopherol in an oil in water emulsion such as described in PCT publication No. WO 1995/17210. In one embodiment a composition of the invention comprises the adjuvant Montanide ISA720 (M-ISA-720; Seppic, Fairfield, N.J.), an adjuvant based on a natural metabolisable oil.

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Preferably, the adjuvant is a mucosal adjuvant effective in enhancing mucosal immunity and/or systemic immunity to immunogenic components administered via the mucosal route. Mucosal adjuvants may be broadly classified as those that facilitate vaccine delivery (e.g. liposomes, cochleates, live-attenuated vectors, poly D,L-lactide-co-glycolide or PLGA, chitans, DNA vaccines, mucoadhesives) to enhance the induction of protective immunity induced by other immunogenic components of the vaccine, and those having an immunostimulatory role (e.g. innate immunity associated toxin-based, cytokine-based etc.). Without limitation to a particular mechanism, it is postulated that the advantageous effects of mucosal adjuvants partially derive from an ability to assist the passage of immunogenic components in the vaccine across the mucosal barrier. Upon traversing the mucosal barrier, the mucosal adjuvant may enhance immunity, for example, by complement activation, the induction of cytokines, stimulation of antibody production or antibody type switching, stimulating antigen presenting cells, and/or influencing MHC class I and/or class II expression.

Routes of Administration

Vaccines of the invention may be administered to a recipient by standard routes, including, but not limited to, parenteral (e.g. intradermal, intravenous, intraspinal, intraperitoneal, subcutaneous or intramuscular), oral, topical, or mucosal routes (e.g. intranasal).

For example, the vaccines may be administered by a mucosal route. Non-limiting examples of acceptable routes of mucosal vaccine administration including intranasal, occular, buccal, genital tract (vaginal), rectal, intratracheal, skin, and the gastrointestinal tract.

In some embodiments, vaccines of the invention are administered by the intranasal route. Without limitation to theory or particular mode(s) of action, intranasal administration of the vaccines may be advantageous for enhancing immunity against certain streptococcal infections in which bacteria infect the host via mucosal surfaces of the upper and/or lower respiratory tracts. In addition, mucosal vaccination (e.g. intranasal vaccination) may induce mucosal immunity not only in the respiratory tracts but also in distant mucosal sites including the genital mucosa.

Intranasal vaccines of the invention can be formulated, for example, in liquid form as nose drops, spray, or suitable for inhalation, as powder, as cream, or as emulsion. Nebulised or aerosolised intranasal vaccines may also be utilised. Administration of vaccines to mucosa of the upper and/or lower respiratory tract via inhalation of mists, powders, or sprays, or by

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intranasal administration of nose drops, swabs, powders, sprays, mists, aerosols, and the like is also contemplated.

In one embodiment, the vaccines for intranasal administration are provided in a freeze-dried powder form capable of re-constitution immediately prior to use. Powder vaccine formulations of vaccines and compositions of the present invention provide a means of overcoming refrigerated storage and distribution requirements associated with liquid-based vaccine stability and delivery. Dry powder formulations offer the advantage of being more stable and also do not support microbial growth.

The freeze-dried vaccines may induce levels of heterosubtypic immunity similar to that of non freeze-dried vaccines. The vaccines may be freeze-dried using any suitable technique known in the art. For example, liquid preparations of photon-irradiated streptococcal bacteria and/or derivatives thereof may be frozen in a dry ice - isopropanol slurry and lyophilized in a freeze Dryer (e.g. Virtis Model 10-324 Bench, Gardiner, NY) for a suitable time period (e.g. 24 hours).

In one embodiment, a dry powder nasal vaccine of the invention is produced by generating spray-freeze-drying (SFD) particles (see, for example, Costantino *et al.*, (2002), *"Protein spray freeze drying. 2. Effect of formulation variables on particle size and stability"*, J Pharm Sci., 91:388-395; Costantino, *et al.*, (2000), *"Protein spray-freeze drying. Effect of atomization conditions on particle size and stability"*, Pharm Res.,17:1374-1383; Maa *et al.*, (1999), *"Protein inhalation powders: spray drying vs spray freeze drying"*, Pharm Res, 16:249-254; Carrasquillo *et al.*, (2001); *"Non-aqueous encapsulation of excipient-stabilized spray-freeze dried BSA into poly(lactide-co-glycolide) microspheres results in release of native protein"*, J Control Release,76: 199-208; Carrasquillo *et al.*, (2001), *"Reduction of structural perturbations in bovine serum albumin by non-aqueous microencapsulation",]* Pharm Pharmacol., 53:115-120; and United States Patent No. 6,569,458).

Preferred devices for intranasal administration of the vaccines are nasal spray devices (e.g. devices available commercially from Pfeiffer GmBH, Valois and Becton Dickinson). Non-limiting examples of suitable devices are described, for example, in Bommer, (1999), *"Advances in Nasal drug delivery Technology"*, Pharmaceutical Technology Europe, p26-33. Intranasal devices may produce droplets in the range 1 to 500 μιη. Preferably, only a small percentage of droplets (e.g. <5%) are below 10 μιη to minimise the chance of inhalation. Intranasal devices may be capable of bi-dose delivery, that is, the delivery of two subdoses of a single vaccination dose, one sub-dose to each nostril.

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A vaccine of the invention may be administered to a recipient in isolation or in combination with other additional therapeutic agent(s). In embodiments where the vaccine is administered with therapeutic agent(s), the administration may be simultaneous or sequential (i.e. vaccine administration followed by administration of the agent(s) or *vice versa).* Thus, where a vaccine of the invention is administered to a subject in conjunction with another agent, both may be administered in a single composition at the same time, in separate compositions at the same time, or separately at different times.

Dosages

In general, vaccines of the invention are administered in a manner compatible with the route of administration and physical characteristics of the recipient (including health status) and in such a way that it is elicits the desired effect(s) (i.e. therapeutically effective, immunogenic and/or protective).

For example, the appropriate dosage of a given vaccine may depend on a variety of factors including, but not limited to, a subject's physical characteristics (e.g. age, weight, sex), whether the compound is being used as single agent or adjuvant therapy, the progression (i.e. pathological state) of a given streptococcal infection, and other factors that may be recognized by one skilled in the art. Various general considerations that may be considered when determining an appropriate dosage of a given vaccine of the invention are described, for example, in Gennaro *et al.* (Eds), (1990), *"Remington's Pharmaceutical Sciences",* Mack Publishing Co., Easton, Pennsylvania, USA; and Gilman *et al.,* (Eds), (1990), *"Goodman And Gilman's: The Pharmacological Bases of Therapeutics",* Pergamon Press.

In general, vaccines of the invention may be administered to a patient in an amount of from about 50 micrograms to about 5 mg of active component(s) (i.e. photon-irradiated streptococcal bacteria and/or derivatives thereof). Dosage in an amount of from about 50 micrograms to about 500 micrograms is especially preferred.

One skilled in the art would be able, by routine experimentation, to determine an effective, non-toxic amount of photon-irradiated streptococcal bacteria or derivatives thereof to include in a vaccine of the invention for the desired therapeutic outcome.

Generally, an effective dosage is expected to be in the range of about O.OOOlmg to about lOOOmg of active component(s) (i.e. photon-irradiated streptococcal bacteria or derivatives thereof) per kg body weight per 24 hours; typically, about O.OOlmg to about 750mg per kg body weight per 24 hours; about O.Olmg to about 500mg per kg body weight per 24 hours; about O.lmg to about 500mg per kg body weight per 24 hours; about O.lmg to

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about 250mg per kg body weight per 24 hours; about 1.Omg to about 250mg per kg body weight per 24 hours. More typically, an effective dose range is expected to be in the range about 1.Omg to about 200mg per kg body weight per 24 hours; about 1.Omg to about 1OOmg per kg body weight per 24 hours; about 1.Omg to about 50mg per kg body weight per 24 hours; about 1.Omg to about 25mg per kg body weight per 24 hours; about 5.0mg to about 50mg per kg body weight per 24 hours; about 5.0mg to about 20mg per kg body weight per 24 hours; about 5.0mg to about 15mg per kg body weight per 24 hours.

Alternatively, an effective dosage may be up to about 500mg/m^2 of active component(s) (i.e. photon-irradiated streptococcal bacteria or derivatives thereof). Generally, an effective dosage is expected to be in the range of about 25 to about 500mg/m^2, preferably about 25 to about 350mg/m^2, more preferably about 25 to about 300mg/m^2, still more preferably about 25 to about 250mg/m^2, even more preferably about 50 to about 250mg/m^2, and still even more preferably about 75 to about 150mg/m^2.

Typically, in therapeutic applications, the treatment would be for the duration of the infection, disease state or condition. Further, it will be apparent to one of ordinary skill in the art that the optimal quantity and spacing of individual dosages will be determined by the nature and extent of the infection, disease state or condition being treated, the form, route and site of administration, and the nature of the particular individual being treated. Also, such optimum conditions can be determined by conventional techniques.

In many instances, it will be desirable to have several or multiple administrations of a vaccine of present invention. For example, vaccines of the invention may be administered 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more times. The administrations may be from about one to about twelve week intervals, and in certain embodiments from about one to about four week intervals. Periodic re-administration may be desirable in the case of recurrent exposure to a particular pathogen targeted by a vaccine of the invention.

It will also be apparent to one of ordinary skill in the art that the optimal course of treatment can be ascertained using conventional course of treatment determination tests.

The methods described herein may comprise administering a priming dose of a vaccine of the invention. The priming dose may be followed by a booster dose. The booster may be for the purpose of revaccination. In various embodiments, the vaccine is administered at least once, twice, three times or more. Vaccines of the invention may be administered to naïve recipients, being individuals seronegative for particular target strain(s) of streptococcal bacteria. Alternatively, the vaccines may be administered to primed recipients, being individuals seropositive for particular target strain(s) of streptococcal bacteria.

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It will be appreciated by persons of ordinary skill in the art that numerous variations and/or modifications can be made to the present invention as disclosed in the specific embodiments without departing from the spirit or scope of the present invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

Examples

The present invention will now be described with reference to specific Example(s), which should not be construed as in any way limiting.

Example 1: generation of a pneumococcal strain suitable for testing as a universal vaccine.

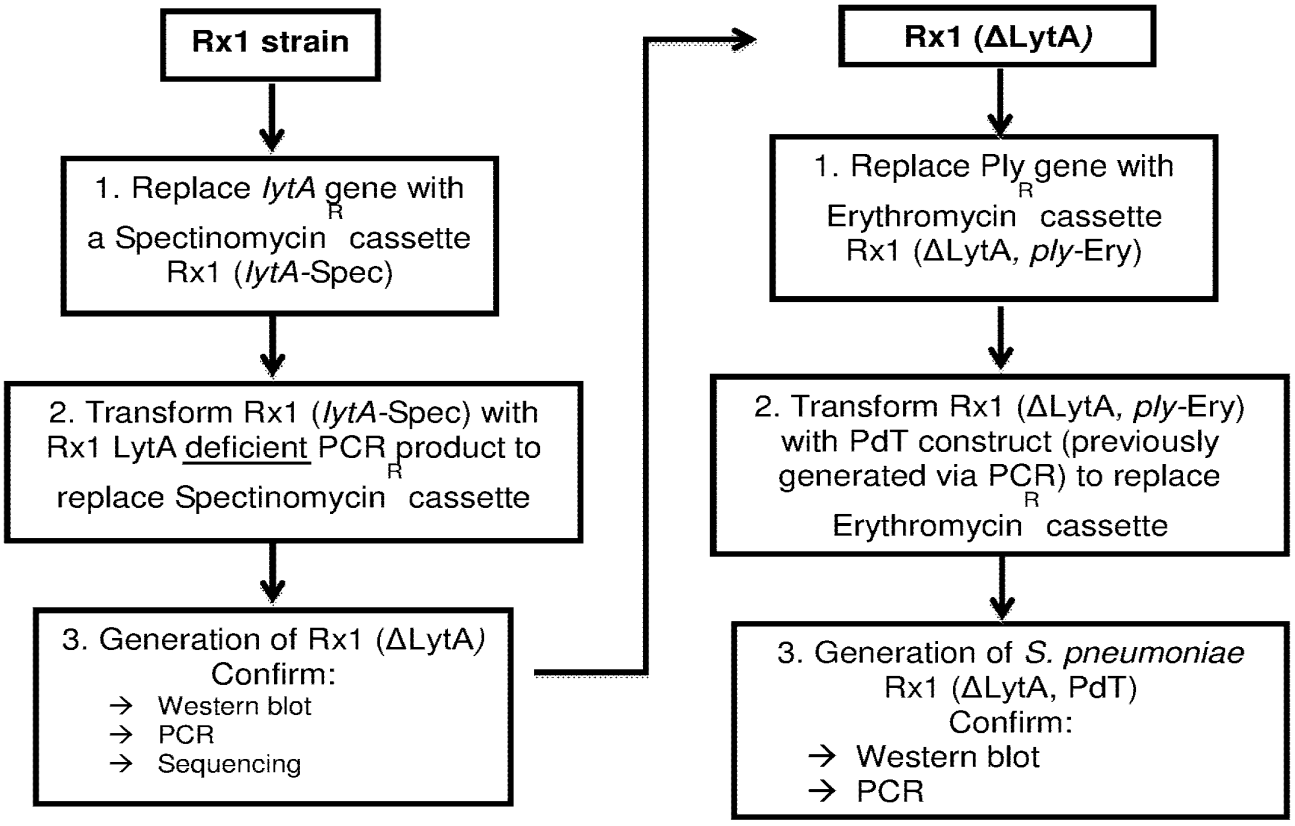
Streptococcus pneumoniae strain Rxl is a derivative of serotype 2 (D39), which lacks the outer capsule of the bacterium. This strain was genetically modified to remove the autolysin gene *(lytA)* as shown in the flow chart below. The resultant Rxl (ALytA) strain was further modified by replacing the pneumolysin gene *(ply)* with a toxoid version of Ply, designated as PdT, as shown in the flow chart below.

PCR and Western blot were performed after each transformation step to further confirm successful transformations, and sequencing confirmed the successful generation of a *lytA* deficient, pneumolysin mutant (PdT) Rxl strain, designated *S. pneumoniae* Rxl (ALytA, PdT) **(Figure 1).**

A haemolytic assay was performed on each derivative to confirm the lack of haemolytic activity in the derivative strain carrying the PdT mutant form of pneumolysin **(Figure 2).**

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Flow Chart: procedure adopted to modify strain Rx1 to remove the *lytA* gene coding for autolysin and to replace the *ply* gene coding for pneumolysin with a mutant derivative of pneumolysin expressing PdT.



Western blot, PCR and sequencing thus confirmed the successful generation of *Streptococcus pneumoniae* strain *S. pneumoniae* Rxl (ALytA, PdT).

Example Two: secondary structure analysis of mRNA transcriptfrom the AlytA gene.

Analyses were performed to predict the secondary structure of the *AlytA* gene mRNA transcript.

The DNA sequence of the *lytA* gene of strain Rxl, prior to deletion, including flanking regions, is shown in **SEQ ID NO: 1** (see below). The entire coding region of the *lytA* gene from the ATG (start codon) through to the TAA (stop codon) was deleted, in-frame, by splice-overlap extension PCR. The resultant *AlytA* gene sequence is shown in **SEQ ID NO: 2** (see below). The mRNA transcript coded by the *AlytA* gene sequence is shown in **SEQ ID NO: 3** (see below).

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The secondary structure of the mRNA transcript was predicted using RNAfold (RNAfold Webserver ([http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi]) and is shown in **Figure 3.** The predicted secondary structure of the mRNA transcript shown in **Figure 3** is anticipated to have a minimum free energy of -52.80 kcal/mol. The predicted double-stranded termination stem-loop sequence shown underlined in black, bold in **SEQ ID NO: 3** is predicted to have a minimum free energy of -18.10 kcal/mol.

SEP ID NO: 1

LytA gene sequence, including flanking regions, of Streptococcus pneumoniae

TGAAAATAGTTTAACAGACTTTTGACTTCTTTTATATGATATAATAAAGTATAGTA

-35

TTTATGAAAAGGACATATAGAGACTGTAAAAATATACTT**TTGAAA**ATCTTTTTAGT

-10

CTGGGG**TGTTATTGT**AGATAG**A**ATGCAGACCTTGTCAGTCCTATTTACAGTGTCAAAA

TAGTGCGTTTTGAAGTTCTATCTACAAGCCTAATCGTGACTAAGATTGTCTTCTTTGTA

AGGTAGAAATAAAGGAGTTTCTGGTTCTGGATTGTAAAAAATGAGTTGTTT

lytA start

TAATT**GATAAGGAGTAGAAT*ATG***GAAA TTAA TGTGAG TAAATTAAGAACAGATTTGCC

TCAAGTCGGCGTGCAACCATATAGGCAAGTACACGCACACTCAACTGGGAATCCGCATT

CAACCG TACAGAA TGAAGCGGA TTA TCAC TGGCGGAAAGACCCAGAA TTAGGTTTTTTC

TCGCACA TTGT TGGGAACGG TTGCA TCA TGCAGGTAGGACCTGT TGA TAA TGG TGCCTG

GGACGTTGGGGGCGGTTGGAATGCTGAGACCTATGCAGCGGTTGAACTGATTGAAAGCC

ATTCAACCAAAGAAGAGTTCATGACGGACTACCGCCTTTATATCGAACTCTTACGCAAT

CTAGCAGATGAAGCAGGTTTGCCGAAAACGCTTGATACAGGGAGTTTAGCTGGAATTAA

AACGCACGAGTATTGCACGAATAACCAACCAAACAACCACTCAGACCACGTTGACCCTT

A TCCA TA TCT TGCTAAA TGGGGCA TTAGCCG TGAGCAGTTTAAGCA TGA TA T TGAGAAC

GGCT TGACGA TTGAAA CAGGC TGGCAGAAGAA TGA CA CTGGC TA CTGG TA CGTACATTC

AGACGGCTCTTATCCAAAAGACAAGTTTGAGAAAATCAATGGCACTTGGTACTACTTTG

ACAGTTCAGGCTATATGCTTGCAGACCGCTGGAGGAAGCACACAGACGGCAACTGGTAC

TGGT TCGA CAAC TCAGGCGAAA TGGCTA CAGGC TGGAAGAAAA TCGC TGA TAAG TGG TA

CTATTTCAACGAAGAAGGTGCCATGAAGACAGGCTGGGTCAAGTACAAGGACACTTGGT

ACTACTTAGACGCTAAAGAAGGCGCCATGGTATCAAATGCCTTTATCCAGTCAGCGGAC

GGAACAGGCTGGTACTACCTCAAACCAGACGGAACACTGGCAGACAAGCCAGAATTCAC

AGTAGAGCCAGATGGCTTGATTACAGTAAAATAm **AAT*GG*AAT*GT*CTTT*C***AAA T CA GA

lytA stop

ACAG**CGCATATTATTAGGTCTTGAAAAAGCTTAATAGTATGCG**

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The *lytA* coding sequence for autolysin (LytA) is shown in italic/underline with the ATG start codon and TAA stop codon highlighted in bold/italics. The -35 and -10 and sigma70 promoter recognition sequences are shaded in light and dark grey, respectively. Squared/unbold nucleotides denote a potential transcription binding site(s) (TFBs) for RpoD. The bold **A** in the middle of the TFBs represent a predicted Transcription Start Site (TSS) position for the lytA mRNA. The upstream and downstream nucleotides that immediately flank the ATG start codon and TAA stop codon are squared/bold.

The entire coding region of the *lytA* gene was deleted, in-frame, by slice-overlap extension PCR, such that the squared/bold nucleotide sequences in **SEQ ID NO: 1** upstream and downstream of *lytA* are fused together.

SEP ID NO: 2

Gene sequence in Streptococcus pneumoniae strain RxlALytA (and derivatives) following deletion of the lytA gene

TGAAAATAGTTTAACAGAC TTTTGACTTCTTTTATATGATATAATAAAGTATAGTA

TTTATGAAAAGGACATATAGAGACTGTAAAAATATACTTTTGAAAA"TCTTTTTAGT

CTGGGCTGTTATTGTAGATAGAATGCAGACCTTGTCAGTCCTATTTACAGTGTCAAAA

TAGTGCGTTTTGAAGTTCTATCTACAAGCCTAATCGT GACTAAGATTGTCTTCTTTGTA

AGGTAGAAATAAAGGAGTTTCTGGTTCTGGATTGTAAAAAAT GAGTTGTTTTAATTGA

TAAGGAGTAGAATTAATGGAATGTCTTTCIAAAT CAGAACAGCGCATATTAT TAGGTCT

TGAAAAAGCTTAATAGTATGCG

The messenger RNA (mRNA) transcript coded by the *AlytA* gene is shown in **SEQ ID NO: 3.** Ribonucleotides shown in bold black and underlined are predicted to form a typical double-stranded (ds) stem-loop structure permitting Rho-dependent termination.

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SEQ ID NO: 3

Messenger RNA (mRNA) transcript coded by the AlytA gene

5' -AAUGCAGACCUUGUCAGUCCUAUUUACAGUGUCAAAAUAGUGCGUUUUGAAGUUC
UAUCUACAAGCCUAAUCGUGACUAAGAUUGUCUUCUUUGUAAGGUAGAAAUAAAGGAGU
UUCUGGUUCUGGAUUGUAAAAAAUGAGUUGUUUUAAUU**GAUAAGGAGUAGAAUUAAUG**
GAAUGUCUUUCAAAUCAGAACAG**CGCAUAUUAUUAGGUCUUGAAAAAGCUUAAUAGUA**
UGCG-3'

Example Three: effect of gamma-irradiation doses and conditions on the viability and morphology of S. pneumoniae Rxl (ALytA, PdT).

The effect of gamma-irradiation on the viability and morphology of concentrated vaccine samples at different irradiation doses and conditions was tested.

S. pneumoniae Rx1 (ALytA, PdT) was cultured in THY broth to obtain a cell density of 10^8 colony forming units (CFU)/ml. The bacteria were concentrated via centrifugation, washed with PBS, re-centrifuged and resuspended in PBS-10% glyerol at a final concentration of 1 X 10^{10} CFU/ml. The stock *S. pneumoniae* Rxl (ALytA, PdT) (1 X 10^{10} CFU/ml) was gamma-irradiated at various doses of irradiation (0.5-25 kGy) and temperature conditions, on ice or dry ice (DI). Post gamma-irradiation, samples of the vaccine were plated out onto blood agar plates to assess viability and confirm inactivation (**Figures** 4A and **Figure** 4B). The Gram stain and scanning electron microscopy were performed to determine the effect of gamma-irradiation on the physical morphology of the bacteria (**Figures** 5 and **Figure** 6, respectively).

Gamma-irradiation did not affect the morphology of the bacteria. Left hand image shows un-irradiated control bacteria whereas the right hand image shows the morphology of bacteria after irradiation at 12 kGy, which is the minimum dose required for complete inactivation of the sample irradiated on dry ice.

Example Four: duration of survival in mice vaccinated with gamma-irradiated S. pneumoniae Rxl (ALytA, PdT) following challenge with live Streptococcus pneumoniae strain D39.

Experiments were performed to determine if gamma-irradiated Rxl (ALytA, PdT prepared using gamma-irradiation at 12KGY on DI is able to confer protection against a lethal challenge with live *Steptococccus pneumoniae* strain D39 (serotype 2).

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Mice were vaccinated intranasally with two doses of gamma-irradiated *S. pneumoniae* Rx1 (ALytA, PdT) (12 KGY DI) (1 X 10^{5} CFU/dose) at a two week interval. Two weeks after the second vaccination dose, mice were challenged with 1 X 10^{6} CFU of *Steptococccus pneumoniae* D39 and monitored for time of survival and percent survival over a period of 21 days **(Figure 7A and Figure 7B).**

The vaccine was able to provide significant protection against lethal challenge with *Steptococccus pneumoniae* D39. Gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT) was thus observed to afford protection against intranasal challenge with live *Streptococcus pneumoniae* strain D39.

Example Five: immunity in mice vaccinated with S. pneumoniae* Rxl *(ALytA,* PdT) *following heterotypic challenge with either Streptococcus pneumoniae strains EF3030 (serotype 19F) or P9 (serotype 6A).

To determine if gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT) (12KGY DI) is able to confer protection against heterotypic challenge with EF3030 (serotype 19F) or P9 (serotype 6A), mice were vaccinated intranasally with two doses of *S. pneumoniae* Rxl (ALytA, PdT) (12 KGY DI) (1 X 10^{5} CFU/dose). Two weeks after the second vaccination dose, mice were infected with 1 X 10^{7} CFU of EF3030 or 5 X 10^{6} CFU of P9. Following EF3030 challenge, 96 hours post infection, the lungs and nasopharynx were harvested to determine bacterial counts (see **Figures 8A** and **Figure 8B,** respectively). Following P9 challenge, mice were monitored for survival for 21 days **(Figure 8C).**

The vaccine was able to provide protection against heterotypic challenge as shown by a significant decrease in *S. pneumoniae* EF3030 counts in the lungs and a significant difference in median survival following *S. pneumoniae* P9 challenge.

Example Six: induction of S. pneumoniae-specific serum antibody responses in mice intranasally vaccinated with S. pneumoniae Rxl (ALytA, PdT).

Analyses were conducted to determine whether gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT) (12KGY DI) is able to induce *S. pneumoniae*-specific antibody responses following intranasal vaccination.

Mice were vaccinated intranasally with two doses of gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT) (12 KGY DI) (1 X 10^{5} CFU/dose) and blood was collected two weeks after the second vaccination dose. ELISA was conducted to determine antigen specific IgG responses in the serum for the following antigens: Ply, NanA, CbpA and GlpO. In addition,

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ELISA was used to determine the IgG and IgA antibody responses to PspA and to un-irradiated whole Rxl (ALytA, PdT) bacterial cells.

There were detectable antibody responses to CbpA, GlpO, PspA and to the whole pneumococcus following intranasal vaccination with gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT) **(Figure 9).**

Example Seven: induction of S. pneumoniae specific antibody responses in mice vaccinated with S. pneumoniae Rxl (ALytA, PdT) intraperitoneal^.

Further experiments were conducted to determine if intraperitoneal vaccination is able to induce high-level *Streptococcus pneumoniae-specific* antibody responses.

Mice were vaccinated intraperitoneally with two doses of gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT) (12 KGY DI) (lxlO ^{8} CFU/dose) and blood was collected two weeks after the second vaccination dose. ELISA was used to determine antigen specific IgG titres in the serum for the following antigens: a whole cell lysate of *Streptococcus pneumoniae* Rxl (ALytA, PdT) cells (WC) **(Figure 10A),** Ply **(Figure 10B)** and CbpA **(Figure IOC).**

The vaccine induced significant high-level antibody titres when injected intraperitoneally .

Example Eight: role of B lymphocytes in protective immunity induced by intranasally administered S. pneumoniae Rxl (ALytA, PdT).

The involvement of B-cells in immune responses induced by *S. pneumoniae* Rxl (ALytA, PdT) was assessed.

Wild-type C57BL/6 mice (WT) and B-cell deficient C57BL/6 (μMT) mice (that are B-cell deficient) were vaccinated intranasally with two doses of gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT) (12 KGY DI) (1 X 10^{8} CFU/dose). Two weeks after the second vaccination dose the mice were challenged intranasally with a lethal dose of *Streptococcus pneumoniae* D39 and survival was monitored for 21 days. Percentage survival is shown in **Figure 11.**

Vaccination of WT mice with gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT) resulted in significant protection against lethal challenge with D39 in comparison to the relative WT control mice (that received PBS rather than the vaccine). In contrast, vaccination of μMT mice with gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT) showed no evidence of increased protection relative to the respective μMT control mice (that received PBS rather

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than the vaccine). These results indicate that B-cells are therefore essential for the intranasally administered gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT) vaccine to induce protective immunity against *Streptococcus pneumoniae.*

Example Nine: analysis of T-lymphocyte responses in protective immunity induced by intranasal administration of gamma-irradiated S. pneumoniae Rxl (ALytA, PdT).

T-lymphocyte responses were assessed following vaccination with gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT).

Mice were vaccinated intranasally with two doses of gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT) (12 KGY DI) (1 X 10^8 CFU/dose) and spleens were harvested two weeks after the second vaccination dose. Splenocytes were then stimulated with either the vaccine antigen, MalX protein or Media alone (- control) for 72 hours. Post 72 hours, the supernatants from cell cultures were collected to determine cytokine levels by ELISA (IL-17A and IFN-γ) **(Figure 12A** and **Figure 12B).**

Intracellular cytokine staining was performed to examine the proportion of Thl cells (using IFN-γ+), Th2 cells (IL-4+), Thl7 cells (IL-17+) and T-reg cells (Foxp3+) following antigen stimulation **(Figure 12C** and **Figure 12D).**

Vaccination with gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT) appears to provide no increase in IL-17A or IFN-γ levels in the supernatant following stimulation of splenocytes with the vaccine antigen or MalX as confirmed by ELISA. Similarly, there were no alterations in the proportions of Thl, Th2, Thl7 or Tregs as determined by intracellular cytokine staining.

Example Ten: challenge with influenza virus following intranasal administration of gamma-irradiated S. pneumoniae Rxl (ALytA, PdT)

Analyses were conducted to determine if prior vaccination with gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT) has any adverse effect on challenge with influenza.

Mice were vaccinated intranasally with two doses of gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT) (12 KGY DI) (1 X 10^8 CFU/dose). Two weeks after the second vaccination dose, mice were challenged with influenza virus A/PR8 (-100 TCID50) and monitored for weight loss and survival **(Figure 13B).** Control mice were given PBS instead of being vaccinated with gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT) and then challenged with influenza virus A/PR8 (-100 TCID50) and monitored for weight loss and survival **(Figure 13A).**

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Prior vaccination with gamma-irradiated *S. pneumoniae* Rx1 (ALytA, PdT) showed no adverse effects when mice were subsequently challenged with live influenza virus. There is some evidence that prior vaccination with gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT) offers some protection to influenza challenge, as indicated by all vaccinated mice surviving challenge with influenza strain A/PR8. This suggests some beneficial bystander effects.

Example Eleven: effect of cholera toxin adjuvant on S. pneumoniae Rxl (ALytA, PdT) efficacy

Gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT) was combined with the prototype adjuvant Cholera Toxin (CT) to assess whether adjuvants can increase the efficacy of the vaccine.

Mice were vaccinated intranasally with two doses of 1μg CT (as the control) or two doses of gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT) (12 KGY DI) (1 X 10^8 CFU/dose) plus 1μg CT.

Two weeks after the second vaccination dose, mice were challenged with 1 X 10^7 CFU of *S. pneumoniae* EF3030, with 1 X 10^6 CFU of *S. pneumoniae* D39 or with 5 X 10^6 CFU of *S. pneumoniae* P9. Following EF3030 infection, seven days later the lungs and nasopharynx were harvested to determine bacterial counts **(Figure 14A** and **Figure 14B).**

Following D39 or P9 challenge, mice were monitored for survival for 21 days **(Figure 14C** and **Figure 14D)**.

Vaccination with gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT) with CT adjuvant resulted in significant decreases in bacterial counts in the lungs compared to the non-vaccinated control, post infection with live EF3030. The vaccine with CT afforded significant protection to lethal challenge with live D39 and P9.

To determine the type of immune responses induced by the gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT) vaccine with cholera toxin as the adjuvant, spleens and serum were harvested two weeks after the second vaccination dose from mice vaccinated intranasally with two doses of gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT) (12 KGY DI) (1 X 10^8 CFU/dose), with or without the addition of 1μg CT. Splenocytes were stimulated with either the vaccine antigen, MalX or Media alone (- control) for 72 hours. Post 72 hours, intracellular cytokine staining was performed to look for the proportion of Thl cells (using IFN-γ +), Th2 cells (IL-4+), Thl 7 cells (IL-17+) & T-reg cells (Foxp3+) induced after

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72h of stimulation. **(Figures 15A** and **Figure 15B).** The supernatants from cell cultures were harvested to look for cytokines (IL-17A and IFN-γ) **(Figures 15C** and **Figure 15D).**

Intranasal vaccination with gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT) with CT polarised a Thl7 response as indicated by an increase in the number of Thl7 cells evidenced by intracellular cytokine staining and an increase in IL-17A in the supernatant following stimulation with gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT) or MalX.

Serum IgA & IgG titres to whole Rxl (ALytA, PdT) cells were determined by ELISA **(Figure 16).** Mice immunised with gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT) in the presence of CT adjuvant showed elevated antibody responses compared to mice immunised with vaccine strain alone.

Example Twelve: role of cytokines in protective immunity induced by intranasally administered gamma-irradiated S. pneumoniae Rxl (ALytA, PdT).

The involvement of IFN-γ and IL-7 in vaccine efficacy in immune responses induced by gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT) was assessed.

Wild-type C57BL/6 mice (WT) were vaccinated intranasally with two doses of gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT) (1×10^8 CFU/dose). Two weeks after the second vaccination dose, mice were injected with 200 μg of neutralising antibodies to IFN-γ or IL-17, or relevant isotype control antibodies at 24 h before challenge, 6 h post challenge and 48 h post challenge **(Figure 17A** and **17B).** Mice were challenged intranasally with a lethal dose of *Streptococcus pneumoniae* D39 and survival was monitored for 21 days. Percentage survival is shown.

Administration of either isotype control antibodies did not alter the protective efficacy of the vaccine against D39 challenge. Importantly, vaccination induced significant protection in immunized mice despite IFN-γ neutralisation when compared to their relative control **(Figure 17A).** In contrast, this protection was abolished by IL-17A neutralisation **(Figure 17B).** These data demonstrate that the mechanisms of protection elicited by gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT) are IL-17A-dependent and IFN-y-independent.

Example Thirteen: gamma-irradiated S. pneumoniae Rxl (ALytA, PdT) induces innate IL-17 responses.

The source of IL-17 dependent protection was accessed. Wild-type C57BL/6 mice (WT) were vaccinated intranasally with two doses of gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT) (1×10^8 CFU/dose). Two weeks after the second vaccination dose, the mice

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were challenged with *Streptococcus pneumoniae* D39. 24 and 48 hours post challenge, the lung was harvested and analysed for the proportion of T effector cells (Thl and Thl7), γδ T cells (γδTI and γδT17) and phagocytic cells (macrophages and neutrophils). Gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT) vaccination did not alter the total number of T effector cells or the relative populations of Thl or Thl7 cells in the lung 24 hours post D39 challenge **(Figure 18A).** Similar results were observed at 48 hours for Total T effector cells and Thl7 cells. There was a significant decrease in Thl cell numbers in immunized mice relative to the PBS-treated controls at 48 hours post challenge. In contrast to T effector cells, intranasal vaccination with the gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT) induced significant changes in γδ T cell populations in the lung post D39 challenge **(Figure 18A).** Whilst the total number of γδ T cells in the lung of vaccinated mice was not significantly different relative to non-immunized mice, the data indicates that immunized mice specifically enhanced γδT17 cell numbers in the lungs at 24 hours which was further enhanced at 48 hours post challenge. There was a decrease in γδTI cell numbers in vaccinated mice at 24 hours, leading to a significant difference detected at 48 hours relative to the control animals. Overall, these data demonstrate that the gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT) vaccine promotes a significant increase in γδT17 cell responses associated with a significant decrease in Thl and γδTI cells and no difference in the Thl7 population. Therefore, the data suggests that γδT17 cells may be a potential innate source of IL-17A involved in mediating protective immunity in vaccinated mice.

The relative numbers of macrophages and neutrophils in the lungs of mice 24 h and 48 h after challenge with *S. pneumoniae* D39 was compared in mice that had been vaccinated with gamma-irradiated *S. pneumoniae* Rxl (ALytA, PdT) or not vaccinated (PBS controls) **(Figure 18B).** The numbers of either cell type remained similar at 24 h and 48 h post-challenge in the vaccinated animals, whereas in the PBS control mice there was a significant increase in macrophages in the lungs between 24 h and 48 h post-challenge. This was also apparent for neutrophils.

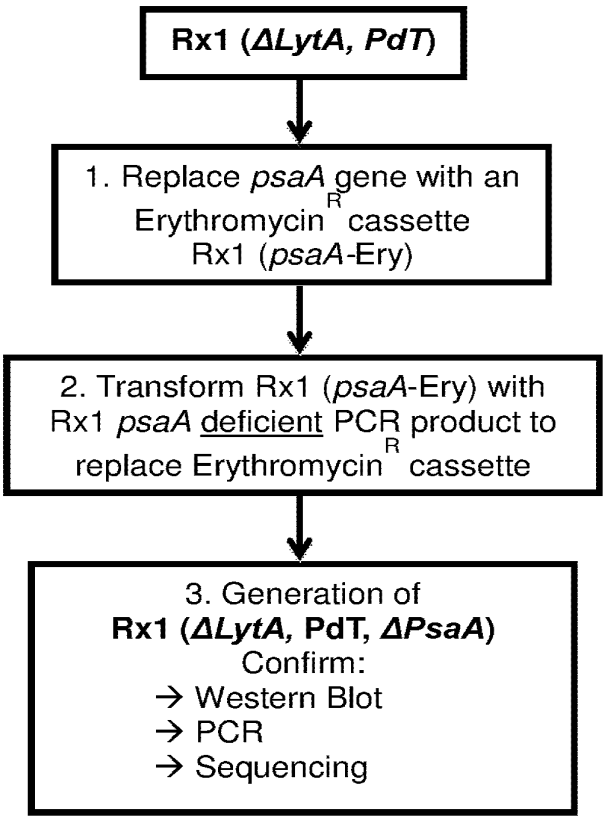
Example Fourteen: generation of a pneumococcal derivative with a genetic alteration in a gene encoding a virulence determinant.

Streptococcus pneumoniae strain Rxl (ALytA, PdT) was further genetically modified by removing the gene encoding the pneumococcal surface antigen A (PsaA), as shown in the flow chart below.

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PCR, sequencing and Western Blot were performed after each transformation step to confirm successful transformations had occurred and to confirm the generation of Rxl (ALytA, PdT, APsaA) strain **(Figure 19A** and **Figurel9B). Figure 19C** confirms the successful deletion of the *psaA* gene from Rxl (ALytA, PdT) to generate Rxl (ALytA, PdT, APsaA) and demonstrates the growth defect of the Rxl (ALytA, PdT, APsaA) strain in media not supplemented with Mn^{2+}.

Flow Chart: procedure adopted to modify strain Rxl (ALytA, PdT) to remove the *psaA* gene coding for pneumococcal surface antigen A.



Western blot, PCR and sequencing thus confirmed the successful generation of *Streptococcus pneumoniae* strain Rxl (ALytA, PdT, APsaA).

Example Fifteen: secondary structure analysis of mRNA transcriptfrom the ApsaA gene.

Analyses were performed to predict the secondary structure of the *ApsaA* gene mRNA transcript.

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The DNA sequence of the *psaA* gene of strain Rxl (ALytA, PdT), prior to deletion, including flanking regions, is shown in **SEQ ID NO: 4** (see below). A total of 865 nucleotides were deleted by splice-overlap extension PCR mutagenesis resulting in the deletion of the region from transcription start site through to the sequence beginning GTAAA, 56 nucleotides upstream of the stop codon. The resultant *ApsaA* gene sequence is shown in **SEQ ID NO: 5** (see below). The mRNA transcript coded by the *ApsaA* gene sequence is shown in **SEQ ID NO: 6** (see below).

The secondary structure of the mRNA transcript was predicted using RNAfold (RNAfold Webserver ([http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi]) and is shown in **Figure 20.** The predicted secondary structure of the mRNA transcript encoded by the *ApsaA* gene shown in **Figure 20** is anticipated to have a minimum free energy of -20.20 kcal/mol.

SEP ID NO: 4

PsaA gene sequence, including flanking regions, of Streptococcus pneumoniae

CAGTTTTGGGACTCTTTATTGGCTATAGTTTTAATGTTGCGGCAGGTTCTAGTATCGTGC

TTACAGCTGCTAGTTTCTTTCTCATTAGCTTCTTTATCGCTCCCAAACAACGATATTTGA

AAC TGAAAAATAAACAT T TGTTAAAAT AAGGGGCAAAGCCCTAA TAAATTG GAGGATCTA

ATGAAAAAATTAGGTACATTACTCGTTCTCTTTCTTTCTGCAATCATTCTTGTAGCATGT

psaA start

GCTAGCGGAAAAAAAGATACAACTTCTGGTCAAAAACTAAAAGTTGTTGCTACAAACTCA

ATCATCGCTGATATTACTAAAAATATTGCTGGTGACAAAATTGACCTTCATAGTATCGTT

CCGATTGGGCAAGACCCACACGAATACGAACCACTTCCTGAAGACGTTAAGAAAACTTCT

GAGGCTGATTTGATTTTCTATAACGGTATCAACCTTGAAACAGGTGGCAATGCTTGGTTT

ACAAAATTGGTAGAAAATGCCAAGAAAACTGAAAACAAAGACTACTTCGCAGTCAGCGAC

GGCGTTGATGTTATCTACCTTGAAGGTCAAAATGAAAAAGGAAAAGAAGACCCACACGCT

TGGCTTAACCTTGAAAACGGTATTATTTTTGCTAAAAATATCGCCAAACAATTGAGCGCC

AAAGACCCTAACAATAAAGAATTCTATGAAAAAAATCTCAAAGAATATACTGATAAGTTA

GACAAACTTGATAAAGAAAGTAAGGATAAATTTAATAAGATCCCTGCTGAAAAGAAACTC

ATTGTAACCAGCGAAGGAGCATTCAAATACTTCTCTAAAGCCTATGGTGTTCCAAGTGCC

TACATCTGGGAAATCAATACTGAAGAAGAAGGAACTCCTGAACAAATCAAGACCTTGGTT

GAAAAACTTCGCCAAACAAAAGTTCCATCACTCTTTGTAGAATCAAGTGTGGATGACCGT

CCAATGAAAACTGTTTCTCAAGACACAAACATCCCAATCTACGCACAAATCTTTACTGAC

TCTATCGCAGAACAMGGTAAAGAAGGCGACMGCTACTACAGCATGATGAAATACAACCTT

GACAAGATTGCTGAAGGATTGGCAAAA TAA GCC TCTGAAA**AACGTCATTCTCATGTGAGC**

psaA stop

TGGCG

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The *psaA* coding sequence for pneumococcal surface antigen A (PsaA) is shown in italic/underline with the ATG start codon and TAA stop codon highlighted in bold/italics. The -35 and -10 and sigma70 promoter recognition sequences are shaded in light and dark grey, respectively. The bold G 9 nucleotides upstream of the ATG start codon is the predicted Transcription Start Site (TSS) for the PsaA mRNA. The nucleotides immediately upstream of the ATG start codon and 56 nucleotides upstream of TAA stop codon are squared/bold and constitute the sites for PCR primer annealing.

The coding region of the *psaA* gene between the primer annealing sites was deleted by splice-overlap extension PCR, such that the squared/bold nucleotide sequences in **SEQ** ID **NO:** 4 in *psaA* are fused together.

SEP ID **NO: 5**

Gene sequence in Streptococcus pneumoniae strain Rxl ALytA, PdT, APsaA following deletion of the psaA gene.

CAGT TTTGGGACTCT TTAT TGGCTATAGT TTTAATGT TGCGGCAGGT TCTAGTATCGTGC

−35

TTACAGCTGCTAGT TTCT TTCTCAT TAGCT TCT TTATCGCTCCCAAACAACGATATTTGA

−10

AAC TGAAAAAT AAACAT T|TGTTAAAAATAAGIGGGCAAAGCCCTAA|TAAATTGGGTAAAGA ¾|

\GGCGAC21GCTACTACAGCATGA TGAAA TACAACCTTGACAAGATTGCTGAAGGATTGGCA

AAATAAGCCTCT**GAAAAACGTCAT TCTCATGTGAGC TGGCG**

psaA stop

The messenger RNA (mRNA) transcript coded by the *ApsaA* gene is shown in **SEQ** ID **NO: 6.** Ribonucleotides shown in bold black and underlined are predicted to form a typical double-stranded (ds) stem-loop structure permitting Rho-dependent termination.

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SEP ID NO: 6

Messenger RNA (mRNA) transcript coded by the ApsaA gene

5 ' -*GGUAAAGAM*

GGCGACMGCUACUACAGCAUGAUGAAAUACAACCUUGACAAGAUUGCUGAAGGAUUGGCA

AAAJ7AAGCCUCUGAAAAACGUCAUUCUCAUGUGAGCUGGCG-3 '

psaA stop

Example Sixteen: effect of gamma-irradiation doses and conditions on the viability and morphology of S. pneumoniae Rxl (ALytA, PdT, APsaA).

The effect of gamma-irradiation on the viability and morphology of concentrated vaccine samples at different irradiation doses.

S. pneumoniae Rxl (ALytA, PdT, APsaA) was cultured in THY broth to obtain a cell density of 10^8 colony forming units (CFU)/ml. The bacteria were concentrated via centrifugation, washed with PBS, re-centrifuged and resuspended in PBS-10% glycerol at a final concentration of 1 X 10^{10} CFU/ml. The stock *S. pneumoniae* Rxl (ALytA, PdT, APsaA) (1 X 10^{10} CFU/ml) was gamma-irradiated at various doses of irradiation (0.5-25 kGy) on dry ice (DI). Post gamma-irradiation samples of the vaccine strain were plated out onto blood agar plates to assess viability and confirm inactivation **(Figures 21A).** Scanning electron microscopy of the physical morphology of *S. pneumoniae* Rxl (ALytA, PdT, APsaA) that had either not been irradiated **(Figure 21B)** or irradiated at 25 kGy **(Figure 21C)** are shown. Gamma-irradiation did not affect the gross morphology of the bacteria.

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CLAIMS:

1. A method for preventing or treating an infection by streptococcal bacteria in a subject, the method comprising administering a therapeutically effective amount of photon-irradiated streptococcal bacteria to the subject to thereby prevent or treat the infection.

2. The method according to claim 1, wherein the method prevents or treats infection by a plurality of different streptococcal species and/or serotypes.

3. The method according to claim 2, wherein the photon-irradiated streptococcal bacteria comprise different: *Streptococcus* species, streptococcal serotypes, and/or streptococcal derivatives.

4. The method according to any one of claims 1 to 3, wherein the streptococcal infection comprises infection by one or more serotypes of *Streptococcus pneumoniae*.

5. The method according to any one of claims 1 to 4, wherein the infection by streptococcal bacteria is any one or more of a respiratory tract infection, pneumonia, an ear infection, an ear-ache, a middle ear infection, otitis media, sinusitis, meningitis, conjunctivitis, bacteraemia, septicaemia, a joint infection, a bone infection, septic arthritis, sepsis, osteomyelitis, a soft tissue infection, cellulitis, myositis, periorbital cellulitis, an abscess, necrotising fasciitis, impetigo, peritonitis, a cardiac infection, endocarditis, and/or pericarditis.

6. A method of inducing an immune response against streptococcal bacteria in a subject, the method comprising administering a therapeutically effective amount of photon-irradiated streptococcal bacteria to the subject to thereby induce the immune response.

7. The method according to claim 6, wherein the immune response comprises a heterotypic immune response against a different streptococcal species and/or serotype to that administered to the subject.

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8. The method according to claim 6 or claim 7, wherein the therapeutically effective amount of photon-irradiated streptococcal bacteria comprise different: *Streptococcus* species, streptococcal serotypes, and/or streptococcal derivatives.

9. The method according to claim 7 or claim 8, wherein the different streptococcal species and/or serotype is a *Streptococcus pneumoniae* serotype.

10. The method according to any one of claims 6 to 9, wherein the immune response comprises any one or more of:

(i) a B-lymphocyte response;

(ii) an innate immune response induced at least in part by interaction of double-stranded RNA from the photon-irradiated streptococcal bacteria with Toll-like receptors;

(iii) a T-lymphocyte response.

11. The method according to any one of claims 1 to 10, wherein the photon-irradiated streptococcal bacteria comprise at least one *Streptococcus pneumoniae* serotype, or at least one unencapsulated derivative of a *Streptococcus pneumoniae* serotype.

12. The method according to any one of claims 1 to 11, wherein the photon-irradiated streptococcal bacteria comprise mutant streptococcal bacteria comprising:

(i) one or more defective DNA repair proteins; and/or

(ii) a genetic alteration causing defective DNA repair capacity.

13. The method according to claim 12, wherein the photon-irradiated mutant streptococcal bacteria comprise a defect in at least one gene that encodes a DNA repair protein.

14. The method according to claim 13, wherein the photon-irradiated mutant streptococcal bacteria are *Streptococcus pneumoniae* mutants comprising a defect in one or more genes selected from a gene encoding a DNA alkylation repair protein, a gene encoding DNA polymerase 4, *hexA, hexB, mutS, radC, recA, recF, recN, recO, uvrA, uvrB, uvrC* or *uvrD*.

15. The method according to any one of claims 12 to 14, wherein the defective DNA repair protein is mutated, truncated or absent in the photon-irradiated mutant streptococcal bacteria.

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16. The method according to any one of claims 12 to 15, wherein the photon-irradiated mutant streptococcal bacteria comprise streptococcal Rx1 strain derivatives in which:

(i) any one or more of autolysin, hemolysin, pneumolysin, and/or PsaA protein is/are defective; and/or

(ii) any one or more of autolysin, hemolysin, and/or a PsaA protein is/are absent.

17. The method according to claim 16, wherein the photon-irradiated streptococcal Rxl strain derivatives:

(i) comprise any one or more of a gene encoding a defective autolysin protein, a gene encoding defective hemolysin, a gene encoding defective pneumolysin, and/or a gene encoding defective PsaA protein; or

(ii) are derivatives in which any one or more of a gene encoding autolysin, a gene encoding hemolysin, and/or a gene encoding PsaA protein, is/are absent.

18. The method according to claim 16 or claim 17, wherein the autolysin is LytA.

19. The method according to any one of claims 16 to 18, wherein the photon-irradiated streptococcal Rxl strain derivatives:

(i) comprise a gene encoding defective LytA protein, and a gene encoding defective pneumolysin; or

(ii) do not contain the *lytA* gene, and comprise a gene encoding a defective pneumolysin.

20. The method according to any one of claims 12 to 19, wherein the photon-irradiated mutant streptococcal bacteria require a reduced dosage of photon-irradiation for inactivation compared to that required by corresponding non-mutant streptococcal bacteria.

21. The method according to any one of claims 1 to 20, wherein some or all of the photon-irradiated streptococcal bacteria comprise modified streptococcal bacteria comprising a sequence of DNA encoding an RNA transcript, wherein the RNA transcript comprises a region of self-complementarity capable of forming a double-stranded portion after transcription.

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22. The method according to claim 21, wherein the portion of the double-stranded RNA transcript is at least: 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65 or 70 base pairs in length.

23. The method according to claim 21 or claim 22, wherein the double-stranded RNA transcript initiates an innate immune response in the subject comprising Toll-like receptor activation.

24. The method according to claim 23, wherein the Toll-like receptor is Toll-like receptor-3 (TLR-3).

25. The method according to any one of claims 1 to 24, wherein the photon-irradiated streptococcal bacteria comprise auxotrophic mutant streptococcal bacteria.

26. The method according to any one of claims 1 to 24, wherein the photon-irradiated streptococcal bacteria comprise at least one recombinant DNA portion encoding an antigen or a component thereof that:

(i) inactivates or attenuates the bacteria; and/or

(ii) induces or enhances an immune response in the subject.

27. The method according to claim 26, wherein the recombinant DNA portion replaces or disrupts an endogenous gene necessary for pathogenicity, infection, multiplication, growth *in vivo*, or any combination thereof.

28. The method according to any one of claims 1 to 27, wherein the photon-irradiated streptococcal bacteria comprise whole killed streptococcal bacteria.

29. The method according to any one of claims 1 to 28, wherein the photon-irradiated streptococcal bacteria are administered to the subject mucosally or intranasally.

30. The method according to any one of claims 1 to 29, wherein the photon-irradiated streptococcal bacteria are administered to the subject in combination with an adjuvant.

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31. The method according to any one of claims 1 to 30, wherein one, two or three separate doses of the photon-irradiated streptococcal bacteria are administered to the subject.

32. The method according to any one of claims 1 to 31, wherein the subject is a human.

33. The method according to any one of claims 1 to 32, wherein the photon-irradiated streptococcal bacteria are administered a total dosage of at least: 8 kilogray (kGy), 9kGy, lOkGy, llkGy, 12kGy, 15kGy, 20kGy, 25kGy, 30 kGy, 40kGy, or 50 kGy of photon-irradiation.

34. The method according to any one of claims 1 to 33, wherein the photon-irradiated streptococcal bacteria comprise gamma-irradiated streptococcal bacteria, X-irradiated streptococcal bacteria, or a combination thereof.

35. The method according to any one of claims 1 to 33, wherein the photon-irradiated streptococcal bacteria comprise X-irradiated streptococcal bacteria.

36. The method according to any one of claims 1 to 33, wherein the photon-irradiated streptococcal bacteria comprise a combination of gamma-irradiated streptococcal bacteria and X-irradiated streptococcal bacteria.

37. The method according to claim 20 or claim 33, wherein the photon-irradiation is gamma-irradiation.

38. The method according to claim 20 or claim 33, wherein the photon-irradiation is X-irradiation.

39. The method according to claim 20 or claim 33, wherein the photon-irradiation is a combination of gamma-irradiation and X-irradiation.

40. The method according to any one of claims 1 to 39, wherein the photon-irradiated streptococcal bacteria comprise streptococcal Rx1 strain derivatives:

(i) comprise a gene encoding a defective LytA protein, a gene encoding defective pneumolysin, and a gene encoding defective PsaA protein;

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(ii) comprise a gene encoding a defective LytA protein, a gene encoding defective pneumolysin, and in which a gene encoding PsaA protein is absent;

(iii) comprise a gene encoding a defective PsaA protein, a gene encoding defective pneumolysin, and in which a gene encoding LytA protein is absent; or

(iv) comprise a gene encoding defective pneumolysin, in which a gene encoding LytA protein is absent, and in which a gene encoding PsaA protein is absent.

41. A vaccine composition comprising photon-irradiated streptococcal bacteria and a pharmaceutically-acceptable excipient, diluent and/or carrier, wherein:

(i) the photon-irradiated streptococcal bacteria compr se. mutant streptococcal bacteria comprising one or more defective DNA repair proteins, and/or mutant streptococcal bacteria in which at least one type of DNA repair protein is absent; and/or

(ii) the photon-irradiated streptococcal bacteria comprise modified streptococcal bacteria comprising a sequence of DNA encoding an RNA transcript, wherein the RNA transcript comprises a region of self-complementarity capable of forming a double-stranded portion after transcription.

42. The vaccine composition according to claim 41, wherein the mutant streptococcal bacteria are *Streptococcus pneumoniae* mutants comprising a defect in one or more genes selected from. a gene encoding a DNA alkylation repair protein, a gene encoding DNA polymerase 4, *hexA, hexB, mutS, radC, recA, recF, recN, recO, uvrA, uvrB, uvrC* or *uvrD.*

43. The vaccine composition according to claim 4 1 or claim 42, wherein the modified streptococcal bacteria are modified *Streptococcus pneumoniae* bacteria and the portion of the double-stranded RNA transcript is at least. 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65 or 70 base pairs in length.

44. The vaccine composition according to any one of claims 4 1 to 43, wherein the photon-irradiated streptococcal bacteria comprise at least one recombinant DNA portion encoding an antigen or a component thereof that:

(i) inactivates or attenuates the bacteria; or

(ii) induces or enhances an immune response in the subject.

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45. The vaccine composition according to claim 44, wherein the recombinant DNA portion replaces or disrupts an endogenous gene necessary for pathogenicity, infection, reproduction or any combination thereof.

46. The vaccine according to any one or more of claims 41 to 45, wherein the photon-irradiated streptococcal bacteria comprise streptococcal Rxl strain derivative strains in which:

(i) any one or more of autolysin, hemolysin, pneumolysin, and/or PsaA protein is/are defective; and/or

(ii) any one or more of autolysin, hemolysin, and/or a PsaA protein is/are absent.

47. The vaccine according to claim 46, wherein the photon-irradiated streptococcal Rxl strain derivatives:

(i) comprise any one or more of a gene encoding a defective autolysin protein, a gene encoding defective hemolysin, a gene encoding defective pneumolysin, and/or a gene encoding defective PsaA protein; or

(ii) are derivatives in which any one or more of a gene encoding autolysin, a gene encoding hemolysin, and/or a gene encoding PsaA protein, is/are absent.

48. The vaccine according to any one of claims 41 to 47, wherein the photon-irradiated streptococcal Rxl strain derivatives:

(i) comprise a gene encoding defective LytA protein, and a gene encoding defective pneumolysin; or

(ii) do not contain the *lytA* gene, and comprise a gene encoding a defective pneumolysin.

49. The vaccine according to any one of claims 41 to 48, wherein the photon-irradiated mutant streptococcal bacteria comprise streptococcal Rxl strain derivatives:

(i) comprise a gene encoding a defective LytA protein, a gene encoding defective pneumolysin, and a gene encoding defective PsaA protein;

(ii) comprise a gene encoding a defective LytA protein, a gene encoding defective pneumolysin, and in which a gene encoding PsaA protein is absent;

(iii) comprise a gene encoding a defective PsaA protein, a gene encoding defective pneumolysin, and in which a gene encoding LytA protein is absent; or

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(iv) comprise a gene encoding defective pneumolysin, in which a gene encoding LytA protein is absent, and in which a gene encoding PsaA protein is absent.

50. The vaccine composition according to any one of claims 41 to 49, wherein the photon-irradiated streptococcal bacteria comprise whole-attenuated or whole-killed streptococcal bacteria.

51. The vaccine composition according to any one of claims 41 to 50, further comprising an adjuvant.

52. The vaccine composition according to any one of claims 41 to 51, wherein the vaccine composition is formulated for mucosal or intranasal administration or formulated for injection intramuscularly, subcutaneously or intradermally.

53. The vaccine composition according to any one of claims 41 to 52, wherein the photon-irradiated streptococcal bacteria comprise gamma-irradiated mutant streptococcal bacteria and/or gamma-irradiated modified streptococcal bacteria.

54. The vaccine composition according to any one of claims 41 to 52, wherein the photon-irradiated streptococcal bacteria comprise X-irradiated mutant streptococcal bacteria and/or X-irradiated modified streptococcal bacteria.

55. The vaccine composition according to any one of claims 41 to 52, wherein the photon-irradiated streptococcal bacteria comprise:

(i) a combination of gamma-irradiated and X-irradiated mutant streptococcal bacteria; and/or

(ii) a combination of gamma-irradiated and X-irradiated modified streptococcal bacteria.

56. A method for preparing the vaccine composition according to any one of claims 41 to 55, the method comprising:

(i) photon-irradiating a preparation of streptococcal bacteria to thereby attenuate or kill the bacteria; and

(ii) combining the photon-irradiated streptococcal bacteria with a pharmaceutically-acceptable excipient, diluent and/or carrier.

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57. The method according to claim 56, wherein said photon-irradiating the preparation of streptococcal bacteria comprises exposing the bacteria to gamma-radiation.

58. The method according to claim 56, wherein said photon-irradiating the preparation of streptococcal bacteria comprises exposing the bacteria to X-radiation.

59. The method according to claim 56, wherein said photon-irradiating the preparation of streptococcal bacteria comprises exposing the bacteria to gamma-radiation and X-radiation.

60. A vaccine composition prepared by the method of any one of claims 56 to 59.

61. A vaccine composition according to any one of claims 41 to 55 or 60 for use in preventing or treating an infection by streptococcal bacteria.

62. Use of photon-irradiated streptococcal bacteria in the preparation of a medicament for preventing or treating an infection by streptococcal bacteria, wherein the medicament is the vaccine composition according to any one of claims 41 to 55 or 60.

63. The vaccine composition according to claim 61 or the use according to claim 62, wherein the infection by streptococcal bacteria is any one or more of a respiratory tract infection, pneumonia, ear infection, earache, middle ear infection, otitis media, sinusitis, meningitis, conjunctivitis, bacteraemia, septicaemia, a joint infection, a bone infection, septic arthritis, osteomyelitis, a soft tissue infection, cellulitis, myositis, periorbital cellulitis, an abscess, peritonitis, a cardiac infection, endocarditis, and pericarditis.

Petitioner Merck Ex-1004, 1064

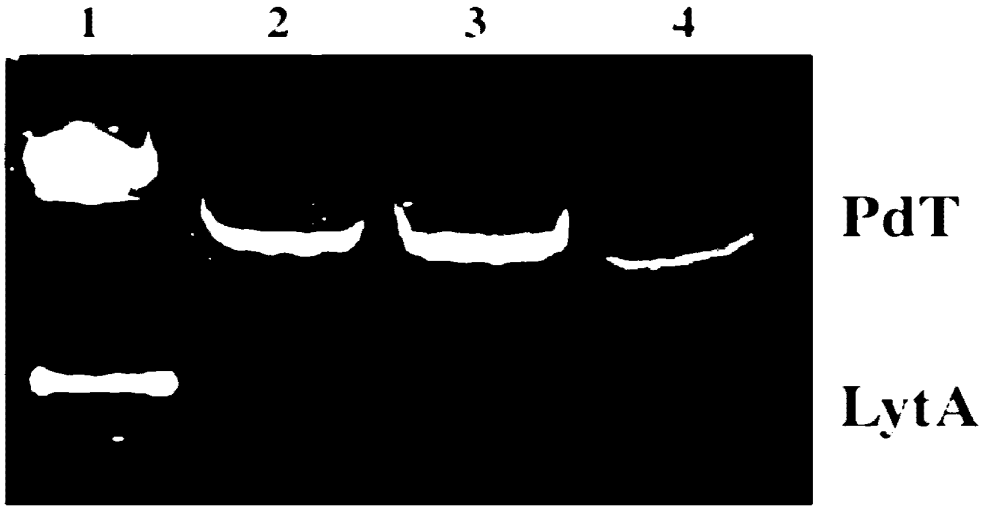


FIGURE ONE

Petitioner Merck Ex-1004, 1065

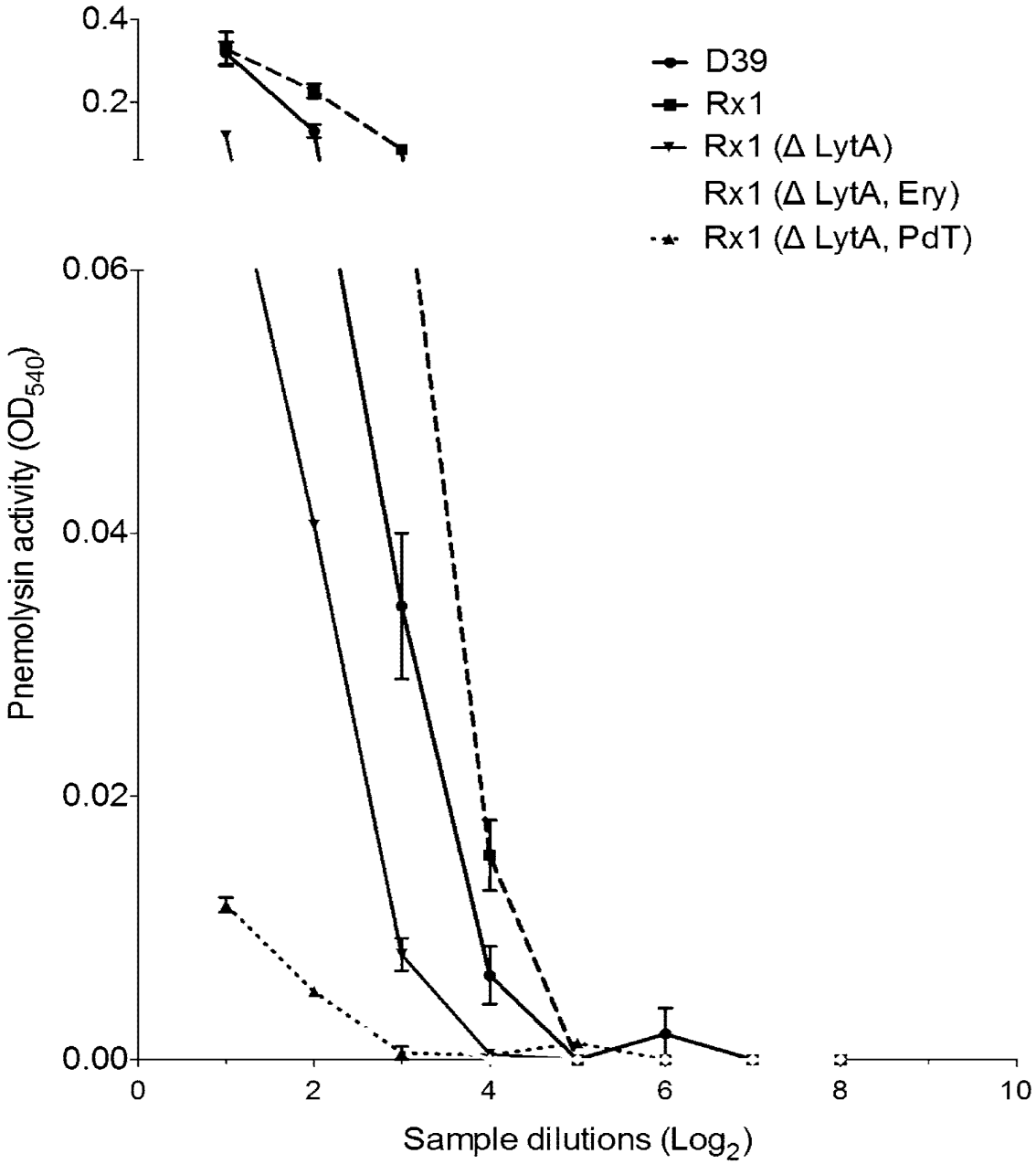


FIGURE TWO

Petitioner Merck Ex-1004, 1066

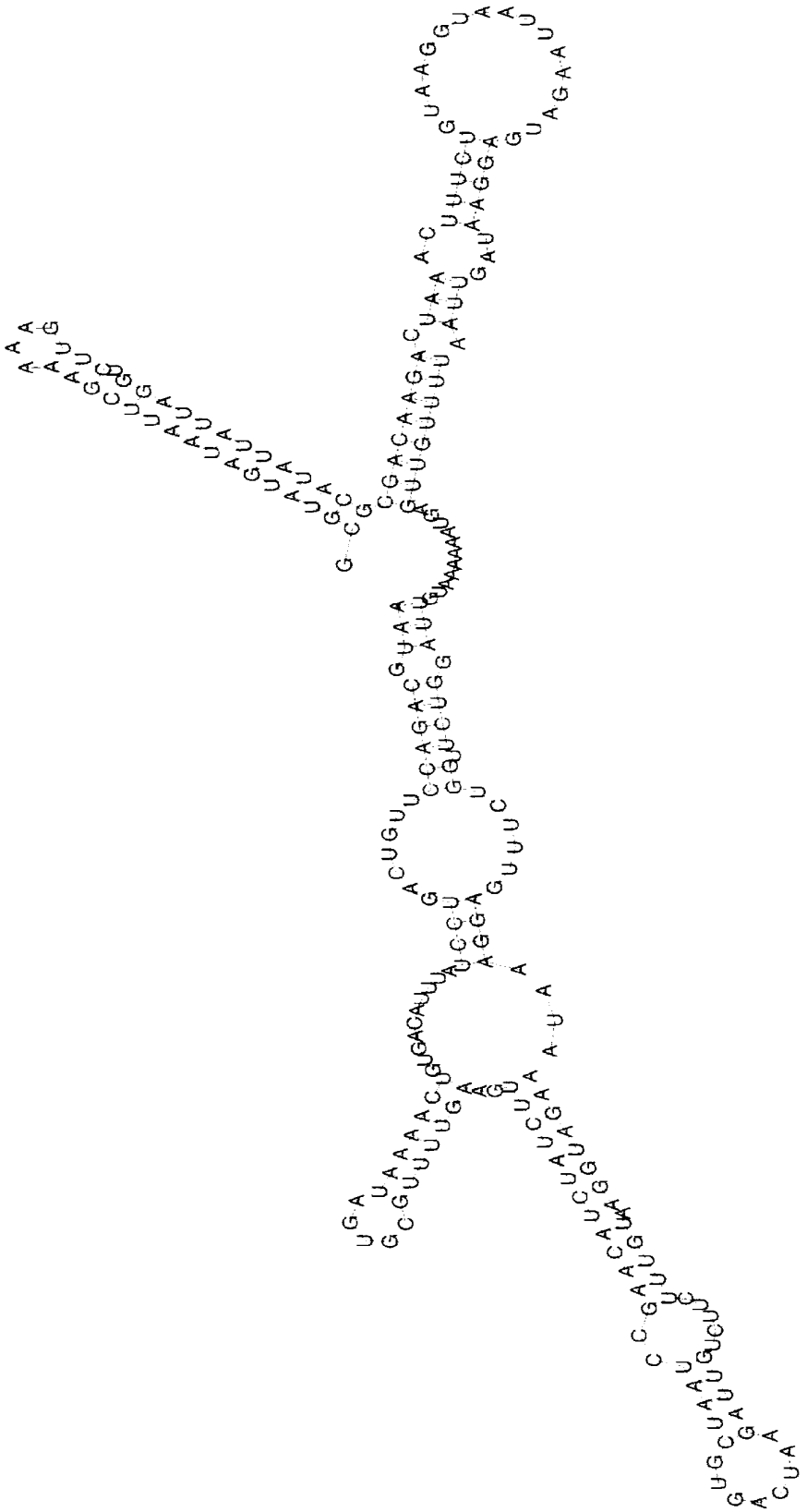


FIGURE THREE

Petitioner Merck Ex-1004, 1067

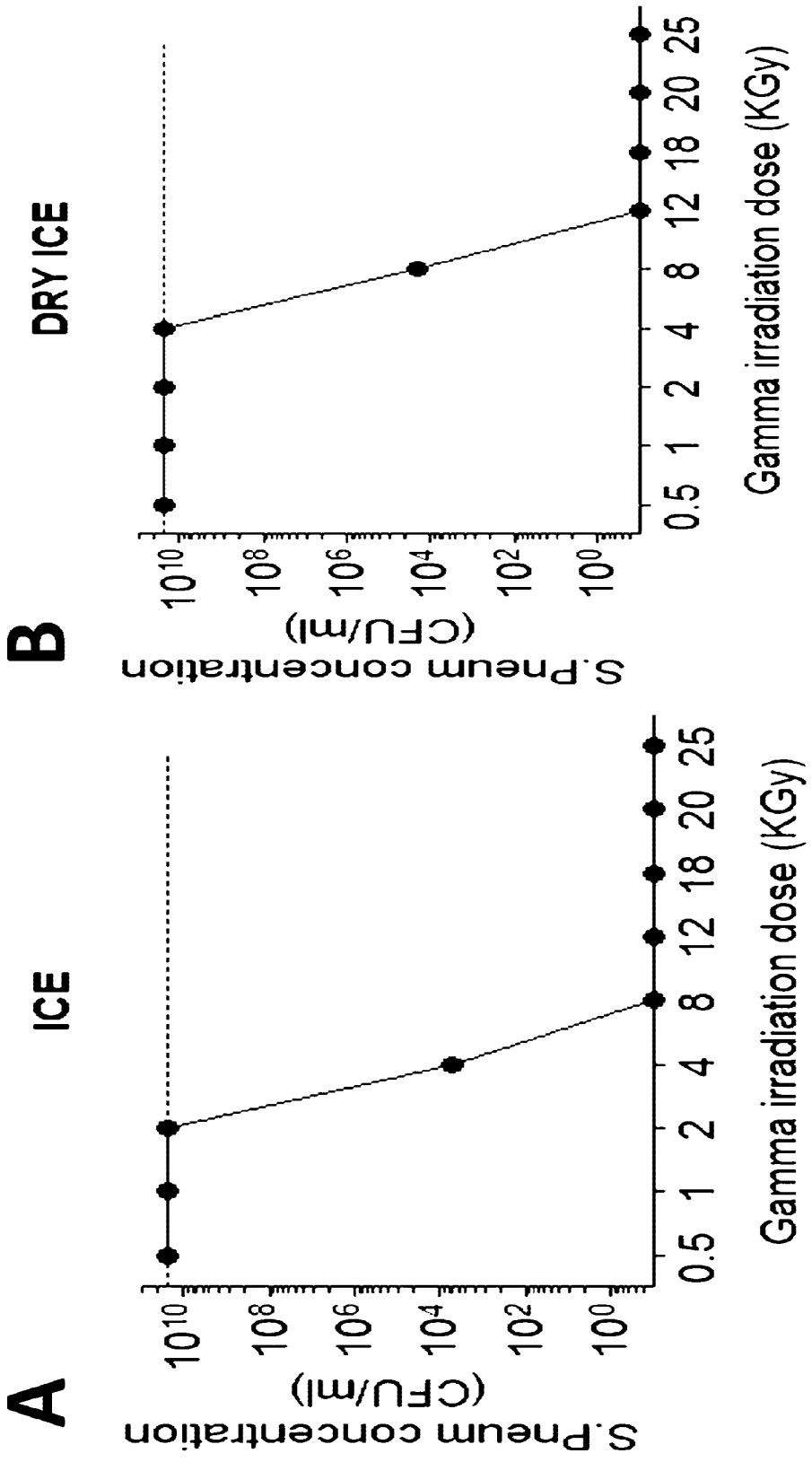


FIGURE FOUR

Petitioner Merck Ex-1004, 1068

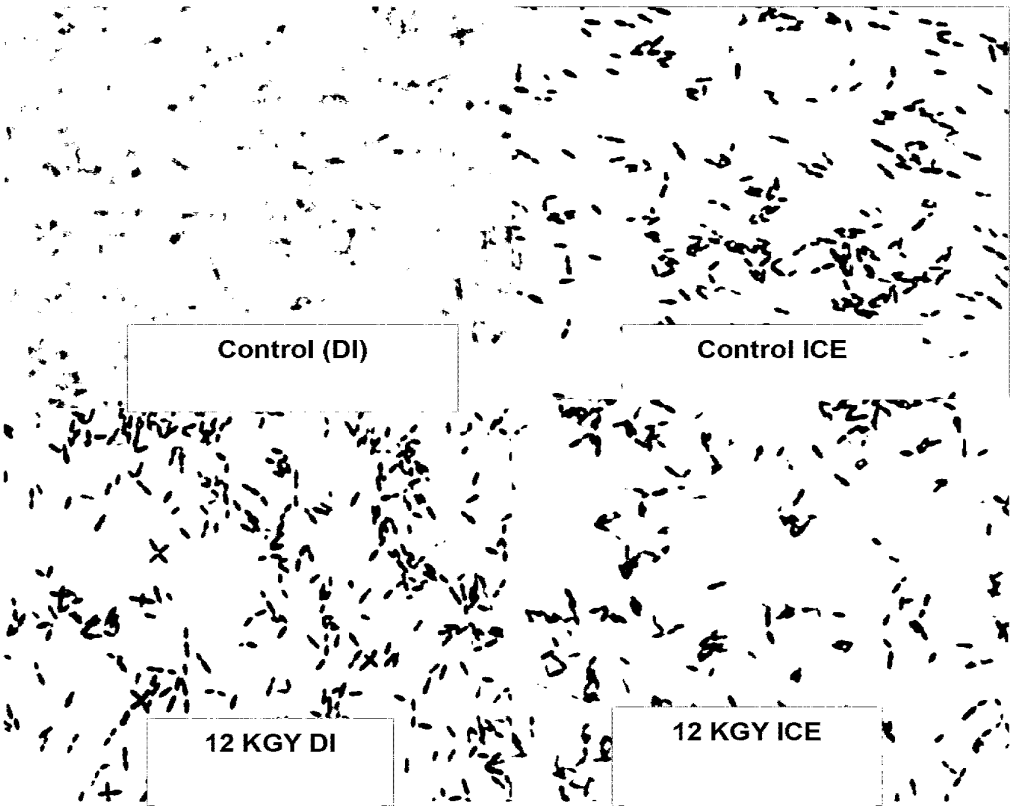


FIGURE FIVE

Petitioner Merck Ex-1004, 1069

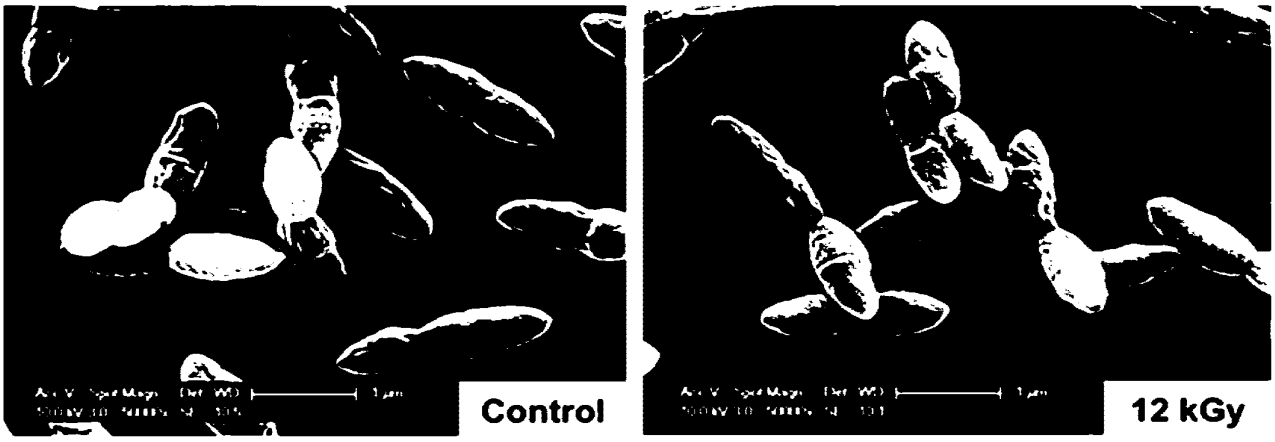


FIGURE SIX

Petitioner Merck Ex-1004, 1070

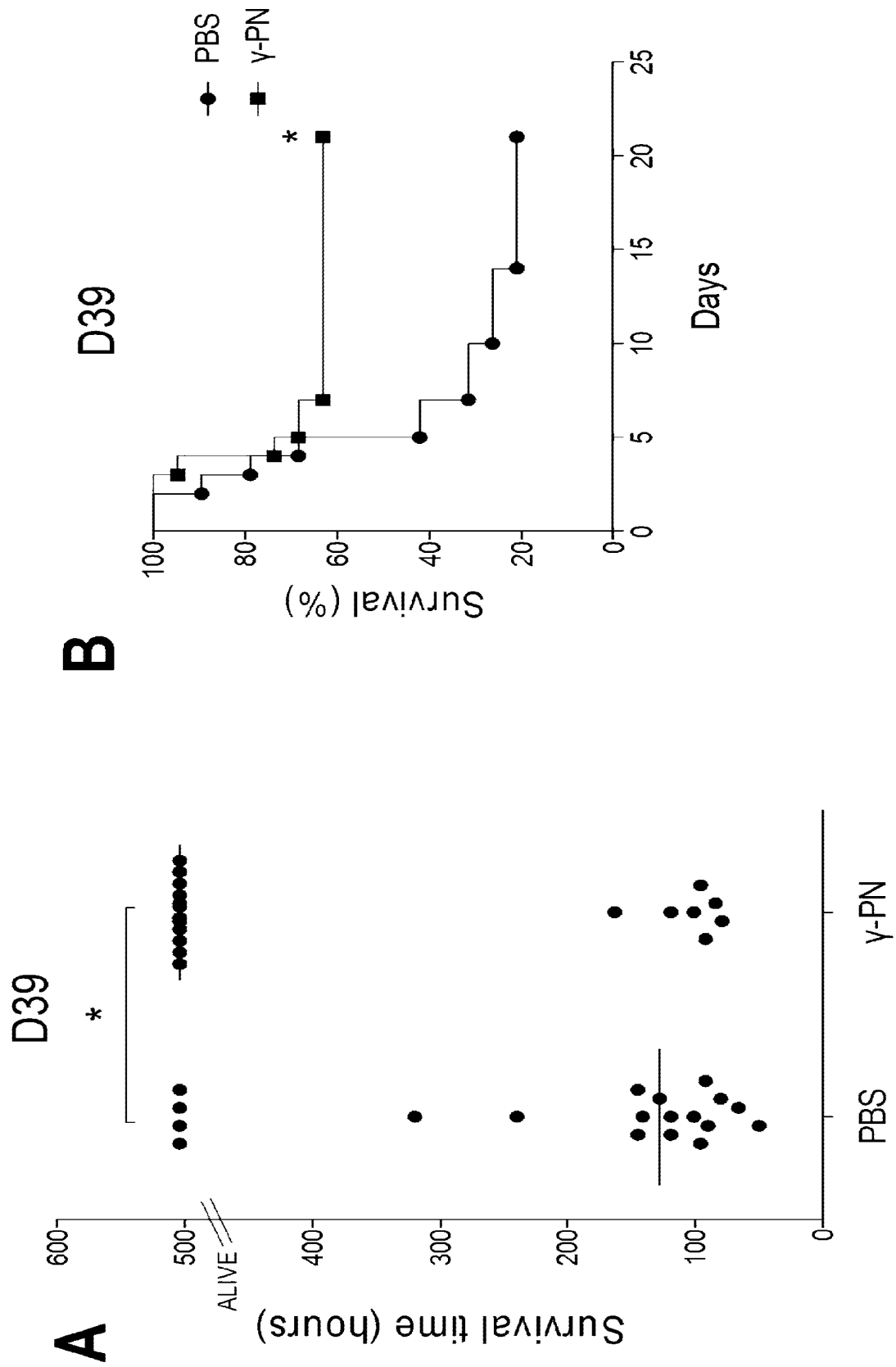


FIGURE SEVEN

Petitioner Merck Ex-1004, 1071

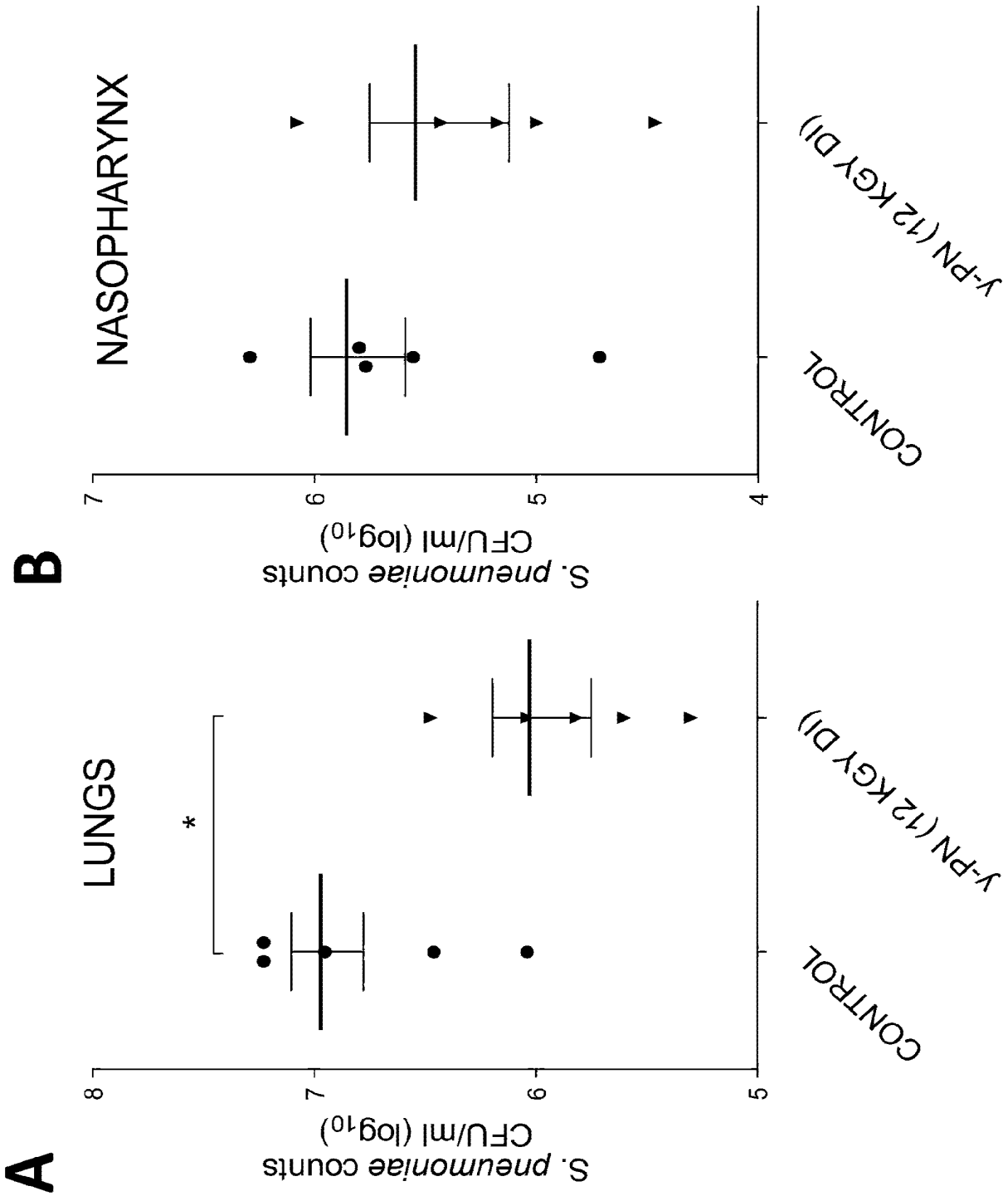


FIGURE EIGHT

Petitioner Merck Ex-1004, 1072

C

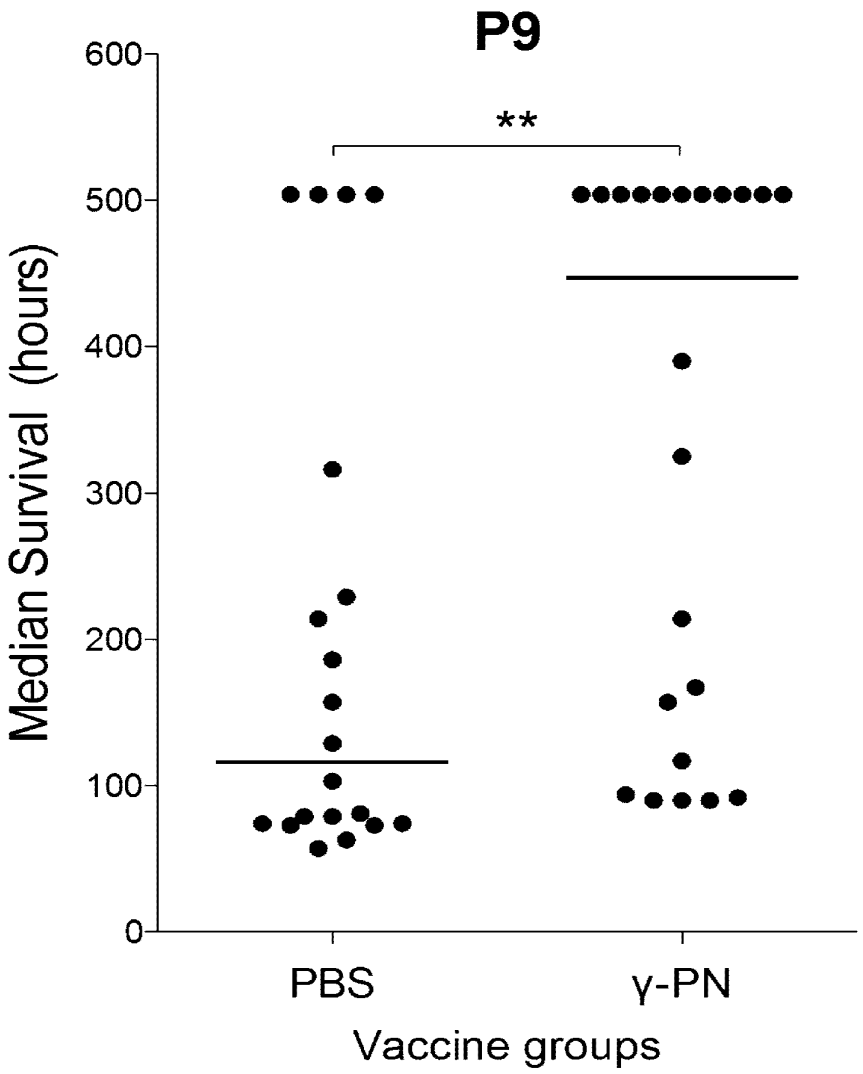


FIGURE EIGHT (CONTINUED)

Petitioner Merck Ex-1004, 1073

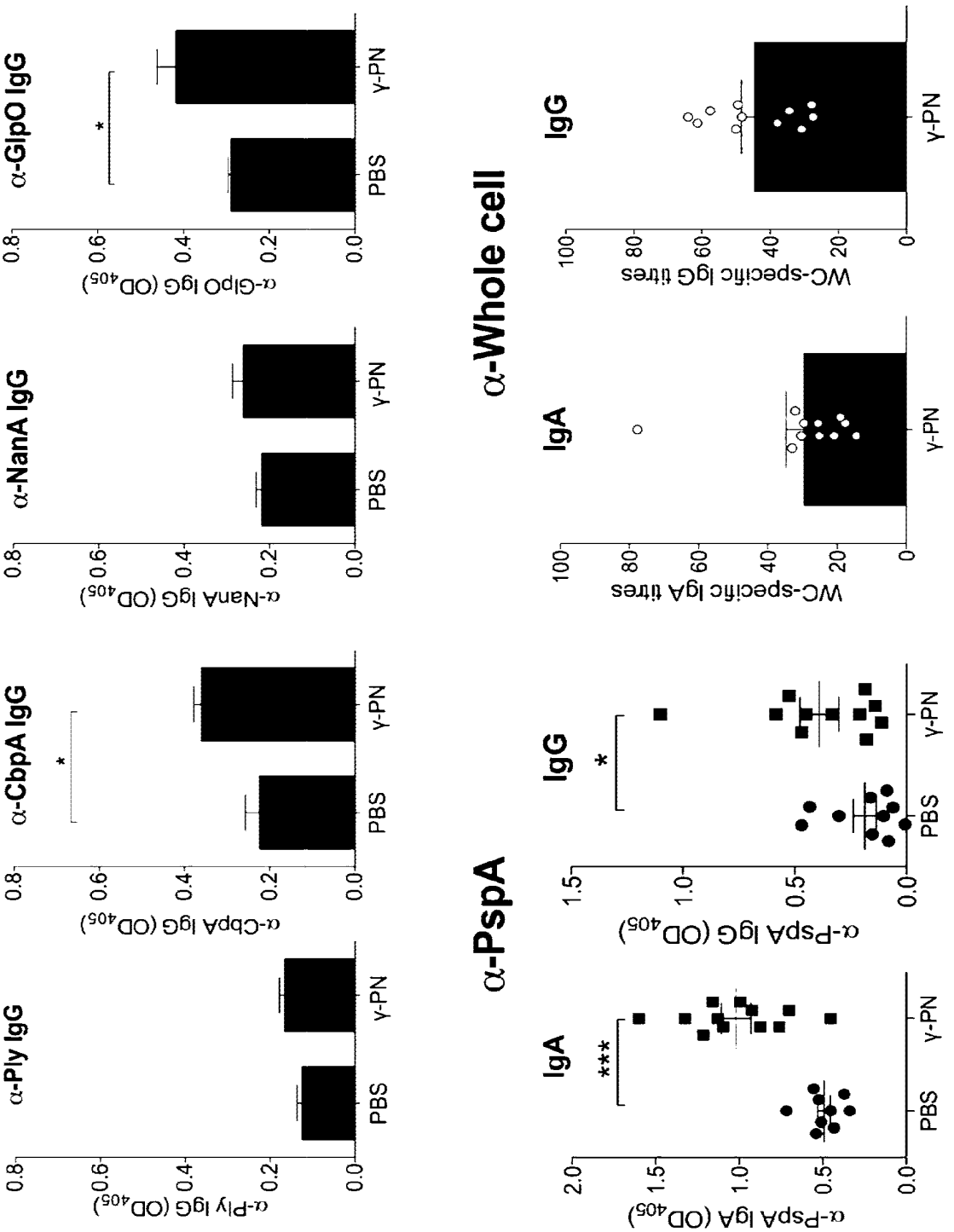


FIGURE NINE

Petitioner Merck Ex-1004, 1074

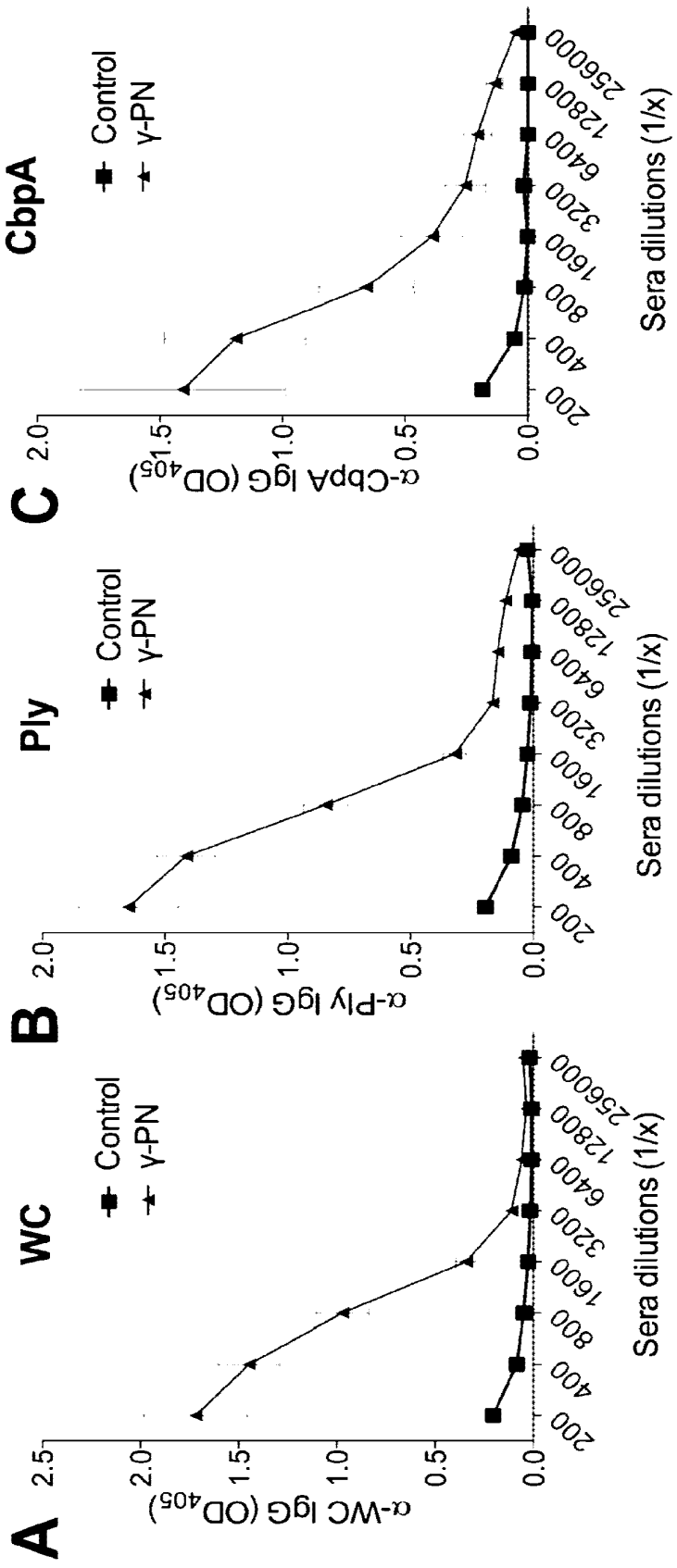


FIGURE TEN

Petitioner Merck Ex-1004, 1075

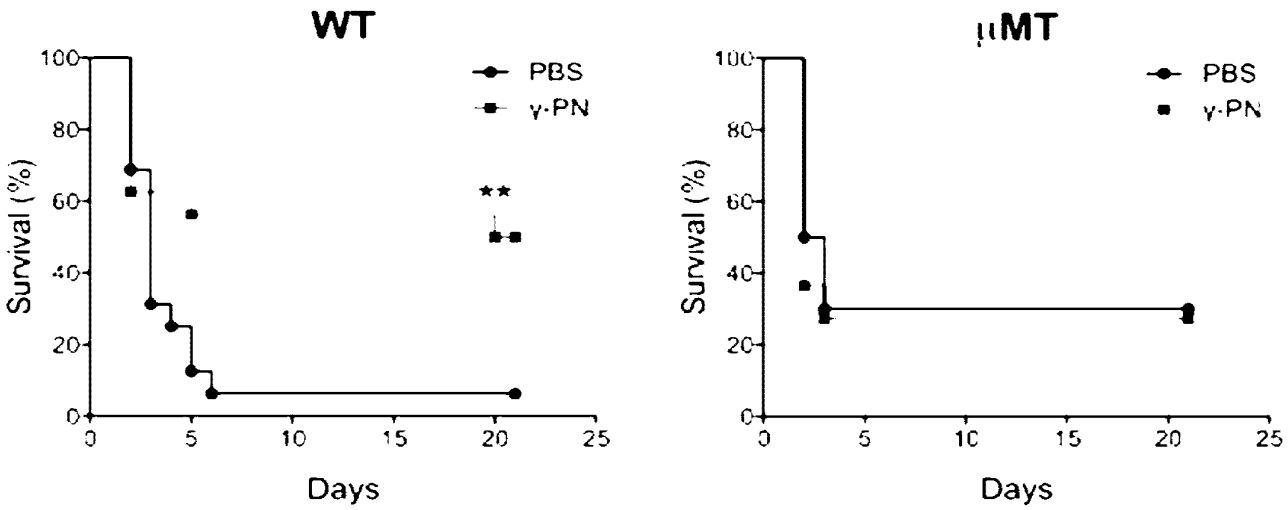


FIGURE ELEVEN

Petitioner Merck Ex-1004, 1076

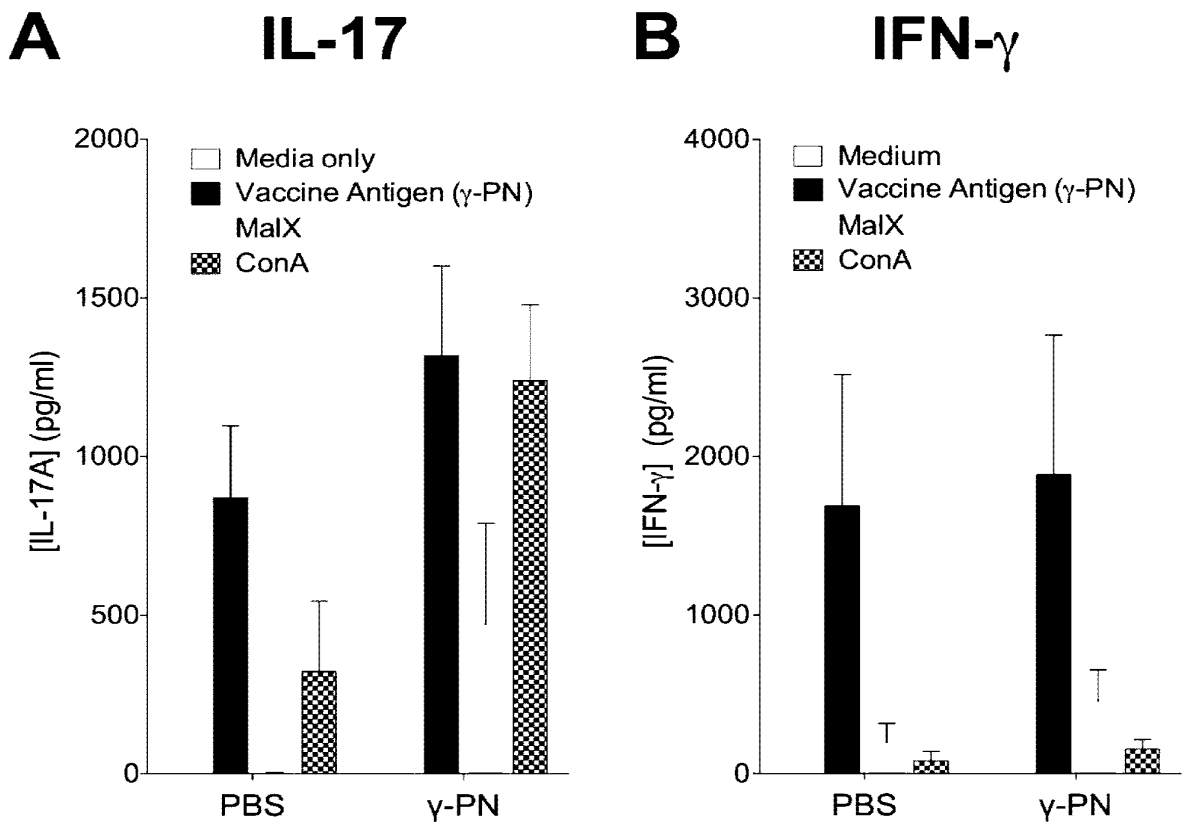


FIGURE TWELVE

Petitioner Merck Ex-1004, 1077

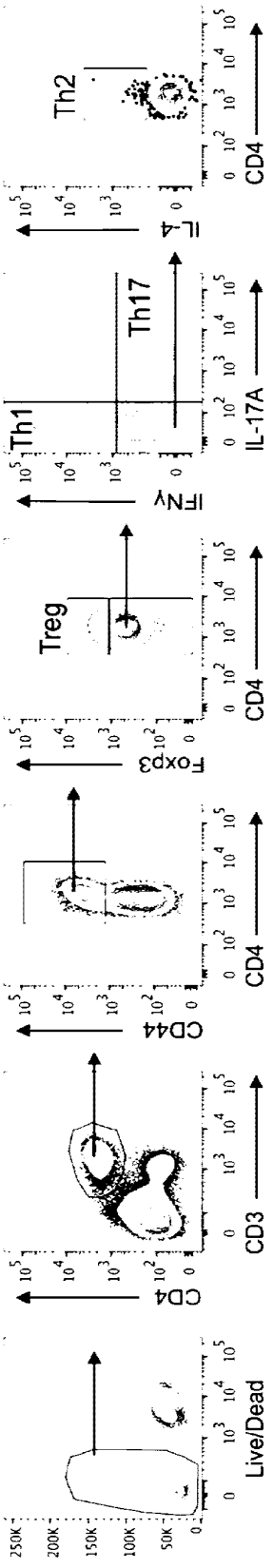


FIGURE TWELVE (CONTINUED)

Petitioner Merck Ex-1004, 1078

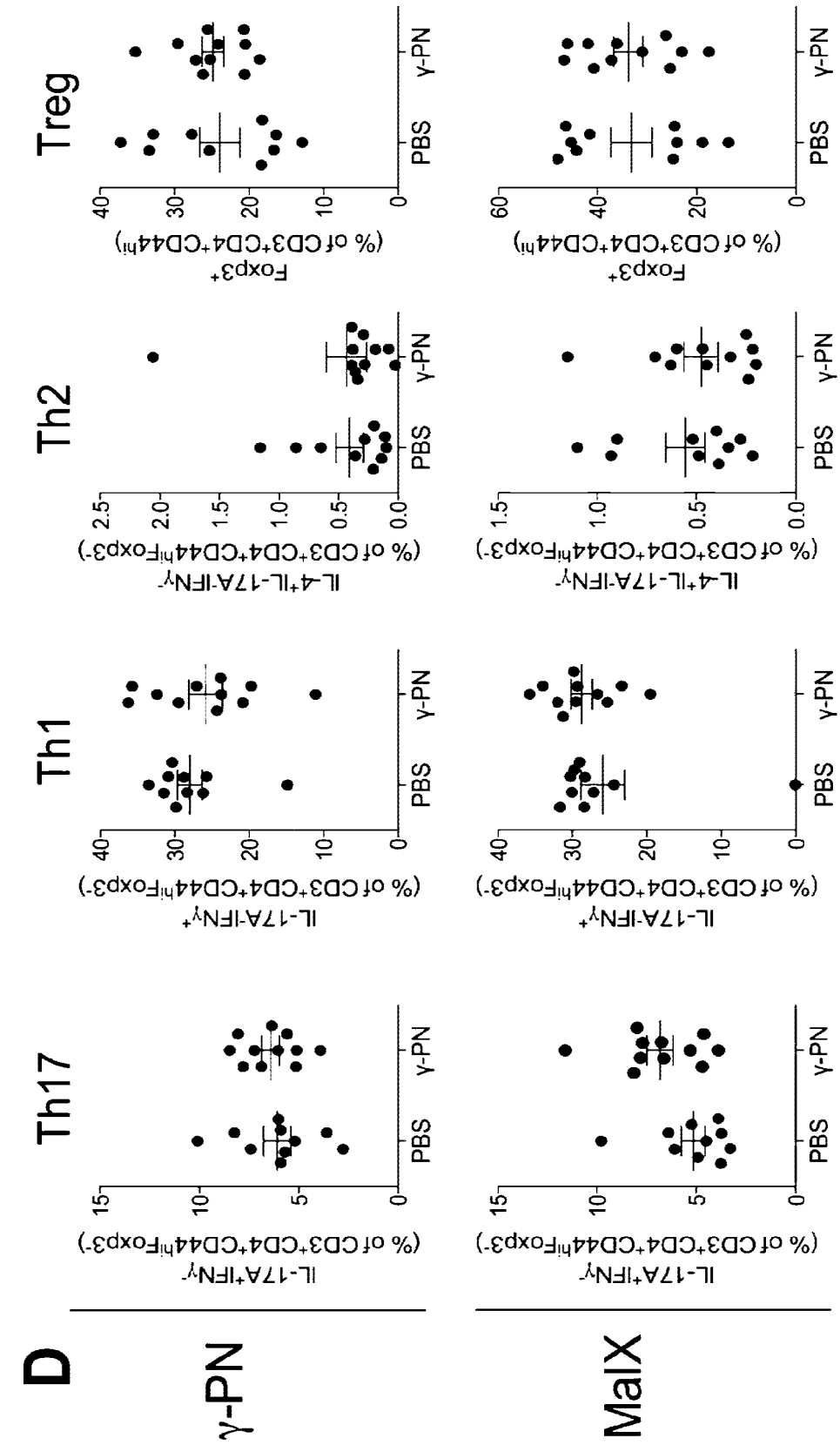


FIGURE TWELVE (CONTINUED)

Petitioner Merck Ex-1004, 1079

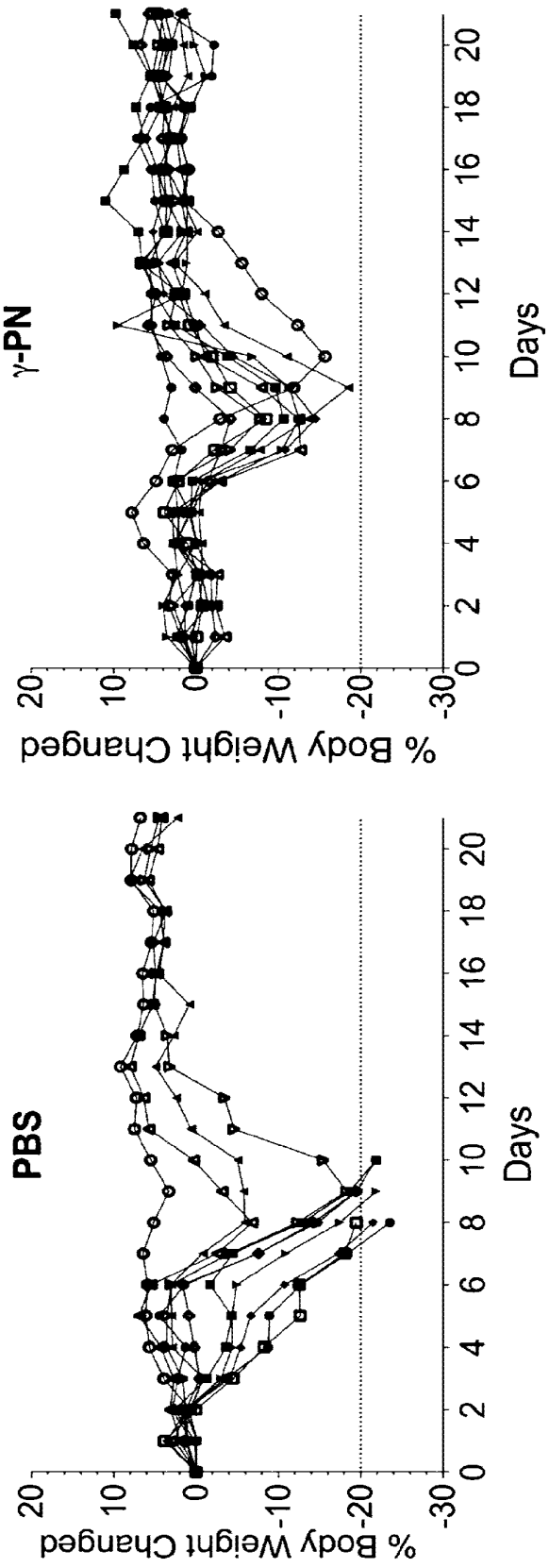


FIGURE THIRTEEN

Petitioner Merck Ex-1004, 1080

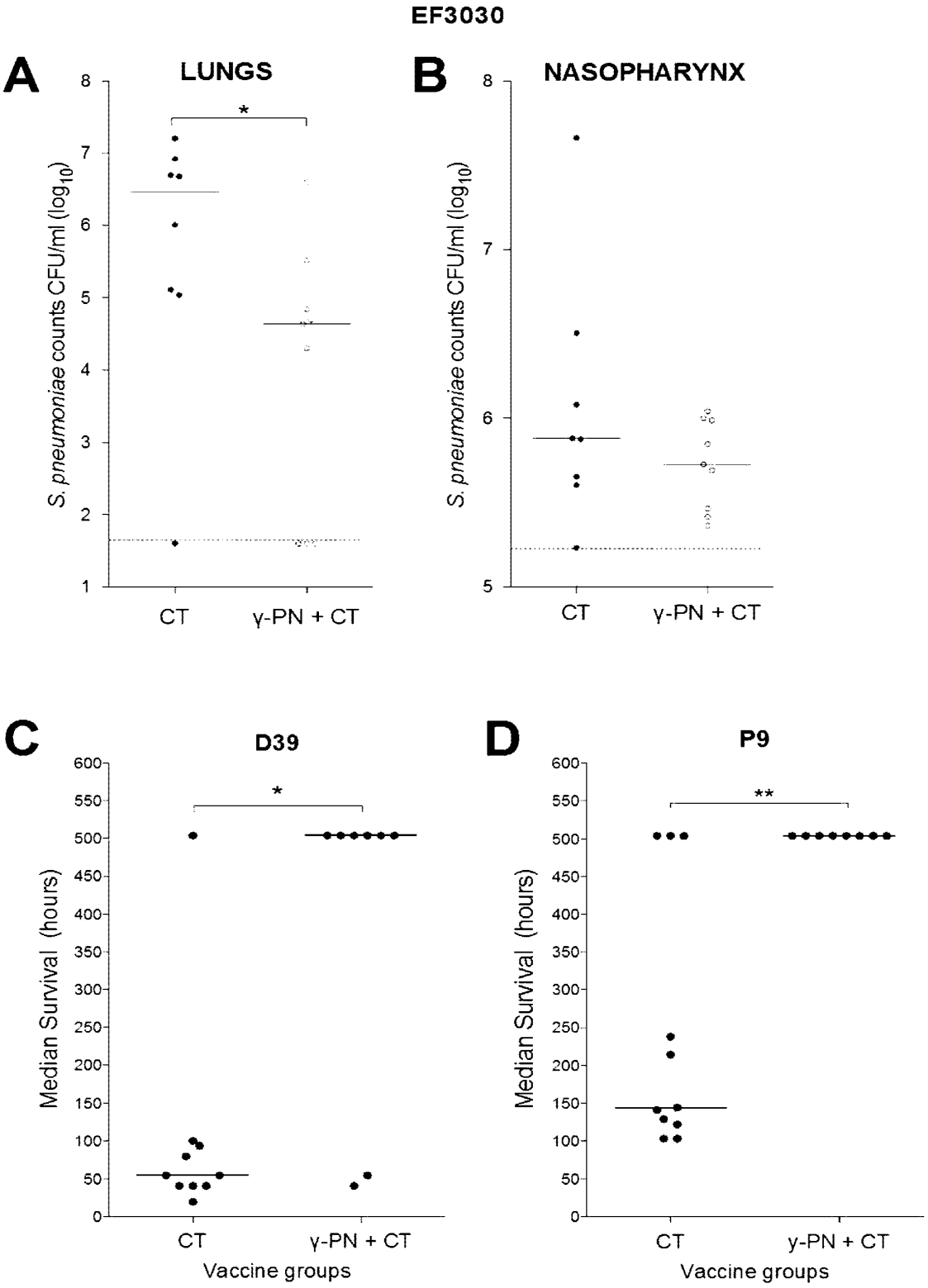


FIGURE FOURTEEN

Petitioner Merck Ex-1004, 1081

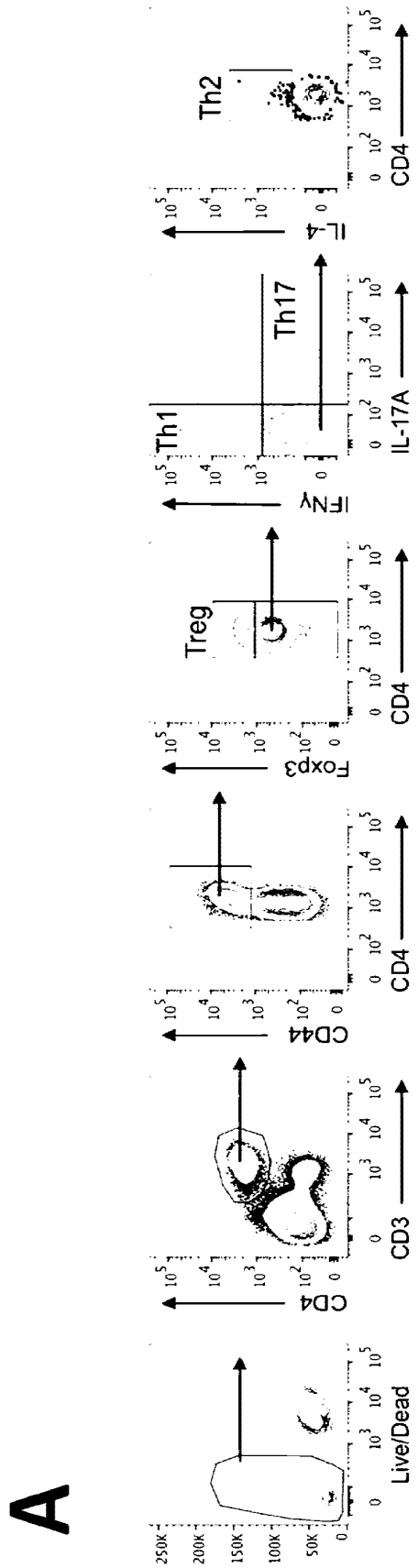


FIGURE FIFTEEN

Petitioner Merck Ex-1004, 1082

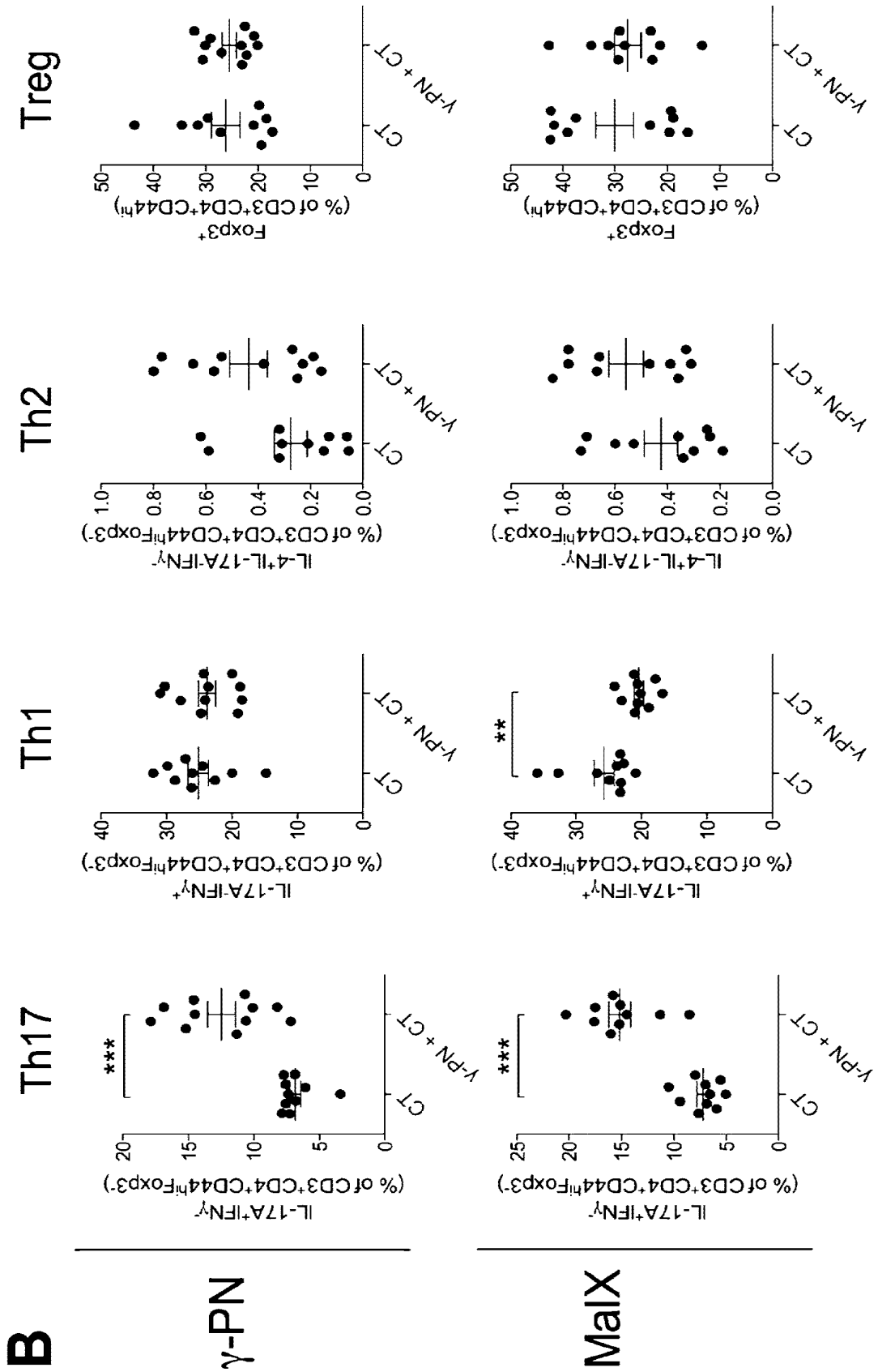


FIGURE FIFTEEN (CONTINUED)

Petitioner Merck Ex-1004, 1083

C

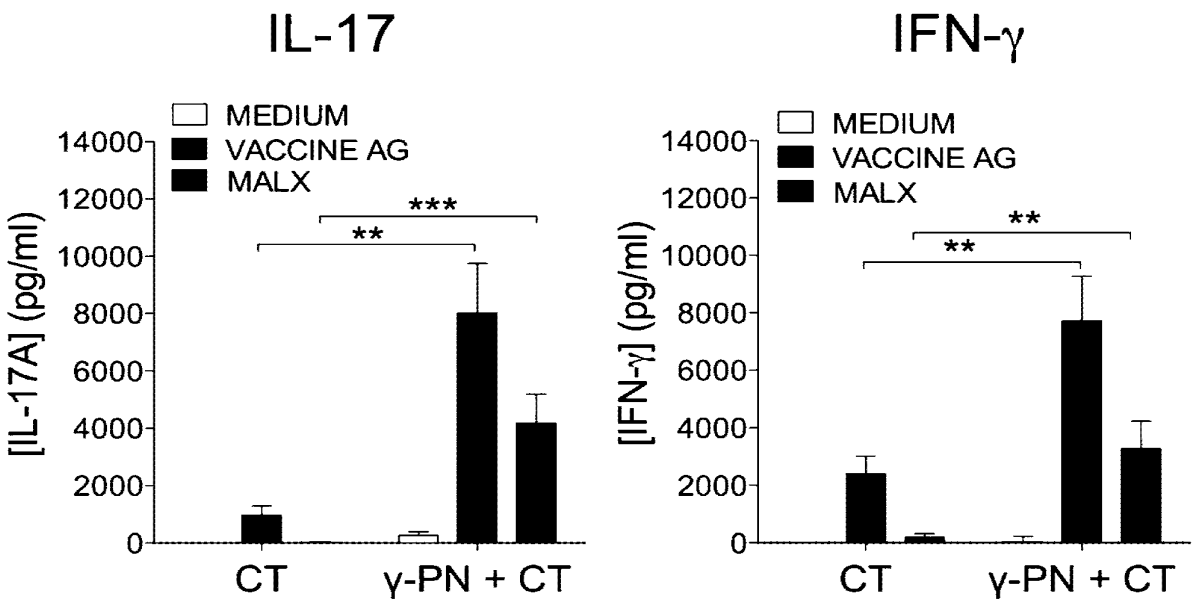


FIGURE FIFTEEN (CONTINUED)

Petitioner Merck Ex-1004, 1084

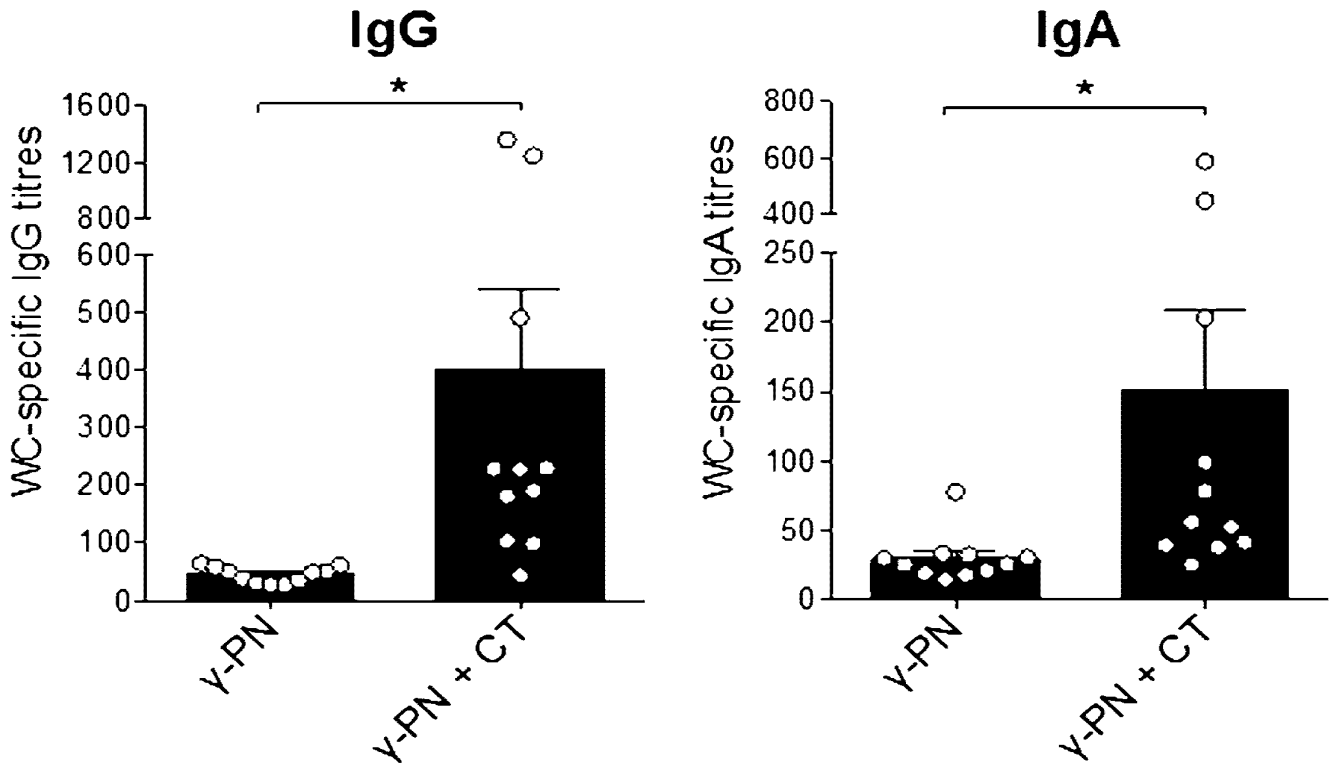


FIGURE SIXTEEN

Petitioner Merck Ex-1004, 1085

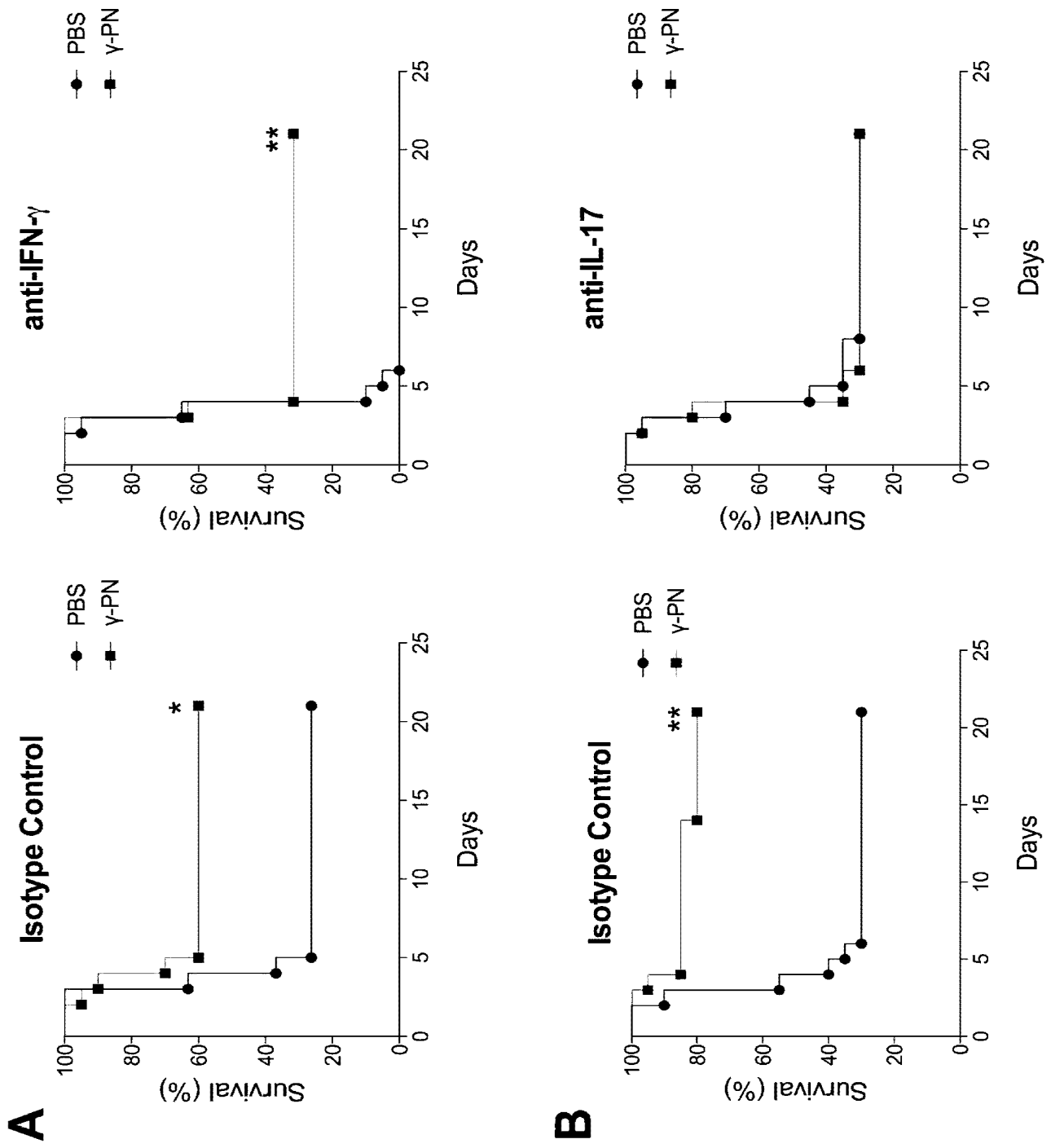


FIGURE SEVENTEEN

Petitioner Merck Ex-1004, 1086

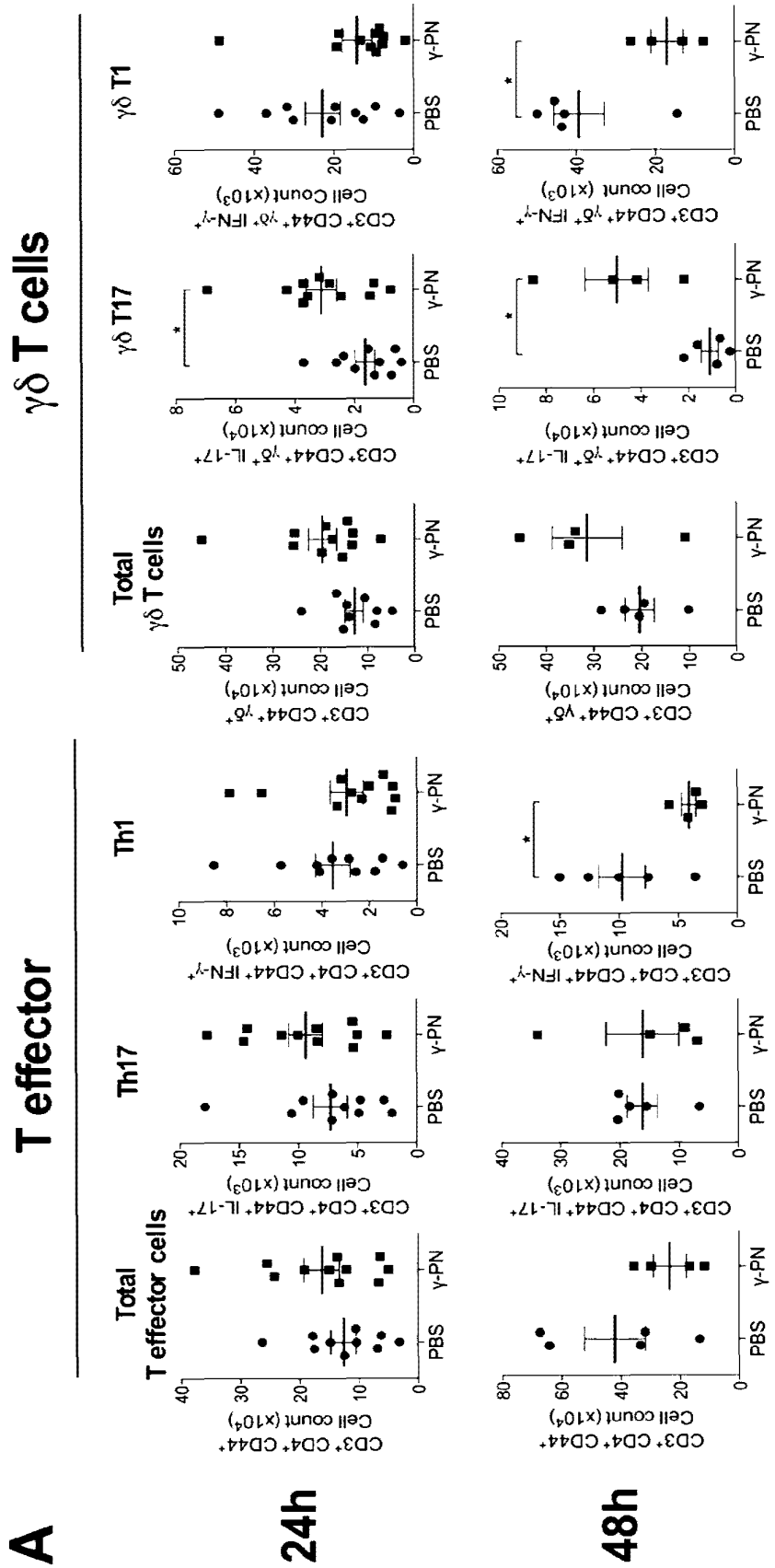


FIGURE EIGHTEEN

Petitioner Merck Ex-1004, 1087

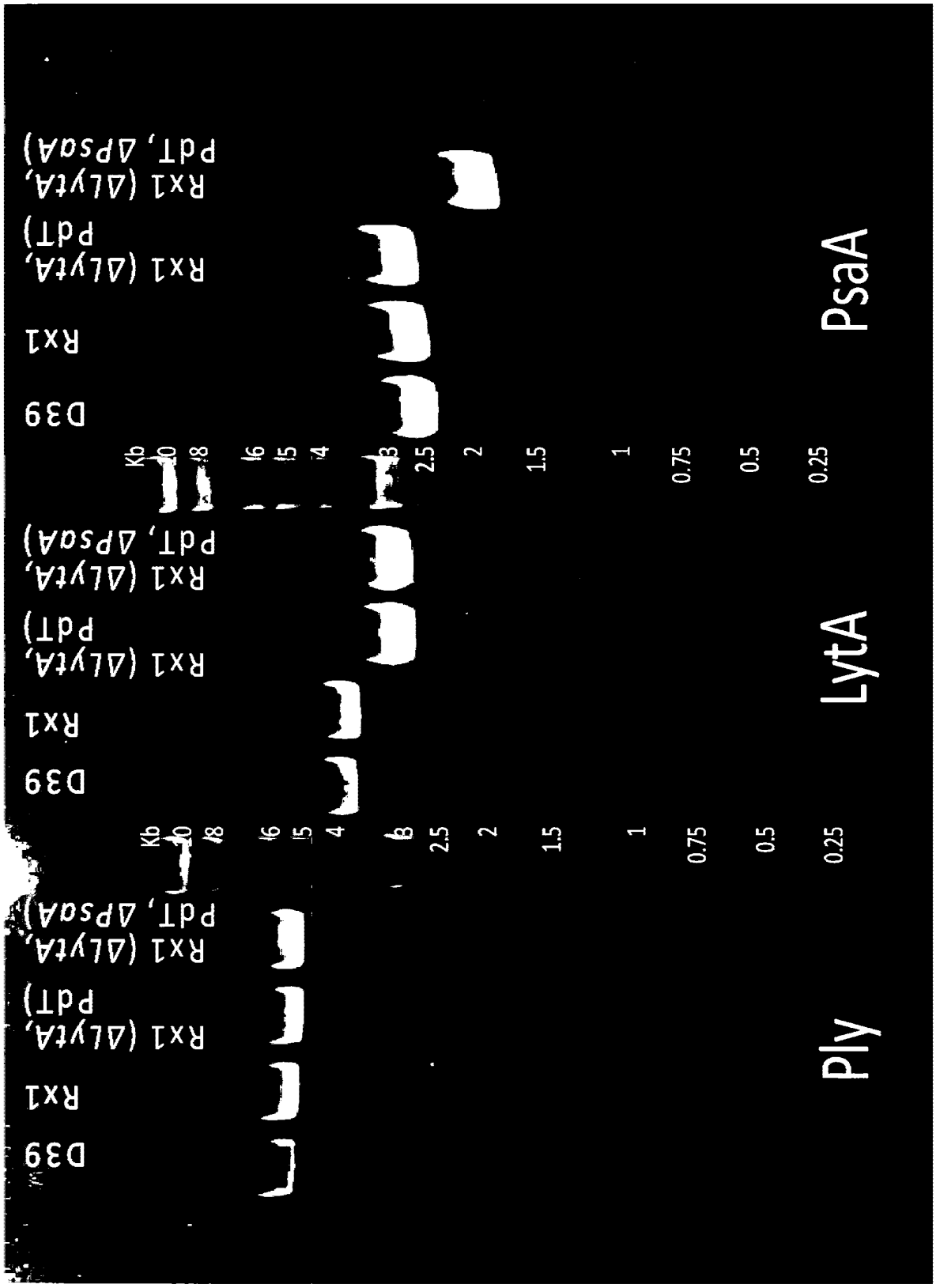


FIGURE NINETEEN

Petitioner Merck Ex-1004, 1088

B

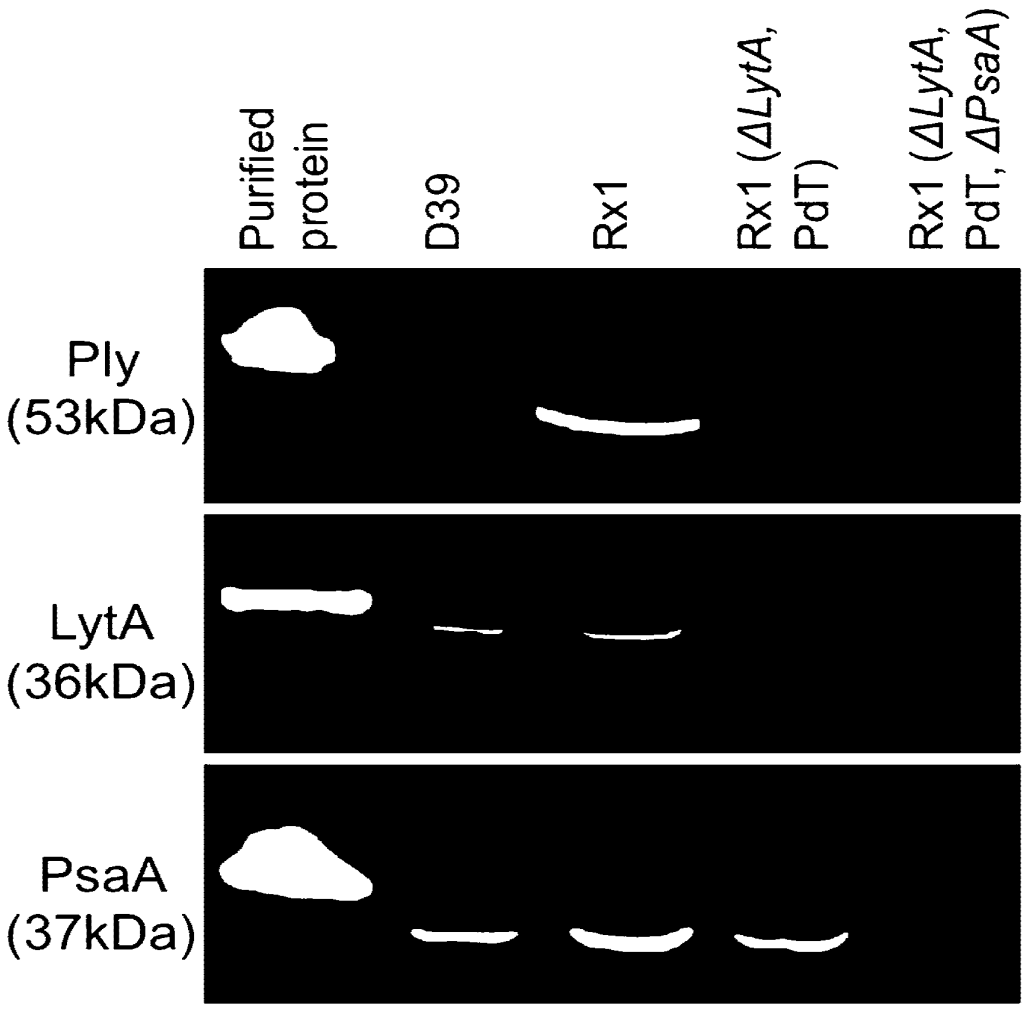


FIGURE NINETEEN (CONTINUED)

Petitioner Merck Ex-1004, 1089

C

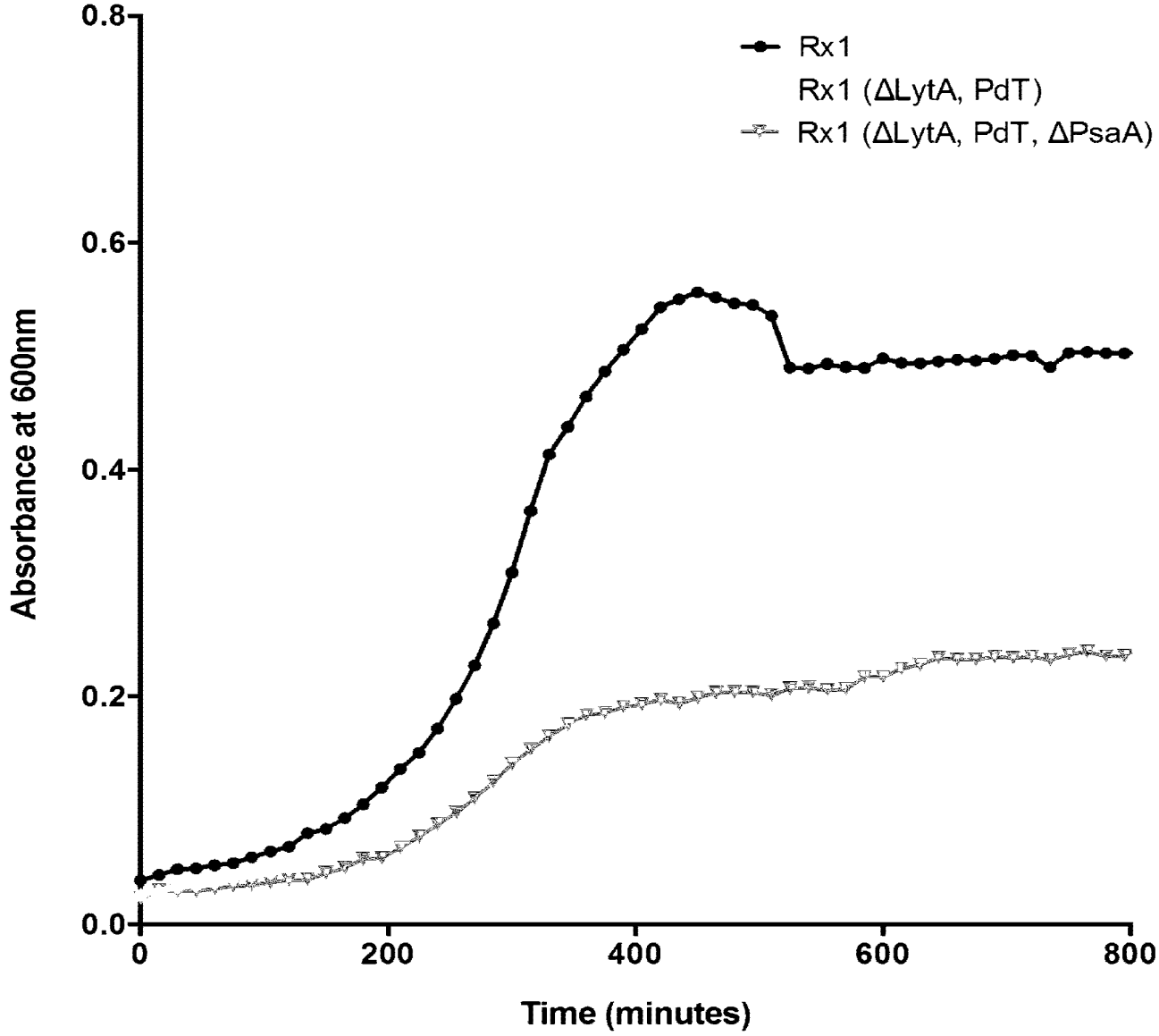


FIGURE NINETEEN (CONTINUED)

Petitioner Merck Ex-1004, 1090

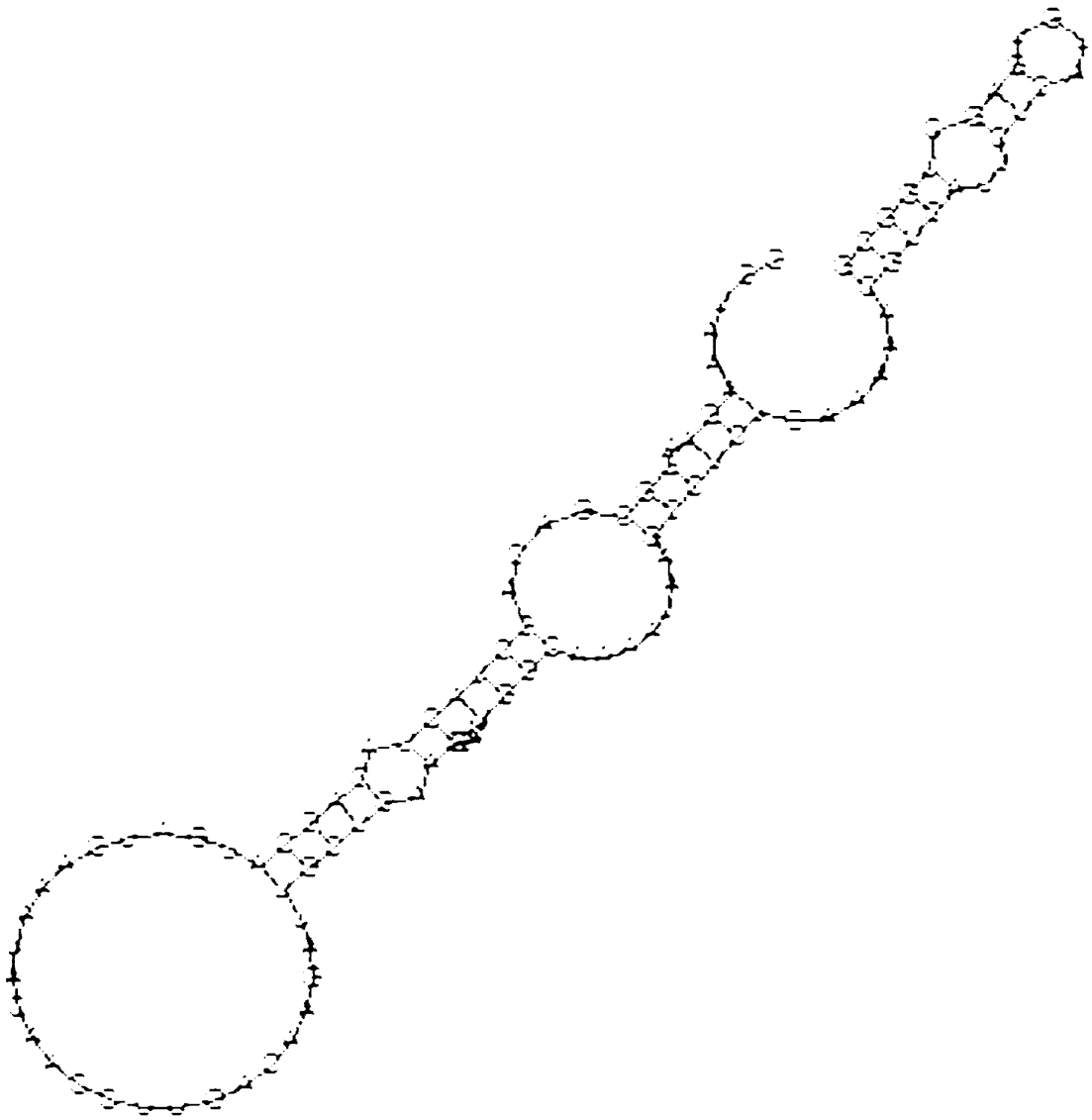


FIGURE TWENTY

Petitioner Merck Ex-1004, 1091

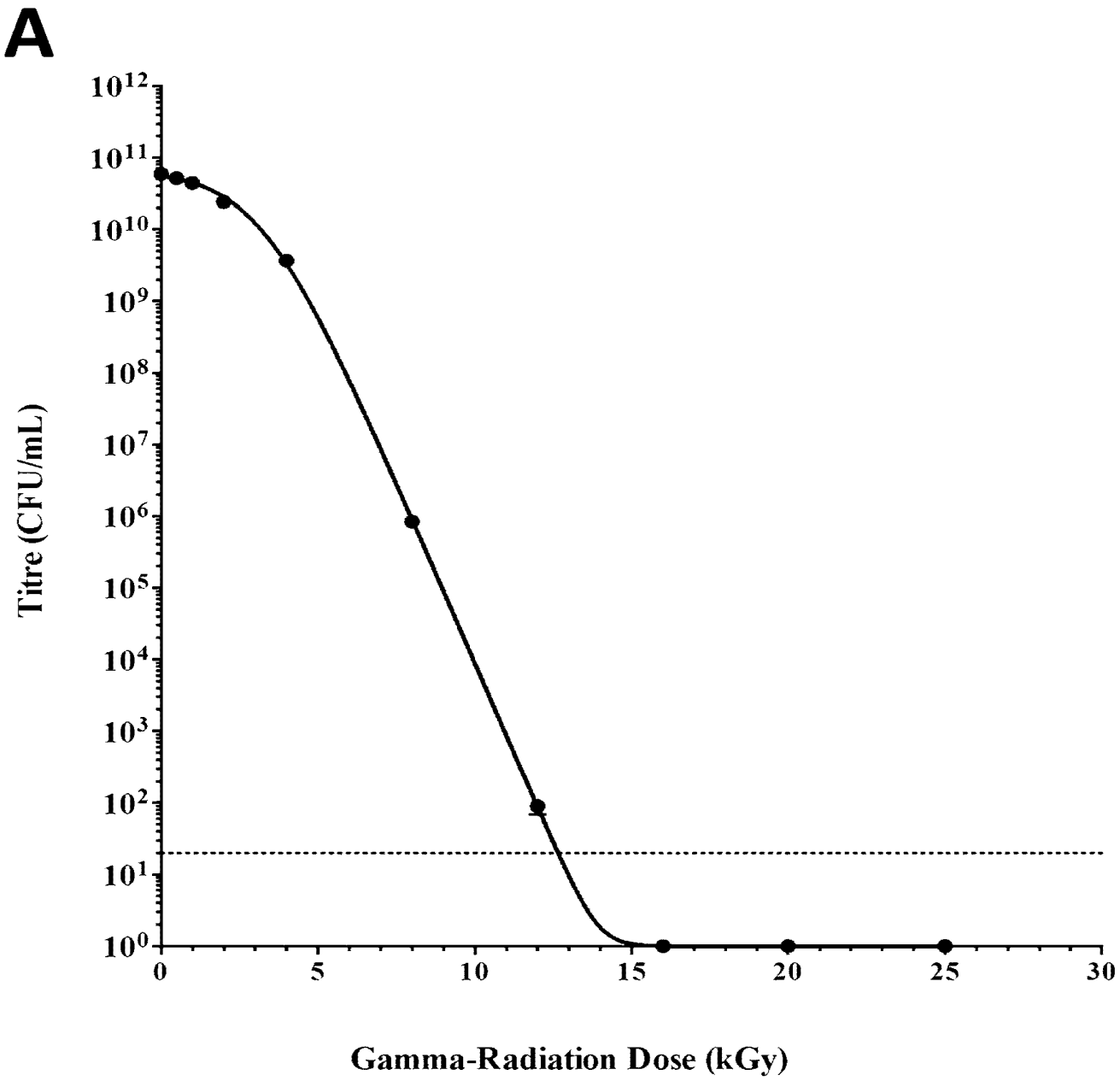


FIGURE TWENTY-ONE

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FIGURE TWENTY-ONE (CONTINUED)

Petitioner Merck Ex-1004, 1093

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2016/050231

A. CLASSIFICATION OF SUBJECT MATTER

A61K 39/09 (2006.01) A61P 11/00 (2006.01) A61P 31/04 (2006.01)

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)

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)

ESPACENET, PUBMED, Internal databases provided by IP Australia (INTESS AND PAMS NOSE), and keywords (Gamma Vaccines, Babb, Alsharifi, Ogunniyi)

WPIAP, EPODOC, MEDLINE, BIOSIS, EMBASE, and keywords (irradiated, gamma ray, X ray, streptococcus, mutant, DNA repair, double stranded RNA, Rx1 strain, and related terms)

GENOMEQUEST and sequences (SEQ ID NOs: 2 and 5)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

[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
6 June 2016	06 June 2016
Name and mailing address of the ISA/AU	**Authorised officer**
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustralia.gov.au	Margaret Chang AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. 0262832631

Form PCT/ISA/210 (fifth sheet) (July 2009)

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2016/050231

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)

   [ ] on paper

   [X] in electronic form

   b. (time)

   [ ] in the international application as filed

   [X] together with the international application in electronic form

   [ ] subsequently to this Authority for the purposes of search

2. [ ] 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 in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

Petitioner Merck Ex-1004, 1095

INTERNATIONAL SEARCH REPORT C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		International application No. PCT/AU2016/050231
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MURRAY, M. "THE ANTIGENIC PROPERTIES OF STREPTOCOCCI KILLED BY ULTRAVIOLET LIGHT", *Journal of Laboratory and Clinical Medicine,* 1939, Volume 24, Pages 1245-1246 Whole document	1-11, 28-34
X	TUASIKAL, B.J.,"Kandidat vaksin iradiasi *Streptococcus agalactiae* untuk pencegahan mastitis subklinis pada ruminansia *{Streptococcus agalactiae* irradiated vaccine candidate for subclinical mastitis prevention in ruminants]" (Doctoral dissertation), 2012, [retrieved from the internet on 2 June 2016] <URL: http://repository.ipb.ac.id/handle/123456789/61095> Abstract (in English)	1, 6, 10, 28-34, 37
Y	LU, Y.-J., *et al.,* "Options for Inactivation, Adjuvant, and Route of Topical Administration of a Killed, Unencapsulated Pneumococcal Whole-Cell Vaccine", *Clinical and Vaccine Immunology,* June 2010, Volume 17, Number 6, Pages 1005-1012 Whole document; Abstract; Page 1006	1-63
Y	DATTA, S.K., *et al.,* "Vaccination with Irradiated *Listeria* Induces Protective T Cell Immunity", *Immunity,* July 2006, Volume 25, Issue 1, Pages 143-152 Whole document; Page 149	1-63
Y	WO 2012/100302 A1 (GAMMA VACCINES PTY LIMITED) 02 August 2012 Abstract; Examples; Page 33	1-63
A	MALLEY, R., *et al.,* "Intranasal Immunization with Killed Unencapsulated Whole Cells Prevents Colonization and Invasive Disease by Capsulated Pneumococci", *Infection and Immunity,* August 2001, Volume 69, Number 8, Pages 4870-4873 Whole document	1-63
A	BERRY, A.M., *et al.,* "Comparative Virulence of *Streptococcus pneumoniae* Strains with Insertion-Duplication, Point, and Deletiion Mutations in the Pneumolysin Gene", *Infection and Immunity,* February 1999, Volume 67, Number 2, Pages 981-985 Whole document	1-63
A	JOHNSON, M., *et al.,* "Murine Model of Interleukin-8-Induced Otitis Media", *The Laryngoscope,* October 1997, Volume 107, Number 10, Pages 1405-1408 Whole document	1-63
T	BABB, R. *et al.,* "Intranasal vaccination with gamma-irradiated *Streptococcus pneumoniae* whole-cell vaccine provides serotype-independent protection mediated by B-cells and innate IL-17 responses", *Clinical Science,* published online 1 February 2016, Volume 130, Number 9, Pages 697-710 Whole document	1-63

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INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/AU2016/050231**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		**Patent Family Member/s**	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
WO 2012/100302 A1	02 August 2012	WO 2012100302 A1	02 Aug 2012
		AU 201221 1043 A1	11 Jul 2013
		CA 2825403 A1	02 Aug 2012
		EP 2667891 A1	04 Dec 2013
		US 2014079732 A1	20 Mar 2014

End of Annex

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

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(54) Title: VACCINE

(57) Abstract: The present invention is in the field of pneumococcal capsular saccharide conjugate vaccines. Specifically, the present invention relates to sized Streptococcus pneumoniae serotype 6A capsular polysaccharides, in particular Streptococcus pneumoniae serotype 6A capsular polysaccharides having the average size (e.g. M_w) of the Streptococcus pneumoniae serotype 6A capsular polysaccharide is between 100-1000 kDa, suitably conjugated to a carrier protein.

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VACCINE

Field of the Invention

The present invention relates to sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharides, in particular *Streptococcus pneumoniae* serotype 6A capsular polysaccharides having an average size (e.g. M_w) between 100-1000, 110-750, 150-500, 180-600, 210-490, 210-450, 180-400, 210-400, 210-370, 220-360, 230-350, 240-340, 240-320, 240-310 or 250-310 kDa. It additionally relates to sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharides conjugated to a carrier protein, immunogenic compositions, vaccines and processes for making the sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharides. It also relates to the use of the immunogenic compositions and vaccines of the invention in therapy and methods of immunising against *Streptococcus pneumoniae* infection.

Background of the Invention

Streptococcus pneumoniae (S. pneumoniae) is a Gram-positive bacterium responsible for considerable morbidity and mortality (particularly in infants and the elderly), causing invasive diseases such as bacteraemia and meningitis, pneumonia and other non-invasive diseases, such as acute otitis media. About 800,000 children die annually due to pneumococcal disease, especially in emerging countries (O-Brien *et al.* 2009 Lancet 374:893-902). The increasing number of antibiotic-resistant strains (Linares *et al.* 2010 Cin. Microbiol. Infect. 16:402-410) and the severity of pneumococcal diseases make vaccination the most effective intervention.

The major clinical syndromes caused by S. *pneumoniae* are widely recognized and discussed in all standard medical textbooks (Fedson DS, Muscher DM. In: Plotkin SA, Orenstein WA, editors. Vaccines. 4th edition. Philadelphia WB Saunders Co, 2004a: 529-588). For instance, Invasive pneumococcal disease (IPD) is defined as any infection in which S. *pneumoniae* is isolated from the blood or another normally sterile site (Musher DM. Streptococcus pneumoniae. In Mandell GL, Bennett JE, Dolin R (eds). Principles and Practice of Infectious diseases (5th ed). New York, Churchill Livingstone, 2001 , p2128-2147). Chronic obstructive pulmonary disease (COPD) is recognised as encompassing several conditions (airflow obstruction, chronic bronchitis, bronchiolitis or small airways disease and emphysema) that often coexist (Wilson et al., Eur. Respir. J. 2001 ; 17: 995-1007). Patients suffer exacerbations of their condition that are usually associated with increased breathlessness, and often have increased cough that may be productive of mucus or purulent sputum (Wilson, Eur Respir J 2001 17:995-1007). COPD is defined

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physiologically by the presence of irreversible or partially reversible airway obstruction in patients with chronic bronchitis and/or emphysema (Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease. American Thoracic Society. Am J Respir Crit Care Med. 1995 Nov;152(5 Pt 2):S77-121). Exacerbations of COPD are often caused by bacterial (e.g. pneumococcal) infection (Sethi S, Murphy TF. Bacterial infection in chronic obstructive pulmonary disease in 2000: a state-of-the-art review. Clin Microbiol Rev. 2001 Apr;14(2):336-63).

Pneumococcus is encapsulated with a chemically linked polysaccharide which confers serotype specificity. There are more than 90 known serotypes of pneumococci, and the capsule is the principle virulence determinant for pneumococci, as the capsule not only protects the inner surface of the bacteria from complement, but is itself poorly immunogenic. An anti-polysaccharide antibody level has been regarded as predictive of the protection against invasive pneumcoccal disease (Jodar et al. Vaccine, (21) 2003, p. 3264-3272). After initial licensure of a 7-valent conjugate vaccine containing serotypes 4, 6B, 9V, 14, 18C, 19F, 23F (PCV7), two pneumococcal conjugate vaccines (PCVs) designed to broaden coverage have been licensed. The 10-valent pneumococcal *Haemophilus influenzae* protein D conjugate vaccine (PCV10) contains serotypes 1, 4, 5, 6B, 7F, 9V, 14 and 23F conjugated to nontypeable *H. influenzae* protein D, plus serotype 18C conjugated to tetanus toxoid and serotype 19F conjugated to diphtheria toxoid. The 13-valent pneumococcal conjugate vaccine (PCV13) contains the PCV7 (4, 6B, 9V, 14, 18C, 19F, 23F) serotypes plus serotypes 1, 3, 5, 6A, 7F and 19A, conjugated to cross-reactive material CRM197.

It is an object of the present invention to develop improved *Streptococcus pneumoniae* polysaccharides and improved *Streptococcus pneumoniae* polysaccharide conjugate vaccines.

S. *pneumoniae* serogroup 6 isolates, including isolates of serotypes 6A, 6B, 6C, and 6D, are important because they are commonly found in infections (Song *et al.* JOURNAL OF CLINICAL MICROBIOLOGY, May 201 1, p. 1758-1764).

WO2009/000826A2 discloses a *Streptococcus pneumoniae* serotype 6A capsular polysaccharide having a size 1100-1540 (Da x 10^3) conjugated to protein D and a *Streptococcus pneumoniae* serotype 6B capsular polysaccharide having a size 1069-1391 (Da x 10^3) conjugated to protein D (see Table 2 of WO2009/000826A2). Both the *Streptococcus pneumoniae* serotype 6A capsular polysaccharide and the *Streptococcus pneumoniae* serotype 6B capsular polysaccharide described in WO2009/000826A2 were native polysaccharides. The chemical structure of serotypes 6A and 6B only differs by the link between rhamnose and ribitol units. As the structure of polysaccharide 6A is very

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similar to polysaccharide 6B it was originally thought that a native PS6A (polysaccharide 6A) should be used for conjugation as a native PS6B (polysaccharide 6B) is used for conjugation. In the FinIP trial, PHiD-CV10 containing 6B conjugated to protein D was demonstrated to be effective against serotype 6B (Palmu *et al.* Lancet 2013; 381 : 214-22). However, surprisingly, the present invention provides a sized *Streptococcus pneumoniae* 6A polysaccharide with improved properties. The inventors have found that by using a *Streptococcus pneumoniae* serotype 6A capsular polysaccharide (PS6A) of a particular size, a conjugate may be obtained having both high immunogenicity and which is filterable.

Brief description of Figures:

Figure 1 Evaluation of PS6A conjugates in 14-valent (14V) AIP04 formulation in the Balb/c mouse with co- administration of Infanrix™ Hexa model. ELISA anti-PS6A.

Figure 2(A) Immunogenicity of PS6A-CRM197 in the Balb/c mouse model ELISA results

Figure 2(B) Immunogenicity of PS6A-CRM197 in the Balb/c mouse model OPA results

Figure 3(A) Evaluation of PS6A-CRM197 ELISA results

Figure 3(B) Evaluation of PS6A-CRM197 OPA results

Description of the Invention

The present invention provides a sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide. In one aspect, the present invention provides a *Streptococcus pneumoniae* serotype 6A capsular polysaccharide wherein the average size (M_w) of the S. *pneumoniae* serotype 6A polysaccharide is between 100-1000, 110-750, 150-500, 180-600, 210-490, 210-450, 180-400, 210-400, 210-370, 220-360, 230-350, 240-340, 240-320, 240-310 or 250-310 kDa.

The term "polysaccharide" throughout this specification refers to a complex carbohydrate composed of a chain of saccharides joined together by glycosidic bonds. The polysaccharide may contain at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40 or 50 or more saccharides.

For the purposes of the invention, "native polysaccharide" refers to a polysaccharide that has not been subjected to a process (e.g. post-purification), the purpose of which is to reduce the size of the polysaccharide. A polysaccharide can become slightly reduced in size during normal purification procedures or by degradation during conjugation. Such a

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saccharide is still native. Only if the polysaccharide has been subjected to sizing techniques would the polysaccharide not be considered native.

For the purposes of the invention, "sized polysaccharide" refers to a polysaccharide that has been subjected to a process (e.g. post-purification), the purpose of which is to reduce the size of the polysaccharide. Polysaccharides may be sized by mechanical or chemical sizing techniques. Mechanical sizing techniques that may be used include high pressure techniques (such as microfluidization , Emulsiflex™, high pressure homogenization , or Gaulin homogenization) and sonication. Chemical sizing techniques that may be used include acid hydrolysis (e.g. treatment with acetic acid) or treatment with periodate. The term "periodate" includes both periodate and periodic acid ; the term also includes both metaperiodate (IOv) and orthoperiodate ($IO\beta^{5-}$) and the various salts of periodate (e.g., sodium periodate and potassium periodate). The molecular weight ranges described herein refer to purified polysaccharides before conjugation (e.g. before activation where activation is carried out).

For the purposes of the invention , "sized by a factor up to x2" means that the saccharide is subject to a process intended to reduce the size of the saccharide but to retain a size more than half the size of the native polysaccharide. Terms such as "sized by a factor up to" x3, x4 etc. are to be interpreted in the same way, i.e. the saccharide is subject to a process intended to reduce the size of the polysaccharide but to retain a size more than a third, a quarter etc., respectively, the size of the native polysaccharide.

The term "Molecular weight" or "average molecular weight" or "average size" of a polysaccharide as used herein refers to the weight-average molecular weight (M_w) of the polysaccharide measured prior to conjugation measured by MALLS (Multi-Angle Laser Light Scattering).

The MALLS technique is known in the art and is typically carried out as described in below. For MALLS analysis of pneumococcal polysaccharides, two columns (TSKG6000 and 5000PWxl) may be used in combination and the polysaccharides are eluted in 0.2M NaCl. Polysaccharides are detected using a light scattering detector (for instance Wyatt Dawn DSP (Digital Signal Processing) equipped with a 10mW argon laser at 488nm) and an interferometric refractometer (for instance Wyatt Otilab DSP (Digital Signal Processing) equipped with a P100 cell and a red filter at 498nm).

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The laser light scattering detector measures the light intensities scattered at 16 angles by the macromolecular solution and on the other hand, an interferometric refractometer placed on-line allows the determination of the quantity of sample eluted. From these intensities, the size and shape of the macromolecules in solution can be determined.

The mean molecular weight in weight (M_w) is defined as the sum of the weights of all the species multiplied by their respective molecular weight and divided by the sum of weights of all the species.

a) Weight-average molecular weight: -M_w-

$$M_w = \frac{\sum W_i . M_i}{\sum W_i} = \frac{m_2}{m_1}$$

b) Number-average molecular weight: -M_n-

$$M_n = \frac{\sum N_i . M_i}{\sum N_i} = \frac{m_1}{m_0}$$

c) Root mean square radius: -Rw- and r^2w is the square radius defined by:

$$R^2w \text{ or } (r^2)w = \frac{\sum m_i . r_i^2}{\sum m_i}$$

(-mi- is the mass of a scattering centre i and -n- is the distance between the scattering centre i and the center of gravity of the macromolecule).

d) The polydispersity is defined as the ratio -M_w / M_n-.

As used herein, the term "treatment" (including variations thereof, e.g. "treat" or "treated") means any one or more of the following: (i) the prevention of infection or re-infection, as in a traditional vaccine, (ii) the reduction in the severity of, or, in the elimination of symptoms, (iii) the delay in recurrence of symptoms, and (iii) the substantial or complete elimination

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of the pathogen or disorder in question in a subject. Hence, treatment may be effected prophylactically (prior to infection) or therapeutically (following infection).

For the purposes of this invention, "treatment or prevention of exacerbations of COPD" or "reduction in severity of COPD exacerbations" refers to a reduction in incidence or rate of COPD exacerbations (for instance a reduction in rate of 0.1 , 0.5, 1, 2, 5, 10, 20% or more) or a reduction in severity of COPD exacerbations (e.g. airflow obstruction, chronic bronchitis, bronchiolitis or small airways disease and emphysema), for instance within a patient group immunized with the immunogenic compositions or vaccines of the invention.

Sized *Streptococcus pneumoniae* Serotype 6A Capsular Polysaccharides

Streptococcus pneumoniae serotype 6A capsular polysaccharides may be sized by mechanical or chemical sizing techniques. In an embodiment, the *Streptococcus pneumoniae* serotype 6A capsular polysaccharides of the invention are sized by a chemical sizing technique. Chemical sizing techniques that may be used include treatment with acetic acid or treatment with periodate. In one aspect, the *Streptococcus pneumoniae* serotype 6A capsular polysaccharides of the invention are sized by treatment with periodate. The term "periodate" includes both periodate and periodic acid; the term also includes both metaperiodate (IO_4^-) and orthoperiodate ($IO6^{5-}$) and the various salts of periodate (e.g., sodium periodate and potassium periodate). In another aspect, the *Streptococcus pneumoniae* serotype 6A capsular polysaccharides of the invention are sized by treatment with acetic acid. In an embodiment, the *Streptococcus pneumoniae* serotype 6A capsular polysaccharides of the invention are sized by a mechanical sizing technique, for example using a high pressure technique. High pressure techniques include microfluidization, high pressure homogenization, or Gaulin homogenization. In one aspect, the *Streptococcus pneumoniae* serotype 6A capsular polysaccharide of the invention is sized by high pressure homogenization. High pressure homogenization achieves high shear rates by pumping the process stream through a flow path with sufficiently small dimensions. The shear rate is increased by using a larger applied homogenization pressure, and exposure time can be increased by recirculating the feed stream through the homogenizer. The technique of high pressure homogenization is described in Cho et al. *(Int. J. Mol. Sci.* **2014,** *15).* In another aspect of the invention, the *Streptococcus pneumoniae* serotype 6A capsular polysaccharide of the invention is sized by Gaulin homogenization. Gaulin homogenization may be carried out using the technique described in Lander *et al. (Biotechnol. Prog.* **2000,** *16,* 80-85). In another aspect, the

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Streptococcus pneumoniae serotype 6A capsular polysaccharide of the invention is sized by microfluidization (for example as described in the Examples below).

Sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharides of the present invention have an average size (M_w) between 100-1 000, 110-750, 150-500, 180-600, 210-490, 210-450, 180-400, 210-400, 210-370, 220-360, 230-350, 240-340, 240-320, 240-310 or 250-310 kDa. Sizing is by a factor of no more than x20, x10, x8, x6, x5, x4, x3 or x2. For example, sizing may be from a factor of between x2 to x6, x2 to x5, x2 to x4, or x3 to x6, x3 to x5 or x3 to x4. In one aspect, the *Streptococcus pneumoniae* serotype 6A capsular polysaccharide of the invention is sized by a factor of no more than x5. The molecular weight ranges described herein refer to molecular weight of the purified *Streptococcus pneumoniae* serotype 6A capsular polysaccharides before conjugation (e.g., before activation where activation is carried out).

In an embodiment, the sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide of the invention is antigenic (as determined by ELISA), for example having an antigenicity index of between 70 to 200 %, preferably between 90% and 150% (e.g. between 120 and 144%). As explained in the Examples below, the antigenicity index is measured relative to native *Streptococcus pneumoniae* serotype 6A capsular polysaccharide which is assigned an antigenicity index of 100% (also represented as 1.0 in the tables below).

Conjugated *Streptococcus pneumoniae* Serotype 6A Capsular Polysaccharides

Suitably, the sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide of the invention is conjugated to a carrier protein. The carrier protein may be selected from the group consisting of TT (tetanus toxoid), DT (diphtheria toxoid), CRM 197, fragment C of TT, PhtD (pneumococcal histidine triad protein D), PhtDE fusions (a fusion of PhtD and PhtE (pneumococcal histidine protein E) particularly those described in WO 01/98334 and WO 03/54007), detoxified pneumolysin and protein D. In one aspect of the invention, the sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide is conjugated to a carrier protein selected from the group consisting of TT (tetanus toxoid), DT (diphtheria toxoid), CRM197, fragment C of TT, and PhtD (pneumococcal histidine triad protein D). In another aspect of the invention, the sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide of the invention is conjugated DT or CRM197. In another aspect

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of the invention, the sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide of the invention is conjugated to CRM197.

CRM197 is a non-toxic form of the diphtheria toxin but is immunologically indistinguishable from the diphtheria toxin (DT). Genetically detoxified analogues of diphtheria toxin include CRM197 and other mutants described in US 4,709,017, US 5,843,71 1, US 5,601 ,827, and US 5,917,017. CRM197 is produced by *C. diphtheriae* infected by the nontoxigenic phase β197ioχ- created by nitrosoguanidine mutagenesis of the toxigenic carynephage b (Uchida et al Nature New Biology (1971) 233; 8-1 1). The CRM197 protein has the same molecular weight as the diphtheria toxin but differs from it by a single base change in the structural gene. This leads to a glycine to glutamine change of amino acid at position 52 which makes fragment A unable to bind NAD and therefore non-toxic (Pappenheimer 1977, Ann Rev, Biochem. 46; 69-94, Rappuoli Applied and Environmental Microbiology Sept 1983 p560-564).

Fragment C of TT is the non-toxic carboxy-terminal fragment of the tetanus toxin heavy chain. Tetanus toxin is a single peptide of approximately 150 kDa, which consists of 1315 amino-acid residues. Cleavage of tetanus-toxin by papain yields two fragments; one of them, fragment C, is approximately 50 kDa. Fragment C of TT is further described in Neubauer et al. *Biochim. Biophys.Acta* **1981** , 27, 141-148.

In an embodiment, the sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide of the invention is conjugated to the carrier protein via a linker, for instance a bifunctional linker (having two reactive ends). The linker is optionally heterobifunctional (having different reactive groups at either end) or homobifunctional (having identical reactive groups at either end of a spacer arm), for example a reactive amino group and a reactive carboxylic acid group, 2 reactive amino groups or two reactive carboxylic acid groups. The linker has for example between 4 and 20, 4 and 12, 5 and 10 carbon atoms. A possible linker is ADH (adipic acid dihydrazide). Other linkers include B-propionamido (WO 00/10599), nitrophenyl-ethylamine (Gever et al (1979) Med. Microbiol. Immunol. 165; 171-288), haloalkyl halides (US4057685), glycosidic linkages (US4673574, US4808700), hexane diamine and 6-aminocaproic acid (US4459286). In another embodiment, the sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide of the invention is directly conjugated to the carrier protein. In one aspect, the sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide of the invention is linked to carrier protein via an isourea link. An isourea link is formed by the reaction between a

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cyanate ester on polysaccharide and an amino group on the carrier. Reference to an "isourea link" herein refers to a stable link.

Immunogenic Compositions

In an embodiment, the present invention provides an immunogenic composition comprising a sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide (or conjugate) of the invention.

Typically the immunogenic compositions of the invention will comprise capsular polysaccharide antigens (suitably conjugated), wherein the polysaccharides are derived from at least ten serotypes of S. *pneumoniae.* The number of S. *pneumoniae* capsular polysaccharides can range from 10 different serotypes (or valences "v") to 23 different serotypes (23v, a 23 valent composition). In one embodiment the immunogenic composition comprises 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more or 15 or more capsular polysaccharides from different S. *pneumoniae* serotypes. In one embodiment, there are 10, 11, 12, 13, 14 or 15 different S. *pneumoniae* serotypes. In another embodiment, the immunogenic composition of the invention may comprise conjugated S. *pneumoniae* polysaccharides and unconjugated S. *pneumoniae* polysaccharides. In an embodiment, the total number of saccharide serotypes is less than or equal to 23.

In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F, although it is appreciated that one or two other serotypes could be substituted depending on the age of the recipient receiving the vaccine and the geographical location where the vaccine will be administered. In an embodiment, the vaccine may be an 11-valent vaccine. For example, a 11-valent vaccine may comprise polysaccharides from serotypes 1, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19F and 23F. In an embodiment, the vaccine may be an 12-valent or 13-valent vaccine. A 12 or 13-valent paediatric (infant) vaccine may also include the 11 valent formulation supplemented with serotypes 19A, or 22F, or 15, or 19A and 22F, or 19A and 15, or 22F and 15, whereas a 13-valent elderly vaccine may include the 11 valent formulation supplemented with serotypes 19A and 22F, 8 and 12F, or 8 and 15, or 8 and 19A, or 8 and 22F, or 12F and 15, or 12F and 19A, or 12F and 22F, or 15 and 19A, or 15 and 22F. In an embodiment, the vaccine may be a 14-valent or

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15-valent vaccine. A 14 or 15-valent paediatric vaccine may include the 11 valent formulation described above supplemented with serotypes 3, 19A and 22F; serotypes 8, 19A and 22F; serotypes 12F, 19A and 22F; serotypes 15, 19A and 22F; serotypes 3, 8, 19A and 22F; serotypes 3, 12F, 19A and 22F; serotypes 3, 15, 19A and 22F. In an embodiment, the vaccine may be a 16-valent vaccine. A 16 valent vaccine may include the 11 valent formulation described above supplemented with serotypes 3, 15B, 19A, 22F and 23F. A 16 valent vaccine may include the 11 valent formulation described above supplemented with serotypes 3, 15B, 19A, 22F and 33F. In an embodiment, the vaccine may be a 19-valent vaccine. A 19 valent vaccine may include the 11 valent formulation described above supplemented with serotypes 8, 10A, 11A, 12F, 15B, 19A, 22F and 23F. A 19 valent vaccine may include the 11 valent formulation described above supplemented with serotypes 8, 10A, 11A, 12F, 15B, 19A, 22F and 33F. In an embodiment, the vaccine may be a 20-valent vaccine. A 20 valent vaccine may include the 11 valent formulation described above supplemented with serotypes 3, 8, 10A, 11A, 12F, 15B, 19A, 22F and 23F. A 20 valent vaccine may include the 11 valent formulation described above supplemented with serotypes 3, 8, 10A, 11A, 12F, 15B, 19A, 22F and 33F. In an embodiment, the vaccine may be a 21-valent vaccine.

In one embodiment, the immunogenic composition of the invention comprises capsular polysaccharides derived from serotypes 1, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19F and 23F (suitably conjugated). In a further embodiment of the invention at least 12 saccharide antigens (suitably conjugated) are included, for example capsular polysaccharides derived from serotypes 1, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F. In a further embodiment of the invention at least 12 saccharide antigens (suitably conjugated) are included, for example capsular polysaccharides derived from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19F and 23F. In a further embodiment of the invention, at least 13 polysaccharide antigens (suitably conjugated) are included, for example a vaccine may comprise capsular polysaccharides derived from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F, although further saccharide antigens, for example 23 valents (such as serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F), are also contemplated by the invention. In a further embodiment of the invention, at least 15 saccharide antigens (suitably conjugated) are included, for example capsular polysaccharides derived from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F. In a further embodiment of the invention, at least 15 saccharide antigens (suitably conjugated) are included, for example capsular polysaccharides derived from serotypes 1, 4, 5, 6A, 6B, 7F, 9V, 14, 15B, 18C, 19A, 19F

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22F, 23F and 33F. In a further embodiment of the invention, at least 16 saccharide antigens (suitably conjugated) are included, for example capsular polysaccharides derived from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 15B, 18C, 19A, 19F 22F, 23F and 33F. In another embodiment, the immunogenic composition comprises (conjugated) capsular (poly)saccharide of serotype 33F of S. *pneumoniae.* In another embodiment, the immunogenic composition comprises (conjugated) capsular (poly)saccharide of serotype 15C of S. *pneumoniae.* In another embodiment, the immunogenic composition comprises (conjugated) capsular (poly)saccharide of serotype 12F of S. *pneumoniae.* In an embodiment, there are 10 to 23 different S. *pneumoniae* capsular polysaccharide serotypes (suitably conjugated).

Carrier Proteins

Examples of carrier proteins which may be used in the present invention are DT (Diphtheria toxoid), TT (tetanus toxoid) or fragment C of TT, DT, CRM197 other DT point mutants, such as CRM176, CRM228, CRM 45 (Uchida et al J. Biol. Chem. 218; 3838-3844, 1973); CRM 9, CRM 45, CRM102, CRM 103 and CRM107 and other mutations described by Nicholls and Youle in Genetically Engineered Toxins, Ed: Frankel, Maecel Dekker Inc, 1992; deletion or mutation of Glu-1 48 to Asp, Gin or Ser and/or Ala 158 to Gly and other mutations disclosed in US 4709017 or US 4950740; mutation of at least one or more residues Lys 516, Lys 526, Phe 530 and/or Lys 534 and other mutations disclosed in US 5917017 or US 6455673; or fragment of DT disclosed in US 584371 1, pneumococcal pneumolysin (Kuo et al (1995) Infect Immun 63; 2706-13) including pneumolysin (Ply) detoxified in some fashion for example GMBS detoxified pneumolysin (dPly-GMBS) (WO 04081515, PCT/EP2005/010258) or formaldehyde detoxified pneumolysin (dPly-formol), Pht (pneumococcal histidine triad) family proteins (PhtX), including PhtA, PhtB, PhtD, PhtE and fusions of Pht proteins for example PhtDE fusions, PhtBE fusions (WO 01/98334 and WO 03/54007), (Pht A-E are described in more detail below) OMPC (meningococcal outer membrane protein - usually extracted from *N. meningitidis* serogroup B - EP0372501), *Neisseria meningitidis* porin PorB, PD *(Haemophilus influenzae* protein D - see, e.g., EP 0 594 610 B), or immunologically functional equivalents thereof, synthetic peptides (EP0378881 , EP0427347), heat shock proteins (WO 93/17712, WO 94/03208), pertussis proteins (WO 98/58668, EP0471 177), cytokines, lymphokines, growth factors or hormones (WO 91/01 146), artificial proteins comprising multiple human CD4+ T cell epitopes from various pathogen derived antigens (Falugi et al (2001) Eur J Immunol 31; 3816-3824) such as N19 protein (Baraldoi et al

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(2004) Infect Immun 72; 4884-7) pneumococcal surface protein PspA (WO 02/091998), iron uptake proteins (WO 01/72337), toxin A or B of *Clostridium difficile* (WO 00/61761).

In an embodiment, in the immunogenic composition of the invention each *Streptococcus pneumoniae* capsular saccharide is conjugated to a carrier protein independently selected from the group consisting of DT, CRM 197, TT, Fragment C of TT, dPly (detoxified pneumolysin), PhtA, PhtB, PhtD, PhtE, PhtDE OmpC, PorB and *Haemophilus influenzae* Protein D. In a further embodiment, each *Streptococcus pneumoniae* capsular saccharide is conjugated to a carrier protein independently selected from the group consisting of TT, DT, CRM197, fragment C of TT, PhtD, PhtDE fusions (particularly those described in WO 01/98334 and WO 03/54007), detoxified pneumolysin and protein D. In a further embodiment, each *Streptococcus pneumoniae* capsular saccharide is conjugated to a carrier protein independently selected from the group consisting of TT, DT, CRM197, PhtD, detoxified pneumolysin and protein D. In a further embodiment, each *Streptococcus pneumoniae* capsular saccharide is conjugated to a carrier protein independently selected from the group consisting of TT, DT, CRM197, PhtD and protein D. In a further embodiment, each *Streptococcus pneumoniae* capsular saccharide is conjugated to a carrier protein independently selected from the group consisting of TT, DT, CRM197 and protein D.

In an embodiment, the immunogenic composition of the invention comprises two or more different carrier proteins. In an embodiment, the immunogenic composition of the invention comprises 2, 3, 4, 5 or 6 different carrier proteins. Each type of carrier protein may act as carrier for more than one polysaccharide, which polysaccharides may be from the same or different serotypes. In one embodiment, two or more different polysaccharide serotypes may be conjugated to the same carrier protein, either to the same molecule of carrier protein (carrier molecules having 2 or more different polysaccharide serotypes conjugated to it) [see for instance WO 04/083251] or to different molecules of the same carrier protein (each molecule of protein carrier only having one serotype of saccharide conjugated to it).

In an embodiment, the immunogenic composition of the invention comprises protein D from *Haemophilus influenzae* (PD), for example, protein D sequence from Figure 9 (Figure 9a and 9b together, 364 amino acids) of EP 0594610 (SEQ ID NO: 1). Inclusion of this protein in the immunogenic composition may provide a level of protection against *Haemophilus influenzae* related otitis media (Pyrmula et al Lancet 367; 740-748 (2006)). Protein D may be used as a full length protein or as a fragment (for example, Protein D

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may be as described in WO0056360). For example, a protein D sequence may comprise (or consist) of the protein D fragment described in EP0594610 which begins at the sequence SSHSSNMANT (SerSerHisSerSerAsnMetAlaAsnThr) (SEQ ID NO. 3), and lacks the 19 N-terminal amino acids from Fig 9 of EP0594610, optionally with the tripeptide MDP from NS1 fused to the N-terminal of said protein D fragment (348 amino acids) (SEQ ID NO:2). In one aspect, the protein D or fragment of protein D is unlipidated. The protein D could be present in the immunogenic composition as a free protein or as a carrier protein. In one aspect, protein D is present in the immunogenic composition as free protein. In another aspect, protein D is present both as a carrier protein and as free protein. In a further aspect, protein D is present as a carrier protein for one or more of the polysaccharides. In a further aspect, 2-9 of the capsular polysaccharides selected from different serotypes are conjugated to protein D. In a further aspect, protein D is present as a carrier protein for the majority of the polysaccharides, for example 6, 7, 8, 9 or more of the polysaccharides may be conjugated to protein D.

In an embodiment, the immunogenic composition of the invention contains 2-8, 2-7, 2-6, 2-5, 3-5, 4-5, 2-4, 2-3, 3-4 or 2, 3, 4, 5, 6, 7 or 8 capsular saccharide serotype conjugates in which protein D is the carrier protein. For example, 2-8, 2-7, 2-6, 2-5, 3-5, 4-5, 2-4, 2-3, 3-4 or 2, 3, 4, 5, 6, 7 or 8 polysaccharides selected from serotype 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F and 23F are conjugated to protein D. For example, polysaccharides from serotypes 1, 4, 5, 6B, 7F, 9V, 14 and 23F are conjugated to protein D.

In an embodiment, polysaccharides from at least serotypes 1 and 3, 1 and 4, 1 and 5, 1 and 6A, 1 and 6B, 1 and 7, 1 and 9V, 1 and 14, 1 and 22F, 1 and 23F, 3 and 4, 3 and 5, 3 and 6A, 3 and 6B, 3 and 7F, 3 and 9V, 3 and 14, 3 and 22F, 3 and 23F, 4 and 5, 4 and 6A, 4 and 6B, 4 and 7F, 4 and 9V, 4 and 14, 4 and 22F, 4 and 23F, 5 and 6A, 5 and 6B, 5 and 7F, 5 and 9V, 5 and 14, 5 and 22F, 5 and 23F, 6A and 6B, 6A and 7F, 6A and 9V, 6A and 14, 6A and 22F, 6A and 23F, 6B and 7F, 6B and 9V, 6B and 14, 6B and 22F, 6B and 23F, 7F and 9V, 7F and 14, 7F and 22F, 7F and 23F, 9V and 14, 9V and 22F, 9V and 23F, 14 and 22F, 14 and 23F or 22F and 23F are conjugated to protein D.

In an embodiment, polysaccharides from at least serotypes 1, 3 and 4; 1, 3 and 5; 1, 3 and 6A; 1, 3 and 6B; 1, 3 and 7F; 1, 3 and 9V; 1, 3 and 14; 3, 4 and 7F; 3, 4 and 5; 3, 4 and 7F; 3, 4 and 9V; 3, 4 and 14; 4, 5 and 7F; 4, 5 and 9V; 4, 5, and 14; 5, 7F and 9V; 5, 7F and 14; 7F, 9V and 14; 1, 3, 4 and 5; 3, 4, 5 and 7F; 4, 5, 7F and 9V; 4, 5, 7F and 14;

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4, 5, 9V and 14; 4, 7F, 9V and 14; 5, 7F, 9V and 14; or 4, 5, 7F, 9V and 14 are conjugated to protein D.

For example, in a 10 valent S. *pneumoniae* immunogenic composition, 2, 3, 4, 5, 6, 7 or 8 of the capsular polysaccharides from different serotypes are conjugated to protein D. For example, in a 11 valent S. *pneumoniae* immunogenic composition, 2, 3, 4, 5, 6, 7 or 8 of the capsular polysaccharides from different serotypes are conjugated to protein D. For example, in a 12 valent S. *pneumoniae* immunogenic composition, 2, 3, 4, 5, 6, 7 or 8 of the capsular polysaccharides from different serotypes are conjugated to protein D. For example, in a 13 valent S. *pneumoniae* immunogenic composition, 2, 3, 4, 5, 6, 7 or 8 of the capsular polysaccharides from different serotypes are conjugated to protein D. For example, in a 14 valent S. *pneumoniae* immunogenic composition, 2, 3, 4, 5, 6, 7 or 8 of the capsular polysaccharides from different serotypes are conjugated to protein D. For example, in a 15 valent S. *pneumoniae* immunogenic composition, 2, 3, 4, 5, 6, 7 or 8 of the capsular polysaccharides from different serotypes are conjugated to protein D. For example, in a 16 valent S. *pneumoniae* immunogenic composition, 2, 3, 4, 5, 6, 7 or 8 of the capsular polysaccharides from different serotypes are conjugated to protein D. For example, in a 17 valent S. *pneumoniae* immunogenic composition, 2, 3, 4, 5, 6, 7 or 8 of the capsular polysaccharides from different serotypes are conjugated to protein D. For example, in a 18 valent S. *pneumoniae* immunogenic composition, 2, 3, 4, 5, 6, 7, 8 or 9 of the capsular polysaccharides from different serotypes are conjugated to protein D. For example, in a 19 valent S. *pneumoniae* immunogenic composition, 2, 3, 4, 5, 6, 7, 8 or 9 of the capsular polysaccharides from different serotypes are conjugated to protein D. Optionally, the serotypes conjugated to protein D are selected from the groups described above.

In an embodiment, the immunogenic composition of the invention comprises at least one capsular saccharide conjugated to tetanus toxoid (TT). In another embodiment, capsular saccharide 18C is conjugated to TT, optionally wherein 18C is the only saccharide in the composition conjugated to tetanus toxoid (TT).

In an aspect of the present invention, serotype 19F is conjugated to DT or CRM197. In another aspect, serotype 19F is conjugated to DT. In one aspect, the remaining saccharide serotypes of the immunogenic composition may all be conjugated to one or more carrier proteins that are not DT (i.e. only 19F is conjugated to DT). In one embodiment, 19F is conjugated to DT or CRM197, and the remaining serotypes are

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conjugated to carrier proteins independently selected from PhtD, PD (Protein D), TT (Tetanus Toxoid), DT (Diphtheria Toxoid) and CRM197. In another embodiment, 19F is conjugated to DT or CRM 197, and the remaining serotypes are conjugated to carrier proteins independently selected from PD, TT, DT and CRM197. In a further embodiment, 19F is conjugated to DT or CRM 197, and the remaining serotypes are conjugated to carrier proteins independently selected from PD, TT and CRM197 (for example as described in WO2007/071710A2 and WO2007/071707A2).

In an embodiment, the sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide of the invention and *Streptococcus pneumoniae* serotype 6B capsular polysaccharide are conjugated to different carrier proteins. In a further embodiment, the sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide of the invention is conjugated to CRM 197. In a further embodiment a *Streptococcus pneumoniae* serotype 6B capsular polysaccharide conjugate is present, but is not conjugated to DT or CRM 197. In a further embodiment, the sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide is conjugated to CRM197 and *Streptococcus pneumoniae* serotype 6B capsular polysaccharide *Streptococcus pneumoniae* serotype is conjugated to a carrier protein other than CRM197. In a further embodiment, the sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide is conjugated to CRM197 and *Streptococcus pneumoniae* serotype 6B capsular polysaccharide *Streptococcus pneumoniae* serotype is conjugated to a carrier protein selected from PhtD, PD (Protein D), TT (Tetanus Toxoid) or DT (Diphtheria Toxoid). In a further embodiment, the sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide is conjugated to CRM197 and *Streptococcus pneumoniae* serotype 6B capsular polysaccharide is conjugated to protein D.

In an embodiment of the invention, the carrier protein conjugated to one or more of the S. *pneumoniae* capsular polysaccharides is a member of the polyhistidine triad family (Pht) proteins, fragments or fusion proteins thereof. The PhtA, PhtB, PhtD or PhtE proteins may have an amino acid sequence sharing 80%, 85%, 90%, 95%, 98%, 99% or 100% identity with a sequence disclosed in WO 00/37105 or WO 00/39299 (e.g. with amino acid sequence 1-838 or 21-838 of SEQ ID NO: 4 of WO 00/37105 for PhtD). For example, fusion proteins are composed of full length or fragments of 2, 3 or 4 of PhtA, PhtB, PhtD, PhtE. Examples of fusion proteins are PhtA/B, PhtA/D, PhtA/E, PhtB/A, PhtB/D, PhtB/E. PhtD/A. PhtD/B, PhtD/E, PhtE/A, PhtE/B and PhtE/D, wherein the proteins are linked with the first mentioned at the N-terminus (see for example WO01/98334).

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Where fragments of Pht proteins are used (separately or as part of a fusion protein), each fragment optionally contains one or more histidine triad motif(s) and/or coiled coil regions of such polypeptides. A histidine triad motif is the portion of polypeptide that has the sequence HxxHxH where H is histidine and x is an amino acid other than histidine. A coiled coil region is a region predicted by "Coils" algorithm Lupus, A et al (1991) Science 252; 1162-1 164. In an embodiment, the fragment includes one or more histidine triad motif as well as at least one coiled coil region. In an embodiment, the fragment contains exactly or at least 2, 3, 4 or 5 histidine triad motifs (optionally, with native Pht sequence between the 2 or more triads, or intra-triad sequence that is more than 50, 60, 70, 80, 90 or 100 % identical to a native pneumococcal intra-triad Pht sequence - e.g. the intra-triad sequence shown in SEQ ID NO: 4 of WO 00/37105 for PhtD). In an embodiment, the fragment contains exactly or at least 2, 3 or 4 coiled coil regions. In an embodiment, a Pht protein disclosed herein includes the full length protein with the signal sequence attached, the mature full length protein with the signal peptide (for example 20 amino acids at N-terminus) removed, naturally occurring variants of Pht protein and immunogenic fragments of Pht protein (e.g. fragments as described above or polypeptides comprising at least 15 or 20 contiguous amino acids from an amino acid sequence in WO00/37105 (SEQ ID NOs 4, 6, 8 or 10) or WO00/39299 (SEQ ID NOs 2, 4, 6, 8, 10 or 14) wherein said polypeptide is capable of eliciting an immune response specific for said amino acid sequence in WO00/37105 or WO00/39299).

In particular, the term "PhtD" as used herein includes the full length protein with the signal sequence attached, the mature full length protein with the signal peptide (for example 20 amino acids at N-terminus) removed, naturally occurring variants of PhtD and immunogenic fragments of PhtD (e.g. fragments as described above or polypeptides comprising at least 15 or 20 contiguous amino acids from a PhtD amino acid sequence in WO00/37105 or WO00/39299) wherein said polypeptide is capable of eliciting an immune response specific for said PhtD amino acid sequence in WO00/37105 or WO00/39299 (e.g. SEQ ID NO: 4 of WO 00/37105 or SEQ ID NO: 14 of WO 00/39299 for PhtD). All forms of PhtD mentioned above can be used in the present invention.

Conjugation Processes

The saccharide conjugates present in the immunogenic compositions of the invention may be prepared by the conjugation methods of the present invention or any known coupling

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technique. The conjugation method may rely on activation of the saccharide with 1-cyano-4-dimethylamino pyridinium tetrafluoroborate (CDAP) to form a cyanate ester. The activated saccharide may thus be coupled directly or via a spacer (linker) group to an amino group on the carrier protein. For example, the spacer could be cystamine or cysteamine to give a thiolated polysaccharide which could be coupled to the carrier via a thioether linkage obtained after reaction with a maleimide-activated carrier protein (for example using GMBS (4-Maleimidobutyric acid N-hydroxysuccinimide ester)) or a haloacetylated carrier protein (for example using SIAB (succinimidyl (4-iodoacetyl)aminobenzoate), or SIA (succinimidyl iodoacetate), or SBAP (succinimidyl-3-(bromoacetamide)propionate)). In an embodiment, the cyanate ester (optionally made by CDAP chemistry) is coupled with hexane diamine or ADH (adipic acid dihydrazide) and the amino-derivatised saccharide is conjugated to the carrier protein using carbodiimide (e.g. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC or EDO)) chemistry via a carboxyl group on the protein carrier. Such conjugates are described in PCT published application WO 93/15760 Uniformed Services University and WO 95/08348 and WO 96/29094.

In an embodiment, at least one of the S. *pneumoniae* capsular polysaccharides is directly conjugated to a carrier protein (e.g. using one of the chemistries described above). In an embodiment, at least one of the S. *pneumoniae* capsular polysaccharides is directly conjugated by 1-cyano-4-dimethylaminopyridinium tetrafluoroborate (CDAP). In an embodiment, the majority of the capsular polysaccharides for example 5, 6, 7, 8, 9 or more are directly linked to the carrier protein by CDAP (see WO 95/08348 and WO 96/29094)

In an embodiment, the *Streptococcus pneumoniae polysaccharide* is conjugated to the carrier protein via a linker, for instance a bifunctional linker. The linker is optionally heterobifunctional or homobifunctional, having for example a reactive amino group and a reactive carboxylic acid group, 2 reactive amino groups or two reactive carboxylic acid groups. The linker has for example between 4 and 20, 4 and 12, 5 and 10 carbon atoms. A possible linker is ADH (adipic acid dihydrazide). Other linkers include B-propionamido (WO 00/10599), nitrophenyl-ethylamine (Gever et al (1979) Med. Microbiol. Immunol. 165; 171-288), haloalkyl halides (US4057685), glycosidic linkages (US4673574, US4808700), hexane diamine and 6-aminocaproic acid (US4459286). In an embodiment, the immunogenic composition of the invention may comprise 18C capsular polysaccharide conjugated to the carrier protein via a linker, optionally the linker is ADH. In an

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embodiment, the immunogenic composition of the invention may comprise 22F capsular polysaccharide conjugated to the carrier protein via a linker, optionally the linker is ADH (for example as described in WO2007/07171 1A2).

Other suitable techniques use carbodiimides, hydrazides, active esters, norborane, p-nitrobenzoic acid, N-hydroxysuccinimide, N-hydroxysulfosuccinimide (S-NHS), EDC, O-(N-Succinimidyl)-1 ,1 ,3,3-tetramethyl-uronium tetrafluoroborate (TSTU). Many are described in WO 98/42721 . Conjugation may involve a carbonyl linker which may be formed by reaction of a free hydroxyl group of the polysaccharide with Carbonyldiimidazole (CDI) (Bethell et al J. Biol. Chem. 1979, 254; 2572-4, Hearn et al J. Chromatogr. 1981 . 218; 509-18) followed by reaction of with a protein to form a carbamate linkage. This may involve reduction of the anomeric terminus to a primary hydroxyl group, optional protection/deprotection of the primary hydroxyl group reaction of the primary hydroxyl group with CDI to form a CDI carbamate intermediate and coupling the CDI carbamate intermediate with an amino group on a protein.

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

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

In an embodiment, at least one S. *pneumoniae* polysaccharide is conjugated to a carrier protein via a linker using CDAP and EDAC. For example, 18C or 22F may be conjugated to a protein via a linker (for example those with two hydrazino groups at its ends such as ADH) using CDAP and EDAC as described above. When a linker is used, CDAP may be used to conjugate the polysaccharide to a linker and EDAC may then be used to conjugate the linker to a protein or, alternatively EDAC may be used first to conjugate the linker to the protein, after which CDAP may be used to conjugate the linker to the saccharide.

In an embodiment, a hydroxyl group (suitably an activated hydroxyl group for example a hydroxyl group activated to make a cyanate ester [e.g. with CDAP]) on a polysaccharide

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

A combination of techniques may also be used, with some saccharide-protein conjugates being prepared by CDAP, and some by reductive amination.

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

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

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

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

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

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

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F) Guanidyl group (for instance via arginine).

G) Indolyl group (for instance via tryptophan).

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

Direct coupling approaches:

Saccharide-OH + CNBr or CDAP -----> cyanate ester + NH2-Protein — > conjugate
Saccharide-aldehyde + NH2-Protein — > Schiff base + NaCNBH3 — > conjugate
Saccharide-COOH + NH_2-Protein + EDAC — > conjugate
Saccharide-NH_2 + COOH-Protein + EDAC — > conjugate

Indirect coupling via spacer (linker) approaches:

Saccharide-OH + CNBr or CDAP —> cyanate ester + NH_2— NH_2 — > saccharide — NH_2 + COOH-Protein + EDAC -----> conjugate

Saccharide-OH + CNBr or CDAP — > cyanate ester + NH_2-----SH -----> saccharide — SH + SH-Protein (native Protein with an exposed cysteine or obtained after modification of amino groups of the protein by SPDP for instance) — > saccharide-S-S-Protein

Saccharide-OH + CNBr or CDAP —> cyanate ester + NH_2— SH -------> saccharide — SH + maleimide-Protein (modification of amino groups) — > conjugate

Saccharide-OH + CNBr or CDAP —> cyanate ester + NH_2-----SH —> Saccharide-SH + haloacetylated-Protein — > Conjugate

Saccharide-COOH + EDAC + NH_2-----NH_2 ---> saccharide ------NH_2 + EDAC + COOH-Protein — > conjugate

Saccharide-COOH + EDAC+ NH_2— SH -----> saccharide — SH + SH-Protein (native Protein with an exposed cysteine or obtained after modification of amino groups of the protein by SPDP for instance) -----> saccharide-S-S-Protein

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Saccharide-COOH + EDAC+ NH_2— SH -----> saccharide— SH + maleimide-Protein (modification of amino groups) — > conjugate

Saccharide-COOH + EDAC + NH_2— SH —> Saccharide-SH + haloacetylated-Protein — > Conjugate

Saccharide-Aldehyde + NH_2-----NH_2 — > saccharide— NH2 + EDAC + COOH-Protein — > conjugate

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

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

In an embodiment, the *Streptococcus pneumoniae* serotype 6A capsular polysaccharide of the invention is conjugated to the carrier protein (e.g. CRM-197) using CDAP chemistry. In one aspect, the CDAP chemistry uses a CDAP:PS6A ratio between 1:2 to 3:1 , 1:1.5 to 2:1 , e.g. 1:1. In another aspect, the CDAP conjugation is carried out using a coupling time of between 50-130 minutes, 60-130 minutes, or 110-130 minutes. Thus the present invention also provides, a process for preparing a *Streptococcus pneumoniae* serotype 6A capsular polysaccharide conjugate (e.g. a sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide of the invention) comprising conjugating a sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide to a carrier protein (e.g. CRM-197) or to a linker (e.g. ADH) using CDAP chemistry using a CDAP:PS (polysaccharide) ratio between 1:2 to 3:1 , 1:1.5 to 2:1 , e.g. 1:1.

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In another aspect, the present invention provides, a process for preparing a *Streptococcus pneumoniae* serotype 6A capsular polysaccharide conjugate (e.g. a sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide of the invention) comprising conjugating a sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide to a carrier protein (e.g. CRM-197) using CDAP chemistry using a CDAP:PS (polysaccharide) ratio between 1:2 to 3:1 , 1:1.5 to 2:1 , e.g. 1:1. In one aspect, the present invention also provides, a process for preparing a *Streptococcus pneumoniae* serotype 6A capsular polysaccharide conjugate (e.g. a sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide of the invention, such as a *Streptococcus pneumoniae* serotype 6A capsular polysaccharide having an average size (e.g. M_w) between 100-1000, 110-750, 150-500, 180-600, 210-490, 210-450, 180-400, 210-400, 210-370, 220-360, 230-350, 240-340, 240-320, 240-310 or 250-310 kDa) comprising conjugating a sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide to a carrier protein (e.g. CRM-197) or to a linker (e.g. ADH) using CDAP chemistry using a coupling time of between 50-130 minutes, 60-130 minutes, or 110-130 minutes.

In another aspect, the present invention provides, a process for preparing a *Streptococcus pneumoniae* serotype 6A capsular polysaccharide conjugate (e.g. a sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide of the invention, such as a *Streptococcus pneumoniae* serotype 6A capsular polysaccharide having an average size (e.g. M_w) between 100-1000, 110-750, 150-500, 180-600, 210-490, 210-450, 180-400, 210-400, 210-370, 220-360, 230-350, 240-340, 240-320, 240-310 or 250-310 kDa) comprising conjugating a sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide to a carrier protein (e.g. CRM-197) using CDAP chemistry using a coupling time of between 50-130 minutes, 60-130 minutes, or 110-130 minutes. In one aspect, the pH for activation and coupling is between pH 8 to pH9, suitably pH9.5. In another aspect, the conjugation is carried out in the presence of NaCl. For example, in 0.1-3M NaCl, 0.1-2.5M NaCl, 1.5-2.5M NaCl or 2M NaCl.

The present invention also provides, a process for preparing a *Streptococcus pneumoniae* serotype 6A capsular polysaccharide conjugate comprising (a) conjugation of a *Streptococcus pneumoniae* serotype 6A capsular polysaccharide (e.g. a sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide of the invention) to a carrier protein (e.g. CRM-197) and (b) diafiltration against a solution having a concentration of NaCl below 150mM (e.g. below 100mM NaCl, below 50mM NaCl, below 10mM NaCl) or using water (e.g. WFI, water for injection).

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In another aspect, the present invention provides, a solution comprising 6A-CRM197 in less than 150mM NaCl. For example, less than 100mM NaCl, less than 50mM NaCl, less than 10nM NaCl, or in the absence of sodium chloride. In another aspect, the present invention provides, an immunogenic composition of the invention (e.g. 6A-CRM197) comprising less than 150mM NaCl. For example, less than 100mM NaCl, less than 50mM NaCl, less than 10mM NaCl, or in the absence of sodium chloride.

Ratio of Carrier Protein to Polysaccharide

In an embodiment, the ratio of carrier protein to S. *pneumoniae polysaccharide* is between 1:5 and 5:1 ; e.g. between 1:0.5-4:1 , 1:1-3.5:1 , 1.2:1-3:1 , 1.5:1-2.5:1 ; e.g. between 1:2 and 2.5:1 ; 1:1 and 2:1 (w/w; weight/weight). In an embodiment, the majority of the conjugates, for example 6, 7, 8, 9 or more of the conjugates have a ratio of carrier protein to polysaccharide that is greater than 1:1, for example 1.1:1, 1.2:1 , 1.3:1 , 1.4:1 , 1.5:1 or 1.6:1 .

In an embodiment, the ratio of carrier protein to *Streptococcus pneumoniae* serotype 6A capsular polysaccharide in immunogenic compositions of the invention is between 5:1 and 1:5, 4:1 and 1:1 or 2:1 and 1:1, 1.5:1 and 1:1, 1.4:1 and 1.3:1 (for example 1.2:1 , 1.5:1) (w/w).

The ratio of polysaccharide to carrier protein (w/w) in a conjugate may be determined using the conjugate. The amount of protein is determined using a Lowry assay (for example Lowry *et al.* (1951) J. Biol. Chem. 193, 265-275 or Peterson *et al.* Analytical Biochemistry 100, 201-220 (1979)) and the amount of polysaccharide is determined using resorcinol assay (Monsigny *et al.* (1988) Anal. Biochem. 175, 525-530). The final Protein/Polysaccharide ratio (w/w) on the sterilized conjugate is determined by the ratio of the Lowry/resorcinol concentrations.

Size of Capsular Polysaccharides in the Immunogenic Composition

Capsular polysaccharides of *Streptococcus pneumoniae* comprise repeating oligosaccharide units which may contain up to 8 sugar residues. For a review of the oligosaccharide units for the key *Streptococcus pneumoniae* serotypes see JONES, Christopher. **Vaccines based on the cell surface carbohydrates of pathogenic bacteria.** *An. Acad. Bras. Cienc,* June 2005, vol.77, no.2, p.293-324. Table II ISSN 0001-

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3765. In one embodiment, a capsular polysaccharide may be a full length polysaccharide, however in others it may be a shorter than native length polysaccharide chain of repeating units. In one embodiment, the *Streptococcus pneumoniae* serotype capsular polysaccharide conjugates post conjugation should be readily filterable through a 0.2 micron filter such that a yield of more than 95% is obtained post filtration compared with the pre filtration sample.

In addition to the sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide conjugate of the invention, the immunogenic composition of the invention may comprise one or more (poly)saccharide conjugates from *Streptococcus pneumoniae* serotypes other than 6A (e.g. 6B and/or 23F) wherein the average size (e.g. weight-average molecular weight; M_w) of the (poly)saccharide before conjugation is above 80kDa, 100kDa, 200kDa, 300kDa, 400kDa, 500kDa, 700kDa or 1000kDa. For example, the immunogenic composition of the invention may comprise one or more (poly)saccharide conjugates from *Streptococcus pneumoniae* serotypes other than 6A wherein the average size (e.g. weight-average molecular weight; M_w) of the (poly)saccharide before conjugation is between 80-100kDa, 100-200kDa, 200-300kDa, 300-400kDa, 400-500kDa, 500-1 OOOkDa or 1000-1 400kDa. In one embodiment, the immunogenic composition comprises (i) sized a *Streptococcus pneumoniae* serotype 6A capsular polysaccharide conjugate and (ii) one or more (poly)saccharide conjugates with an average size of saccharide before conjugation of 50-1600, 80-1400, 100-1000, 150-500, or 200-400 kDa (note that where average size is M_w , 'kDa' units should be replaced herein with '$x10^3$').

In an embodiment, the immunogenic composition of the invention comprises a serotype 1 S. *pneumoniae* polysaccharide having an average size (M_w) of between 100-1000, 200-800, 250-600, or 300-400 kDa. In an embodiment, the immunogenic composition of the invention comprises a serotype 4 S. *pneumoniae* polysaccharide having an average size (Mw) of between 50-500, 60-300, 70-150, or 75-125 kDa. In an embodiment, the immunogenic composition of the invention comprises a serotype 5 S. *pneumoniae* polysaccharide having an average size (M_w) of between 100-1000, 100-700, 100-350, or 150-300 kDa. In an embodiment, the immunogenic composition of the invention comprises a serotype 6B S. *pneumoniae* polysaccharide having an average size (M_w) of between 200-1800, 500-1800, 600-1800, 900-1660, or 1000-1400 kDa. In an embodiment, the immunogenic composition of the invention comprises a serotype 7F S. *pneumoniae* polysaccharide having an average size (M_w) of between 50-1000, 100-750, 150-500, or 200-300 kDa. In an embodiment, the immunogenic composition of the

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invention comprises a serotype 9V S. *pneumoniae* polysaccharide having an average size (Mw) of between 50-1000, 100-750, 150-500, 200-400, or 250-300 kDa. In an embodiment, the immunogenic composition of the invention comprises a serotype 14 S. *pneumoniae* polysaccharide having an average size (M_w) of between 50-1000, 100-750, 150-500, or 200-250 kDa. In an embodiment, the immunogenic composition of the invention comprises a 18C S. *pneumoniae* polysaccharide having an average size (M_w) of between 50-1000, 50-750, 50-500, 50-190, 50-150 or 80-1 10 kDa. In an embodiment, the immunogenic composition of the invention comprises a 19A S. *pneumoniae* polysaccharide having an average size (M_w) of between 50-800, 110-700, 110-300, 120-200, 130-180, 140-160 or 80-130 kDa. In an embodiment, the immunogenic composition of the invention comprises a serotype 19F S. *pneumoniae* polysaccharide having an average size (M_w) of between 50-1000, 100-750, 100-500, 100-190 or 120-180 kDa. In an embodiment, the immunogenic composition of the invention comprises a serotype 23F S. *pneumoniae* polysaccharide having an average size (M_w) of between 500-1500, 600-1500, 700-1300, 900-1250, 800-1 100, or 900-1000 kDa. In an embodiment, the immunogenic composition of the invention comprises a serotype 22F S. *pneumoniae* polysaccharide having an average size (M_w) of between 50- 800, 110- 700, 110-300, 120-200, 130-1 80, 150-1 70, 100-1 90, 100-1 50, 95-1 25 or 100-1 15 kDa.

In an embodiment, the immunogenic composition of the invention comprises 1 or more, native capsular polysaccharides from different S. *pneumoniae* serotypes. In another embodiment, the immunogenic composition comprises *Streptococcus pneumoniae* polysaccharides from at least 10 serotypes conjugated to a carrier protein, wherein at least 1, 2, 3, 4, 5, 6, 7, 8, or 9 of the S. *pneumoniae* polysaccharide serotypes is native polysaccharide. In another embodiment, the immunogenic composition of the invention comprises native *Streptococcus pneumoniae* capsular serotype 6B polysaccharide. In another embodiment, the immunogenic composition of the invention comprises native *Streptococcus pneumoniae* capsular serotype 23F polysaccharide.

In an aspect of the invention, the immunogenic composition comprises *Streptococcus pneumoniae* polysaccharides from at least 10 serotypes conjugated to a carrier protein, wherein at least 1, 2, 3, 4, 5, 6, 7, 8, or 9 of the S. *pneumoniae* polysaccharide serotypes is sized by a factor up to x2, x3, x4, x5, x6, x7, x8, x9 or x10. In one embodiment of this aspect, the majority of the polysaccharides, for example 6, 7, 8 or more of the polysaccharide serotypes are sized by a factor up to x2, x3, x4, x5, x6, x7, x8, x9 or x 10.

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For example, sizing may be from a factor of between x2 to x6, x2 to x5, x2 to x4, or x3 to x6, x3 to x5 or x3 to x4.

In an embodiment, the majority of S. *pneumoniae* polysaccharides in the immunogenic composition are sized. In an embodiment, the majority of S. *pneumoniae* polysaccharide serotypes in the immunogenic composition are sized. In one aspect, S. *pneumoniae* polysaccharides in the immunogenic composition are sized mechanical cleavage, for instance by microfluidisation or sonication. In another aspect, S. *pneumoniae* polysaccharides in the immunogenic composition are sized by chemical cleavage, e.g. treatment with acetic acid or periodate. Sizing is by a factor of no more than x20, x10, x8, x6, x5, x4, x3 or x2.

In an embodiment, the immunogenic composition comprises S. *pneumoniae* conjugates that are a mixture of native polysaccharides and polysaccharides that are sized by a factor of no more than x20, x10, x8, x6, x5, x4, x3 or x2. In one aspect of this embodiment, the majority of the polysaccharides, for example 6, 7, 8, 9, 10 or more of the polysaccharides are sized by a factor of up to x2, x3, x4, x5 or x6.

Dosage

In general, the immunogenic composition of the invention may comprise a dose of each saccharide conjugate between 0.1 and 20μg, 1 and 10μg or 1 and 3μg of saccharide.

In an embodiment, in the immunogenic composition of the present invention the dose of the *Streptococcus pneumoniae* 6A polysaccharide conjugate is between 1 and 10 μg, 1 and 5 μg, or 1 and 3 μg of saccharide (e.g. 2μg).

In an embodiment, the immunogenic composition of the invention contains each S. *pneumoniae* capsular saccharide at a dose of between 0.1-20μg; 0.5-10μg; 0,5- 5μg or 1-3μg of saccharide. In an embodiment, capsular polysaccharides may be present at different dosages, for example some capsular polysaccharides may be present at a dose of around or exactly 1μg or some capsular polysaccharides may be present at a dose of around or exactly 3μg. In an embodiment, polysaccharides from serotypes 3, 18C and 19F are present at a higher dose than other polysaccharides. In an embodiment, polysaccharides from serotypes 4, 18C and 19F are present at a higher dose than other

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polysaccharides. In one aspect of this embodiment, serotypes 3, 18C and 19F are present at a dose of around or exactly 3 μg whilst other polysaccharides in the immunogenic composition are present at a dose of around or exactly ^g. In one aspect of this embodiment, serotypes 4, 18C and 19F are present at a dose of around or exactly 3 μg whilst other polysaccharides in the immunogenic composition are present at a dose of around or exactly ^g.

"Around" or "approximately" are defined as within 10% more or less of the given figure for the purposes of the invention.

***Streptococcus pneumoniae* Proteins**

The immunogenic composition of the invention may also comprise *Streptococcus pneumoniae* proteins, herein termed *Streptococcus pneumoniae* proteins of the invention. Such proteins may be used as carrier proteins, or may be present as free proteins, or may be present both as carrier proteins and as free proteins. In an embodiment, the immunogenic composition of the invention further comprises one or more unconjugated or conjugated S. *pneumoniae* proteins. In an embodiment, the immunogenic composition of the invention further comprises one or more unconjugated S. *pneumoniae* proteins. For example, immunogenic compositions of the invention may comprise unconjugated pneumolysin, e.g. dPly, and unconjugated pneumococcal PhtD.

The *Streptococcus pneumoniae* proteins of the invention are either surface exposed, at least during part of the life cycle of the pneumococcus, or are proteins which are secreted or released by the pneumococcus. In an embodiment, the proteins of the invention are selected from the following categories, such as proteins having a Type II Signal sequence motif of LXXC (where X is any amino acid, e.g., the polyhistidine triad family (PhtX)), choline binding proteins (e.g. CbpX, PcpA), proteins having a Type I Signal sequence motif (e.g., Sp101), proteins having a LPXTG motif (where X is any amino acid, e.g., Sp128, Sp130), and toxins (e.g., Ply). Preferred examples within these categories (or motifs) are the following proteins, or immunologically functional equivalents thereof. Thus, the immunogenic composition of the invention may comprise one or more S. *pneumoniae* proteins selected from Poly Histidine Triad family (PhtX), Choline Binding Protein family (CbpX), CbpX truncates, pneumococcal autolysin LytX family (LytA (/V-acetylmuramoyl-l-alanine amidase), LytB, LytC), LytX truncates, CbpX truncate-LytX truncate chimeric

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proteins, detoxified pneumolysin (Ply), PspA, PsaA, Sp128, Sp101 , Sp130, Sp125 and Sp133. In a further embodiment, the immunogenic composition of the invention comprises 2 or more proteins selected from the group consisting of the Poly Histidine Triad family (PhtX), Choline Binding Protein family (CbpX), CbpX truncates, LytX family, LytX truncates, CbpXtruncate-LytXtruncate chimeric proteins (or fusions), pneumolysin (Ply), PspA, PsaA, and Sp128. In a further embodiment, the immunogenic composition comprises 2 or more proteins selected from the group consisting of the Poly Histidine Triad family (PhtX), Choline Binding Protein family (CbpX), CbpX truncates, LytX family, LytX truncates, CbpX truncate-LytX truncate chimeric proteins (or fusions), pneumolysin (Ply), and Sp128.

The Pht (Poly Histidine Triad) family comprises proteins PhtA, PhtB, PhtD, and PhtE. The family is characterized by a lipidation sequence, two domains separated by a proline-rich region and several histidine triads, possibly involved in metal or nucleoside binding or enzymatic activity, (3-5) coiled-coil regions, a conserved N-terminus and a heterogeneous C terminus. It is present in all strains of pneumococci tested. Homologous proteins have also been found in other Streptococci and Neisseria. In one embodiment of the invention, the immunogenic composition comprises PhtD. It is understood, however, that the terms Pht A, B, D, and E refer to proteins having sequences disclosed in the citations below as well as variants thereof that have a sequence homology that is at least 90% identical to the proteins described below, e.g. amino acids 21-838 of SEQ ID NO: 4 of WO00/37105. In an embodiment it is at least 95% identical and in another embodiment it is 97% identical.

With regards to the PhtX proteins, PhtA is disclosed in WO 98/18930, and is also referred to Sp36. As noted above, it is a protein from the polyhistidine triad family and has the type II signal motif of LXXC. PhtD is disclosed in WO 00/37105, and is also referred to Sp036D. As noted above, it also is a protein from the polyhistidine triad family and has the type II LXXC signal motif. PhtB is disclosed in WO 00/37105, and is also referred to Sp036B. Another member of the PhtB family is the C3-Degrading Polypeptide, as disclosed in WO 00/17370. This protein also is from the polyhistidine triad family and has the type II LXXC signal motif. A preferred immunologically functional equivalent is the protein Sp42 disclosed in WO 98/18930. A PhtB truncate (approximately 79kD) is disclosed in W099/15675 which is also considered a member of the PhtX family. PhtE is disclosed in WO00/30299 and is referred to as BVH-3. Where any Pht protein is referred to herein, it is meant that immunogenic fragments or fusions thereof of the Pht protein can

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be used. For example, a reference to PhtX includes immunogenic fragments or fusions thereof from any Pht protein.

In one embodiment, the S. *pneumoniae* protein selected from member(s) of the Polyhistidine Triad family is PhtD. The term "PhtD" as used herein includes the full length protein with the signal sequence attached or the mature full length protein with the signal peptide (for example 20 amino acids at N-terminus) removed, and immunogenic fragments, variants and/or fusion proteins thereof, e.g. SEQ ID NO: 4 of WOOO/37105. In one aspect, PhtD is the full length protein with the signal sequence attached e.g. SEQ ID NO: 4 of WOOO/37105. In another aspect, PhtD is a sequence comprising the mature full length protein with the signal peptide (for example 20 amino acids at N-terminus) removed, e.g. amino acids 21-838 of SEQ ID NO: 4 of WOOO/37105. Suitably, the PhtD sequence comprises an N-terminal methionine. The present invention also includes PhtD polypeptides which are immunogenic fragments of PhtD, variants of PhtD and/or fusion proteins of PhtD. For example, as described in WOOO/37105, WOOO/39299, US6699703 and WO09/12588.

Where immunogenic fragments of PhtD proteins are used (separately or as part of a fusion protein), these immunogenic fragments will be at least about 15, at least about 20, at least about 40, or at least about 60 contiguous amino acid residues in length, e.g from a PhtD amino acid sequence in WOOO/37105 or WOOO/39299, such as SEQ ID NO: 4 of WOOO/37105. In an embodiment of the invention, immunogenic fragments of PhtD protein comprise at least about 15, at least about 20, at least about 40, or at least about 60 contiguous amino acid residues of the sequence shown in SEQ ID NO: 4 of WOOO/37105, wherein said polypeptide is capable of eliciting an immune response specific for said amino acid sequence. In an embodiment, the immunogenic composition of the invention comprises an immunogenic fragment of PhtD, for example described in WO09/12601 , WO01/98334 and WO09/12588. Where immunogenic fragments of PhtD proteins are used (separately or as part of a fusion protein), each immunogenic fragment optionally contains one or more histidine triad motif(s) of such polypeptides. A histidine triad motif is the portion of polypeptide that has the sequence HxxHxH where H is histidine and x is an amino acid other than histidine. In an embodiment of the present invention, the or each immunogenic fragment contains exactly or at least 2, 3, 4 or 5 histidine triad motifs (optionally, with native PhtD sequence between the 2 or more triads, or intra-triad sequence) where the immunogenic fragment is more than 50, 60, 70, 80, 90 or 100% identical to a native pneumococcal intra-triad PhtD sequence (e.g. the intra-triad

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sequence shown in SEQ ID NO: 4 of WO00/37105). Immunogenic fragments of PhtD proteins optionally contain one or more coiled coil regions of such polypeptides. A coiled coil region is a region predicted by "Coils" algorithm Lupus, A et al (1991) Science 252; 1162-1 164. In an embodiment of the present invention, each immunogenic fragment contains exactly or at least 2, 3 or 4 coiled coil regions. In an embodiment of the present invention, the or each immunogenic fragment contains exactly or at least 2, 3 or 4 coiled coil regions where the immunogenic fragment is more than 50, 60, 70, 80, 90, 95, 96 or 100% identical to a native pneumococcal PhtD sequence (e.g. the sequence shown in SEQ ID NO: 4 of WO00/37105). In another embodiment of the present invention, the immunogenic fragment includes one or more histidine triad motif as well as at least 1, 2, 3 or 4 coiled coil regions.

In the case where the PhtD polypeptide is a variant, the variation is generally in a portion thereof other than the histidine triad residues and the coiled-coil region, although variations in one or more of these regions may be made. In accordance with the present invention, a polypeptide variant includes sequences in which one or more amino acids are substituted and/or deleted and/or inserted compared to the wild type sequence. Amino acid substitution may be conservative or non-conservative. In one aspect, amino acid substitution is conservative. Substitutions, deletions, insertions or any combination thereof may be combined in a single variant so long as the variant is an immunogenic polypeptide. Variants of PhtD typically include any immunogenic fragment or variation of PhtD which shares at least 80, 90, 95, 96, 98, or 99% amino acid sequence identity with a wild-type PhtD sequence, e.g. SEQ ID NO: 4 of WO00/37105. In an embodiment, the present invention includes immunogenic fragments and/or variants in which several, 5 to 10, 1 to 5, 1 to 3, 1 to 2 or 1 amino acid(s) are substituted, deleted, or added in any combination. In another embodiment, the present invention includes immunogenic fragments and/or variants which comprise a B-cell or T-cell epitope. Such epitopes may be predicted using a combination of 2D-structure prediction, e.g. using the PSIPRED program (from David Jones, Brunei Bioinformatics Group, Dept. Biological Sciences, Brunei University, Uxbridge UB8 3PH, UK) and antigenic index calculated on the basis of the method described by Jameson and Wolf (CABIOS 4:181-186 [1988]).

In an embodiment of the invention, PhtD and its immunogenic fragments, variants and/or fusion proteins thereof comprise an amino acid sequence sharing at least 80, 85, 90, 95, 96, 97, 98, 99 or 100% identity with amino acid sequence 21 to 838 of SEQ ID NO:4 of WO00/37105. In another embodiment of the invention, PhtD and its immunogenic

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fragments, variants and/or fusion proteins thereof have an amino acid sequence sharing at least 80, 85, 90, 95, 96, 97, 98, 99 or 100% identity with amino acid sequence 21 to 838 of SEQ ID NO:4 of WO00/37105. Suitably, PhtD and its immunogenic fragments, variants and/or fusion proteins thereof comprise an amino acid sequence having an N-terminal methionine. In another embodiment of the invention, PhtD and its immunogenic fragments, variants and/or fusion proteins thereof comprise at least about 15, at least about 20, at least about 40, or at least about 60 or at least about 100, or at least about 200, or at least about 400 or at least about 800 contiguous amino acid residues of the sequence shown in SEQ ID NO: 4 of WO00/37105.

Pneumolysin (Ply) is a multifunctional toxin witha distinct cytolytic (hemolytic) and complement activation activities (Rubins et al., Am. Respi. Cit Care Med, 153:1339-1346 (1996)). The toxin is not secreted by pneumococci, but it is released upon lysis of pneumococci under the influence of autolysin. Its effects include e.g., the stimulation of the production of inflammatory cytokines by human monocytes, the inhibition of the beating of cilia on human respiratory epithelial, the decrease of bactericidal activity and migration of neutrophils, and in the lysis of red blood cells, which involves binding to cholesterol. Because it is a toxin, it needs to be detoxified (i.e., non-toxic to a human when provided at a dosage suitable for protection) before it can be administered *in vivo*. Expression and cloning of wild-type or native pneumolysin is known in the art. See, for example, Walker et al. (Infect Immun, 55:1 184-1 189 (1987)), Mitchell et al. (Biochim Biophys Acta, 1007:67-72 (1989) and Mitchell et al (NAR, 18:4010 (1990)). Detoxification of Ply can be conducted by chemical means, e.g., subject to formalin or glutaraldehyde treatment or a combination of both (WO 04081515, PCT/EP2005/010258). Such methods are known in the art for various toxins. Alternatively, Ply can be genetically detoxified. Thus, the invention encompasses derivatives of pneumococcal proteins which may be, for example, mutated proteins. The term "mutated" is used herein to mean a molecule which has undergone deletion, addition or substitution of one or more amino acids using known techniques for site directed mutagenesis or any other conventional method. For example, as described above, a mutant Ply protein may be altered so that it is biologically inactive whilst still maintaining its immunogenic epitopes, see, for example, WO90/06951 , Berry et al. (Infect Immun, 67:981-985 (1999)) and WO99/03884.

As used herein, it is understood that the term "Ply" encompasses mutated pneumolysin and detoxified pneumolysin (dPly) suitable for medical use (i.e., non toxic).

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Concerning the Choline Binding Protein family (CbpX), members of that family were originally identified as pneumococcal proteins that could be purified by choline-affinity chromatography. All of the choline-binding proteins are non-covalently bound to phosphorylcholine moieties of cell wall teichoic acid and membrane-associated lipoteichoic acid. Structurally, they have several regions in common over the entire family, although the exact nature of the proteins (amino acid sequence, length, etc.) can vary. In general, choline binding proteins comprise an N terminal region (N), conserved repeat regions, a proline rich region (P) and a conserved choline binding region (C), made up of multiple repeats, that comprises approximately one half of the protein. As used in this application, the term "Choline Binding Protein family (CbpX)" is selected from the group consisting of Choline Binding Proteins as identified in W097/41 151 , , Choline binding protein A, CbpA (also referred to as PbcA (C3-binding protein A), SpsA *(Streptococcus pneumoniae* secretory IgA binding protein), PspC (pneumococcal surface protein C)), Choline binding protein D (CbpD), and Choline binding protein G (CbpG). CbpA is disclosed in W097/41 151 . CbpD and CbpG are disclosed in WO00/29434. PspC is disclosed in WO97/09994. PbcA is disclosed in W098/21337. SpsA is a Choline binding protein disclosed in WO 98/39450. In an embodiment, the Choline Binding Proteins is CbpA. Another Choline Binding Protein is pneumococcal choline-binding protein A (PcpA) (Sanchez-Beato et al FEMS Microbiology Letters 164 (1998) 207-214).

Another preferred embodiment is CbpX truncates wherein "CbpX" is CbpA, CbpD or CbpG and "truncates" refers to CbpX proteins lacking 50% or more of the Choline binding region (C). Another preferred embodiment is PcpA truncates wherein "truncates" refers to PcpA proteins lacking 50% or more of the Choline binding region (C). In an embodiment, CbpX truncates or PcpA truncates lack the entire choline binding region. In another embodiment, the CbpX truncates or PcpA truncates lack (i) the choline binding region and (ii) a portion of the N-terminal half of the protein as well, yet retain at least one repeat region. In another embodiment, the truncate has at least 2 repeat regions. Examples of such preferred embodiments are illustrated in W099/51266 or W099/51 188, however, other choline binding proteins lacking a similar choline binding region are also contemplated within the scope of this invention.

The LytX family is membrane associated proteins associated with cell lysis. The N-terminal domain comprises choline binding domain(s), however the LytX family does not have all the features found in the CbpA family noted above and thus for the present invention, the LytX family is considered distinct from the CbpX family. In contrast with the

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CbpX family, the C-terminal domain contains the catalytic domain of the LytX protein family. The family comprises LytA, LytB and LytC. With regards to the LytX family, LytA is disclosed in Ronda et al., Eur J Biochem, 164:621-624 (1987). LytB is disclosed in WO 98/18930, and is also referred to as Sp46. LytC is also disclosed in WO 98/18930, and is also referred to as Sp91 . A preferred member of that family is LytC.

Another preferred embodiment are LytX truncates wherein "LytX" is LytA, LytB or LytC and "truncates" refers to LytX proteins lacking 50% or more of the Choline binding region. Suitably such proteins lack the entire choline binding region. Yet another preferred embodiment of this invention are CbpX truncate-LytX truncate chimeric proteins (or fusions). In an embodiment, the CbpX truncate-LytX truncate chimeric protein comprises the repeat regions of CbpX and the C-terminal portion (Cterm, i.e., lacking the choline binding domains) of LytX (e.g., LytCCterm or Sp91 Cterm). In another embodiment, CbpX is selected from the group consisting of CbpA, PbcA, SpsA and PspC. In another embodiment, it is CbpA. In an embodiment, LytX is LytC (also referred to as Sp91). Another embodiment of the present invention is a PspA or PsaA truncates lacking the choline binding domain (C) and expressed as a fusion protein with LytX. In an embodiment, LytX is LytC.

PsaA and transmembrane deletion variants thereof have been described by Berry & Paton, Infect Immun 1996 Dec;64(12):5255-62. PspA and transmembrane deletion variants thereof have been disclosed in, for example, US 5804193, WO 92/14488, and WO 99/53940.

Sp128 and Sp130 are disclosed in WO00/76540. Sp125 is an example of a pneumococcal surface protein with the Cell Wall Anchored motif of LPXTG (i.e. leucine-pro//ne-X-threonine-glycine where X is any amino acid). Any protein within this class of pneumococcal surface protein with this motif has been found to be useful within the context of this invention, and is therefore considered a further protein of the invention. Sp125 itself is disclosed in WO 98/18930, and is also known as ZmpB - a zinc metalloproteinase. Sp101 is disclosed in WO 98/06734 (where it has the reference # y85993). It is characterized by a Type I signal sequence. Sp133 is disclosed in WO 98/06734 (where it has the reference # y85992). It is also characterized by a Type I signal sequence.

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The proteins of the invention may also be beneficially combined. By combined is meant that the immunogenic composition comprises all of the proteins from within the following combinations, either as carrier proteins or as free proteins or a mixture of the two. For example, in a combination of two proteins as set out hereinafter, both proteins may be used as carrier proteins, or both proteins may be present as free proteins, or both may be present as carrier and as free protein, or one may be present as a carrier protein and a free protein whilst the other is present only as a carrier protein or only as a free protein, or one may be present as a carrier protein and the other as a free protein. Where a combination of three proteins is given, similar possibilities exist. Preferred combinations include, but are not limited to PhtD + CbpX repeat regions, , PhtD + Ply, PhtD + Sp128, PhtD + PsaA, PhtD + PspA, PhtA + CbpX repeat regions , PhtA + CbpX repeat regions - Sp91Cterm chimeric or fusion proteins, PhtA + Ply, PhtA + Sp128, PhtA + PsaA, PhtA + PspA, CbpX repeat regions + LytC, CbpX repeat regions + PspA, CbpX repeat regions + PsaA, CbpX repeat regions + Sp128, CbpX repeat regions + LytC, CbpX repeat regions + PspA, CbpX repeat regions + PsaA, CbpX repeat regions + Sp128, CbpX repeat regions + PhtD, CbpX repeat regions + PhtA. In an embodiment, CbpX repeat regionsis from CbpA. In another embodiment, it is from CbpA. Other combinations include 3 protein combinations such as PhtD + CbpX repeat regions + Ply, and PhtA + CbpX repeat regions + PhtD. In one embodiment, the immunogenic composition comprises detoxified pneumolysin and PhtD or PhtDE as carrier proteins. In a further embodiment, the immunogenic composition comprises detoxified pneumolysin and PhtD or PhtDE as free proteins.

The total content of protein antigens in the vaccine will typically be in the range 1-100μg, or 5-80μg, e.g. in the range 50-70μg. For example, in one aspect, the immunogenic composition of the invention comprises 26μg-45μg (for example 26μg-40μg, 28μg-35μg or around 30μg) of each S. pneumoniae protein, per human dose. In another aspect, the immunogenic composition comprises 26μg-45μg (for example 26μg-40μg, 28μg-35μg or around 30μg) of PhtD, per human dose. In another aspect, the immunogenic composition of the invention comprises 26μg-45μg (for example 26μg-40μg, 28μg-35μg or around 30μg) of pneumolysin (e.g. dPly), per human dose.

By the term "human dose" is meant a dose which is in a volume suitable for human use. Generally this is between 0.25 and 1.5 ml. In one embodiment, a human dose is 0.5 ml. In a further embodiment, a human dose is higher than 0.5 ml, for example 0.6, 0.7, 0.8, 0.9 or 1 ml. In a further embodiment, a human dose is between 1 ml and 1.5 ml. In another

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embodiment, in particular when the immunogenic composition is for the paediatric population, a human dose may be less than 0.5 ml such as between 0.25 and 0.5 ml.

Adjuvants

The immunogenic compositions of the present invention may be adjuvanted, particularly when intended for use in an elderly population but also for use in infant populations. Thus, a further aspect is an immunogenic composition of the invention which further comprises an adjuvant. Suitable adjuvants include an aluminum salt such as aluminum hydroxide gel or aluminum phosphate or alum, but a suitable adjuvant may also be a salt of calcium, magnesium, iron or zinc, or may be an insoluble suspension of acylated tyrosine, or acylated sugars, cationically or anionically derivatized polysaccharides, or polyphosphazenes. In one aspect of the invention, the adjuvant is an aluminium salt, e.g. aluminium phosphate. In a further aspect, the adjuvant comprises (per 0.5 mL dose) 100-750, 200-500, or 300-400 μg Al (aluminium) as aluminium phosphate.

In one embodiment, the adjuvant is a preferential inducer of a Th1 type of response. Such high levels of Th1-type cytokines tend to favour the induction of cell mediated immune responses to a given antigen, whilst high levels of Th2-type cytokines tend to favour the induction of humoral immune responses to the antigen. Suitable adjuvant systems which promote a predominantly Th1 response include: Monophosphoryl lipid A or a derivative thereof, particularly 3-de-O-acylated monophosphoryl lipid A (3D-MPL) (for its preparation see GB 222021 1 A); and a combination of monophosphoryl lipid A, suitably 3-de-O-acylated monophosphoryl lipid A, together with either an aluminum salt (for instance aluminum phosphate or aluminum hydroxide) or an oil-in-water emulsion. In such combinations, antigen and 3D-MPL are contained in the same particulate structures, allowing for more efficient delivery of antigenic and immunostimulatory signals. Studies have shown that 3D-MPL is able to further enhance the immunogenicity of an alum-adsorbed antigen [Thoelen *et al.* Vaccine (1998) 16:708-14; EP 689454-B1].

The immunogenic composition may comprise a sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide (and optionally an immunostimulant) adsorbed onto a metal salt (such as an aluminium salt, for example Aluminium phosphate or Aluminium hydroxide). The term "immunostimulant" as used herein means a substance that stimulates an immune response, in particular an adjuvant which stimulates the immune system of a host animal (e.g. human) to which it is administered and thereby increases

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the protective effect produced by a antigen administered to that animal, as compared to the effect which would be produced by administration of the antigen alone. For aluminium based vaccine formulations wherein the antigen is typically adsorbed onto aluminium salt for one hour at room temperature under agitation.

Adjuvants comprising Additional Immunostimulants

The adjuvant of the invention may comprise immunostimulants, such as saponins (e.g. QS21) and/or 3D-MPL. Examples of immunostimulants are described herein and in "Vaccine Design - The Subunit and Adjuvant Approach" 1995, Pharmaceutical Biotechnology, Volume 6, Eds. Powell, M.F., and Newman, M.J., Plenum Press, New York and London, ISBN 0-306-44867-X. In one aspect of the present invention the adjuvant comprises QS21 , monophosphoryl lipid A (MPL), phospholipid and sterol, presented in the form of a liposome.

QS-21 is a purified saponin fraction from the bark extracts of the South American tree *Quillaja saponaria.* QS21 typically comprises two principal isomers that share a triterpene, a branched trisaccharide, and a glycosylated pseudodimeric acyl chain. The two isomeric forms differ in the constitution of the terminal sugar within the linear tetrasaccharide segment, wherein the major isomer, QS-21 -Api incorporates a β-D-apiose residue, and the minor isomer, QS-21 -Xyl terminates in a β-D-xylose substituent. (Cleland, J. L.et al. J. Pharm. Sci. 1996, 85, 22-28). QS21 may be prepared by HPLC purification from Quil A™. Quil A™ was described as having adjuvant activity by Dalsgaard *et al.* in 1974 ("Saponin adjuvants", Archiv. fur die gesamte Virusforschung, Vol. 44, Springer Verlag, Berlin, p243-254). Methods for production of QS21 are described in US5057540 (where QS21 is described as QA21) and EP0362278. In an embodiment, immunogenic compositions of the invention contain QS21 in substantially pure form, that is to say, the QS21 comprises at least 90%, for example at least 95%, or at least 98% of the immunogenic composition (i.e. the QS21 composition contains at least 90%, for example at least 95%, or at least 98% QS21). The dose of QS21 is suitably able to enhance an immune response to an antigen in a human. In particular a suitable QS21 amount is that which improves the immunological potential of the composition compared to the unadjuvanted composition, or compared to the composition adjuvanted with another QS21 amount, whilst being acceptable from a reactogenicity profile. QS21 can be used, for example, at an amount of 1 to 100 μg per composition dose, for example in an amount of 10 to 50 μg per composition dose.

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Monophosphoryl lipid A (MPL) is a nontoxic derivative of the lipopolysaccharide (LPS) of gram-negative bacteria, e.g. *Salmonella minnesota* R595. It retains adjuvant properties of the LPS while demonstrating a reduced toxicity (Johnson et al. 1987 Rev. Infect. Dis. 9 Suppl:S512-S516). MPL is composed of a series of 4'-monophosphoryl lipid A species that vary in the extent and position of fatty acid substitution. It may be prepared by treating LPS with mild acid and base hydrolysis followed by purification of the modified LPS. For example, LPS may be refluxed in mineral acid solutions of moderate strength (e.g. 0.1 M HCl) for a period of approximately 30 minutes. This process results in dephosphorylation at the 1 position, and decarbohydration at the 6' position. The term "monophosphoryl lipid A (MPL)" as used herein includes derivatives of monophosphoryl lipid A. Derivatives of monophosphoryl lipid A include 3D-MPL and synthetic derivatives.

3D-MPL is 3-O-deacylated monophosphoryl lipid A (or 3 De-O-acylated monophosphoryl lipid A). Chemically it is a mixture of 3- deacylated monophosphoryl lipid A with 4, 5 or 6 acylated chains. 3D-MPL is available under the trademark MPL® by GlaxoSmithKline Biologicals North America. 3-O-deacylated monophosphoryl lipid A (3D-MPL). It has a further reduced toxicity while again maintaining adjuvanticity, and may typically be prepared by mild alkaline hydrolysis, see for example US4912094. Alkaline hydrolysis is typically performed in organic solvent, such as a mixture of chloroform/methanol, by saturation with an aqueous solution of weak base, such as 0.5 M sodium carbonate at pH 10.5. For further information on the preparation of 3D-MPL see GB222021 1A and WO02078637 (Corixa Corporation). In one aspect of the present invention small particle 3 D-MPL may be used. Small particle 3D-MPL has a particle size such that it may be sterile-filtered through a 0.22μηι filter. Such preparations are described in International Patent Application No. W094/21292. In an embodiment, immunogenic compositions of the invention comprise 3-O-Deacylated monophosphoryl lipid A (3D-MPL).

The dose of monophosphoryl lipid A (MPL), e.g. 3D-MPL, is suitably able to enhance an immune response to an antigen in a human. In particular a suitable monophosphoryl lipid A (MPL), e.g. 3D-MPL, amount is that which improves the immunological potential of the composition compared to the unadjuvanted composition, or compared to the composition adjuvanted with another MPL amount, whilst being acceptable from a reactogenicity profile. Monophosphoryl lipid A (MPL), e.g. 3D-MPL, can be used, for example, at an amount of 1 to 100 μg per composition dose, for example in an amount of 10 to 50 μg per composition dose.

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Liposomes may be made from phospholipids (such as dioleoyl phosphatidyl choline, DOPC) and sterol, e.g. cholesterol, using techniques known in the art. Such liposome carriers may carry the QS21 and/or monophosphoryl lipid A (MPL), e.g. 3D-MPL. Suitable compositions of the invention are those wherein liposomes are initially prepared without MPL (as described in W096/33739), and MPL is then added, suitably as small particles of below 100 nm particles or particles that are susceptible to sterile filtration through a 0.22 μη membrane. The MPL is therefore not contained within the vesicle membrane (known as MPL out). Compositions where the MPL is contained within the vesicle membrane (known as MPL in) also form an aspect of the invention. The unconjugated S. *pneumoniae* proteins can be contained within the vesicle membrane or contained outside the vesicle membrane. Suitably soluble antigens are outside and hydrophobic or lipidated antigens are either contained inside or outside the membrane. Encapsulation within liposomes is described in US4235877.

The liposomes of the present invention may comprise a phospholipid, for example a phosphatidylcholine, which may be non-crystalline at room temperature, for example egg yolk phosphatidylcholine, dioleoyl phosphatidylcholine or dilauryl phosphatidylcholine. Suitably, the phospholipid is dioleoylphosphatidylcholine (DOPC). A further aspect is an immunogenic composition of the invention comprising 0.1 to 10mg, 0.2 to 7, 0.3 to 5, 0.4 to 2, or 0.5 to 1 mg (e.g. 0.4 to 0.6, 0.9 to 1.1, 0.5 or 1 mg) phospholipid.

The liposomes of the present invention may comprise a sterol. The sterol increases the stability of the liposome structure. Suitable sterols include β-sitosterol, stigmasterol, ergosterol, ergocalciferol and cholesterol. These sterols are described in the art, for example cholesterol is disclosed in the Merck Index, 11th Edn., page 341 , as a naturally occurring sterol found in animal fat. In one particular embodiment of the invention, the sterol is cholesterol. Typically, the sterol may be added during formulation of the antigen preparation using QS21 quenched with the sterol as described in W096/33739. In an embodiment, the immunogenic compositions of the invention comprise 0.025 to 2.5, 0.05 to 1.5, 0.075 to 0.75, 0.1 to 0.3, or 0.125 to 0.25 mg (e.g. 0.2 to 0.3, 0.1 to 0.15, 0.25 or 0.125 mg) sterol.

In one embodiment the the adjuvant comprises (per 0.5 mL dose) 0.1-10mg, 0.2-7, 0.3-5, 0.4-2, or 0.5-1 mg (e.g. 0.4-0.6, 0.9-1 .1, 0.5 or 1 mg) phospholipid (for instance DOPC), 0.025-2.5, 0.05-1 .5, 0.075-0.75, 0.1-0.3, or 0.125-0.25 mg (e.g. 0.2-0.3, 0.1-0.15, 0.25 or

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0.125 mg) sterol (for instance cholesterol), 5-60, 10-50, or 20-30 μg (e.g. 5-15, 40-50, 10, 20, 30, 40 or 50 μg) lipid A derivative (for instance 3D-MPL), and 5-60, 10-50, or 20-30 μg (e.g. 5-15, 40-50, 10, 20, 30, 40 or 50 μg) saponin (for instance QS21).

Liposomes of the invention will suitably be comprised in a liquid medium. The liquid medium comprises physiologically acceptable liquids such as water, aqueous salt solutions and buffer solutions, e.g phosphate buffered saline (PBS) etc. For example, immunogenic compositions of the invention may comprise water and PBS.

In one aspect of the invention, the adjuvant is AS01 B (see e.g. W096/33739). In another aspect of the invention, the adjuvant is AS01 E (see e.g. WO2007/068907).

In some cases it may be advantageous that the immunogenic compositions and vaccines of the present invention will further contain a stabiliser, for example other emulsifiers/surfactants, including caprylic acid (Merck index 10th Edition, entry no. 1739), of which Tricaprylin is particularly preferred.

Oil in Water Emulsion Adjuvants

Oil in water emulsion adjuvants *per se* have been suggested to be useful as adjuvant compositions (EP 0 399 843B), also combinations of oil in water emulsions and other active agents have been described as adjuvants for vaccines (WO 95/17210; WO 98/56414; WO 99/12565; WO 99/1 1241). Other oil emulsion adjuvants have been described, such as water in oil emulsions (US 5,422,109; EP 0 480 982 B2) and water in oil in water emulsions (US 5,424,067; EP 0 480 981 B). All of which form preferred oil emulsion systems (in particular when incorporating tocols) that are suitable as adjuvants for use in compositions of the present invention.

A suitable oil emulsion (for example an oil-in-water emulsion) comprises a metabolisible, non-toxic oil, such as squalane, a tocopherol such as alpha tocopherol and optionally an emulsifier (or surfactant) such as polysorbate 80 (Tween™ 80). A sterol (for example cholesterol) may also be included. In one aspect of the invention, there is provided a vaccine or immunogenic composition comprising a sized S. *pnemoniae* serotype 6A capsular polysaccharide and an adjuvant composition comprising an oil in water emulsion, wherein the oil in water emulsion comprises 0.5 - 11mg metabolisable oil, (such as

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squalene), 0.5 - 12 mg tocol (such as alpha-tocopherol) and 0.4 - 5 mg emulsifying agent (such as polyoxyethylene sorbitan monooleate), per human dose.

In order for any oil in water composition to be suitable for human administration, the oil phase of the emulsion system has to comprise a metabolisable oil. The meaning of the term metabolisable oil is known in the art. Metabolisable can be defined as 'being capable of being transformed by metabolism' (Dorland's Illustrated Medical Dictionary, W.B. Sanders Company, 25th edition (1974)). The oil may be any vegetable oil, fish oil, animal oil or synthetic oil, which is not toxic to the recipient and is capable of being transformed by metabolism. Nuts, seeds, and grains are common sources of vegetable oils. Synthetic oils are also part of this invention and can include commercially available oils such as NEOBEE® and others. A particularly suitable metabolisable oil is squalene. Squalene (2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22-tetracosahexaene) is an unsaturated oil which is found in large quantities in shark-liver oil, and in lower quantities in olive oil, wheat germ oil, rice bran oil, and yeast, and is a particularly preferred oil for use in this invention. Squalene is a metabolisable oil, that it is an intermediate in the biosynthesis of cholesterol (Merck index, 10th Edition, entry no.8619). Suitably the metabolisable oil is present in the adjuvant composition in an amount of 0.5-10 mg, for example 1-10, 2-10, 3-9, 4-8, 5-7, or 5-6 mg (e.g. 2-3, 5-6, or 9-10mg).

The oil in water emulsion suitably comprises a tocol. Tocols are known in the art and are described in EP0382271 . Suitably the tocol is alpha-tocopherol or a derivative thereof such as alpha-tocopherol succinate (also known as vitamin E succinate). Said tocol is suitably present in the adjuvant composition in an amount of 0.5-11 mg, for example 1-11, 2-10, 3-9, 4-8, 5-7, 5-6 (e.g. 10-11, 5-6, 2.5-3.5 or 1-3 mg). In a specific embodiment the tocol is present in an amount of 5.94 mg or 2.38 mg. In a further embodiment, said tocol is suitably present in the vaccine (or immunogenic) composition in an amount of 0.5-11 mg, for example 1-11, 2-10, 3-9, 4-8, 5-7, 5-6 (e.g. 10-11, 5-6, 2.5-3.5 or 1-3 mg).

The oil in water emulsion may further comprise an emulsifying agent. The emulsifying agent may suitably be polyoxyethylene sorbitan monooleate. In a particular embodiment the emulsifying agent may be selected from the group comprising: Polysorbate 80 (Tween™ 80). Said emulsifying agent is suitably present in the adjuvant composition in an amount of 0.1-5, 0.2-5, 0.3-4, 0.4-3 or 2-3 mg (e.g. 0.4-1.2, 2-3 or 4-5 mg) emulsifying agent.

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The method of producing oil-in-water emulsions is known to the person skilled in the art. Commonly, the method comprises mixing the tocol-containing oil phase with a surfactant such as a PBS/Tween™80 solution, followed by homogenisation using a homogenizer.A method comprising passing the mixture twice through a syringe needle would be suitable for homogenising small volumes of liquid. Equally, the emulsification process in microfluidiser (M1 10S Microfluidics machine, maximum of 50 passes, for a period of 2 minutes at maximum pressure input of 6 bar (output pressure of about 850 bar)) could be adapted by the man skilled in the art to produce smaller or larger volumes of emulsion.

In an oil in water emulsion, the oil and emulsifier should be in an aqueous carrier. The aqueous carrier may be, for example, phosphate buffered saline.

In an embodiment, the oil-in-water emulsion systems of the present invention have a small oil droplet size in the sub-micron range. Suitably the droplet sizes will be in the range 120 to 750 nm, or from 120 to 600 nm in diameter. In an embodiment, the oil-in water emulsion contains oil droplets of which at least 70% by intensity are less than 500 nm in diameter, or at least 80% by intensity are less than 300 nm in diameter, or at least 90% by intensity are in the range of 120 to 200 nm in diameter.

The oil droplet size, i.e. diameter, according to the present invention is given by intensity. There are several ways of determining the diameter of the oil droplet size by intensity. Intensity is measured by use of a sizing instrument, suitably by dynamic light scattering such as the Malvern Zetasizer 4000 or suitably the Malvern Zetasizer 3000HS. A first possibility is to determine the z average diameter (ZAD) by dynamic light scattering (PCS, Photon correlation spectroscopy); this method additionally gives the polydispersity index (PDI), and both the ZAD and PDI are calculated with the cumulants algorithm. These values do not require the knowledge of the particle refractive index. A second mean is to calculate the diameter of the oil droplet by determining the whole particle size distribution by another algorithm, either the Contin, or non-negative least squares (NNLS), or the automatic "Malvern" one (the default algorithm provided for by the sizing instrument). Most of the time, as the particle refractive index of a complex composition is unknown, only the intensity distribution is taken into consideration, and if necessary the intensity means originating from this distribution.

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Lipopolysaccharide (LPS) or lipooligosaccharide (LOS) derivatives or mutations or lipid A derivatives described herein are designed to be less toxic (e.g. 3D-MPL) than native lipopolysaccharides

In one embodiment the adjuvant used for the compositions of the invention comprises an oil in water emulsion made from a metabolisable oil (such as squalene), an emulsifier (such as Tween™ 80) and optionally a tocol (such as alpha tocopherol). In one embodiment the adjuvant comprises (per 0.5 mL dose) 0.5-15, 1-13, 2-11, 4-8, or 5-6mg (e.g. 2-3, 5-6, or 10-11 mg) metabolisable oil (such as squalene), 0.1-10, 0.3-8, 0.6-6, 0.9-5, 1-4, or 2-3 mg (e.g. 0.9-1.1, 2-3 or 4-5 mg) emulsifier (such as Tween™ 80) and optionally 0.5-20, 1-15, 2-12, 4-10, 5-7 mg (e.g. 11-13, 5-6, or 2-3 mg) tocol (such as alpha tocopherol).

The adjuvant may optionally further comprise 5-60, 10-50, or 20-30 μg (e.g. 5-15, 40-50, 10, 20, 30, 40 or 50 μg) lipid A derivative (for instance 3D-MPL).

The adjuvant may optionally contain 0.025-2.5, 0.05-1.5, 0.075-0.75, 0.1-0.3, or 0.125-0.25 mg (e.g. 0.2-0.3, 0.1-0.15, 0.25 or 0.125 mg) sterol (for instance cholesterol), 5-60, 10-50, or 20-30 μg (e.g. 5-15, 40-50, 10, 20, 30, 40 or 50 μg) lipid A derivative (for instance 3D-MPL), and 5-60, 10-50, or 20-30 μg (e.g. 5-15, 40-50, 10, 20, 30, 40 or 50 μg) saponin (for instance QS21).

In one embodiment, the adjuvant used for the compositions of the invention comprises aluminium phosphate and a lipid A derivative (such as 3D-MPL). This adjuvant may comprise (per 0.5 mL dose) 100-750, 200-500, or 300-400 μg Al as aluminium phosphate, and 5-60, 10-50, or 20-30 μg (e.g. 5-15, 40-50, 10, 20, 30, 40 or 50 μg) lipid A derivative (for instance 3D-MPL).

Method of Administration

The vaccine preparations containing immunogenic compositions of the present invention may be used to protect or treat a mammal susceptible to infection, by means of administering said vaccine via a systemic or mucosal route. These administrations may include injection *via* the intramuscular (IM), intraperitoneal (IP), intradermal (ID) or subcutaneous (SC) routes; or *via* mucosal administration to the oral/alimentary,

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respiratory, genitourinary tracts. Although the vaccine of the invention may be administered as a single dose, components thereof may also be co-administered together at the same time or at different times (for instance pneumococcal saccharide conjugates could be administered separately, at the same time or 1-2 weeks after the administration of the any bacterial protein component of the vaccine for optimal coordination of the immune responses with respect to each other). For co-administration, the optional Th1 adjuvant may be present in any or all of the different administrations. In addition to a single route of administration, 2 different routes of administration may be used. For example, polysaccharide conjugates may be administered IM (or ID) and bacterial proteins may be administered IN (or ID). In addition, the vaccines of the invention may be administered IM for priming doses and IN for booster doses.

Following an initial vaccination, subjects may receive one or several booster immunizations adequately spaced.

Vaccine

The present invention further provides a vaccine containing the immunogenic compositions of the invention and a pharmaceutically acceptable excipient or carrier.

Pharmaceutically acceptable excipients and carriers are well known and can be selected by those of skill in the art. For example, the pharmaceutically acceptable excipient or carrier can include a buffer, such as Tris (trimethamine), phosphate (e.g. sodium phosphate), acetate, borate (e.g. sodium borate), citrate, glycine, histidine and succinate (e.g. sodium succinate), suitably sodium chloride, histidine, sodium phosphate or sodium succinate. The pharmaceutically acceptable excipient may include a salt, for example sodium chloride, potassium chloride or magnesium chloride. Optionally, the pharmaceutically acceptable excipient contains at least one component that stabilizes solubility and/or stability. Examples of solubilizing/stabilizing agents include detergents, for example, laurel sarcosine and/or tween (e.g. Tween 80). Examples of stabilizing agents also include poloxamer (e.g. poloxamer 124, poloxamer 188, poloxamer 237, poloxamer 338 and poloxamer 407). The pharmaceutically acceptable excipient may include a non-ionic surfactant, for example polyoxyethylene sorbitan fatty acid esters, Polysorbate-80 (Tween 80), Polysorbate-60 (Tween 60), Polysorbate-40 (Tween 40) and Polysorbate-20 (Tween 20), or polyoxyethylene alkyl ethers (suitably polysorbate-80). Alternative solubilizing/stabilizing agents include arginine, and glass forming polyols (such

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as sucrose, trehalose and the like). The pharmaceutically excipient may be a preservative, for example phenol, 2-phenoxyethanol, or thiomersal. Other pharmaceutically acceptable excipients include sugars (e.g. lactose, sucrose), and proteins (e.g. gelatine and albumin). Pharmaceutically acceptable carriers include water, saline solutions, aqueous dextrose and glycerol solutions. Numerous pharmaceutically acceptable excipients and carriers are known in the art and are described, e.g., in Remington's Pharmaceutical Sciences, by E. W. Martin, Mack Publishing Co., Easton, PA, 5th Edition (975).

According to a further aspect of the invention there is provided a process for making the immunogenic composition or vaccine of the invention comprising the step of mixing S. *pneumoniae* capsular polysaccharide (conjugates) of the invention, optionally with a pharmaceutically acceptable excipient or carrier.

Vaccine preparation is generally described in Vaccine Design ("The subunit and adjuvant approach" (eds Powell M.F. & Newman M.J.) (1995) Plenum Press New York). Encapsulation within liposomes is described by Fullerton, US Patent 4,235,877.

The vaccines of the present invention may be stored in solution or lyophilized. In an embodiment, the solution is lyophilized in the presence of a sugar such as sucrose or lactose. It is still further preferable that they are lyophilized and extemporaneously reconstituted prior to use. Lyophilizing may result in a more stable composition (vaccine) and may possibly lead to higher antibody titers in the presence of 3D-MPL and in the absence of an aluminium based adjuvant.

In one aspect of the invention is provided a vaccine kit, comprising a vial containing an immunogenic composition of the invention, optionally in lyophilised form, and further comprising a vial containing an adjuvant as described herein. It is envisioned that in this aspect of the invention, the adjuvant will be used to reconstitute the lyophilised immunogenic composition.

Although the vaccines of the present invention may be administered by any route, administration of the described vaccines into the skin (ID) forms one embodiment of the present invention. Human skin comprises an outer "horny" cuticle, called the stratum corneum, which overlays the epidermis. Underneath this epidermis is a layer called the dermis, which in turn overlays the subcutaneous tissue. Researchers have shown that

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injection of a vaccine into the skin, and in particular the dermis, stimulates an immune response, which may also be associated with a number of additional advantages. Intradermal vaccination with the vaccines described herein forms a preferred feature of the present invention.

The conventional technique of intradermal injection, the "mantoux procedure", comprises steps of cleaning the skin, and then stretching with one hand, and with the bevel of a narrow gauge needle (26-31 gauge) facing upwards the needle is inserted at an angle of between 10-15°. Once the bevel of the needle is inserted, the barrel of the needle is lowered and further advanced whilst providing a slight pressure to elevate it under the skin. The liquid is then injected very slowly thereby forming a bleb or bump on the skin surface, followed by slow withdrawal of the needle.

More recently, devices that are specifically designed to administer liquid agents into or across the skin have been described, for example the devices described in WO 99/34850 and EP 1092444, also the jet injection devices described for example in WO 01/13977; US 5,480,381 , US 5,599,302, US 5,334,144, US 5,993,412, US 5,649,912, US 5,569,189, US 5,704,91 1, US 5,383,851 , US 5,893,397, US 5,466,220, US 5,339,163, US 5,312,335, US 5,503,627, US 5,064,413, US 5,520, 639, US 4,596,556, US 4,790,824, US 4,941 ,880, US 4,940,460, WO 97/37705 and WO 97/13537. Alternative methods of intradermal administration of the vaccine preparations may include conventional syringes and needles, or devices designed for ballistic delivery of solid vaccines (WO 99/27961), or transdermal patches (WO 97/48440; WO 98/28037); or applied to the surface of the skin (transdermal or transcutaneous delivery WO 98/20734 ; WO 98/28037).

When the vaccines of the present invention are to be administered to the skin, or more specifically into the dermis, the vaccine is in a low liquid volume, particularly a volume of between about 0.05 ml and 0.2 ml.

The content of the immunogenic composition in the skin or intradermal vaccines of the present invention may be similar to conventional doses as found in intramuscular vaccines (see above). However, it is a feature of skin or intradermal vaccines that the formulations may be "low dose". Accordingly the protein antigens in "low dose" vaccines are suitably present in as little as 0.1 to 10μg, or 0.1 to 5 μg per dose; and the

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polysaccharide (suitably conjugated) antigens may be present in the range of 0.01 -^g, and suitably between 0.01 to 0.5 μg of saccharide per dose.

As used herein, the term "intradermal delivery" means delivery of the vaccine or immunogenic composition to the region of the dermis in the skin. However, the vaccine or immunogenic composition will not necessarily be located exclusively in the dermis. The dermis is the layer in the skin located between about 1.0 and about 2.0 mm from the surface in human skin, but there is a certain amount of variation between individuals and in different parts of the body. In general, it can be expected to reach the dermis by going 1.5 mm below the surface of the skin. The dermis is located between the stratum corneum and the epidermis at the surface and the subcutaneous layer below. Depending on the mode of delivery, the vaccine or immunogenic composition may ultimately be located solely or primarily within the dermis, or it may ultimately be distributed within the epidermis and the dermis.

The present invention further provides an improved vaccine for the prevention or amelioration of otitis media caused by *Haemophilus influenzae* by the addition of *Haemophilus influenzae* proteins, for example protein D in conjugated form. One or more *Moraxella catarrhalis* protein antigens can also be included in the vaccine or immunogenic composition of the invention in a free or conjugated form. Thus, the present invention is an improved method to elicit an immune response against otitis media in infants.

Examples of preferred *Moraxella catarrhalis* protein antigens which can be included in a combination vaccine or immunogenic composition of the invention (especially for the prevention of otitis media) are: outer membrane protein 106 (OMP106) [WO 97/41731 (Antex) & WO 96/34960 (PMC)]; outer membrane protein 21 (OMP21) or fragments thereof (WO 0018910); lactoferrin binding protein A (LbpA) &/or lactoferrin binding protein B (LbpB) [WO 98/55606 (PMC)]; transferrin binding protein A (TbpA) &/or transferring binding protein B (TbpB) [WO 97/13785 & WO 97/32980 (PMC)]; *Moraxella catarrhalis* CopB protein [Helminen ME, *et al.* (1993) Infect. Immun. 61:2003-2010]; ubiquitous surface protein A1 (UspA1) and/or ubiquitous surface protein A2 (UspA2) [WO 93/03761 (University of Texas)]; outer membrane protein CD (OmpCD); HasR (PCT/EP99/03824); PilQ (PCT/EP99/03823); outer membrane protein 85 (OMP85) (PCT/EPOO/01468); lipo06 (GB 9917977.2); lipo10 (GB 9918208.1); lipo1 1 (GB 9918302.2); lipo18 (GB 9918038.2); outer membrane protein P6 (P6) (PCT/EP99/03038); D15 surface antigen (D15) (PCT/EP99/03822); outer membrane protein A1 (OmpA1) (PCT/EP99/06781); Hly3

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(PCT/EP99/03257); and outer membrane protein E (OmpE). Examples of non-typeable *Haemophilus influenzae* proteins or fragments thereof which can be included in a combination vaccine (especially for the prevention of otitis media) include: Fimbrin protein [(US 5766608 - Ohio State Research Foundation)] and fusions comprising peptides therefrom [eg LB1 (f) peptide fusions; US 5843464 (OSU) or WO 99/64067]; outer membrane protein 26 (OMP26) [WO 97/01638 (Cortecs)]; P6 [EP 281673 (State University of New York)]; TbpA and/or TbpB; *H. influenzae* adhesin (Hia); *Haemophilus* surface fibrils (Hsf); *Haemophilus influenzae* Hin47 protein; *Haemophilus influenzae* Hif protein; *Haemophilus influenzae* Hmw1 protein; *Haemophilus influenzae* Hmw2 protein; *Haemophilus influenzae* Hmw3 protein; *Haemophilus influenzae* Hmw4 protein; *Haemophilus influenzae* autotransporter adhesin (Hap); D15 (WO 94/12641); P2; and P5 (WO 94/26304).

Methods of Treatment and Use

The present invention provides a method for the treatment or prevention of *Streptococcus pneumoniae* infection in a subject in need thereof comprising administering to said subject a therapeutically effective amount of an immunogenic composition or the vaccine of the invention. The present invention also provides a method of immunising a human host against *Streptococcus pneumoniae* infection comprising administering to the host an immunoprotective dose of the immunogenic composition or vaccine of the invention. The present invention also provides a method of inducing an immune response to *Streptococcus pneumoniae* (e.g. *Streptococcus pneumoniae* serotype 6A) in a subject, the method comprising administering a therapeutically effective amount of the immunogenic composition or vaccine of the invention.

In an embodiment, the present invention is an improved method to elicit an immune response in infants (defined as 0-2 years old in the context of the present invention) by administering a therapeutically effective amount of an immunogenic composition or vaccine of the invention. In one embodiment, the immune response is protective (i.e. it can prevent or reduce infection caused by S. *pneumoniae).* In one embodiment, the vaccine is a paediatric vaccine.

In an embodiment, the present invention is an improved method to elicit a (protective) immune response in the elderly population (in the context of the present invention a patient is considered elderly if they are 50 years or over in age, typically over 55 years

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and more generally over 60 years) by administering a therapeutically effective amount of the immunogenic composition or vaccine of the invention.

In one embodiment, the present invention provides a method of protecting a subject against a disease caused by infection with *Streptococcus pneumoniae,* or a method of preventing infection with *Streptococcus pneumoniae,* or a method of reducing the severity of or delaying the onset of at least one symptom associated with an infection caused by *Streptococcus pneumoniae,* the methods comprising administering to a subject an immunogenic amount of an immunogenic composition or vaccine of the invention.

In an embodiment, the present invention provides immunogenic compositions and vaccines of the invention for use in the prevention or treatment of a disease caused by S. *pneumoniae* infection. In an embodiment, the present invention provides the use of an immunogenic composition or vaccine of the invention in the manufacture of a medicament for the prevention (or treatment) of a disease caused by S. *pneumoniae* infection.

The disease caused by *Streptococcus pneumoniae* infection may be selected from pneumonia, invasive pneumococcal disease (IPD), exacerbations of chronic obstructive pulmonary disease (COPD), otitis media, meningitis, bacteraemia, pneumonia and/or conjunctivitis. Where the human host is an infant, the disease may be selected from otitis media, meningitis, bacteraemia, pneumonia and/or conjunctivitis. Where the human host is elderly, the disease may be selected from pneumonia, invasive pneumococcal disease (IPD), and/or exacerbations of chronic obstructive pulmonary disease (COPD).

Embodiments herein relating to "vaccine compositions" of the invention are also applicable to embodiments relating to "immunogenic compositions" of the invention, and vice versa.

Embodiments of the invention are further described in the subsequent numbered paragraphs:

Paragraph 1: A sized Streptococcus pneumoniae serotype 6A capsular polysaccharide wherein the average size (Mw) of the Streptococcus pneumoniae serotype 6A capsular polysaccharide is between 180-400, 210-400, 210-370, 220-360, 230-350, 240-340, 240-320, 240-310 or 250-31 OkDa.

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Paragraph 2: A sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide according to paragraph 1 which has been sized by a mechanical sizing technique.

Paragraph 3: A sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide according to paragraph 1 or paragraph 2 conjugated to a carrier protein (e.g. CRM-197).

Paragraph 4: A sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide according to paragraph 3 wherein the *Streptococcus pneumoniae* serotype 6A capsular polysaccharide is directly conjugated to the carrier protein.

Paragraph 5: A sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide according to any one of paragraphs 3-4 wherein the 6A polysaccharide is conjugated to the carrier protein or to a linker using CDAP chemistry.

Paragraph 6: A sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide according to any one of paragraphs 3-5 wherein the serotype 6A capsular polysaccharide (PS6A) is conjugated to the carrier protein or to a linker using CDAP chemistry using a CDAP:PS6A ratio between 1:2 to 3:1 , 1:1.5 to 2:1 , or 1:1 .

Paragraph 7: A sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide according to any one of paragraphs 3-6 wherein the serotype 6A capsular polysaccharide is conjugated to the carrier protein or to a linker using CDAP chemistry wherein the reaction was carried out using a coupling time of between 50-130 minutes, 60-130 minutes, or 110-130 minutes.

Paragraph 8: A sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide according to any one of paragraphs 3-7 wherein the ratio of carrier protein to serotype 6A capsular polysaccharide is between 5:1 and 1:5, 4:1 and 1:1 or 2:1 and 1:1 , 1.5:1 and 1:1 , 1.4:1 and 1.3:1 (for example 1.2:1 , 1.5:1) (w/w).

Paragraph 9: A sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide according to paragraphs 1-8 wherein the 6A capsular polysaccharide is sized by a factor of no more than x5.

Paragraph 10: A process for preparing a *Streptococcus pneumoniae* serotype 6A capsular polysaccharide conjugate comprising conjugating a sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide to a carrier protein or to a linker using CDAP chemistry using a CDAP:PS ratio between 1:2 to 3:1 , 1:1.5 to 2:1 , or 1:1 .

Paragraph 11: A process according to paragraph 10 wherein the reaction was carried out using a coupling time of between 50-130 minutes, 60-130 minutes, or 110-130 minutes.

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Paragraph 12: A process for preparing a *Streptococcus pneumoniae* serotype 6A capsular polysaccharide conjugate comprising (a) conjugation of a sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide to a carrier protein and (b) diafiltration against a solution having a concentration of NaCl below 150mM.

Paragraph 13: An immunogenic composition comprising a sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide according to paragraphs 1-9 or *Streptococcus pneumoniae* serotype 6A capsular polysaccharide obtained by a process of any one of paragraphs 10-12 conjugated to a carrier protein.

Paragraph 14: An immunogenic composition according to paragraph 13 comprising 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, or 16 or more capsular polysaccharides conjugates from different S. *pneumoniae* serotypes.

Paragraph 15: An immunogenic composition according to paragraph 13 or paragraph 14 comprising 1 or more, native capsular polysaccharides conjugates from S. *pneumoniae.*

Paragraph 16: An immunogenic composition according to paragraph 15 comprising native *Streptococcus pneumoniae* capsular serotype 6B polysaccharide.

Paragraph 17: An immunogenic composition according to paragraph 15 or 16 comprising native *Streptococcus pneumoniae* capsular serotype 23F polysaccharide.

Paragraph 18: An immunogenic composition according to any one of paragraphs 13 to 17 which comprises *Streptococcus pneumoniae* capsular serotype 6B polysaccharide having an average size (M_w) of between 500-1800, 900-1660, or 1000- 1400 kDa.

Paragraph 19: An immunogenic composition according to any one of paragraphs 13 to 18 which comprises *Streptococcus pneumoniae* capsular serotype 23F polysaccharide having an average size (M_w) of between 500-1500, 600-1500, 700-1300, 900-1250, 800-1100, or 900-1 000 kDa.

Paragraph 20: An immunogenic composition according to any one of paragraphs 13 to 19 which comprises *Streptococcus pneumoniae* capsular polysaccharide from: (a) serotype 1 having an average size (M_w) of between 100-1000, 200-800, 250-600, or 300-400 kDa; (b) serotype 4 having an average size (M_w) of between 50-500, 60-300, 70-150, or 75-125 kDa; (c) serotype 5 having an average size (M_w) of between 100-1000, 100-700, 100-350, or 150-300 kDa; (d) serotype 7F having an average size (M_w) of between 50-1000, 100-750, 150-500, or 200-300 kDa; (e) serotype 9V having an average size (M_w) of between 50-1000, 100-750, 150-500, 200-400, or 250-300 kDa; (f) serotype 14 having an average size (Mw) of between 50-1000, 100-750, 150-500, or 200-250 kDa; (g) 18C having an

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average size (M_w) of between 50-1000, 50-750, 50-500, 50-190, 50-150 or 80-1 10 kDa (h) serotype 19F having an average size (M_w) of between 50-1000, 100-750, 100-500, 100-190 or 120-180 kDa; and/or (i) serotype 19A having an average size (M_w) of between 50-800kDa, 110-700, 110-300, 120-200, 130-180, 140-160 or 80-130 kDa.

Paragraph 21: An immunogenic composition according to any one of paragraphs 13 to 20 which further comprises *Streptococcus pneumoniae* capsular serotype 22F having an average size (M_w) of between 50 and 800 kDa, 110 and 700 kDa, 110-300, 120-200, 130-180, 150-170, 100-190, 100-150, 95-125 or 100-1 15 kDa.

Paragraph 22: An immunogenic composition according to any one of paragraphs 13 to 21 comprising 2, 3, 4, 5 or 6 different carrier proteins.

Paragraph 23: An immunogenic composition according to any one of paragraphs 13 to 22 wherein one or more or all carrier proteins is selected from the group consisting of: diphtheria toxoid (DT), CRM197, tetanus toxoid (TT), Fragment C of TT, dPly, PhtA, PhtB, PhtD, PhtE, PhtDE OmpC, PorB and *Haemophilus influenzae* Protein D.

Paragraph 24: An immunogenic composition of any one of paragraphs 13 to 23 wherein the *Streptococcus pneumoniae* capsular serotype 6B polysaccharide is conjugated to a different carrier protein (e.g. protein D) than the carrier protein to which *Streptococcus pneumoniae* serotype 6A capsular polysaccharide is conjugated.

Paragraph 25: An immunogenic composition of any one of paragraphs 13 to 24 comprising serotype 1 saccharide conjugated to protein D, serotype 4 saccharide conjugated to protein D, serotype 5 saccharide conjugated to protein D, serotype 6B saccharide conjugated to protein D, serotype 7F saccharide conjugated to protein D, serotype 9V saccharide conjugated to protein D, serotype 14 saccharide conjugated to protein D and serotype 23F saccharide conjugated to protein D.

Paragraph 26: An immunogenic composition according to any one of paragraphs 13 to 25 comprising serotype 19F conjugated to Diphtheria toxoid.

Paragraph 27: An immunogenic composition according to any one of paragraphs 13 to 26 wherein the composition comprises capsular saccharide 18C conjugated to tetanus toxoid (TT), optionally wherein 18C is the only saccharide in the composition conjugated to tetanus toxoid (TT), optionally via an ADH linker

Paragraph 28: An immunogenic composition according to any of paragraphs 13 to 27 further comprising S. *pneumoniae* capsular saccharide(s) of one or more of: serotype 33F, serotype 15 and serotype 12F, conjugated to carrier protein(s).

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Paragraph 29: An immunogenic composition according to any of paragraphs 13 to 28 wherein the dose of the 6A saccharide conjugate is between 1 and 10 μg, 1 and 5 μg, or 1 and 3 μg of saccharide (e.g. 2 μg).

Paragraph 30: An immunogenic composition according to any one of paragraphs 13 to 29 which further comprises one or more unconjugated or conjugated *S pneumoniae* proteins selected from: Poly Histidine Triad family (PhtX), Choline Binding Protein family (CbpX), CbpX truncates, LytX family, LytX truncates, CbpX truncate-LytX truncate chimeric proteins, detoxified pneumolysin (Ply), PspA, PsaA, Sp128, Sp101 , Sp130, Sp125 and Sp133.

Paragraph 31: An immunogenic composition according to any one of paragraphs 13 to 30 which further comprises an adjuvant.

Paragraph 32: An immunogenic composition of paragraph 31 wherein the adjuvant comprises (per 0.5 mL dose) 100-750, 200-500, or 300-400 μg Al (aluminium) as aluminium phosphate.

Paragraph 33: A vaccine comprising the immunogenic composition of any one of paragraphs 13 to 32 and a pharmaceutically acceptable excipient or carrier.

Paragraph 34: A process for making the vaccine according to paragraph 33 which comprises the step of mixing the immunogenic composition of any of paragraphs 13 to 32 with a pharmaceutically acceptable excipient or carrier.

Paragraph 35: A method for the treatment or prevention of *Streptococcus pneumoniae* infection in a subject in need thereof comprising administering to said subject a therapeutically effective amount of an immunogenic composition of any of paragraphs 13 to 32 or the vaccine of paragraph 33.

Paragraph 36: A method of immunising a human host against *Streptococcus pneumoniae* infection comprising administering to the host an immunoprotective dose of the immunogenic composition of any of paragraphs 13 to 32 or vaccine of paragraph 34.

Paragraph 37: A method of inducing an immune response to *Streptococcus pneumoniae* serotype 6A in a subject, the method comprising administering a therapeutically or prophylactically effective amount of the immunogenic composition of any of paragraphs 13 to 32 or the vaccine of paragraph 33.

Paragraph 38: The immunogenic composition of paragraphs 13 to 32 or vaccine of paragraph 33 for use in the treatment or prevention of disease caused by *Streptococcus pneumoniae* infection.

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Paragraph 39: A use of the immunogenic composition of paragraphs 12 to 32 or vaccine of paragraph 33 in the manufacture of a medicament for the treatment or prevention of a disease caused by *Streptococcus pneumoniae* infection.

All references or patent applications cited within this patent specification are incorporated by reference herein.

In order that this invention may be better understood, the following examples are set forth. These examples are for purposes of illustration only, and are not to be construed as limiting the scope of the invention in any manner.

Examples

Example 1 - preparation of sized polysaccharide

Sizing

A homogenizer EMULSIFLEX C-50 apparatus was used to reduce the molecular weight and the viscosity of the polysaccharide before the activation step (microfluidization). The efficiency of the sizing depended on the circuit pressure and on the total cycle's number. The homogenizing cell of EMULSIFLEX C-50 was replaced by a cell with a fixed geometry (Microfluidics F20Y-75μm interaction chamber for E01 to E07; Microfluidics F30Y-125μm interaction chamber for E08). The aim of the sizing was to reduce the molecular weight and the viscosity of the polysaccharide without a critical decrease of its antigenicity.

The size reduction was followed *in-process* by viscosimetry (Brookfield Programmable DV III + rheometer). When the target in viscosity was reached, the sized polysaccharide was characterized by HP-SEC-RI (high-performance size-exclusion chromatography, refractive index).

In-process characterizations of the M_w were performed by HP-SEC-RI (TSK5000PWxi_ column + guard column). The elution was realised using a 50mM **Na/K2P0$_4$,** 500mM NaCl pH 7.6 at a flow rate of 0.6ml/min. The detection was realised by a refractive index detector. This characterization is based on the determination of a relative retention time

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(RRT). The RRT is calculated with regard to calibrated dextrans having a molecular weight in the linear range of separation of a TSK5000PWxi_ column. The RRT is defined by the following formula:

$$RRT = \frac{RT_{(sized\ PS)} - RT_{(HMW\ dext)}}{RT_{(LMW\ dext)} - RT_{(HMW\ dext)}}$$

$RT_{(sized\ ps)}$ = retention time of sized PS (polysaccharide)

$RT_{(HMw\ dext)}$ = retention time of high molecular weight dextran (dextran 1730 Kda)

$RT_{(LMw\ dext)}$ = retention time of low molecular weight dextran (dextran 150 Kda)

The native serotype 6A polysaccharide was dissolved at 15 mg/ml during 4 hours in WFI (water for injection) at room temperature. After 4 hours of dissolution, the pH of the solution was adjusted at 6.5 +/- 0.5 before its transfer in a cold room to pursue dissolution overnight. Before the sizing, the solution of native serotype 6A polysaccharide was clarified on 5 μη filter.

The serotype 6A polysaccharide was then sized by Emulsiflex with a Microfluidics F30Y-125 μη homogenizing cell at a pressure of between 2900 and 3800. The sizing was stopped when the viscosity of the polysaccharide reached the targeted value for the viscosity (8.35 or 12.4). The number of cycles required depended on the target. For example, 32.5 cycles were needed to reach a viscosity of 12.4 cps using a pressure of 2900 psi. For this sample, in process determination of relative retention time by HP-SEC-RI gave a value of 0.28.

The sized 6A polysaccharide was filtered on a Millipak 20 (5 g scale) membrane (cut-off 0.22 μη) at a flow-rate of 10 ml/min.

The polysaccharide content of sized serotype 6A polysaccharide solution was accurately determined by colorimetric method (resorcinol) before its use in conjugation. Sized polysaccharide was characterized either by HP-SEC-MALLS or estimated by dextrans calibration curve and determination of antigenic activity (Table 1).

Antigenicity

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Test and reference samples were incubated in microtiter plates previously coated with monoclonal antibodies raised against S. *pneumoniae* polysaccharide serotype 6A (PS6A). Rabbit polyclonal anti-PS6A antibodies were then added. Antigen-antibody complex were revealed using a goat anti-rabbit Ig peroxidase linked. Colour development was then performed by the system Ortho-phenylenediamine/H202 reacting with peroxidase. Coloration was measured by spectrophotometry (absorbances at 490 and 620 nm). The polysaccharide curve was compared to the reference curve (one native polysaccharide lot used as reference while another one native polysaccharide lot was used as internal control) in order to determine the polysaccharide concentration.

Measurement of Molecular weight and polydispersity by MALLS

The molecular weight (M_w) was determined by Laser Light Scattering (SEC-MALLS). In a first time, the analyses were performed on a TSK5000PWxi_ (+guard column) using NaCl 0.2M + 0.02% azide solution as elution buffer. Last analyses were performed on a TSKGMPWXL column (+guard column) with a loading of 100 µI of polysaccharide (1 mg/ml) using 50 mM Na/K_2P0_4, 200 mM NaCl pH 7.0 as elution buffer and a flow-rate of 0.75 ml/min.

The detection was realised with a laser spectrophotometer and an interferometric refractometer (Wyatt Otilab DSP equipped with a P100 cell and a red filter at 498nm).
The average molecular weight in number (M_n) was also obtained by MALLS. The polydispersity of the sized polysaccharide was obtained as the M_w/M_n ratio.
A theoretical dn/dc of 0.14 was first used. When determined, the experimental value of 0.151 was used.

Table 1: Sized PS6A characterizations

PS6A Lot	PS content (µg/ml)	RRT $_{150-1730}$ [1]	Viscosity (cp)	M_W (kDa) [2] (estimation versus dextran calibration curve)	MALLS - M_W (kDa) - R_w (nm) - Polydispersity	Antigenicity by ELISA (%)
E01	13650	0.45	8.35	575	ND	124
E02	8570	0.50	6.03	514	ND	ND

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E03	14210	0.24	15.6	968	ND	ND
E04	12000	0.21	17.3	1018		120 [3]
E05	-	-	-	-	-	-
E06	13910	0.24	12.1	916	T13M / -70°C 256 25.6 1.124	130 [3]
E07	14700	0.24	12.2	929	307.4 28.1 1.112	120 [3]
E08	13590	0.20	12.3	1004	T12M / -70°C 277 27.7 1.094	144 [3]

[1] RRT: relative retention time (HP-SEC-RI) -[2] HP-SEC-RI - [3] relative value versus corresponding native polysaccharide. Remark: No MALLS values available at TO for lots E06 and E08. Value at T13M (time = 13 minutes) or T12M (time = 12 minutes) could be considered as representative insofar as relative retention time and viscosity did not change between TO (time = 0 minutes) and T13M or T12M for both samples. ND means "not determined".

Example 2: Production of 6A conjugates with different carriers

a) Native S. *pneumoniae* serotype 6A polysaccharide vs. sized S. *pneumoniae* serotype 6A polysaccharide

The activation and coupling conditions used to produce different 6A conjugates are given in Table 2.

Table 2: Specific activation/coupling/quenching conditions of S. *pneumoniae* serotype 6A polysaccharide -Protein D/PhtD conjugates

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Carrier	PD (lot PD003) (PS6A-PD conjugate)	PhtD (lot PhtD004) (PS6A-PhtD conjugate)	PhtD (lot PhtD008) (PS6A-PhtD conjugate)	dPly (lot dPly010) (PS6A-dPly conjugate)
PS6A cone, (mg/ml)	5.5 Native PS	5.5 Native PS	10.0 Sized PS	10 Sized PS
PS6A dissolution	NaCl 2M	NaCl 2M	NaCl 2M	NaCl 2M
Carrier protein concentration (mg/ml)	5.0	10.0	20.0	10.0
Initial Carrier protein/PS6A Ratio (w/w)	1/1	3/1	3/1	3/1
CDAP/PS6A ratio (mg/mg PS)	0.75	1.5	1.5	1.5
Coupling time	60 min	45 min	150 min	180 min
pHa/pHc/pHq	9.5/9.5/9.0	9.5/9.5/9.0	9.5/9.5/9.0	9.5/9.5/9.0

The final protein/PS6A ratio (w/w) for the resulting conjugates were:

PS6A-PD003: 0.6/1 (Native PS6A, M_w by MALLS 1106kDa)

PS6A-PhtD004: 1.4/1 (Native PS6A, M_w by MALLS 1106kDa)

PS6A-PhtD008: 2.7/1 (Sized PS6A, lot E01)

PS6A-dPly010: 1.65/1 (Sized PS6A, lot E01)

b) Sized polysaccharide conjugated to different carriers

The activation and coupling conditions used to produce different PS6A conjugates are given in Table 3.

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Table 3: Specific activation/coupling/quenching conditions of PS6A-Protein D/CRM197/PhtD conjugates

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Carrier	PD (lot PDLS001 and lot PDLS002) (PS6A-PD conjugate)	CRM197 (lot CRM025) (PS6A-CRM197 conjugate)
PS6A conc. (mg/ml)	10.0	10.0
PS6A dissolution	NaCl 2M	NaCl 2M
Carrier protein concentration (mg/ml)	10.0	10.0
Initial Carrier protein /PS6A Ratio (w/w)	1.5/1	1.5/1
CDAP conc. (mg/mg PS)	1.5	1
Coupling time	120 min	120 min
$pH_a/pH_c/pH_q$	9.5/9.5/9.0	9.5/9.5/9.0

The final protein/PS6A ratio (w/w) for the resulting conjugates were:
PS6A-PD/LS001 and PS6A-PD/LS002: 1.6/1 (Sized PS6A, lot E06)
PS6A-CRM1 97/025: 1.3/1 (Sized PS6A, lot E03)

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PS6A $_{AH}$-PhtD conjugate (lot 6A-PhtD106)

In a second conjugation method PS6A was linked to the carrier protein PhtD via a linker — Adipic acid dihydrazide (ADH); this conjugate is designated PS6AAH-PhtD.

PS6A derivatization

Activation and coupling were performed at 25°C under continuous stirring in a temperature-controlled waterbath. Microfluidized PS6A was diluted to obtain a final polysaccharide concentration of 10 mg/ml in water for injection (WFI) and the solution was adjusted at pH 6.0 ± 0.2 with 0.1 N HCl. CDAP solution (100 mg/ml freshly prepared in acetonitrile/WFI, 50/50) was added to reach the appropriate CDAP/PS ratio (1/1 , w/w).

The pH was raised up to the activation pH 9.00 ±0.05 by the addition of 0.2M NaOH. After 3 minutes, ADH was added to reach the appropriate ADH/PS ratio (8.9/1 w/w); the pH was regulated to coupling pH 9.0. The solution was left for 1 hour under pH regulation. The PSAH derivative was then dialysed against 0.2M NaCl.

Coupling

PhtD at 7.5 mg/ml in 0.2M NaCl was added to the PS6A $_{AH}$ derivative (PS6A with the ADH linker) in order to reach a PhtD/PS6AAH ratio of 3/1 (w/w). The pH was adjusted to 5.0 ± 0.05 with HCl. The EDAC solution (50 mg/ml in 0.1 M Tris-HCl pH 7.5) was added manually in 10 min (20 µl/ min) to reach 0.5 mg EDAC/mg PS6AAH. The resulting solution was incubated for 45 min at room temperature under stirring and pH regulation. The solution was neutralized by addition of 1ml of 1M Tris-HCl pH 7.5 and let 30 min at room temperature.

Prior to the elution on Sephacryl S400HR, the conjugate was clarified using a 5µm Minisart filter. The resulting conjugate PS6A $_{AH}$-PhtD106 had a final PhtD/PS6A ratio of 2.84 (w/w).

A target in relative retention time was chosen (RRT $_{150\text{-}1730}$ around 0.50). Several conjugates (6A-PhtD004 (native polysaccharide) and 6A-PhtD008 (sized polysaccharide)

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(as described above) were produced with sized polysaccharide and were compared to conjugates produced with native PS6A.

Example 3 : Preclinical evaluation of Anti-PS6A Responses

Groups of 40 mice were immunized IM at days 0, 14 and 28 with 14 valent (14V) formulations containing PS6A conjugates (at 1/10 of human dose) using $AlPO_4$ as adjuvant. Anti-PS6A ELISA IgG titers and Opsonophagocytosis (OP) titers were measured in individual sera collected at day 42.

The 14V-formulation contained the following conjugates :
1-PD, 3-PD, 4-PD, 5-PD, 6B-PD, 7F-PD, 9V-PD, 14-PD, 18C-TT_{AH}, 19A-dPly, 19F-DT, 22F-PhtD, 23F-PD. The serotype 6A was conjugated with PD, PhtD or dPly as carrier.

Results are summarized in Figure 1 ELISA anti-PS6A response.

Conclusions:
The highest anti-PS6A ELISA IgG titers were obtained with the PS6A-PD conjugate produced with a native polysaccharide (1106kDa). The evaluation showed a trend to a lower immunogenicity for conjugates with sized polysaccharide. Using PhtD as carrier, a lower anti-PS ELISA IgG titer was observed with the conjugate produced with a sized polysaccharide (14V (6A-PhtD/008 using lot E01). Subsequently, as described in the following examples, a change in sizing target was investigated to produce a PS6A with robustness of process and immunogenicity of resulting conjugates. The following experiments were carried out to evaluate sized polysaccharide having a higher molecular weight than PS6A from lot E01 .

Example 4 : Evaluation of Sized PS6A-CRM197 Conjugates

For each conjugate, conjugation parameters are given in Table 4 (PS6A and carrier concentrations, initial carrier/PS6A ratio(w/w), coupling time and CDAP/PS6A ratio (w/w)

Preparation of the solutions

- CDAP solution

Just before activation, a cyanodiaminopyridinium tetrafluoroborate (CDAP) solution was prepared at 100 mg/ml in acetonitrile/water for injection (50/50 (v/v))

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Activation

Native or sized PS6A was diluted at a defined concentration and the pH of the solution was set to 6.0 ± 0.2. At time 0, the CDAP solution was added manually in order to obtain a defined CDAP/PS6A ratio (w/w).

After 1.5 minutes the pH was raised up to activation pH value by addition of NaOH.
At time 4.5 minutes, the protein solution (at a defined concentration and buffer) was added in order to obtain a fixed protein/PS6A ratio (w/w). The pH of the solution was regulated at coupling pH value during a defined timing (coupling time - See Table 4).
At time T=coupling time + 4min 30, a solution of Glycine 2M pH 9.0 was added to quench the reaction. After 30 minutes of quenching, the conjugate was directly injected on purification column or let overnight under continuous stirring at +2 to+8°C before purification.

Purification

Before purification, the conjugate solution was filtered through a 5 µm or 10 µm membrane in order to remove aggregates and particles. Conjugate was then purified on Sephacryl S400HR column (bed height: 100 cm +/- 10 cm) using NaCl 150 mM as eluent. The conjugate was then sterile filtered on 0.22 µm PVDF (polyvinylidene difluoride) membrane. The sterilised bulk (Conjugated Bulk) was stored at +2-8°C until formulation.

Characterization

The final Protein/Polysaccharide ratio (w/w) on the sterilized conjugate was determined by the ratio of the Lowry/resorcinol concentrations. Antigenicity and free PS (polysaccharide) content were determined using methods described here below. Data are shown in Table 5.

Antigenicity test

Native polysaccharide was assigned an antigenicity index value of 100%. Insofar as correlation between antigenicity index and immunogenicity had not been established, the determination of sized polysaccharide antigenicity was performed as indicative value. The objective was to keep this value as high as possible. This value was determined by ELISA.

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The antigenicity of polysaccharide was determined in a sandwich-type ELISA (see Example 1 for measurement of antigenicity).

Free PS content by ELISA

After reaction of the conjugate with anti-carrier serum, the complex was precipitated using saturated ammonium sulphate (SAS). After centrifugation, the free PS06A content was performed by ELISA (anti-PS/anti-PS, see part 2.8.1 .2) on the supernatant. The percentage of free PS was calculated proportionally to the total PS content measured by resorcinol method.

The absence of conjugate in the supernatant was also controlled by a a-carrier/a-PS06A ELISA.

Table 4 : Process conditions for PS6A-CRM197 conjugates at 50mg scale

Conjugate	PS6A	PS Dissolution (M NaCl)	[mg/ ml]	Carrier Dissolution buffer	[mg/ ml]
PS6A-CRM197/015	native	2	5	K/K_2PO_4 10mM pH7 2NaCl 0.2M	10
PS6A-CRM197/016	native	2	5	K/K_2PO_4 10mM pH7 2NaCl 0.2M	10
PS6A-CRM197/017	E01	2	10	K/K_2PO_4 10mM pH7 2NaCl 0.2M	10
PS6A-CRM197/018	E01	2	10	K/K_2PO_4 10mM pH7 2NaCl 0.2M	10
PS6A-CRM197/019	E01	2	10	K/K_2PO_4 10mM pH7 2NaCl 0.2M	10
PS6A-CRM197/020	E01	2	10	K/K_2PO_4 10mM pH7 2NaCl 0.2M	10
PS6A-CRM197/021	E01	2	10	K/K_2PO_4 10mM pH7	10

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				2NaCl 0.2M	
PS6A-CRM1 97/022	E03	2	10	K/K2PO4 10mM pH7 2NaCl 0.2M	10
PS6A-CRM1 97/023	E03	2	10	K/K2PO4 10mM pH7 2NaCl 0.2M	10
PS6A-CRM1 97/024	E03	2	10	K/K2PO4 10mM pH7 2NaCl 0.2M	10

Conjugate	Initial Ratio (w/w)	CDAP (mg/mg PS)	Coupling Time (min)	pHa/ pHc/ PHq	Remark
PS6A-CRM197/015	1.5/1	1.5/1	60	9.5/9.5/9.0	50 mg
PS6A-CRM197/016	1.5/1	0.75/1	60	9.5/9.5/9	50 mg
PS6A-CRM197/017	1.5/1	0.75/1	120	9.5/9.5/9	50 mg
PS6A-CRM197/018	1.5/1	1.5/1	120	9.5/9.5/9	50 mg
PS6A-CRM197/019	1.5/1	1/1	120	9.5/9.5/9	50 mg
PS6A-CRM197/020	2/1	0.75/1	120	9.5/9.5/9	50 mg
PS6A-CRM197/021	1.5/1	1.5/1	120	9.5/9.5/9	200 mg
PS6A-CRM197/022	1.5/1	1.5/1	120	9.5/9.5/9	50 mg
PS6A-CRM197/023	1.5/1	1.5/1	60	9.5/9.5/9	50 mg
PS6A-CRM197/024	1.5/1	1/1	120	9.5/9.5/9	50 mg

Note: Designation such as "PS6ACRM 197/024" indicates PS6ACRM197 from lot024.

Table 5: Characterization of PS6A-CRM197 conjugates at 50mg-PS scale

Conjugate	Final Ratio Carrier/PS	Free polysaccharide	αPS/ αPS (%)	αprot/ αPS (%)	Yield (%)	Remark

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	(w/w)	ELISA (%) 4°C	4°C	4°C		
PS6A-CRM 197/01 5	0.91/1				58.8	Filtration issue
PS6A-CRM 197/01 6	0.65/1				45.1	
PS6A-CRM 197/01 7	1.23/1				28.2	Tightened pool/bad separation
PS6A-CRM 197/01 8	1.33/1 (NF)				48.3	
PS6A-CRM 197/01 9	1.10/1				50.2	
PS6A-CRM 197/020	1.41/1				50.6	
PS6A-CRM 197/021	1.42/1	0.8	35	125	45.4	
PS6A-CRM 197/022	1.1 1/1	1.1	23	67	69.8	Filtration issue
PS6A-CRM 197/023	1.24/1	0.8	27	98	61.4	
PS6A-CRM 197/024	1.19/1	1.3	31	95	65.3	

The first set of conjugates (PS6A-CRM197/015, PS6A-CRM197/016) were produced with native polysaccharide (Mw by MALLS 990kDa) and showed limitations in term of reachable final CRM/PS ratio and filterability of resulting conjugates (Table 5).

A second set of conjugates (PS6A-CRM197/017, PS6A-CRM197/018, PS6A-CRM197/019, PS6A-CRM1 97/020, PS6A-CRM1 97/021) were produced using sized polysaccharide (lot E01). By increasing CDAP/PS ratio and/or initial CRM/PS ratio, the desired final CRM/PS ratio and polysaccharide yield could be reached. The best conditions (PS6A-CRM1 97/021) were reproduced with a new sized polysaccharide lots but having a higher molecular weight (lot E03) than the previous one. However a filtration

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issue was observed. It was found that by reducing coupling time or CDAP/PS6A ratio, the filtration issue was solved.

A third set of conjugates (PS6A-CRM1 97/022, PS6A-CRM1 97/023, PS6A-CRM1 97/024) were produced using sized serotype 6A polysaccharide (lot E03). The best conditions (PS6A-CRM1 97/024) were the following: a polysaccharide concentration of 10 mg/ml in 2M NaCl , a CDAP/PS ratio of 1/1 (w/w), a CRM concentration of 10 mg/ml in 10 mM K/K_2P0_4 pH 7.2, 0.2M NaCl , an initial CRM/PS ratio of 1.5/1 (w/w), a pH for activation and coupling of 9.5 and a coupling time of 120 min. Resulting conjugates had a final CRM/PS ratio around 1.2/1 (w/w) for a global yield around 65 %.

Example 5: Production of Sized 6A-CRM197 Conjugates at 200mg scale

A scale up at 200mg-PS scale was performed (Table 6 and Table 7).

Table 6: Process conditions for PS6A-CRM197 conjugates at 200mg-PS scale

Conjugate	Lot	Estimated molecular weight (kDa)*	PS Dissolution (M NaCl)	[mg/ml]	Lot	Carrier Dissolution buffer	[mg/ml]
PS6A-CRM197/025	E03	968	2	10	CRM197 -012	K/K_2PO_4 10mM pH7 2NaCl 0.2M	10
PS6A-CRM197/026	E03	968	2	10	CRM197 -012	K/K_2PO_4 10mM pH7 2NaCl 0.2M	10
PS6A-CRM197/027	E03	968	2	10	CRM197 -009	K/K_2PO_4 10mM pH7 2NaCl 0.2M	10
PS6A-CRM197/028	E07	929	2	10	CRM197 -014	K/K_2PO_4 10mM pH7 2NaCl 2M	10
PS6A-CRM197/029	E07	929	2	10	CRM197 -014	K/K_2PO_4 10mM pH7 2NaCl 0.2M	10
PS6A-CRM197/030	E08	1004	2	10	CRM197 -014	K/K_2PO_4 10mM pH7 2NaCl 2M	10
* Estimation realized using a dextran calibration curve (see Table 1 for MALLS data)							

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Conjugate	Initial Ratio (w/w)	CDAP (mg/mg PS)	Coupling Time (min)	pHa/pHc/pHq
PS6A-CRM 197/025	1.5/1	1/1	120	9.5/ 9.5/ 9
PS6A-CRM 197/026	1.5/1	1/1	120	9.5/ 9.5/ 9
PS6A-CRM 197/027	1.5/1	1/1	120	9.5/ 9.5/ 9
PS6A-CRM 197/028	1.5/1	1/1	120	9.5/ 9.5/ 9
PS6A-CRM 197/029	1.5/1	1/1	120	9.5/ 9.5/ 9
PS6A-CRM 197/030	1.5/1	1/1	120	9.5/ 9.5/ 9

Table 7: Characterization of PS6A-CRM conjugates at 200mg-PS scale

Conjugate	F.Ratio Carrier/PS (w/w)	Free PS ELISA (%) 4°C	αPS / αPS (%) 4°C	αprot / αPS (%) 4°C	Yield (%)
PS6A-CRM197/025	1.32/1	0.2	56	132	56.6
PS6A-CRM197/026	1.20/1	0.1	50	125	56.2
PS6A-CRM197/027	1.25/1	0.2	49	113	57.6
PS6A-CRM197/028	1.18/1		44	89	60.2
PS6A-CRM197/029	1.22/1		36	101	67.8

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PS6A-CRM 197/030	1.36/1		28	9 1	56.4
Remark		E6A-417		PS6A-CRM1 97/025	

Data from 200 mg-PS scale lots was consistent with data obtained at small scale. Resulting conjugates had final CRM/PS ratio between 1.25 to 1.32/1 , for a global polysaccharide yield of 56 %. In term of stability, no issue appeared in HP-SEC analyses or free polysaccharide content by ELISA (data not available with chemical method).

Based on these results, further lots (see Table 5 below) were produced in the following conditions: a polysaccharide concentration of 10 mg/ml in 2M NaCl, a CDAP/PS ratio of 1/1 (w/w), a CRM concentration of 10 mg/ml in 10 mM K/K_2P0_4 pH 7.2, 2M NaCl, an initial CRM/PS ratio of 1.5/1 (w/w), a pH for activation and coupling of 9.5 and a coupling time of 120 min.

Example 6 : Immunogenicity of PS6A conjugates in mice

Different sized PS6A conjugates were evaluated in Balb/c mice (as monovalent formulation): 6A-CRM197 (PS6A-CRM025), 6A-PD (PS6A-PDLS001 (E06)), 6A-PD (PS6A-PDLS002(E06)), 6A-PhtD (PS6A-PhtD008) and $6A_{AH}$-PhtD (PS6A-PhtD106 (E04)), PS6A-PhtD (PS6A-PhtD100(E06)) conjugates produced with a sized PS.

34 Balb/c Mice were immunized three times (days 0-14-28) with 0.3 μg of different PS6A conjugates adsorbed onto $AlP0_4$. Anti-PS6A ELISA IgG titers levels and Opsonophagocytosis (OP) titers were measured in individual sera collected at day 42. Results are shown in Figures 2A and 2B. A significantly higher antibody response was induced by PS6A-AH-PhtD (CDAP/EDAC) and PS6A-CRM 025 (CDAP) in ELISA and OPA.

Example 7 : Characterisaton of sized PS6A

CRM197 concentration

Thawing of CRM197

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Purified bulk is stored at -20°C at a concentration of 1.917 mg/ml in 10 mM K/K_2P0_4 pH 7.2. 7.5 g of purified bulk were thawed overnight at +2/+8°C.

Concentration by UF

The ultrafiltration was realized at room temperature on a centramate device.

The ultrafiltration membrane was an OMEGA medium screen membrane of 0.09 m^2 with a 10 kDa cut off (2 membranes). The circulation flow-rate was 1200 ml/min and the transmembrane pressure applied during the run was between 7-10 psi.

The CRM197 bulk was 7.5-fold concentrated in order to reach a concentration of around 15 mg/ml (target for conjugation >10 mg/ml).

Filtration on 0.22 μιτι

After the ultrafiltration, the concentrated bulk was sterile filtered on 0.22 μη Millipack 20 filter (PVDF) at 20 ml/min. It was then stored at -20°C until its use in coupling.

The sized PS6A was characterized by the following test: MALLS and antigen content by ELISA. Stability was evaluated following 2 months at -70°C (T=0 compared to T=2 months). No stability issue observed when the sized PS6A is stored at -70°C. Results of sized PS6A characterization are summarized in (Table 8).

Table 8: Characterization data and stability of sized PS6A

	T =0	**T=2M at -70°C**
Antigen content by resorcinol (IP value)	15487 μg/ml	/
Mean molecular weight in weight by MALLS	302.1 kDa	288.8 kDa
Root mean square radius in weight by MALLS	27.7 nm	27.3 nm
Polydispersity ratio (M_w/Mn) by MALLS	1.102	1.136
Antigen content by ELISA	22250 μg/ml	2291 1 μg/ml
Ratio ELISA/resorcinol (%)	***144 %***	***148 %***

Two conjugates (D06ADJA001 , D06ADJA002) were produced at 2g-PS scale using lot of sized PS6A and lot as carrier. The conditions of conjugation are described hereafter.

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The reaction was performed at 25 +/- 1°C in a 1L-bioreactor.

2g of sized PS6A was diluted at 10 mg/ml in 2M NaCl and the pH of the solution was adjusted at 6.0 +/- 0.2 with 0.05N HCl solution.

At T = 0, 2 g of CDAP in solution (solution at 100 mg/ml in CH_3CN/H_20 50/50) was manually added to the solution (CDAP/PS ratio = 1.0/1 w/w).

At T= 1min 30, pH was increased up to 9.5 +/- 0.05 by addition of 0.5N NaOH. It took approximately 90 sec to reach the target pH.

At T = 4min30, 3g of CRM197 (solution at 10 mg/ml in 10 mM KH2PO4/K2H PO4 pH 7.2, 2M NaCl) was added at the solution of activated PS6A in 1 minute in order to reach a CRM197/PS6A ratio of 1.5/1 w/w. The pH was regulated at 9.5 +/- 0.05 during 120 minutes.

At T =124 min 30, 100 ml of a solution of 2M glycine pH 9.0 was added to quench the conjugate (Ratio Gly/PS = 7.5/1 w/w). After 30 minutes of quenching, the pH of the mixture was adjusted to 6.5 +/- 0.2 using 5N HCl. When pH was stabilized, the conjugate was let overnight under continuous stirring at +2/+8°C before clarification and purification.

Purification

Prior to the elution on Sephacryl S400HR, the conjugate was clarified using a 10 µm Kleenpack HDCII filter at 50 ml/min

The conjugate was then injected on a Sephacryl S400HR. Elution was done with 0.15M NaCl solution and the collection pool was based on a Kd value. Kd is the distribution coefficient ($Kd = (V_e - Vo)/(V_t - V)$ V_e= elution volume, Vo = void volume, V_t = total volume of the column.

The following criteria were used for the pool collection: from OD_{280nm} =0.05 AU to a Kd value of 0.28.

Sterilizing filtration

Before filtration, the bulk was brought back to room temperature.

Lots were filtered on Opticap 4" sterilizing membrane (1900 cm^2, PVDF) at a flow-rate of 40 ml/min. The filter was rinsed by 0.15M NaCl buffer before filtration. After filtration, 200 ml of 0.15M NaCl was passed through filter to limit the loss of material. No issue appeared during filtration.

Preclinical evaluation

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Groups of 48 female Balb/c mice (4-weeks-old) were immunized IM at days 0,14 and 28 with adsorbed conjugates formulations ($AIP0_4$) containing 0.1 µg of either D06ADJA001 , D06ADJA002, PS6A-CRMLS001 (produced with sized PS6A, lot E07), PS6A-CRMLS002 (produced with sized PS6A, lot E07) or PS6A-PDLS001 (as a benchmark).

Anti-PS6A IgG levels (Figure 3A) and opsono-phagocytosis titers (Figure 3B) were measured in individual sera collected at day 42. No significant difference was observed in antibody responses induced by the PS6A-CRM produced according to Example 7 compared to the PS6A-CRM produced according to Example 5 in ELISA and in OPA.

Example 8 : Preparation of 6A Conjugate

Sized 6A conjugate (lot E06AADJA059) was produced at 15 g PS scale in a 15L reactor using the conjugation parameters of the sized 6A produced at 200 mg PS scale (see Example 5). Conjugate was then purified on Sephacryl S400HR column (BPG450 column (GE Healthcare)) using NaCl 150 mM as eluent. The conjugate was then sterile filtered on 0.22 µm PDVF membrane and stored at 2-8°C. The conjugate was concentrated (5x) and diafiltrated (10 diafiltration volume) against WFI (water for injection) using a 10 kDa MWCO OMEGA PES membrane (T-series, PALL). The retentate was then filtered on 0.22 µm membrane.

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SEQ ID NO 1: MetLysLeuLysThrLeuAlaLeuSerLeuLeuAlaAlaGlyValLeuAlaGly
CysSerSerHisSerSerAsnMetAlaAsnThrGlnMetLysSerAspLysIle
HelleAlaHisArgGlyAlaSerGlyTyrLeuProGluHisThrLeuGluSerLysAla
LeuAlaPheAlaGlnGlnAlaAspTyrLeuGluGlnAspLeuAlaMetThrLysAspGly
ArgLeuValVallleHisAspHisPheLeuAspGlyLeuThrAspValAlaLysLysPhe
ProHisArgHisArgLysAspGlyArgTyrTyrVallleAspPheThrLeuLysGlulle
GlnSerLeuGluMetThrGluAsnPheGluThrLysAspGlyLysGlnAlaGlnValTyr
ProAsnArgPheProLeuTrpLysSerHisPheArgIleHisThrPheGluAspGlulle
GluPhelleGlnGlyLeuGluLysSerThrGlyLysLysValGlylleTyrProGlulle
LysAlaProTrpPheHisHisGlnAsnGlyLysAspIleAlaAlaGluThrLeuLysVal
LeuLysLysTyrGlyTyrAspLysLysThrAspMetValTyrLeuGlnThrPheAspPhe
AsnGluLeuLysArgIleLysThrGluLeuLeuProGlnMetGlyMetAspLeuLysLeu
ValGlnLeulleAlaTyrThrAspTrpLysGluThrGlnGluLysAspProLysGlyTyr
TrpValAsnTyrAsnTyrAspTrpMetPheLysProGlyAlaMetAlaGluValValLys
TyrAlaAspGlyValGlyProGlyTrpTyrMetLeuValAsnLysGluGluSerLysPro
AspAsnIleValTyrThrProLeuValLysGluLeuAlaGlnTyrAsnValGluValHis
ProTyrThrValArgLysAspAlaLeuProGluPhePheThrAspValAsnGlnMetTyr
AspAlaLeuLeuAsnLysSerGlyAlaThrGlyValPheThrAspPheProAspThrGly
ValGluPheLeuLysGlylleLys

SEQ ID NO. 2: MetAspProSerSerHisSerSerAsnMetAlaAsnThrGlnMetLysSerAspLysIle
HelleAlaHisArgGlyAlaSerGlyTyrLeuProGluHisThrLeuGluSerLysAla
LeuAlaPheAlaGlnGlnAlaAspTyrLeuGluGlnAspLeuAlaMetThrLysAspGly
ArgLeuValVallleHisAspHisPheLeuAspGlyLeuThrAspValAlaLysLysPhe
ProHisArgHisArgLysAspGlyArgTyrTyrVallleAspPheThrLeuLysGlulle
GlnSerLeuGluMetThrGluAsnPheGluThrLysAspGlyLysGlnAlaGlnValTyr
ProAsnArgPheProLeuTrpLysSerHisPheArgIleHisThrPheGluAspGlulle
GluPhelleGlnGlyLeuGluLysSerThrGlyLysLysValGlylleTyrProGlulle
LysAlaProTrpPheHisHisGlnAsnGlyLysAspIleAlaAlaGluThrLeuLysVal
LeuLysLysTyrGlyTyrAspLysLysThrAspMetValTyrLeuGlnThrPheAspPhe
AsnGluLeuLysArgIleLysThrGluLeuLeuProGlnMetGlyMetAspLeuLysLeu
ValGlnLeulleAlaTyrThrAspTrpLysGluThrGlnGluLysAspProLysGlyTyr
TrpValAsnTyrAsnTyrAspTrpMetPheLysProGlyAlaMetAlaGluValValLys
TyrAlaAspGlyValGlyProGlyTrpTyrMetLeuValAsnLysGluGluSerLysPro
AspAsnIleValTyrThrProLeuValLysGluLeuAlaGlnTyrAsnValGluValHis
ProTyrThrValArgLysAspAlaLeuProGluPhePheThrAspValAsnGlnMetTyr
AspAlaLeuLeuAsnLysSerGlyAlaThrGlyValPheThrAspPheProAspThrGly
ValGluPheLeuLysGlylleLys

SEQ ID NO. 3: SerSerHisSerSerAsnMetAlaAsnThr

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CLAIMS

1. A sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide wherein the average size (M_w) of the *Streptococcus pneumoniae* serotype 6A capsular polysaccharide is between 180-400, 210-400, 210-370, 220-360, 230-350, 240-340, 240-320, 240-310 or 250-310 kDa.

2. A sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide according to claim 1 which has been sized by a mechanical sizing technique.

3. A sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide according to claim 1 or claim 2 conjugated to a carrier protein (e.g. CRM-197).

4. A sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide according to claim 3 wherein the *Streptococcus pneumoniae* serotype 6A capsular polysaccharide is directly conjugated to the carrier protein.

5. A sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide according to any one of claims 3-4 wherein the 6A polysaccharide is conjugated to the carrier protein or to a linker using CDAP chemistry.

6. A sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide according to any one of claims 3-5 wherein the serotype 6A capsular polysaccharide (PS6A) is conjugated to the carrier protein or to a linker using CDAP chemistry using a CDAP:PS6A ratio between 1:2 to 3:1 , 1:1.5 to 2:1 , or 1:1 .

7. A sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide according to any one of claims 3-6 wherein the serotype 6A capsular polysaccharide is conjugated to the carrier protein or to a linker using CDAP chemistry wherein the reaction was carried out using a coupling time of between 50-130 minutes, 60-130 minutes, or 110-130 minutes.

8. A sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide according to any one of claims 3-7 wherein the ratio of carrier protein to serotype 6A capsular polysaccharide is between 5:1 and 1:5, 4:1 and 1:1 or 2:1 and 1:1, 1.5:1 and 1:1, 1.4:1 and 1.3:1 (for example 1.2:1 , 1.5:1) (w/w).

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9. A sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide according to claims 1-8 wherein the 6A capsular polysaccharide is sized by a factor of no more than x5.

10. A process for preparing a *Streptococcus pneumoniae* serotype 6A capsular polysaccharide conjugate comprising conjugating a sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide to a carrier protein or to a linker using CDAP chemistry using a CDAP:PS ratio between 1:2 to 3:1 , 1:1 .5 to 2:1 , or 1:1 .

11. A process according to claim 10 wherein the reaction was carried out using a coupling time of between 50-130 minutes, 60-130 minutes, or 110-130 minutes.

12. A process for preparing a *Streptococcus pneumoniae* serotype 6A capsular polysaccharide conjugate comprising (a) conjugation of a sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide to a carrier protein and (b) diafiltration against a solution having a concentration of NaCl below 150mM.

13. An immunogenic composition comprising a sized *Streptococcus pneumoniae* serotype 6A capsular polysaccharide according to claims 1-9 or *Streptococcus pneumoniae* serotype 6A capsular polysaccharide obtained by a process of any one of claims 10-12 conjugated to a carrier protein.

14. An immunogenic composition according to claim 13 comprising 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, or 16 or more capsular polysaccharides conjugates from different S. *pneumoniae* serotypes.

15. An immunogenic composition according to claim 13 or claim 14 comprising 1 or more, native capsular polysaccharides conjugates from S. *pneumoniae.*

16. An immunogenic composition according to claim 15 comprising native *Streptococcus pneumoniae* capsular serotype 6B polysaccharide.

17. An immunogenic composition according to claim 15 or 16 comprising native *Streptococcus pneumoniae* capsular serotype 23F polysaccharide.

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18. An immunogenic composition according to any one of claims 13 to 17 which comprises *Streptococcus pneumoniae* capsular serotype 6B polysaccharide having an average size (M_w) of between 500-1800, 900-1660, or 1000-1400 kDa.

19. An immunogenic composition according to any one of claims 13 to 18 which comprises *Streptococcus pneumoniae* capsular serotype 23F polysaccharide having an average size (M_w) of between 500-1500, 600-1500, 700-1300, 900-1250, 800-1 100, or 900-1 000 kDa.

20. An immunogenic composition according to any one of claims 13 to 19 which comprises *Streptococcus pneumoniae* capsular polysaccharide from: (a) serotype 1 having an average size (M_w) of between 100-1000, 200-800, 250-600, or 300-400 kDa; (b) serotype 4 having an average size (M_w) of between 50-500, 60-300, 70-150, or 75-125 kDa; (c) serotype 5 having an average size (M_w) of between 100-1000, 100-700, 100-350, or 150-300 kDa; (d) serotype 7F having an average size (M_w) of between 50-1000, 100-750, 150-500, or 200-300 kDa; (e) serotype 9V having an average size (M_w) of between 50-1000, 100-750, 150-500, 200-400, or 250-300 kDa; (f) serotype 14 having an average size (M_w) of between 50-1000, 100-750, 150-500, or 200-250 kDa; (g) 18C having an average size (M_w) of between 50-1000, 50-750, 50-500, 50-190, 50-150 or 80-1 10 kDa (h) serotype 19F having an average size (M_w) of between 50-1000, 100-750, 100-500, 100-190 or 120-180 kDa; and/or (i) serotype 19A having an average size (M_w) of between 50-800kDa, 110-700, 110-300, 120-200, 130-180, 140-160 or 80-130 kDa.

21. An immunogenic composition according to any one of claims 13 to 20 which further comprises *Streptococcus pneumoniae* capsular serotype 22F having an average size (M_w) of between 50 and 800 kDa, 110 and 700 kDa, 110-300, 120-200, 130-180, 150-170, 100-190, 100-150, 95-125 or 100-1 15 kDa.

22. An immunogenic composition according to any one of claims 13 to 21 comprising 2, 3, 4, 5 or 6 different carrier proteins.

23. An immunogenic composition according to any one of claims 13 to 22 wherein one or more or all carrier proteins is selected from the group consisting of: diphtheria toxoid (DT), CRM197, tetanus toxoid (TT), Fragment C of TT, dPly, PhtA, PhtB, PhtD, PhtE, PhtDE OmpC, PorB and *Haemophilus influenzae* Protein D.

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24. An immunogenic composition of any one of claims 13 to 23 wherein the *Streptococcus pneumoniae* capsular serotype 6B polysaccharide is conjugated to a different carrier protein (e.g. protein D) than the carrier protein to which *Streptococcus pneumoniae* serotype 6A capsular polysaccharide is conjugated.

25. An immunogenic composition of any one of claims 13 to 24 comprising serotype 1 saccharide conjugated to protein D, serotype 4 saccharide conjugated to protein D, serotype 5 saccharide conjugated to protein D, serotype 6B saccharide conjugated to protein D, serotype 7F saccharide conjugated to protein D, serotype 9V saccharide conjugated to protein D, serotype 14 saccharide conjugated to protein D and serotype 23F saccharide conjugated to protein D.

26. An immunogenic composition according to any one of claims 13 to 25 comprising serotype 19F conjugated to Diphtheria toxoid.

27. An immunogenic composition according to any one of claims 13 to 26 wherein the composition comprises capsular saccharide 18C conjugated to tetanus toxoid (TT), optionally wherein 18C is the only saccharide in the composition conjugated to tetanus toxoid (TT), optionally via an ADH linker

28. An immunogenic composition according to any of claims 13 to 27 further comprising S. *pneumoniae* capsular saccharide(s) of one or more of: serotype 33F, serotype 15 and serotype 12F, conjugated to carrier protein(s).

29. An immunogenic composition according to any of claims 13 to 28 wherein the dose of the 6A saccharide conjugate is between 1 and 10 μg, 1 and 5 μg, or 1 and 3 μg of saccharide (e.g. 2 μg).

30. An immunogenic composition according to any one of claims 13 to 29 which further comprises one or more unconjugated or conjugated S *pneumoniae* proteins selected from: Poly Histidine Triad family (PhtX), Choline Binding Protein family (CbpX), CbpX truncates, LytX family, LytX truncates, CbpX truncate-LytX truncate chimeric proteins, detoxified pneumolysin (Ply), PspA, PsaA, Sp128, Sp101 , Sp130, Sp125 and Sp133.

31. An immunogenic composition according to any one of claims 13 to 30 which further comprises an adjuvant.

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32. An immunogenic composition of claim 31 wherein the adjuvant comprises (per 0.5 mL dose) 100-750, 200-500, or 300-400 μg Al (aluminium) as aluminium phosphate.

33. A vaccine comprising the immunogenic composition of any one of claims 13 to 32 and a pharmaceutically acceptable excipient or carrier.

34. A process for making the vaccine according to claim 33 which comprises the step of mixing the immunogenic composition of any of claims 13 to 32 with a pharmaceutically acceptable excipient or carrier.

35. A method for the treatment or prevention of *Streptococcus pneumoniae* infection in a subject in need thereof comprising administering to said subject a therapeutically effective amount of an immunogenic composition of any of claims 13 to 32 or the vaccine of claim 33.

36. A method of immunising a human host against *Streptococcus pneumoniae* infection comprising administering to the host an immunoprotective dose of the immunogenic composition of any of claims 13 to 32 or vaccine of claim 34.

37. A method of inducing an immune response to *Streptococcus pneumoniae* serotype 6A in a subject, the method comprising administering a therapeutically or prophylactically effective amount of the immunogenic composition of any of claims 13 to 32 or the vaccine of claim 33.

38. The immunogenic composition of claims 13 to 32 or vaccine of claim 33 for use in the treatment or prevention of disease caused by *Streptococcus pneumoniae* infection.

39. A use of the immunogenic composition of claims 12 to 32 or vaccine of claim 33 in the manufacture of a medicament for the treatment or prevention of a disease caused by *Streptococcus pneumoniae* infection.

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Figure 1: Evaluation of PS6A conjugates in 14V AlPO4 formulation in the Balb/c mouse with co-administration of Infanrix Hexa model. ELISA anti-PS6A.

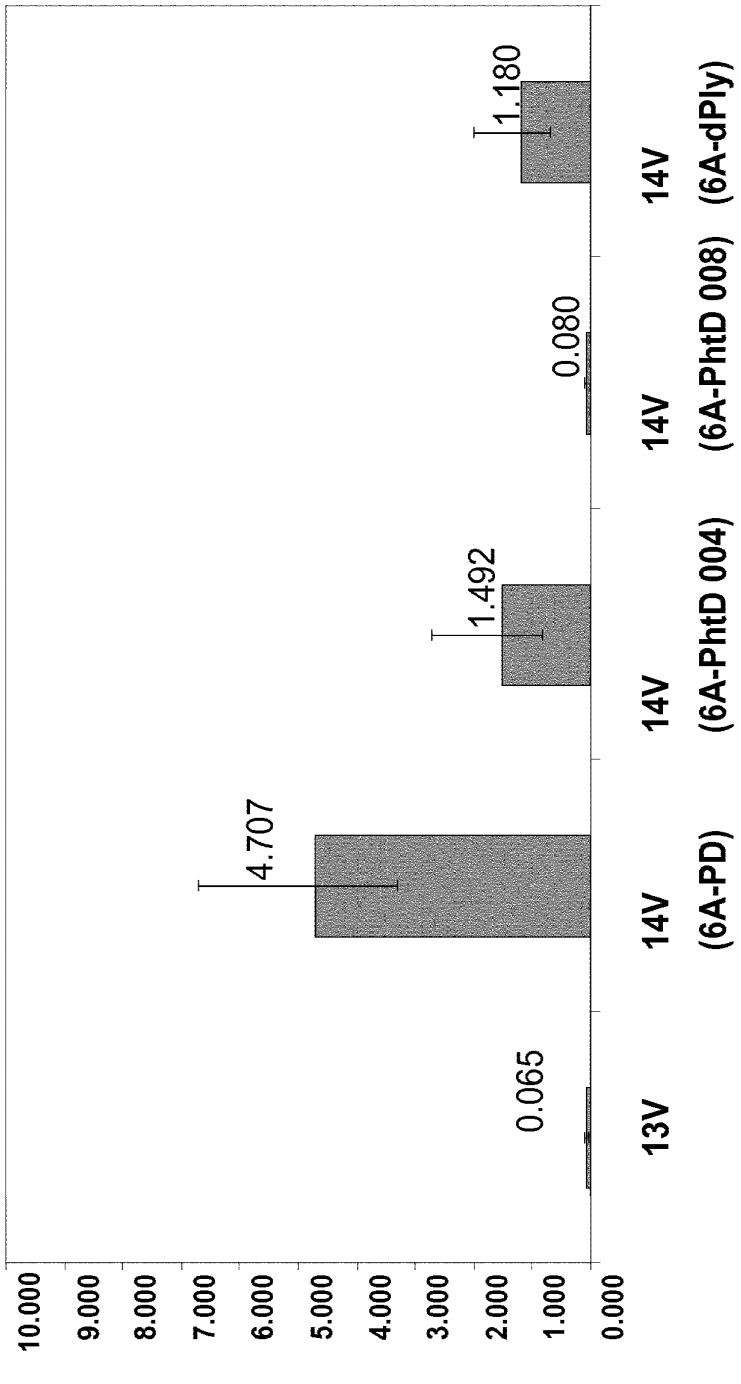
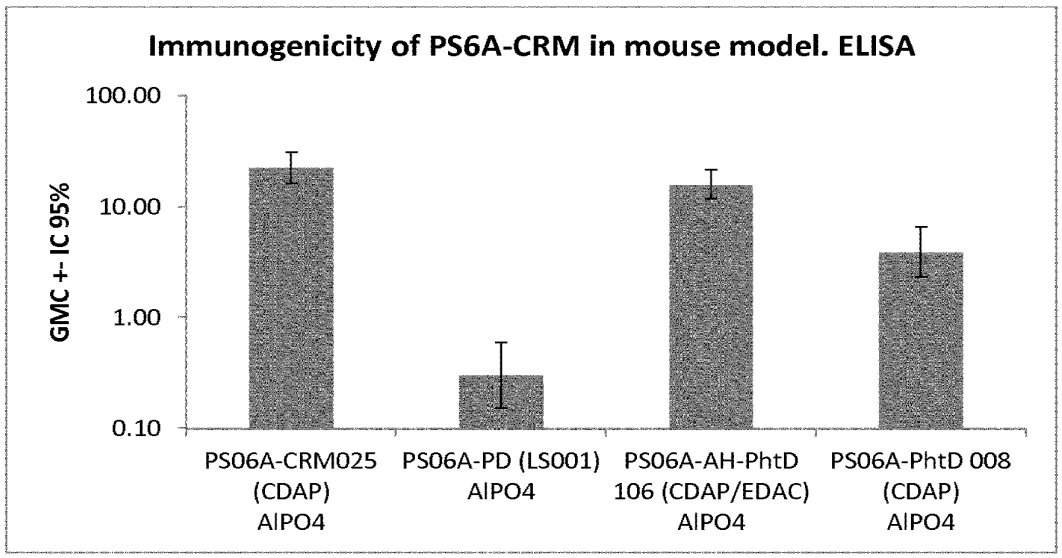


Figure 1

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(A)



(B)

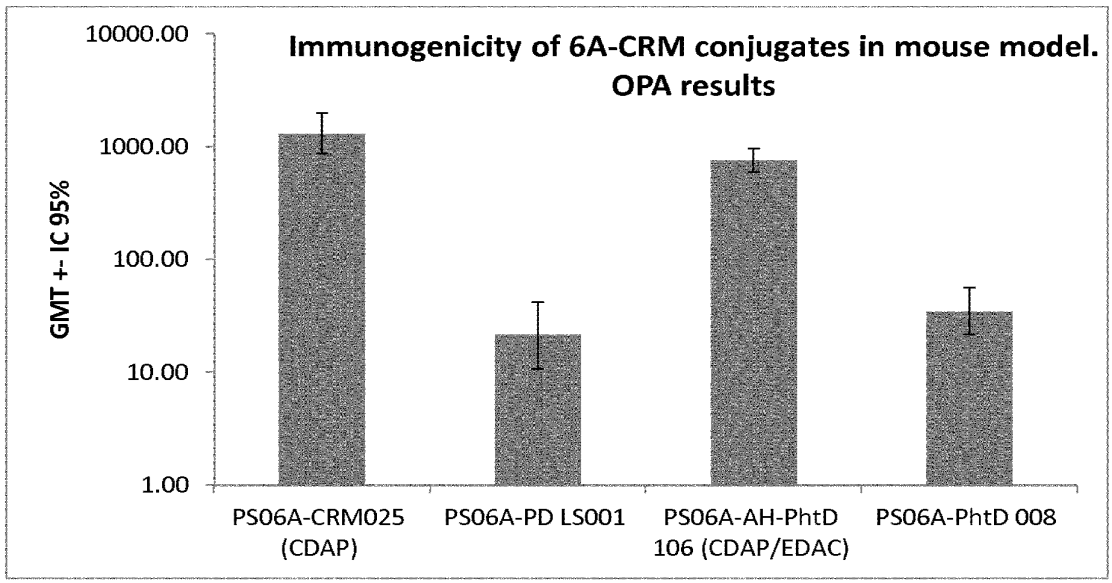


Figure 2

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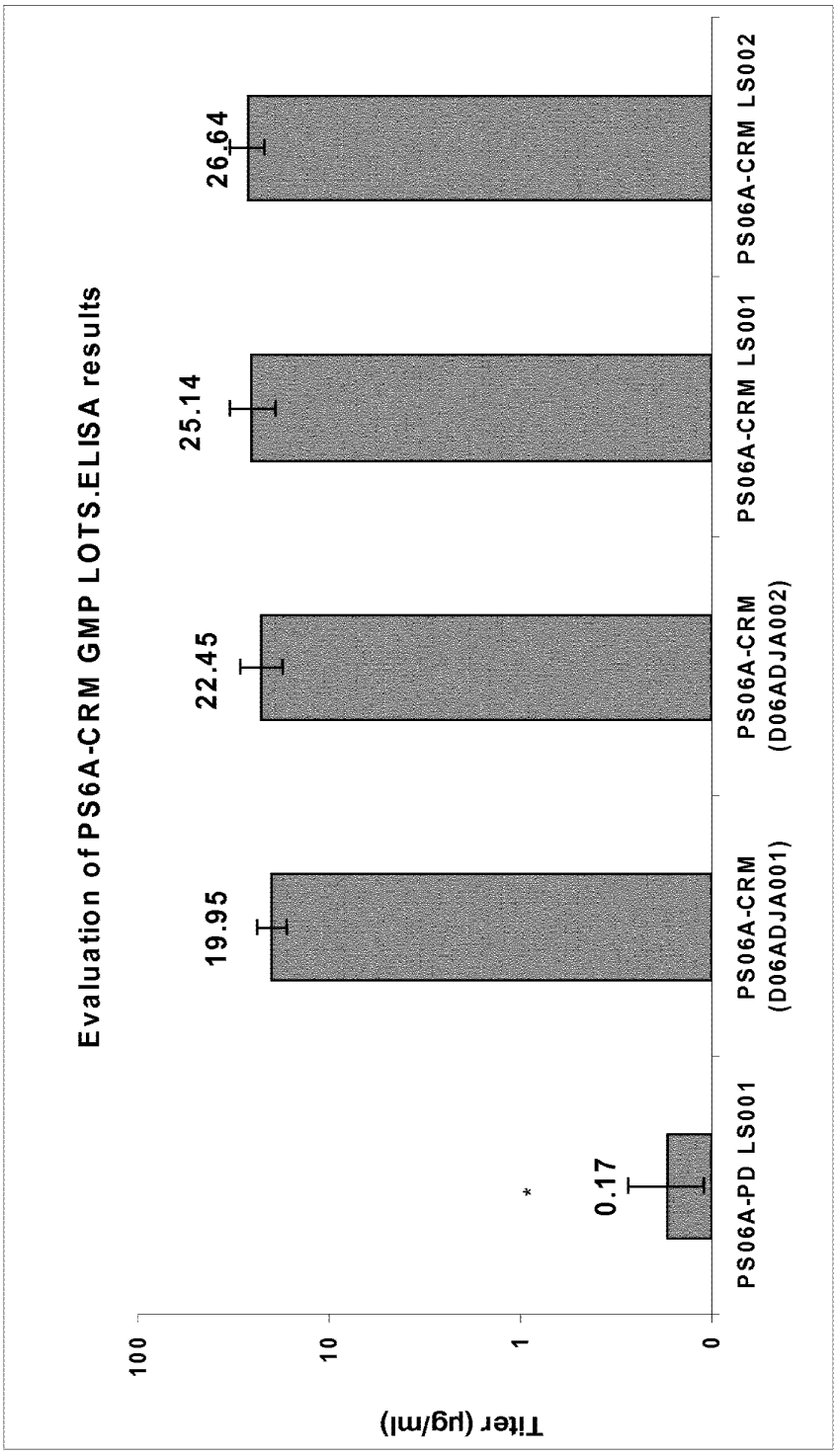


Figure 3A

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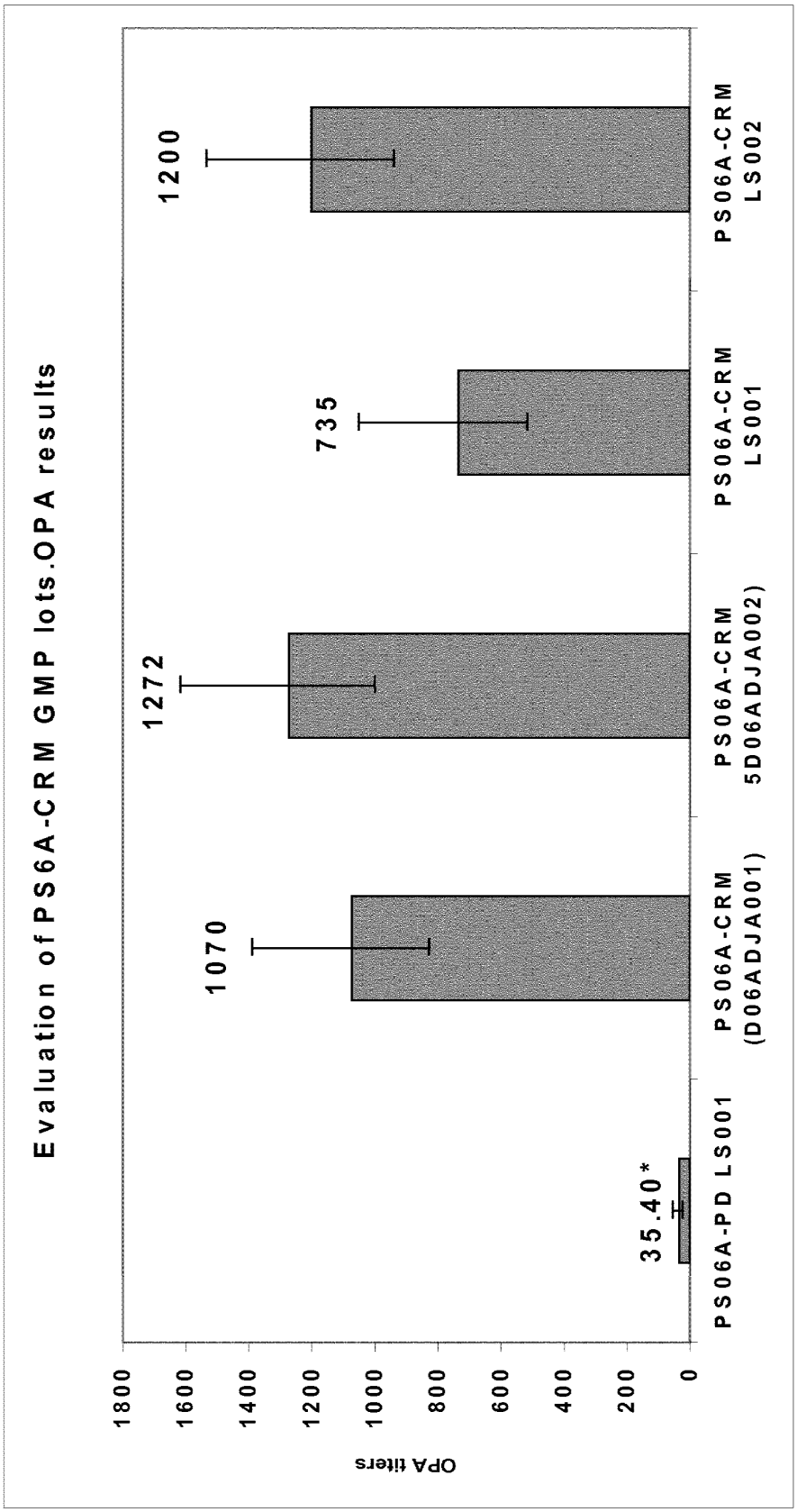


Figure 3B

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/075045

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/09 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, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2011/110241 A1 (GLAXOSMITHKLINE BIOLOG SA [BE] ; BIEMANS RALPH LEON [BE] ; DUVIVIER PIER) 15 September 2011 (2011-09-15) example A11	1-39
X	WO 2009/000826 A1 (GLAXOSMITHKLINE BIOLOG SA [BE] ; BIEMANS RALPH LEON [BE] ; HERMAND PHILI) 31 December 2008 (2008-12-31) example A11	1-39
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X	WO 2014/060383 A1 (GLAXOSMITHKLINE BIOLOG SA [BE]) 24 April 2014 (2014-04-24) example A11	1-39

-/ -

[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) orwhich 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
3 March 2017	13/03/2017

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	Fellows, Edward

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Form PCT/ISA/210 (second sheet) (April 2005)

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/075045

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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3

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2016/075045

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.:
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:

see addi tional sheet

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 an 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.:

I-39(partial ly)

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.:

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.

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-39 (partially)

   A sized Streptococcus pneumoniae serotype 6A capsular polysaccharide wherein the average size (Mw) of said polysaccharide is 180-400 kDa and related subject-matter.

   ---

2. claims: 1-39 (partially)

   A sized Streptococcus pneumoniae serotype 6A capsular polysaccharide wherein the average size (Mw) of said polysaccharide is 210-400 kDa and related subject-matter.

   ---

3. claims: 1-39 (partially)

   A sized Streptococcus pneumoniae serotype 6A capsular polysaccharide wherein the average size (Mw) of said polysaccharide is 210-370 kDa and related subject-matter.

   ---

4. claims: 1-39 (partially)

   A sized Streptococcus pneumoniae serotype 6A capsular polysaccharide wherein the average size (Mw) of said polysaccharide is 220-360 kDa and related subject-matter.

   ---

5. claims: 1-39 (partially)

   A sized Streptococcus pneumoniae serotype 6A capsular polysaccharide wherein the average size (Mw) of said polysaccharide is 230-350 kDa and related subject-matter.

   ---

6. claims: 1-39 (partially)

   A sized Streptococcus pneumoniae serotype 6A capsular polysaccharide wherein the average size (Mw) of said polysaccharide is 240-340 kDa and related subject-matter.

   ---

7. claims: 1-39 (partially)

   A sized Streptococcus pneumoniae serotype 6A capsular polysaccharide wherein the average size (Mw) of said polysaccharide is 240-320 kDa and related subject-matter.

   ---

8. claims: 1-39 (partially)

   A sized Streptococcus pneumoniae serotype 6A capsular

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

polysaccharide wherein the average size (Mw) of said polysaccharide is 240-310 kDa and related subject-matter.

---

9. claims: I-39 (partially)

A sized Streptococcus pneumoniae serotype 6A capsular polysaccharide wherein the average size (Mw) of said polysaccharide is 250-310 kDa and related subject-matter.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2016/075045

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International application No
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Petitioner Merck Ex-1004, 1185

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International application No
PCT/EP2016/075045

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Petitioner Merck Ex-1004, 1186

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International application No
PCT/EP2016/075045

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Petitioner Merck Ex-1004, 1187

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To: KELI HOLZAPFEL
WILSON SONSINI GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304

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)* 11 FEB 2019
Applicant's or agent's file reference 50308-706601	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/US 18/54391	International filing date *(day/month/year)* 04 October 2018 (04.10.2018)
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 two months 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 preparations 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 **22 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 Lee W. Young Telephone No. PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/220 (July 2014)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 50308-706601	FOR FURTHER ACTION	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US 18/54391	International filing date *(day/month/year)* 04 October 2018 (04.10.2018)	(Earliest) Priority Date *(day/month/year)* 04 October 2017 (04.10.2017)
Applicant LIFFEY BIOTECH LIMITED		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 6 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out 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)).

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

c. ☒ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☒ **Certain claims were found unsearchable** (see Box No. II).

3. ☒ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

a. the figure of the **drawings** to be published with the abstract is Figure No. _____

☐ as suggested by the applicant.

☐ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

b. ☒ none of the figures is to be published with the abstract.

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

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/54391

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out 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).

2. ☐ 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.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/54391

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.: 81-169, 174-448
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:

- see extra sheet for Box No. III Observations where unity of invention is lacking -

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.:
1, 28/1, 34-46, 77-80 limited to serotypes 2, 6C, 7F, 7C, 8, 9N, 10A, 11A

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.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/54391

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/09, A61K 39/00 (2018.01)
CPC - A61K 39/092, A61K 47/646, A61K 2039/6087, A61K 2039/62, A61K 2039/70

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	WO 2017/067962 A1 (GLAXOSMITHKLINE BIOLOGICALS S.A.) 27 April 2017 (27.04.2017) pg 9, ln 10-15, pg 9, ln 23-26, pg 11, ln 14-20, pg 28, ln 28-30, pg 43, ln 1-2, pg 43, ln 15-20	1, 77-80 ----- 28/1, 34-46
Y	US 2017/0021006 A1 (PFIZER Inc.) 26 January 2017 (26.01.2017) Claim 1	28/1
Y	WO 2016/149771 A1 (GAMMA VACCINES PTY LIMITED) 29 September 2016 (29.09.2016) pg 18, ln 6-12	34-46

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

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 January 2019	11 FEB 2019
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: Lee W. Young 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/US 18/54391

Continuation of:
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 searched, the appropriate additional search fees must be paid.

Group I+: Claims 1-80, 170-173, drawn to a pharmaceutical composition or vaccine in unit dose form comprising a plurality of unique immunogenic saccharide-polypeptide conjugates. The plurality of unique immunogenic saccharide-polypeptide conjugates will be searched to the extent that the capsular polysaccharides encompasses eight Streptococcus pneumoniae serotypes 2, 6C, 7F, 7C, 8, 9N, 10A, 11A. It is believed that claims 1, 28(in part), 34-46, 77-80 encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass capsular polysaccharide serotypes 2, 6C, 7F, 7C, 8, 9N, 10A, 11A. Additional capsular polysaccharide serotypes will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected capsular polysaccharide serotypes. 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. An exemplary election would be a pharmaceutical composition or vaccine encompasses 9 capsular polysaccharide serotypes of 6C, 15A, 15B, 15C, 17F, 20A, 20B, 23A, 23B (Claims 2, 28(in part), 34-46, 72, 74-80, 170-173).

The inventions listed as Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

The inventions of Groups I+ each include the special technical feature of a specific set of capsular polysaccharide selected from a group of S. pneumoniae serotypes, not required by any of the other inventions of Groups I+.

Common Technical Features

The inventions of Groups I+ share the technical feature of Claims 1, 29, 34, 72, 77, 170.

However, these shared technical features do not represent a contribution over prior art in view of WO 2017/067962 A1 to Glaxosmithkline Biologicals S.A. (hereinafter 'GSK'); WO 2016/149771 A1 to Gamma Vaccines PTY Limited (hereinafter 'GVPL'); and the article "Biochemical, Genetic, and Serological Characterization of Two Capsule Subtypes among Streptococcus pneumoniae Serotype 20 Strains. DISCOVERY OF A NEW PNEUMOCOCCAL SEROTYPE" by Calix et al. (hereinafter 'Calix') (The Journal Of Biological Chemistry VOL. 287, NO. 33, pp. 27885-27894, August 10, 2012).

GSK teaches (instant claim 1) a pharmaceutical composition in unit dose form (pg 43, ln 1-2, the vaccine of the invention may be administered as a single dose.; pg 43, ln 15-20, a vaccine containing the immunogenic compositions of the invention and a pharmaceutically acceptable excipient or carrier), comprising 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 (pg 9, ln 10-15, the invention will comprise capsular polysaccharide antigens (suitably conjugated), wherein the polysaccharides are derived from at least ten serotypes of S. pneumoniae. The number of S. pneumoniae capsular polysaccharides can range from 10 different serotypes (or valences "v") to 23 different serotypes (23v, a 23 valent composition).; pg 11, ln 14-20, Examples of carrier proteins which may be used in the present invention are DT (Diphtheria toxoid), TT (tetanus toxoid) or fragment C of TT, DT, CRM197 other DT point mutants, such as CRM176, CRM228, CRM 45.), wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique Streptococcus pneumoniae serotype selected from a group consisting of 2, 6C, 7F, 7C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, I 7F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35B, 35F, and 38 (pg 9, ln 23-26, In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.), 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 (pg 43, ln 1-2), ranges from about 0.5 ug to about 5 ug (pg 28, ln 28-30, In the immunogenic composition of the invention contains each S. pneumoniae capsular saccharide at a dose of between 0.1-20 ug; 0.5-1.0 ug; 0,5-5 ug or 1-3 ug of saccharide.).

GSK teaches (instant claim 29) a pharmaceutical composition in unit dose form (pg 43, ln 1-2, pg 43, ln 15-20) comprising a plurality of at least 23 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide (pg 9, ln 10-15, pg 11, ln 14-20), 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, 7C, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, I 7F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 35F, 34, 35B, and 38 (pg 9, ln 23-26), 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 (pg 43, ln 1-2), ranges from about 0.5 ug to about 5 ug (pg 28, ln 28-30). GSK does not specifically teaches a composition comprising 29 unique immunogenic saccharide-polypeptide conjugates. GVPL teaches a vaccine composition comprising any one or more of S. pneumoniae serotypes (more than 29 serotypes) (pg 18, ln 6-12, the vaccines may comprise any one of more of S. pneumoniae serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, IOC, 10F, 11A, 11B, 11C, 11D, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20, 21, 22A, 22F, 23A, 23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F, 29, 31, 32A, 32F, 33A, 33B, 33C, 33D, 33F, 34, 35A, 35B, 35C, 35F, 36, 37, 38, 39, 40, 41A, 41F, 42, 43, 44, 45, 46, 47A, 47F, and/or 48.). It would have been obvious to one of ordinary skill in the art to have prepared a vaccine comprising 29 unique immunogenic saccharide-polypeptide conjugates, as taught by GVPL.

----continued on next sheet----

Petitioner Merck Ex-1004, 1193

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/54391

Continuation of:
Box No. III. Observations where unity of invention is lacking

GSK teaches (instant claim 34) a pharmaceutical composition in unit dose form (pg 43, ln 1-2, pg 43, ln 15-20) comprising at least 2 immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide (pg 9, ln 10-15, pg 11, ln 14-20), wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique Streptococcus pneumoniae serotype group, wherein 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, l 7F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35B, 35F, and 38 (pg 9, ln 23-26), 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 (pg 43, ln 1-2), ranges from about 0.5 ug to about 5 ug (pg 28, ln 28-30). GSK does not specifically teaches a composition comprising serotype 6C. GVPL teaches a vaccine composition comprising any one or more of S. pneumoniae serotypes comprising 6C (pg 18, ln 6-12, the vaccines may comprise any one of more of S. pneumoniae serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D,.....). It would have been obvious to one of ordinary skill in the art to have prepared a vaccine comprising serotype 6C, as taught by GVPL.

GSK teaches (instant claim 72) a pharmaceutical composition in unit dose form (pg 43, ln 1-2, pg 43, ln 15-20) comprising at least 2 immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide (pg 9, ln 10-15, pg 11, ln 14-20), wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique Streptococcus pneumoniae serotype group, wherein at least one additional serotype is 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, l7F, 20A, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35B, and 38 (pg 9, ln 23-26), 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 (pg 43, ln 1-2), ranges from about 0.5 ug to about 5 ug (pg 28, ln 28-30). GSK does not specifically teaches a composition comprising serotype 20B. Calix teaches serotype 20B (abstract, We examined the capsule structures of two serologically dissimilar serotype 20 strains, 20alpha and 20beta, by extensive biochemical analysis. 20alpha was composed of the previously described serotype 20 hexasaccharide repeat unit, whereas the 20beta was composed of a novel heptasaccharide repeat unit containing an extra branching alpha-glucose residue.). It would have been obvious to one of ordinary skill in the art to have prepared a vaccine comprising serotype 20B, which was first identified by Calix.

GSK teaches (instant claim 77) a pharmaceutical composition comprising 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, 7C, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, l 7F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35F, 35B, and 38 (pg 9, ln 10-15, pg 9, ln 23-26, pg 11, ln 14-20).

GSK teaches (instant claim 170) a vaccine in unit dose form (pg 43, ln 1-2, pg 43, ln 15-20) comprising at 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 (pg 9, ln 10-15, pg 9, ln 23-26, pg 11, ln 14-20). GSK does not specifically teaches serotypes 23A or 23B, or a combination thereof. GVPL teaches a vaccine composition comprising any one or more of S. pneumoniae serotypes comprising 23A or 23B (pg 18, ln 6-12, the vaccines may comprise any one of more of S. pneumoniae serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D,...23A, 23B....). It would have been obvious to one of ordinary skill in the art to have prepared a vaccine comprising serotype 23A or 23B, as taught by GVPL.

As said technical features were known in the art at the time of the invention, these cannot be considered special technical features that would otherwise unify the groups.

Groups I+ therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: KELI HOLZAPFEL
WILSON SONSINI GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	11 FEB 2019
Applicant's or agent's file reference 50308-706601	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 18/54391	04 October 2018 (04.10.2018)	04 October 2017 (04.10.2017)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - A61K 39/09, A61K 39/00 (2018.01)
CPC - A61K 39/092, A61K 47/646, A61K 2039/6087, A61K 2039/62, A61K 2039/70

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	20 January 2019	Lee W. Young 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/US 18/54391

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/US 18/54391

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. 81-169, 174-448

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. 81-169, 174-448 are so unclear that no meaningful opinion could be formed *(specify)*:

Claims 81-169, 174-448 are improper multiple dependent claims because they are 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. 81-169, 174-448

☐ 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.

Petitioner Merck Ex-1004, 1197

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 18/54391

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:

--------continued in Supplemental Box--------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 searched, the appropriate additional search fees must be paid.

Group I+: Claims 1-80, 170-173, drawn to a pharmaceutical composition or vaccine in unit dose form comprising a plurality of unique immunogenic saccharide-polypeptide conjugates. The plurality of unique immunogenic saccharide-polypeptide conjugates will be searched to the extent that the capsular polysaccharides encompasses eight Streptococcus pneumoniae serotypes 2, 6C, 7F, 7C, 8, 9N, 10A, 11A. It is believed that claims 1, 28(in part), 34-46, 77-80 encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass capsular polysaccharide serotypes 2, 6C, 7F, 7C, 8, 9N, 10A, 11A. Additional capsular polysaccharide serotypes will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected capsular polysaccharide serotypes. 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. An exemplary election would be a pharmaceutical composition or vaccine encompasses 9 capsular polysaccharide serotypes of 6C, 15A, 15B, 15C, 17F, 20A, 20B, 23A, 23B (Claims 2, 28(in part), 34-46, 72, 74-80, 170-173).

The inventions listed as Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

The inventions of Groups I+ each include the special technical feature of a specific set of capsular polysaccharide selected from a group of S. pneumoniae serotypes, not required by any of the other inventions of Groups I+.

Common Technical Features

The inventions of Groups I+ share the technical feature of Claims 1, 29, 34, 72, 77, 170.

However, these shared technical features do not represent a contribution over prior art in view of WO 2017/067962 A1 to Glaxosmithkline Biologicals S.A. (hereinafter 'GSK'); WO 2016/149771 A1 to Gamma Vaccines PTY Limited (hereinafter 'GVPL'); and the article "Biochemical, Genetic, and Serological Characterization of Two Capsule Subtypes among Streptococcus pneumoniae Serotype 20 Strains. DISCOVERY OF A NEW PNEUMOCOCCAL SEROTYPE" by Calix et al. (hereinafter 'Calix') (The Journal Of Biological Chemistry VOL. 287, NO. 33, pp. 27885-27894, August 10, 2012).

--------continued in 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, 28/1, 34-46, 77-80 limited to serotypes 2, 6C, 7F, 7C, 8, 9N, 10A, 11A

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 18/54391

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

1. Statement

Novelty (N)	Claims	28/1, 34-46	YES
	Claims	1, 77-80	NO
Inventive step (IS)	Claims	NONE	YES
	Claims	1, 28/1, 34-46, 77-80	NO
Industrial applicability (IA)	Claims	1, 28/1, 34-46, 77-80	YES
	Claims	NONE	NO

2. Citations and explanations:

Claim 1, 77-80 lacks novelty under PCT Article 33(2) as being anticipated by WO 2017/067962 A1 to GlaxoSmithkline Biologicals S.A. (hereinafter 'GSK').

Regarding claim 1, GSK teaches a pharmaceutical composition in unit dose form (pg 43, ln 1-2, the vaccine of the invention may be administered as a single dose.; pg 43, ln 15-20, a vaccine containing the immunogenic compositions of the invention and a pharmaceutically acceptable excipient or carrier), comprising 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 (pg 9, ln 10-15, the invention will comprise capsular polysaccharide antigens (suitably conjugated), wherein the polysaccharides are derived from at least ten serotypes of S. pneumoniae. The number of S. pneumoniae capsular polysaccharides can range from 10 different serotypes (or valences "v") to 23 different serotypes (23v, a 23 valent composition).; pg 11, ln 14-20, Examples of carrier proteins which may be used in the present invention are DT (Diphtheria toxoid), TT (tetanus toxoid) or fragment C of TT, DT, CRM197 other DT point mutants, such as CRM176, CRM228, CRM 45.), wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique Streptococcus pneumoniae serotype selected from a group consisting of 2, 6C, 7F, 7C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, I 7F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35B, 35F, and 38 (pg 9, ln 23-26, In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.), 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 (pg 43, ln 1-2), ranges from about 0.5 ug to about 5 ug (pg 28, ln 28-30, In the immunogenic composition of the invention contains each S. pneumoniae capsular saccharide at a dose of between 0.1-20 ug; 0.5-1.0 ug; 0,5-5 ug or 1-3 ug of saccharide.).

Regarding claim 77, GSK teaches a pharmaceutical composition comprising an immunogenic saccharide-polypeptide conjugate (pg 43, ln 1-2, the vaccine of the invention may be administered as a single dose.; pg 43, ln 15-20, a vaccine containing the immunogenic compositions of the invention and a pharmaceutically acceptable excipient or carrier.) 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 (pg 9, ln 10-15, the invention will comprise capsular polysaccharide antigens (suitably conjugated), wherein the polysaccharides are derived from at least ten serotypes of S. pneumoniae. The number of S. pneumoniae capsular polysaccharides can range from 10 different serotypes (or valences "v") to 23 different serotypes (23v, a 23 valent composition).; pg 11, ln 14-20, Examples of carrier proteins which may be used in the present invention are DT (Diphtheria toxoid), TT (tetanus toxoid) or fragment C of TT, DT, CRM197 other DT point mutants, such as CRM176, CRM228, CRM 45.), are from a Streptococcus pneumoniae serotype selected from a group consisting of 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, I 7F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35F, 35B, and 38 (pg 9, ln 23-26, In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.).

Regarding claim 78, GSK teaches the pharmaceutical composition of claim 77, 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 6C, 8, 9N, 10A, 11A, I 5A, I 5B, 15C, 20B, 22F, 23A, 23B, 33F, and 35B (pg 9, ln 23-26, In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.).

Regarding claim 79, GSK teaches the pharmaceutical composition of claim 77, 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 6C, 8, 9N, 10A, 11A, I 5A, I 5B, 15C, 22F, 23A, 23B, 33F, and 35B (pg 9, ln 23-26, In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.).

--------continued in Supplemental Box--------

Petitioner Merck Ex-1004, 1199

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 18/54391

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claim 47 is objected to for purporting to depend from claim 4, because claim 4 lacks antecedent basis for "the at least one additional serotype". Claim 47 is construed as being dependent upon claim 34.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 18/54391

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
Box No. IV. Lack of unity of invention

GSK teaches (instant claim 1) a pharmaceutical composition in unit dose form (pg 43, ln 1-2, the vaccine of the invention may be administered as a single dose.; pg 43, ln 15-20, a vaccine containing the immunogenic compositions of the invention and a pharmaceutically acceptable excipient or carrier), comprising 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 (pg 9, ln 10-15, the invention will comprise capsular polysaccharide antigens (suitably conjugated), wherein the polysaccharides are derived from at least ten serotypes of S. pneumoniae. The number of S. pneumoniae capsular polysaccharides can range from 10 different serotypes (or valences "v") to 23 different serotypes (23v, a 23 valent composition).; pg 11, ln 14-20, Examples of carrier proteins which may be used in the present invention are DT (Diphtheria toxoid), TT (tetanus toxoid) or fragment C of TT, DT, CRM197 other DT point mutants, such as CRM176, CRM228, CRM 45.), wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique Streptococcus pneumoniae serotype selected from a group consisting of 2, 6C, 7F, 7C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, I 7F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35B, 35F, and 38 (pg 9, ln 23-26, In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F
and 33F.), 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 (pg 43, ln 1-2), ranges from about 0.5 ug to about 5 ug (pg 28, ln 28-30, In the immunogenic composition of the invention contains each S. pneumoniae capsular saccharide at a dose of between 0.1-20 ug; 0.5-1.0 ug; 0,5-5 ug or 1-3 ug of saccharide.).

GSK teaches (instant claim 29) a pharmaceutical composition in unit dose form (pg 43, ln 1-2, pg 43, ln 15-20) comprising a plurality of at least 23 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide (pg 9, ln 10-15, pg 11, ln 14-20), 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, 7C, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, I 7F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 35F, 34, 35B, and 38 (pg 9, ln 23-26), 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 (pg 43, ln 1-2), ranges from about 0.5 ug to about 5 ug (pg 28, ln 28-30). GSK does not specifically teaches a composition comprising 29 unique immunogenic saccharide-polypeptide conjugates. GVPL teaches a vaccine composition comprising any one or more of S. pneumoniae serotypes (more than 29 serotypes) (pg 18, ln 6-12, the vaccines may comprise any one of more of S. pneumoniae serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, IOC, 10F, 11A, 11B, 11C, 11D, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20, 21, 22A, 22F, 23A, 23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F, 29, 31, 32A, 32F, 33A, 33B, 33C, 33D, 33F, 34, 35A, 35B, 35C, 35F, 36, 37, 38, 39, 40, 41A, 41F, 42, 43, 44, 45, 46, 47A, 47F, and/or 48.). It would have been obvious to one of ordinary skill in the art to have prepared a vaccine comprising 29 unique immunogenic saccharide-polypeptide conjugates, as taught by GVPL.

GSK teaches (instant claim 34) a pharmaceutical composition in unit dose form (pg 43, ln 1-2, pg 43, ln 15-20) comprising at least 2 immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide (pg 9, ln 10-15, pg 11, ln 14-20), wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique Streptococcus pneumoniae serotype group, wherein 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, I 7F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35B, 35F, and 38 (pg 9, ln 23-26), 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 (pg 43, ln 1-2), ranges from about 0.5 ug to about 5 ug (pg 28, ln 28-30). GSK does not specifically teaches a composition comprising serotype 6C. GVPL teaches a vaccine composition comprising any one or more of S. pneumoniae serotypes comprising 6C (pg 18, ln 6-12, the vaccines may comprise any one of more of S. pneumoniae serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D,.....). It would have been obvious to one of ordinary skill in the art to have prepared a vaccine comprising serotype 6C, as taught by GVPL.

GSK teaches (instant claim 72) a pharmaceutical composition in unit dose form (pg 43, ln 1-2, pg 43, ln 15-20) comprising at least 2 immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide (pg 9, ln 10-15, pg 11, ln 14-20), wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique Streptococcus pneumoniae serotype group, wherein at least one additional serotype is 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, I7F, 20A, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35B, and 38 (pg 9, ln 23-26), 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 (pg 43, ln 1-2), ranges from about 0.5 ug to about 5 ug (pg 28, ln 28-30). GSK does not specifically teaches a composition comprising serotype 20B. Calix teaches serotype 20B (abstract, We examined the capsule structures of two serologically dissimilar serotype 20 strains, 20alpha and 20beta, by extensive biochemical analysis. 20alpha was composed of the previously described serotype 20 hexasaccharide repeat unit, whereas the 20beta was composed of a novel heptasaccharide repeat unit containing an extra branching alpha-glucose residue.). It would have been obvious to one of ordinary skill in the art to have prepared a vaccine comprising serotype 20B, which was first identified by Calix.

--------continued in next Supplemental Box--------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 18/54391

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Box No. IV. Lack of unity of invention

GSK teaches (instant claim 77) a pharmaceutical composition comprising 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, 7C, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, I 7F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35F, 35B, and 38 (pg 9, ln 10-15, pg 9, ln 23-26, pg 11, ln 14-20).

GSK teaches (instant claim 170) a vaccine in unit dose form (pg 43, ln 1-2, pg 43, ln 15-20) comprising at 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 (pg 9, ln 10-15, pg 9, ln 23-26, pg 11, ln 14-20). GSK does not specifically teaches serotypes 23A or 23B, or a combination thereof. GVPL teaches a vaccine composition comprising any one or more of S. pneumoniae serotypes comprising 23A or 23B (pg 18, ln 6-12, the vaccines may comprise any one of more of S. pneumoniae serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D,...23A, 23B....). It would have been obvious to one of ordinary skill in the art to have prepared a vaccine comprising serotype 23A or 23B, as taught by GVPL.

As said technical features were known in the art at the time of the invention, these cannot be considered special technical features that would otherwise unify the groups.

Groups I+ therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No. PCT/US 18/54391

Supplemental Box

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

Continuation of:
Box No. V2. Citations and explanation

Regarding claim 80, GSK teaches the pharmaceutical composition of claim 77, further comprising at least one unique immunogenic saccharide-polypeptide conjugate 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, 7C, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, I 7F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 35F, 35B, and 38 (pg 9, In 23-26, In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.).

Claim 28/1 lacks an inventive step under PCT Article 33(3) as being obvious over GSK, in view of US 2017/0021006 A1 to Pfizer Inc. (hereinafter 'Pfizer').

Regarding claim 28/1, GSK teaches the pharmaceutical composition of claim 1, but does not specifically teach wherein the unique Streptococcus pneumoniae serotype is not any of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. Pfizer teaches a pharmaceutical composition comprising at least one unique immunogenic saccharide-polypeptide conjugate, but does not specifically teach wherein the unique Streptococcus pneumoniae serotype is not any of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F (Claim 1, A kit comprising: (a) a first immunogenic composition comprising at least one glycoconjugate selected from the group consisting of a glycoconjugate from Streptococcus pneumoniae serotype 15B, a glycoconjugate from S. pneumoniae serotype 22F, a glycoconjugate from S. pneumoniae serotype 33F, a glycoconjugate from S. pneumoniae serotype 12F, a glycoconjugate from S. pneumoniae serotype 10A, a glycoconjugate from S. pneumoniae serotype 11A and a glycoconjugate from S. pneumoniae serotype 8, wherein said composition is a 1, 2, 3, 4, 5, 6 or 7 -valent pneumococcal conjugate composition.). Thus, it would have been obvious to have applied the glycoconjugate from Streptococcus pneumoniae serotypes 15B, 22F, 33F, 12F, 10A, 11A, 8 of Pfizer as the the glycoconjugate from Streptococcus pneumoniae serotypes of GSK.

Claims 34-46 lack an inventive step under PCT Article 33(3) as being obvious over GSK, in view of WO 2016/149771 A1 to Gamma Vaccines PTY Limited (hereinafter 'GVPL').

Regarding claim 34, GSK teaches a pharmaceutical composition in unit dose form (pg 43, In 1-2, pg 43, In 15-20) comprising at least 2 immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide (pg 9, In 10-15, pg 11, In 14-20), wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique Streptococcus pneumoniae serotype group, wherein 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, I 7F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35B, 35F, and 38 (pg 9, In 23-26, In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.), 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 (pg 43, In 1-2), ranges from about 0.5 ug to about 5 ug (pg 28, In 28-30, In the immunogenic composition of the invention contains each S. pneumoniae capsular saccharide at a dose of between 0.1-20 ug; 0.5-1.0 ug; 0,5-5 ug or 1-3 ug of saccharide.). GSK does not specifically teaches a composition comprising serotype 6C. GVPL teaches a vaccine composition comprising any one or more of S. pneumoniae serotypes comprising 6C (pg 18, In 6-12, the vaccines may comprise any one of more of S. pneumoniae serotypos 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D,.....). It would have been obvlous to one of ordinary skill in the art to have prepared a vaccine comprising serotype 6C, as taught by GVPL.

Regarding claim 35, GSK and GVPL teach the pharmaceutical composition of claim 34, wherein the at least one additional serotype is selected from the group consisting of 1, 3, 4, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 23F, 24F, 33F, 35F, 35B, and 38 (GSK, pg 9, In 23-26, In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.).

Regarding claim 36, GSK and GVPL teach the pharmaceutical composition of claim 34, wherein the at least one additional serotype is selected from the group consisting of 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B (GSK, pg 9, In 23-26, In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.).

Regarding claim 37, GSK and GVPL teach the pharmaceutical composition of claim 34, 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 (GSK, pg 9, In 23-26, In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.).

Regarding claim 38, GSK and GVPL teach the pharmaceutical composition of claim 34, 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 (GSK, pg 9, In 23-26, In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.).

--------continued in next Supplemental Box--------

Petitioner Merck Ex-1004, 1203

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 18/54391

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Box No. V2. Citations and explanation

Regarding claim 39, GSK and GVPL teach the pharmaceutical composition of claim 34, 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 (GSK, pg 9, In 23-26, In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.).

Regarding claim 40, GSK and GVPL teach the pharmaceutical composition of claim 34, wherein the at least one additional serotype is selected from the group consisting of 8, 9N, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 23B, 33F, and 35B (GSK, pg 9, In 23-26, In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.).

Regarding claim 41, GSK and GVPL teach the pharmaceutical composition of claim 34, wherein the at least one additional serotype is selected from the group consisting of 8, 9N, 10A, 11A, 15A, 15B, 15C, 20B, 22F, 23A, 23B, 33F, and 35B (GSK, pg 9, In 23-26, In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.).

Regarding claim 42, GSK and GVPL teach the pharmaceutical composition of claim 34, wherein the at least one additional serotype is selected from the group consisting of 9N, 15A, 15C, 16F, 23A, 23B, and 35B (GSK, pg 9, In 23-26, In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.).

Regarding claim 43, GSK and GVPL teach the pharmaceutical composition of claim 34, wherein the at least one additional serotype is selected from the group consisting of 15A, 15C, 16F, 23A, 23B, and 35B (GSK, pg 9, In 23-26, In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.).

Regarding claim 44, GSK and GVPL teach the pharmaceutical composition of claim 34, wherein the at least one additional serotype is selected from the group consisting of 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 24F, 31, 33F, 34, 35F, and 35B (GSK, pg 9, In 23-26, In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.).

Regarding claim 45, GSK and GVPL teach the pharmaceutical composition of claim 34, wherein the at least one additional serotype is selected from the group consisting of 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 33F, 34, 35F, and 35B (GSK, pg 9, In 23-26, In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.).

Regarding claim 46, GSK and GVPL teach the pharmaceutical composition of claim 34, wherein the at least one additional serotype is selected from the group consisting of 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20A, 22F, 23A, 23B, 33F, and 35B (GSK, pg 9, In 23-26, In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.).

Claims 1, 28/1, 34-46, 77-80 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

Petitioner Merck Ex-1004, 1204

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:

BAKER, C. Hunter
WOLF, GREENFIELD & SACKS, P.C.
600 Atlantic Avenue
Boston, MA 02210
ETATS-UNIS D'AMERIQUE

Date of mailing *(day/month/year)*
16 April 2020 (16.04.2020)

Applicant's or agent's file reference
P1143.70001WO00

IMPORTANT NOTICE

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2018/054391	04 October 2018 (04.10.2018)	04 October 2017 (04.10.2017)

Applicant
LIFFEY BIOTECH LIMITED et al

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

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Authorized officer
Athina Nickitas-Etienne

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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 P1143.70001WO00	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/US2018/054391	International filing date *(day/month/year)* 04 October 2018 (04.10.2018)	Priority date *(day/month/year)* 04 October 2017 (04.10.2017)
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 11 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 07 April 2020 (07.04.2020)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Athina Nickitas-Etienne e-mail: pct.team4@wipo.int

Form PCT/IB/373 (January 2004)

Petitioner Merck Ex-1004, 1206

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: KELI HOLZAPFEL
WILSON SONSINI GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	11 FEB 2019
Applicant's or agent's file reference 50308-706601	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 18/54391	04 October 2018 (04.10.2018)	04 October 2017 (04.10.2017)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - A61K 39/09, A61K 39/00 (2018.01)
CPC - A61K 39/092, A61K 47/646, A61K 2039/6087, A61K 2039/62, A61K 2039/70

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	20 January 2019	Lee W. Young 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/US 18/54391

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/US 18/54391

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. 81-169, 174-448

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. 81-169, 174-448 are so unclear that no meaningful opinion could be formed *(specify)*:

Claims 81-169, 174-448 are improper multiple dependent claims because they are 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. 81-169, 174-448

☐ 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/US 18/54391

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:

--------continued in Supplemental Box--------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 searched, the appropriate additional search fees must be paid.

Group I+: Claims 1-80, 170-173, drawn to a pharmaceutical composition or vaccine in unit dose form comprising a plurality of unique immunogenic saccharide-polypeptide conjugates. The plurality of unique immunogenic saccharide-polypeptide conjugates will be searched to the extent that the capsular polysaccharides encompasses eight Streptococcus pneumoniae serotypes 2, 6C, 7F, 7C, 8, 9N, 10A, 11A. It is believed that claims 1, 28(in part), 34-46, 77-80 encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass capsular polysaccharide serotypes 2, 6C, 7F, 7C, 8, 9N, 10A, 11A. Additional capsular polysaccharide serotypes will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected capsular polysaccharide serotypes. 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. An exemplary election would be a pharmaceutical composition or vaccine encompasses 9 capsular polysaccharide serotypes of 6C, 15A, 15B, 15C, 17F, 20A, 20B, 23A, 23B (Claims 2, 28(in part), 34-46, 72, 74-80, 170-173).

The inventions listed as Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

The inventions of Groups I+ each include the special technical feature of a specific set of capsular polysaccharide selected from a group of S. pneumoniae serotypes, not required by any of the other inventions of Groups I+.

Common Technical Features

The inventions of Groups I+ share the technical feature of Claims 1, 29, 34, 72, 77, 170.

However, these shared technical features do not represent a contribution over prior art in view of WO 2017/067962 A1 to Glaxosmithkline Biologicals S.A. (hereinafter 'GSK'); WO 2016/149771 A1 to Gamma Vaccines PTY Limited (hereinafter 'GVPL'); and the article "Biochemical, Genetic, and Serological Characterization of Two Capsule Subtypes among Streptococcus pneumoniae Serotype 20 Strains. DISCOVERY OF A NEW PNEUMOCOCCAL SEROTYPE" by Calix et al. (hereinafter 'Calix') (The Journal Of Biological Chemistry VOL. 287, NO. 33, pp. 27885-27894, August 10, 2012).

--------continued in 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, 28/1, 34-46, 77-80 limited to serotypes 2, 6C, 7F, 7C, 8, 9N, 10A, 11A

Form PCT/ISA/237 (Box No. IV) (January 2015)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 18/54391

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

1. Statement

Novelty (N)	Claims	28/1, 34-46	YES
	Claims	1, 77-80	NO
Inventive step (IS)	Claims	NONE	YES
	Claims	1, 28/1, 34-46, 77-80	NO
Industrial applicability (IA)	Claims	1, 28/1, 34-46, 77-80	YES
	Claims	NONE	NO

2. Citations and explanations:

Claim 1, 77-80 lacks novelty under PCT Article 33(2) as being anticipated by WO 2017/067962 A1 to GlaxoSmithkline Biologicals S.A. (hereinafter 'GSK').

Regarding claim 1, GSK teaches a pharmaceutical composition in unit dose form (pg 43, ln 1-2, the vaccine of the invention may be administered as a single dose.; pg 43, ln 15-20, a vaccine containing the immunogenic compositions of the invention and a pharmaceutically acceptable excipient or carrier), comprising 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 (pg 9, ln 10-15, the invention will comprise capsular polysaccharide antigens (suitably conjugated), wherein the polysaccharides are derived from at least ten serotypes of S. pneumoniae. The number of S. pneumoniae capsular polysaccharides can range from 10 different serotypes (or valences "v") to 23 different serotypes (23v, a 23 valent composition).; pg 11, ln 14-20, Examples of carrier proteins which may be used in the present invention are DT (Diphtheria toxoid), TT (tetanus toxoid) or fragment C of TT, DT, CRM197 other DT point mutants, such as CRM176, CRM228, CRM 45.), wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique Streptococcus pneumoniae serotype selected from a group consisting of 2, 6C, 7F, 7C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, I 7F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35B, 35F, and 38 (pg 9, ln 23-26, In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.), 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 (pg 43, ln 1-2), ranges from about 0.5 ug to about 5 ug (pg 28, ln 28-30, In the immunogenic composition of the invention contains each S. pneumoniae capsular saccharide at a dose of between 0.1-20 ug; 0.5-1.0 ug; 0,5-5 ug or 1-3 ug of saccharide.).

Regarding claim 77, GSK teaches a pharmaceutical composition comprising an immunogenic saccharide-polypeptide conjugate (pg 43, ln 1-2, the vaccine of the invention may be administered as a single dose.; pg 43, ln 15-20, a vaccine containing the immunogenic compositions of the invention and a pharmaceutically acceptable excipient or carrier.) 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 (pg 9, ln 10-15, the invention will comprise capsular polysaccharide antigens (suitably conjugated), wherein the polysaccharides are derived from at least ten serotypes of S. pneumoniae. The number of S. pneumoniae capsular polysaccharides can range from 10 different serotypes (or valences "v") to 23 different serotypes (23v, a 23 valent composition).; pg 11, ln 14-20, Examples of carrier proteins which may be used in the present invention are DT (Diphtheria toxoid), TT (tetanus toxoid) or fragment C of TT, DT, CRM197 other DT point mutants, such as CRM176, CRM228, CRM 45.), are from a Streptococcus pneumoniae serotype selected from a group consisting of 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, I 7F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35F, 35B, and 38 (pg 9, ln 23-26, In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.).

Regarding claim 78, GSK teaches the pharmaceutical composition of claim 77, 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 6C, 8, 9N, 10A, 11A, I 5A, I 5B, 15C, 20B, 22F, 23A, 23B, 33F, and 35B (pg 9, ln 23-26, In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.).

Regarding claim 79, GSK teaches the pharmaceutical composition of claim 77, 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 6C, 8, 9N, 10A, 11A, I 5A, I 5B, 15C, 22F, 23A, 23B, 33F, and 35B (pg 9, ln 23-26, In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.).

--------continued in Supplemental Box--------

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 18/54391

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
Claim 47 is objected to for purporting to depend from claim 4, because claim 4 lacks antecedent basis for "the at least one additional serotype". Claim 47 is construed as being dependent upon claim 34.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 18/54391

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
Box No. IV. Lack of unity of invention

GSK teaches (instant claim 1) a pharmaceutical composition in unit dose form (pg 43, ln 1-2, the vaccine of the invention may be administered as a single dose.; pg 43, ln 15-20, a vaccine containing the immunogenic compositions of the invention and a pharmaceutically acceptable excipient or carrier), comprising 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 (pg 9, ln 10-15, the invention will comprise capsular polysaccharide antigens (suitably conjugated), wherein the polysaccharides are derived from at least ten serotypes of S. pneumoniae. The number of S. pneumoniae capsular polysaccharides can range from 10 different serotypes (or valences "v") to 23 different serotypes (23v, a 23 valent composition).; pg 11, ln 14-20, Examples of carrier proteins which may be used in the present invention are DT (Diphtheria toxoid), TT (tetanus toxoid) or fragment C of TT, DT, CRM197 other DT point mutants, such as CRM176, CRM228, CRM 45.), wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique Streptococcus pneumoniae serotype selected from a group consisting of 2, 6C, 7F, 7C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, I 7F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35B, 35F, and 38 (pg 9, ln 23-26, In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F
and 33F.), 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 (pg 43, ln 1-2), ranges from about 0.5 ug to about 5 ug (pg 28, ln 28-30, In the immunogenic composition of the invention contains each S. pneumoniae capsular saccharide at a dose of between 0.1-20 ug; 0.5-1.0 ug; 0,5-5 ug or 1-3 ug of saccharide.).

GSK teaches (instant claim 29) a pharmaceutical composition in unit dose form (pg 43, ln 1-2, pg 43, ln 15-20) comprising a plurality of at least 23 unique immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide (pg 9, ln 10-15, pg 11, ln 14-20), 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, 7C, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, I 7F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 35F, 34, 35B, and 38 (pg 9, ln 23-26), 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 (pg 43, ln 1-2), ranges from about 0.5 ug to about 5 ug (pg 28, ln 28-30). GSK does not specifically teaches a composition comprising 29 unique immunogenic saccharide-polypeptide conjugates. GVPL teaches a vaccine composition comprising any one or more of S. pneumoniae serotypes (more than 29 serotypes) (pg 18, ln 6-12, the vaccines may comprise any one of more of S. pneumoniae serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, IOC, 10F, 11A, 11B, 11C, 11D, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20, 21, 22A, 22F, 23A, 23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F, 29, 31, 32A, 32F, 33A, 33B, 33C, 33D, 33F, 34, 35A, 35B, 35C, 35F, 36, 37, 38, 39, 40, 41A, 41F, 42, 43, 44, 45, 46, 47A, 47F, and/or 48.). It would have been obvious to one of ordinary skill in the art to have prepared a vaccine comprising 29 unique immunogenic saccharide-polypeptide conjugates, as taught by GVPL.

GSK teaches (instant claim 34) a pharmaceutical composition in unit dose form (pg 43, ln 1-2, pg 43, ln 15-20) comprising at least 2 immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide (pg 9, ln 10-15, pg 11, ln 14-20), wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique Streptococcus pneumoniae serotype group, wherein 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, I 7F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35B, 35F, and 38 (pg 9, ln 23-26), 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 (pg 43, ln 1-2), ranges from about 0.5 ug to about 5 ug (pg 28, ln 28-30). GSK does not specifically teaches a composition comprising serotype 6C. GVPL teaches a vaccine composition comprising any one or more of S. pneumoniae serotypes comprising 6C (pg 18, ln 6-12, the vaccines may comprise any one of more of S. pneumoniae serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D,.....). It would have been obvious to one of ordinary skill in the art to have prepared a vaccine comprising serotype 6C, as taught by GVPL.

GSK teaches (instant claim 72) a pharmaceutical composition in unit dose form (pg 43, ln 1-2, pg 43, ln 15-20) comprising at least 2 immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide (pg 9, ln 10-15, pg 11, ln 14-20), wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique Streptococcus pneumoniae serotype group, wherein at least one additional serotype is 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, I7F, 20A, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35B, and 38 (pg 9, ln 23-26), 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 (pg 43, ln 1-2), ranges from about 0.5 ug to about 5 ug (pg 28, ln 28-30). GSK does not specifically teaches a composition comprising serotype 20B. Calix teaches serotype 20B (abstract, We examined the capsule structures of two serologically dissimilar serotype 20 strains, 20alpha and 20beta, by extensive biochemical analysis. 20alpha was composed of the previously described serotype 20 hexasaccharide repeat unit, whereas the 20beta was composed of a novel heptasaccharide repeat unit containing an extra branching alpha-glucose residue.). It would have been obvious to one of ordinary skill in the art to have prepared a vaccine comprising serotype 20B, which was first identified by Calix.

--------continued in next Supplemental Box--------

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International application No.
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Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Box No. IV. Lack of unity of invention

GSK teaches (instant claim 77) a pharmaceutical composition comprising 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, 7C, 7F, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, I 7F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35F, 35B, and 38 (pg 9, In 10-15, pg 9, In 23-26, pg 11, In 14-20).

GSK teaches (instant claim 170) a vaccine in unit dose form (pg 43, In 1-2, pg 43, In 15-20) comprising at 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 (pg 9, In 10-15, pg 9, In 23-26, pg 11, In 14-20). GSK does not specifically teaches serotypes 23A or 23B, or a combination thereof. GVPL teaches a vaccine composition comprising any one or more of S. pneumoniae serotypes comprising 23A or 23B (pg 18, In 6-12, the vaccines may comprise any one of more of S. pneumoniae serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D,...23A, 23B....). It would have been obvious to one of ordinary skill in the art to have prepared a vaccine comprising serotype 23A or 23B, as taught by GVPL.

As said technical features were known in the art at the time of the invention, these cannot be considered special technical features that would otherwise unify the groups.

Groups I+ therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

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Supplemental Box

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Continuation of:
Box No. V2. Citations and explanation

Regarding claim 80, GSK teaches the pharmaceutical composition of claim 77, further comprising at least one unique immunogenic saccharide-polypeptide conjugate 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, 7C, 9V, 14, 18C, 19A, 19F, 23F, 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, I 7F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 35F, 35B, and 38 (pg 9, ln 23-26, In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.).

Claim 28/1 lacks an inventive step under PCT Article 33(3) as being obvious over GSK, in view of US 2017/0021006 A1 to Pfizer Inc. (hereinafter 'Pfizer').

Regarding claim 28/1, GSK teaches the pharmaceutical composition of claim 1, but does not specifically teach wherein the unique Streptococcus pneumoniae serotype is not any of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. Pfizer teaches a pharmaceutical composition comprising at least one unique immunogenic saccharide-polypeptide conjugate, but does not specifically teach wherein the unique Streptococcus pneumoniae serotype is not any of 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F (Claim 1, A kit comprising: (a) a first immunogenic composition comprising at least one glycoconjugate selected from the group consisting of a glycoconjugate from Streptococcus pneumoniae serotype 15B, a glycoconjugate from S. pneumoniae serotype 22F, a glycoconjugate from S. pneumoniae serotype 33F, a glycoconjugate from S. pneumoniae serotype 12F, a glycoconjugate from S. pneumoniae serotype 10A, a glycoconjugate from S. pneumoniae serotype 11A and a glycoconjugate from S. pneumoniae serotype 8, wherein said composition is a 1, 2, 3, 4, 5, 6 or 7 -valent pneumococcal conjugate composition.). Thus, it would have been obvious to have applied the glycoconjugate from Streptococcus pneumoniae serotypes 15B, 22F, 33F, 12F, 10A, 11A, 8 of Pfizer as the the glycoconjugate from Streptococcus pneumoniae serotypes of GSK.

Claims 34-46 lack an inventive step under PCT Article 33(3) as being obvious over GSK, in view of WO 2016/149771 A1 to Gamma Vaccines PTY Limited (hereinafter 'GVPL').

Regarding claim 34, GSK teaches a pharmaceutical composition in unit dose form (pg 43, ln 1-2, pg 43, ln 15-20) comprising at least 2 immunogenic saccharide-polypeptide conjugates each comprising individually a capsular polysaccharide, fragment thereof, or combination thereof conjugated to a polypeptide (pg 9, ln 10-15, pg 11, ln 14-20), wherein the capsular polysaccharide, fragment thereof, or combination thereof is from a unique Streptococcus pneumoniae serotype group, wherein 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, I 7F, 20A, 20B, 22F, 23A, 23B, 24F, 24B, 31, 33F, 33B, 34, 35B, 35F, and 38 (pg 9, ln 23-26, In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.), 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 (pg 43, ln 1-2), ranges from about 0.5 ug to about 5 ug (pg 28, ln 28-30, In the immunogenic composition of the invention contains each S. pneumoniae capsular saccharide at a dose of between 0.1-20 ug; 0.5-1.0 ug; 0,5-5 ug or 1-3 ug of saccharide.). GSK does not specifically teaches a composition comprising serotype 6C. GVPL teaches a vaccine composition comprising any one or more of S. pneumoniae serotypes comprising 6C (pg 18, ln 6-12, the vaccines may comprise any one of more of S. pneumoniae serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D,.....). It would have been obvious to one of ordinary skill in the art to have prepared a vaccine comprising serotype 6C, as taught by GVPL.

Regarding claim 35, GSK and GVPL teach the pharmaceutical composition of claim 34, wherein the at least one additional serotype is selected from the group consisting of 1, 3, 4, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 15C, 16F, 19F, 19A, 20A, 20B, 22F, 23A, 23B, 23F, 24F, 33F, 35F, 35B, and 38 (GSK, pg 9, ln 23-26, In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.).

Regarding claim 36, GSK and GVPL teach the pharmaceutical composition of claim 34, wherein the at least one additional serotype is selected from the group consisting of 8, 9N, 10A, 11A, 12F, 15A, 15B, 22F, 23A, 23B, 33F, and 35B (GSK, pg 9, ln 23-26, In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.).

Regarding claim 37, GSK and GVPL teach the pharmaceutical composition of claim 34, 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 (GSK, pg 9, ln 23-26, In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.).

Regarding claim 38, GSK and GVPL teach the pharmaceutical composition of claim 34, 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 (GSK, pg 9, ln 23-26, In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.).

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International application No.
PCT/US 18/54391

Supplemental Box

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Continuation of:
Box No. V2. Citations and explanation

Regarding claim 39, GSK and GVPL teach the pharmaceutical composition of claim 34, 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 (GSK, pg 9, ln 23-26, In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.).

Regarding claim 40, GSK and GVPL teach the pharmaceutical composition of claim 34, wherein the at least one additional serotype is selected from the group consisting of 8, 9N, 10A, 11A, 15A, 15B, 15C, 22F, 23A, 23B, 33F, and 35B (GSK, pg 9, ln 23-26, In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.).

Regarding claim 41, GSK and GVPL teach the pharmaceutical composition of claim 34, wherein the at least one additional serotype is selected from the group consisting of 8, 9N, 10A, 11A, 15A, 15B, 15C, 20B, 22F, 23A, 23B, 33F, and 35B (GSK, pg 9, ln 23-26, In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.).

Regarding claim 42, GSK and GVPL teach the pharmaceutical composition of claim 34, wherein the at least one additional serotype is selected from the group consisting of 9N, 15A, 15C, 16F, 23A, 23B, and 35B (GSK, pg 9, ln 23-26, In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.).

Regarding claim 43, GSK and GVPL teach the pharmaceutical composition of claim 34, wherein the at least one additional serotype is selected from the group consisting of 15A, 15C, 16F, 23A, 23B, and 35B (GSK, pg 9, ln 23-26, In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.).

Regarding claim 44, GSK and GVPL teach the pharmaceutical composition of claim 34, wherein the at least one additional serotype is selected from the group consisting of 9N, 15A, 15C, 16F, 17F, 20B, 23A, 23B, 24F, 31, 33F, 34, 35F, and 35B (GSK, pg 9, ln 23-26, In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.).

Regarding claim 45, GSK and GVPL teach the pharmaceutical composition of claim 34, wherein the at least one additional serotype is selected from the group consisting of 15A, 15C, 16F, 20B, 23A, 23B, 24F, 31, 33F, 34, 35F, and 35B (GSK, pg 9, ln 23-26, In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.).

Regarding claim 46, GSK and GVPL teach the pharmaceutical composition of claim 34, wherein the at least one additional serotype is selected from the group consisting of 8, 9N, 10A, 11A, 15A, 15B, 15C, 16F, 20A, 22F, 23A, 23B, 33F, and 35B (GSK, pg 9, ln 23-26, In one embodiment the multivalent pneumococcal vaccine of the invention will comprise polysaccharides (suitably conjugated) selected from the following serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 15C, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F.).

Claims 1, 28/1, 34-46, 77-80 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

Electronic Acknowledgement Receipt

EFS ID:	41500331
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:	23628
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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-CEW.pdf	26322 f7e0e2b2c377503a9e5d7d193af72fd12287abb7	no	1

Warnings:

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2	Transmittal Letter	P114370000US04-IDSBODY-CEW.pdf	35822 09547b5dc94ab2e4a82c99dbcff34f747ab7893a	no	3
Warnings:					
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3	Information Disclosure Statement (IDS) Form (SB08)	P114370000US04-IDS1449-CEW.pdf	21638 a132611a5a30ab7e5aae0a408b3dcc6d20ff83d0	no	1
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6	Other Reference-Patent/App/Search documents	P114370001WO00_ISR_WO_mailed_02-11-2019.PDF	1181750 8ffdee4036a1220135eafbee16a64150fbf3fb97	no	17
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7	Other Reference-Patent/App/Search documents	P114370001WO00_IPRP_mailed_04-16-2020.PDF	653626 32441f5595e30d8c77d694605007a8d23bf96c53	no	12
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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.

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TRANSMITTAL FORM

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Total Number of Pages in This Submission	

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First Named Inventor	Bruce D. Forrest
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Attorney Docket Number	P1143.70000US04

ENCLOSURES (*Check all that apply*)

- [ ] Fee Transmittal Form
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- [ ] Amendment/Reply
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  - [ ] Affidavits/declaration(s)
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Date	December 28. 2020	Reg. No.	75,713

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Filed:	September 27, 2018
For:	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF
Examiner:	Padmavathi Baskar
Art Unit:	1645

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 28, 2020 Electronic Signature for Kristy J. Alaura: /Kristy J. Alaura/

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

STATEMENT FILED PURSUANT TO THE DUTY OF DISCLOSURE UNDER 37 C.F.R. §§ 1.56, 1.97 AND 1.98

Commissioner:

Pursuant to the duty of disclosure under 37 C.F.R. §§ 1.56, 1.97 and 1.98, the undersigned requests consideration of this Information Disclosure Statement.

PART I: Compliance with 37 C.F.R. § 1.97

This Information Disclosure Statement has been filed before the mailing of a first Office Action on the merits in the above-referenced Application. Therefore, no fee or certification is required.

PART II: Information Cited

The undersigned hereby makes of record in the present Application the information listed on the attached Form PTO-1449 (modified PTO/SB/08). The order of presentation of the references should not be construed as an indication of the importance of the references.

Petitioner Merck Ex-1004, 1221

The undersigned would also like to bring to the Examiner's attention the following application that may contain subject matter related to the present Application:

Serial No.	Filing Date	Inventors	Docket No.
*16/753,597	04-03-2020	Forrest *et al.*	P1143.700001US02

*A copy of this reference is not provided as the Office has waived the requirement under 37 C.F.R. § 1.98(a)(2)(iii) for submitting a copy of a cited U.S. patent application if it is scanned to the Image File Wrapper system and is available on Private PAIR.

This application may include subject matter which is related to subject matter of the present Application and may contain claims to such subject matter. During prosecution, the examiner of this application will search for relevant art and, in some circumstances, takes positions with respect to the art and/or the interpretation of the claims that may be material to the examination of the present Application. Accordingly, the undersigned respectfully requests that the Examiner review the file history of this application to determine whether there is any information that the Examiner believes is relevant to the prosecution of the present Application. It is assumed that the Examiner has access to the file history of this application, and the undersigned does not plan to provide copies of documents from this file history to the Examiner. However, if the Examiner would like copies of any or all of the information included in the file history of this application, or any other information related to this application, the Examiner is respectfully requested to contact the undersigned.

The undersigned would also like to bring to the Examiner's attention the enclosed search report and other communication from a related] international patent application:

Serial No.	Date of Mailing	Type of Communication	Docket No.
PCT/US2018/054391	02-11-2019	International Search Report and Written Opinion	P1143.70001WO00
PCT/US2018/054391	04-16-2020	International Preliminary Report on Patentability	P1143.70001WO00

PART III: Remarks

Documents cited anywhere in the Information Disclosure Statement are enclosed unless otherwise indicated. It is respectfully requested that:

1. The Examiner consider completely the cited information, along with any other information, in reaching a determination concerning the patentability of the present claims;

2. The enclosed Form PTO-1449 (modified PTO/SB/08) be signed by the Examiner to evidence that the cited information has been fully considered by the United States Patent and Trademark Office during the examination of the present Application; and

3. The citations for the information be printed on any patent which issues from the present Application.

By submitting this Information Disclosure Statement, the undersigned makes no representation that a search has been performed, of the extent of any search performed, or that more relevant information does not exist.

By submitting this Information Disclosure Statement, the undersigned makes no representation that the information cited in the Statement is, or is considered to be, material to patentability as defined in 37 C.F.R. § 1.56(b).

By submitting this Information Disclosure Statement, the undersigned makes no representation that the information cited in the Statement is, or is considered to be, in fact, prior art as defined by 35 U.S.C. § 102.

Notwithstanding any statements by the undersigned, the Examiner is urged to form his or her own conclusion regarding the relevance of the cited information.

An early and favorable action is hereby requested.

The Director is hereby authorized to charge any deficiency in the fees due, or credit any overpayment, by the filing of this Information Disclosure Statement to our Deposit Account No. 23/2825, under Docket No. P1143.70000US04, from which the undersigned is authorized to draw.

Respectfully submitted,

Electronic signature: /Chelsea E. Witte-Garcia/
Chelsea E. Witte-Garcia, Ph.D.
Registration No.: 75,713
WOLF, GREENFIELD & SACKS, P.C.
600 Atlantic Avenue
Boston, Massachusetts 02210-2206
617.646.8000

Docket No.: P1143.70000US04
Date: December 28, 2020

PTO/SB/06 (09-11)
Approved for use through 1/31/2014. 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.

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 16/089,040	Filing Date 09/27/2018	☐ To be Mailed

ENTITY: ☑ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA		RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A		N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A		N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*		x $100 =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*		x $460 =	
☐ 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).				
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))					
* If the difference in column 1 is less than zero, enter "0" in column 2.				TOTAL	

APPLICATION AS AMENDED - PART II

		(Column 1)		(Column 2)	(Column 3)			
AMENDMENT	12/28/2020	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 20	Minus	** 20	= 0		x $100 =	0
	Independent (37 CFR 1.16(h))	* 1	Minus	*** 5	= 0		x $480 =	0
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	0

		(Column 1)		(Column 2)	(Column 3)			
AMENDMENT		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=		x $0 =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=		x $0 =	
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.	LIE
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".	/LINDA HUMES/
*** 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 number found in the appropriate box in column 1.	

This collection of information is required by 37 CFR 1.16. 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 12 minutes 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.

Doc Code: ECOMM.AUTH/ECOMM.WTDW
Doc Description: Internet Communications Authorized/Internet Communications Authorization Withdrawn

PTO/SB/439 (11-15)

AUTHORIZATION FOR INTERNET COMMUNICATIONS IN A PATENT APPLICATION OR REQUEST TO WITHDRAW AUTHORIZATION FOR INTERNET COMMUNICATIONS		
	Application No.	16/089,040-Conf. #8702
	Filing Date	September 27, 2018
	First Named Inventor	Bruce D. Forrest
	Art Unit	1645
	Examiner Name	Padmavathi Baskar
	Practitioner Docket No.	P1143.70000US04

To: Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I. To authorize permission for Internet Communications.

[X] Recognizing that Internet communications are not secure, I hereby authorize the USPTO to communicate with the undersigned and practitioners in accordance with 37 CFR 1.33 and 37 CFR 1.34 concerning any subject matter of this application via video conferencing, instant messaging, or electronic mail. I understand that a copy of these communications will be made of record in the application file. (MPEP 502.03)

II. To withdraw authorization for Internet Communications.

[ ] The authorization given on _____, to the USPTO to communicate with the undersigned and any practitioner in accordance with 37 CFR 1.33 and 37 CFR 1.34 concerning any subject matter of this application via Internet communications such as video conferencing, instant messaging, or electronic mail is hereby withdrawn. I understand that the withdrawal is effective when approved rather than when received.

I am the

[ ] applicant.

[X] attorney or agent of record. Registration number 75,713.

[ ] attorney or agent acting under 37 CFR 1.34. Registration number _____.

/Chelsea E. Witte-Garcia/ — Signature

February 25, 2021 — Date

Chelsea E. Witte-Garcia, Ph.D. — Typed or printed name

617.646.8000 — Telephone Number

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. Juristic entities must be represented by a patent practitioner (see 37 CFR 1.31, which is applicable to any paper filed on or after September 16, 2012, that is presented on behalf of a juristic entity, regardless of application filing date). Submit multiple forms if more than one signature is required, see below*.

[ ] * Total of 1 forms are submitted.

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: February 25, 2021 Signature: /Alexandra E. Cinamon/

Petitioner Merck Ex-1004, 1225

Electronic Acknowledgement Receipt

EFS ID:	42025172
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:	23628
Filer:	Chelsea E. Witte-Garcia/Alexandra Cinamon
Filer Authorized By:	Chelsea E. Witte-Garcia
Attorney Docket Number:	P1143.70000US04
Receipt Date:	25-FEB-2021
Filing Date:	27-SEP-2018
Time Stamp:	15:47:09
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-CEW.pdf	26201 61aff86a69fecfc0887cdb365890abe9014c432c	no	1
Warnings:					

Information:					
2	Internet Communications Authorized	P114370000US04-INTAUT-CEW.pdf	54734 7c3801e54e8e5bd819ea881678a51bfdff200a95	no	1
Warnings:					
Information:					
			Total Files Size (in bytes):	80935	

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.

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	1645
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
- [ ] 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):

-Authorization for Internet Communications in a Patent Application

Remarks

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	WOLF, GREENFIELD & SACKS, P.C.		
Signature	/Chelsea E. Witte-Garcia/		
Printed name	Chelsea E. Witte-Garcia, Ph.D.		
Date	February 25, 2021	Reg. No.	75,713

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: February 25, 2021 Signature: /Alexandra E. Cinamon/

Petitioner Merck Ex-1004, 1228

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	1645
Examiner Name	Padmavathi Baskar
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
- [ ] 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):

  Executed Inventors' Declaration

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	March 5, 2021	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: March 5, 2021 Signature: /Alessandra G. Torres Pena/

9019765.1

DECLARATION (37 C.F.R. § 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 C.F.R. § 1.76)

Title of Invention	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF

As the below named inventor, I declare that:

This declaration is directed to:

☐ The attached application, or

☒ United States application or PCT international application number: PCT/US2017/025621

filed on March 31, 2017

The above-identified application was made or authorized to be made by me.

I believe I am the original inventor or an original joint inventor of a claimed invention in the application.

I have reviewed and understand the contents of the above-identified application, including the claims.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in 37 C.F.R. § 1.56.

All statements made herein of my own knowledge are true, and all statements made herein on information and belief are believed to be true.

I hereby acknowledge that any willful false statement and the like made in this declaration is punishable by fine or imprisonment of not more than five (5) years, or both, under 18 U.S.C. § 1001, and may jeopardize the validity of the application or any patent issuing thereon.

LEGAL NAME OF SOLE OR FIRST INVENTOR

Inventor one: Bruce D. Forrest Date: Jan 15th, 2021

Signature:

LEGAL NAME OF ADDITIONAL JOINT INVENTOR, if any

Inventor two: Jack D. Love Date: Jan 14, 2021

Signature:

LEGAL NAME OF ADDITIONAL JOINT INVENTOR, if any

Inventor three: Date:

Signature:

LEGAL NAME OF ADDITIONAL JOINT INVENTOR, if any

Inventor four: Date:

Signature:

☐ Additional inventors are being named on _____ Additional form(s) attached hereto.

Petitioner Merck Ex-1004, 1230

Electronic Acknowledgement Receipt

EFS ID:	42104377
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:	23628
Filer:	Charles Baker/Alessandra Torres Pena
Filer Authorized By:	Charles Baker
Attorney Docket Number:	P1143.70000US04
Receipt Date:	05-MAR-2021
Filing Date:	27-SEP-2018
Time Stamp:	16:29:21
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	26312 70200f40e3d2fd20ce72c368f8b90e9a5142c8ec	no	1

Warnings:

Information:					
2	Oath or Declaration filed	P114370000US04-INVDEC-CHB.pdf	165460 b6e9a2805b4cd775d1a8f57cb5a748e47c5e6b77	no	1
Warnings:					
Information:					
			Total Files Size (in bytes):	191772	

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.

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

NOTICE OF ALLOWANCE AND FEE(S) DUE

23628 7590 03/10/2021
WOLF GREENFIELD & SACKS, P.C.
600 ATLANTIC AVENUE
BOSTON, MA 02210-2206

EXAMINER
BASKAR, PADMAVATHI

ART UNIT	PAPER NUMBER
1645	

DATE MAILED: 03/10/2021

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/089,040	09/27/2018	Bruce D. FORREST	P1143.70000US04	8702

TITLE OF INVENTION: SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	$600	$0.00	$0.00	$600	06/10/2021

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Maintenance fees are due in utility patents issuing on applications filed on or after Dec. 12, 1980. It is patentee's responsibility to ensure timely payment of maintenance fees when due. More information is available at www.uspto.gov/PatentMaintenanceFees.

Petitioner Merck Ex-1004, 1233

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), by mail or fax, or via EFS-Web.

By mail, send to: Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

By fax, send to: (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

23628 7590 03/10/2021
WOLF GREENFIELD & SACKS, P.C.
600 ATLANTIC AVENUE
BOSTON, MA 02210-2206

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being transmitted to the USPTO via EFS-Web or by facsimile to (571) 273-2885, on the date below.

(Typed or printed name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/089,040	09/27/2018	Bruce D. FORREST	P1143.70000US04	8702

TITLE OF INVENTION: SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	$600	$0.00	$0.00	$600	06/10/2021

EXAMINER	ART UNIT	CLASS-SUBCLASS
BASKAR, PADMAVATHI	1645	424-244100

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-09 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 _____
2 _____
3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. Fees submitted: ❑ Issue Fee ❑ Publication Fee (if required) ❑ Advance Order - # of Copies _____

4b. Method of Payment: *(Please first reapply any previously paid fee shown above)*

❑ Electronic Payment via EFS-Web ❑ Enclosed check ❑ Non-electronic payment by credit card (Attach form PTO-2038)

❑ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. _____

5. **Change in Entity Status** (from status indicated above)

❑ Applicant certifying micro entity status. See 37 CFR 1.29

❑ Applicant asserting small entity status. See 37 CFR 1.27

❑ Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.
NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.
NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

Petitioner Merck Ex-1004, 1234

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/089,040	09/27/2018	Bruce D. FORREST	P1143.70000US04	8702

23628 7590 03/10/2021

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

EXAMINER	
BASKAR, PADMAVATHI	
ART UNIT	PAPER NUMBER
1645	

DATE MAILED: 03/10/2021

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

Petitioner Merck Ex-1004, 1235

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. 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 30 minutes 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, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. 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.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability	Application No. 16/089,040	Applicant(s) FORREST et al.	
	Examiner Padmavathi Baskar	Art Unit 1645	AIA (FITF) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☑ This communication is responsive to 12/28/2020.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

3. ☑ The allowed claim(s) is/are See Continuation Sheet . As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see **http://www.uspto.gov/patents/init_events/pph/index.jsp** or send an inquiry to **PPHfeedback@uspto.gov.**

4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

   Certified copies:

   a) ☐ All b) ☐ Some *c) ☐ None of the:

   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____ .

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____ .

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☑ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date 03/10/2020 and 12/28/2020.
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material _____.
4. ☑ Interview Summary (PTO-413), Paper No./Mail Date. 03/04/2021.
5. ☑ Examiner's Amendment/Comment
6. ☑ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

/Padmavathi Baskar/
Primary Examiner, Art Unit 1645

Petitioner Merck Ex-1004, 1237

Continuation of 3. The allowed claim(s) is/are: 67,74-75,78,81,97,106,109,112-114,154,256,297 and 299-303

DETAILED ACTION

Notice of Pre-AIA or AIA Status

1. The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

Response to Restriction Requirement

2. Applicant's response 12/28/2020 to election is acknowledged. Applicant elected Applicant hereby elects Group 4 (claim 67), drawn to a pharmaceutical composition comprising at least two immunogenic polypeptide conjugates, 35B and 23A and 23B.

Status of claims

3. Claims 67, 71, 73-75, 78, 81, 97, 106, 109, 112-114, 154, 256, and 296-300 are pending.

Claims 1, 29, 62, 157, 163, and 256 have been canceled.

Claims 71, 74, 75, 68, 81, 97, 106, 109, 112, 113, and 256 have been amended

New claims 296-300 have been added.

Claims 67, 71, 73-75, 78, 81, 97, 106, 109 and 296-300 are under examination with respect to 35B, 23A and 23B.

Information Disclosure Statement

4. The information disclosure statements (IDS) submitted on 03/10/2020 and 12/28/2020 are in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statements are being considered by the examiner and a signed copy of each is attached to this office action.

Examiner's amendment

5. An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with Chelsea E. Witte-Garcia, on 03/04/2021 and the application has been amended as follows:

Claims 71, 73, 296 and 298 are cancelled.

67. (Currently Amended) A pharmaceutical composition comprising a plurality of at least two unique ~~an~~ immunogenic saccharide-polypeptide conjugates, each comprising~~,~~ individually a~~, at least two unique~~ capsular polysaccharide~~s, fragments thereof, or combinations thereof,~~ conjugated to a polypeptide, wherein each of the ~~at least two unique~~ capsular polysaccharides~~, or fragments thereof,~~ is from a *Streptococcus pneumoniae* serotype selected from a 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, 20A, 20B, 22F,~~ 23A, 23B, ~~24F, 24B, 31, 33F, 34, 35F,~~ and 35B~~, and 38~~.

74. (Currently Amended) The pharmaceutical composition of claim 67, wherein ~~at least one polypeptide of the plurality is an oligopeptide and/or~~ at least one polypeptide comprises a mixture of polypeptides.

78. (Currently Amended) The pharmaceutical composition of claim 67, further comprising an adjuvant; a chelating agent; a surfactant; , an emulsifier~~, or a combination thereof~~; a buffering agent; a preservative; a salt; ~~and/or~~ an anti-fungal compound; or a combination ~~or a salt~~ thereof.

97. (Currently Amended) The pharmaceutical composition of claim 67, wherein at least one of the unique immunogenic saccharide-polypeptide conjugate elicits an opsonophagocytic response.

106. (Currently Amended) The pharmaceutical composition of claim 67, wherein the immunogenic saccharide-polypeptide conjugates ~~conjugate(s)~~ are ~~individually, respectively, or~~ collectively present in an amount of at least ~~about~~ 0.001%, by weight, based on the weight of the pharmaceutical composition.

114. (Currently Amended) The method of claim 113, wherein the second composition comprises an immunogenic saccharide-polypeptide conjugate comprising a capsular polysaccharide ~~or fragment of~~

~~the capsular polysaccharide~~ from a serotype of *Streptococcus pneumoniae* conjugated to ~~the~~ a second polypeptide.

154. (Currently Amended) The method of claim 112 ~~113~~ wherein the subject is a human.

299. (Currently Amended) The pharmaceutical composition of claim 67, wherein each of the immunogenic saccharide-polypeptide conjugates is present in an amount of ~~about:~~ at least 0.001%, by weight, based on the weight of the pharmaceutical composition.

301. (New) The pharmaceutical composition of claim 67, further comprising at least one additional immunogenic saccharide-polypeptide conjugate comprising a capsular polysaccharide from a unique *Streptococcus pneumoniae* serotype selected from the group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 20A, 20B, 22F, and 34.

302. (New) The pharmaceutical composition of claim 67, wherein plurality of unique immunogenic saccharide-polypeptide conjugate comprises individually *Streptococcus pneumoniae* serotype comprising 6C, 9N, 15A, 15C, 16F, 23A, 23B, and 35B.

303. (New) The pharmaceutical composition of claim 67, wherein plurality of unique immunogenic saccharide-polypeptide conjugate comprises individually *Streptococcus pneumoniae* serotype consisting of 6C, 9N, 15A, 15C, 16F, 23A, 23B, and 35B.

Reasons for Allowance

6. The following is an examiner's statement of reasons for allowance:

The claimed pharmaceutical composition comprising capsular polysaccharides from a *Streptococcus pneumoniae* serotype 23A, 23B and 35B of the instant application is novel and nonobvious. The specification teaches (Table 2 and examples) the capsular conjugates and therefore induce an immune response when administered. The claimed composition has not been disclosed or

studied in the prior art and the specification meets 35 U.S.C. 112, first paragraph, requirements because it contains an adequate written description of the invention that enables one of ordinary skill in the art to make and use the invention. The specification also sets forth the best mode to carry out the invention. The claims also meet 35 U.S.C. 112, second paragraph, requirements because the claims point out and distinctly claim the invention.

7. Claims 67, 74-75, 78, 81, 97, 106, 109, 297 and 299-303 are directed to an allowable product. Pursuant to the procedures set forth in MPEP § 821.04(B), claims 112-114, 154 and 256 (group 5), directed to the process of making or using an allowable product, previously withdrawn from consideration as a result of a restriction requirement, 06/29/2020 hereby rejoined and fully examined for patentability under 37 CFR 1.104. Because all claims previously withdrawn from consideration under 37 CFR 1.142 have been rejoined, the restriction requirement as set forth in the Office action mailed on is hereby withdrawn. In view of the withdrawal of the restriction requirement as to the rejoined inventions, applicant(s) are advised that if any claim presented in a continuation or divisional application is anticipated by, or includes all the limitations of, a claim that is allowable in the present application, such claim may be subject to provisional statutory and/or nonstatutory double patenting rejections over the claims of the instant application. Once the restriction requirement is withdrawn, the provisions of 35 U.S.C. 121 are no longer applicable. See In re Ziegler, 443 F.2d 1211, 1215, 170 USPQ 129, 131-32 (CCPA 1971). See also MPEP § 804.01.

Conclusion

8. Claims 67, 74-75, 78, 81, 97, 106, 109, 297, 299-303, 112-114, 154 and 256 are allowed and are numbered as 1-19 respectively.

Correspondence

9. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Padmavathi Baskar whose telephone number is (571)272-0853. The examiner can normally be reached on Mon-Fri 9-5:30

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Nickol can be reached on 571-272-0835. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Respectfully,

/Padmavathi Baskar/
Primary Examiner, Art Unit 1645
Padmavathi Baskar, Ph.D. (Microbiology and Immunology)

Examiner-Initiated Interview Summary	**Application No.** 16/089,040	**Applicant(s)** FORREST et al.		
	Examiner Padmavathi Baskar	**Art Unit** 1645	**AIA (First Inventor to File) Status** Yes	**Page** 1 of 1

All Participants (applicant, applicants representative, PTO personnel)	**Title**	**Type**
Padmavathi Baskar	Primary Examiner	Telephonic
Chelsea E. Witte-Garcia	Attorney of Record	

Date of Interview: 04 March 2021

Issues Discussed:

Other

The examine and the attorney discussed the allowable subject matter of pharmaceutical composition comprising polysaccharide conjugates 35B, 23A and 23B from Streptococcus pneumoniae. These serotypes along with 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 20A, 20B, 22F, and 34 or 6C, 9N, 15A, 15C, 16F, 23A, 23B, and 35B appear to be free of prior art and the specification table 2 has support doe said composition. The applicant agreed to the examiner's amendment as set forth in the attached offfice action.

/Padmavathi Baskar/ Primary Examiner, Art Unit 1645	

Applicant is reminded that a complete written statement as to the substance of the interview must be made of record in the application file. It is the applicants responsibility to provide the written statement, unless the interview was initiated by the Examiner and the Examiner has indicated that a written summary will be provided. See MPEP 713.04
Please further see:
MPEP 713.04
Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews, paragraph (b)
37 CFR § 1.2 Business to be transacted in writing

Applicant recordation instructions: It is not necessary for applicant to provide a separate record of the substance of interview.

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

Petitioner Merck Ex-1004, 1244

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	16/089,040	FORREST et al.
	Examiner	**Art Unit**
	Padmavathi Baskar	1645

CPC						
Symbol					**Type**	**Version**
A61K	/	39	/	092	F	2013-01-01
A61K	/	47	/	646	I	2017-08-01
A61K	/	2039	/	55505	A	2013-01-01
A61K	/	2039	/	6037	A	2013-01-01
A61K	/	2039	/	70	A	2013-01-01

CPC Combination Sets							
Symbol					**Type**	**Set**	**Ranking**	**Version**
	/		/					

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	19	
/Padmavathi Baskar/ Primary Examiner, Art Unit 1645	04 March 2021	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	----------

U.S. Patent and Trademark Office — Part of Paper No.: 20210304

Petitioner Merck Ex-1004, 1245

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	16/089,040	FORREST et al.
	Examiner	**Art Unit**
	Padmavathi Baskar	1645

INTERNATIONAL CLASSIFICATION					
CLAIMED					
A61K39/09	/	39	/	09	
A61K47/64	/	47	/	64	

NON-CLAIMED					
A61K39/00	/	39	/	00	

US ORIGINAL CLASSIFICATION	
CLASS	**SUBCLASS**

CROSS REFERENCES(S)						
CLASS	**SUBCLASS (ONE SUBCLASS PER BLOCK)**					

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	19	
/Padmavathi Baskar/ Primary Examiner, Art Unit 1645	04 March 2021	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	----------

U.S. Patent and Trademark Office — Part of Paper No.: 20210304

Petitioner Merck Ex-1004, 1246

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	16/089,040	FORREST et al.
	Examiner	**Art Unit**
	Padmavathi Baskar	1645

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIMS

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
1	67	10	299	19	256										
2	74	11	300												
3	75	12	301												
4	78	13	302												
5	81	14	303												
6	97	15	112												
7	106	16	113												
8	109	17	114												
9	297	18	154												

NONE (Assistant Examiner)	(Date)	**Total Claims Allowed:** 19	
/Padmavathi Baskar/ Primary Examiner, Art Unit 1645 (Primary Examiner)	04 March 2021 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure ----------

U.S. Patent and Trademark Office

Part of Paper No.: 20210304

Petitioner Merck Ex-1004, 1247

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	16/089,040	FORREST et al.
	Examiner	Art Unit
	Padmavathi Baskar	1645

CPC - Searched*		
Symbol	Date	Examiner
SEE ATTACHED WEST SEARCH HISTORY.	03/04/2021	PB

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	Date	Examiner
INVENTORS NAME SEARCH IS PERFORMED IN PALM/PE2E.	03/04/2021	PB
CONTINUATION DOCUMENTS WERE REVIEWED AND SIGNED. SEE ATTACHED BIB SHEET.	03/04/2021	PB
TEXT AND INVENTORS NAME SEARCH PERFORMED ON WEST USING DATABASES, USPAT, US PUBLICATIONS, JPO, EPO, AND DERWENT. SEE ATTACHED WEST SEARCH HISTORY.	03/04/2021	PB
TEXT AND INVENTORS NAME SEARCH PERFORMED ON STN USING DATABASES MED LINE, BIOSIS, CAPLUS, HCAPLUS, EMBASE, WPIX, JAPIO, PASCAL, DISSABS. SEE ATTACHED STN SEARCH HISTORY.	03/04/2021	PB

Petitioner Merck Ex-1004, 1248

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	16/089,040	FORREST et al.
	Examiner	Art Unit
	Padmavathi Baskar	1645

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner
-	SEE ATTACHED WEST SEARCH HISTORY.	03/04/2021	PB

Petitioner Merck Ex-1004, 1249

Bibliographic Data

Application No: 16/089,040

Foreign Priority claimed: ○ Yes ◉ No

35 USC 119 (a-d) conditions met: ☐ Yes ☑ No ☐ Met After Allowance

Verified and Acknowledged: /Padmavathi Baskar/ Examiner's Signature | Initials

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

FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
09/27/2018	424	1645	P1143.70000US04
RULE			

APPLICANTS

Pogona, LLC, South Nyack, NY, UNITED STATES

INVENTORS

Bruce D. FORREST, South Nyack, NY, UNITED STATES

Jack D. LOVE, Tampa, FL, UNITED STATES

CONTINUING DATA

This application is a 371 of PCT/US17/25621 03/31/2017

PCT/US17/25621 has PRO of 62373807 08/11/2016

PCT/US17/25621 has PRO of 62372263 08/08/2016

PCT/US17/25621 has PRO of 62330245 05/02/2016

PCT/US17/25621 has PRO of 62316555 03/31/2016

FOREIGN APPLICATIONS

IF REQUIRED, FOREIGN LICENSE GRANTED**

01/08/2020

** SMALL ENTITY **

STATE OR COUNTRY

UNITED STATES

ADDRESS

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

FILING FEE RECEIVED

$600

WEST Search History for Application 16089040

Creation Date: 2021030417:30

Prior Art Searches

Query	DB	Hits	Op.	Plur.	Thes.	Date
capsular polysaccharides from serotypes 35B 23A 23B	USPT	0	ADJ	YES		03-04-2021
polysaccharides 35B 23A 23B	USPT	0	ADJ	YES		03-04-2021
FORREST BRUCE D.in.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	10	ADJ	YES		03-04-2021
LOVE, JACK D.in.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	29	ADJ	YES		03-04-2021
6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	2	ADJ	YES		03-04-2021
35B 23A 23B	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	4	ADJ	YES		03-04-2021
6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 34	PGPB, USPT,	2	ADJ	YES		03-04-2021

Petitioner Merck Ex-1004, 1251

	USOC, EPAB, JPAB, DWPI, TDBD, FPRS					
(35B 23A 23B) and (6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 34)	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	0	ADJ	YES		03-04-2021
(35B 23A 23B) and (6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B)	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	0	ADJ	YES		03-04-2021
(LOVE, JACK D.in.) and (35B 23A 23B)	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	0	ADJ	YES		03-04-2021
(LOVE, JACK D.in.) and (35B 23A 23B)	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	0	ADJ	YES		03-04-2021
35B 23A 23B 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 34	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	0	ADJ	YES		03-04-2021

Petitioner Merck Ex-1004, 1252

35B 23A 23B 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	0	ADJ	YES		03-04-2021
35B 23A 23B 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, 35B	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	0	ADJ	YES		03-04-2021
capsular polysaccharides from serotypes 35B 23A 23B	USPT	0	ADJ	YES		03-04-2021
polysaccharides 35B 23A 23B	USPT	0	ADJ	YES		03-04-2021
FORREST BRUCE D.in.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	10	ADJ	YES		03-04-2021
LOVE, JACK D.in.	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	29	ADJ	YES		03-04-2021
6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	2	ADJ	YES		03-04-2021
35B 23A 23B	PGPB, USPT, USOC,	4	ADJ	YES		03-04-2021

Petitioner Merck Ex-1004, 1253

	EPAB, JPAB, DWPI, TDBD, FPRS					
6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 34	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	2	ADJ	YES		03-04-2021
(35B 23A 23B) and (6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 34)	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	0	ADJ	YES		03-04-2021
(35B 23A 23B) and (6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B)	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	0	ADJ	YES		03-04-2021
(LOVE, JACK D.in.) and (35B 23A 23B)	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	0	ADJ	YES		03-04-2021
(LOVE, JACK D.in.) and (35B 23A 23B)	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	0	ADJ	YES		03-04-2021
35B 23A 23B 6C, 8, 9N, 10A, 11A, 12F, 15A,	PGPB,	0	ADJ	YES		03-04-2021

Petitioner Merck Ex-1004, 1254

22F, 23A, 23B, 24F, 33F, and 34	USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS					
35B 23A 23B 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 35B	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	0	ADJ	YES		03-04-2021
35B 23A 23B 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, 35B	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	0	ADJ	YES		03-04-2021
35B 23A 23B 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 24F, 33F, 35B	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	0	ADJ	YES		03-04-2021
35B 23A 23B 6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 24F, 33F, and 34	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	0	ADJ	YES		03-04-2021
(6C, 8, 9N, 10A, 11A, 12F, 15A, 22F, 23A, 23B, 24F, 33F, and 34) and ((A61K2039/55505 | A61K2039/6037 | A61K39/092 | A61K2039/54 | A61K2039/545 | A61K2039/70 | A61K39/39 | A61K45/06 | A61K47/10 | A61K47/183 | A61K47/26 | A61K47/646 | A61K9/08 | A61P31/04 | A61P37/04 | A61P43/00).CPC. or (1/1).CCLS. or (A61K39/09 | A61K39/39 |	PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD, FPRS	2	ADJ	YES		03-04-2021

Petitioner Merck Ex-1004, 1255

A61K47/10 I A61K47/18 I A61K47/26 I A61K47/64 I A61K9/08).IPCR.)						

Petitioner Merck Ex-1004, 1256

FORM PTO-1449/A and B (modified PTO/SB/08)				APPLICATION NO.: 16/089,040	ATTY. DOCKET NO.: P1143.70000US014
INFORMATION DISCLOSURE STATEMENT BY APPLICANT				FILING DATE: September 27, 2018	CONFIRMATION NO.: 8702
				FIRST NAMED INVENTOR: Bruce D. Forrest	
Sheet	1	of	1	GROUP ART UNIT: 1645	EXAMINER: Padmavathi Baskar

U.S. PATENT DOCUMENTS

Examiner's Initials #	Cite No.	U.S. Patent Document		Name of Patentee or Applicant of Cited Document	Date of Publication or Issue of Cited Document MM-DD-YYYY
		Number	Kind Code		
/P.B/		2017-0021006	A1	Watson et al.	01-26-2017

FOREIGN PATENT DOCUMENTS

Examiner's Initials #	Cite No.	Foreign Patent Document			Name of Patentee or Applicant of Cited Document	Date of Publication of Cited Document MM-DD-YYYY	Translation (Y/N)
		Office/ Country	Number	Kind Code			
/P.B/		WO	2016/149771	A1	Gamma Vaccines Pty Limited	09-29-2016	
/P.B/		WO	2017/067962	A1	Glaxosmithkline Biologicals S.A.	04-27-2017	

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)
/P.B/		International Search Report and Written Opinion, mailed February 11, 2019, in connection with Application No. PCT/US2018/054391 (P1143.70001WO00).	
/P.B/		International Preliminary Report on Patentability, mailed April 16, 2020, in connection with Application No. PCT/US2018/054391 (P1143.70001WO00).	

[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:
/Padmavathi Baskar/	01/28/2021

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.

8486076.1

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=> d his ful

     FILE 'REGISTRY' ENTERED AT 07:54:24 ON 08 JAN 2021
                  E CRM197/CN
L1             1 SEA ABB=ON  PLU=ON  "CRM197 PROTEIN"/CN
                  E CRM 197/CN
L2             5 SEA ABB=ON  PLU=ON  ("CRM 197"/CN OR "CRM 197 (173-LYSINE)(
                  SYNTHETIC CATALYTICALLY INACTIVATED DIPHTHERIA TOXIN
                  PRECURSOR)"/CN OR "CRM 197 (CORYNEBACTERIUM DIPHTHERIAE
                  INACTIVE DIPHTHERIA TOXIN CRM197 GENE)"/CN OR "CRM 197
                  (CORYNEBACTERIUM DIPHTHERIAE INACTIVE DIPHTHERIA TOXIN
                  CRM197 PRECURSOR)"/CN OR "CRM 197 (CORYNEBACTERIUM
                  DIPHTHERIAE)"/CN OR "CRM 197 (SYNTHETIC CATALYTICALLY
                  INACTIVATED DIPHTHERIA TOXIN PRECURSOR)"/CN)
L3            34 SEA ABB=ON  PLU=ON  TETANUS TOXOID?/CN
                  E TETANUS TOXIN/CN
L4             1 SEA ABB=ON  PLU=ON  "TETANUS TOXIN"/CN
                  E DIPHTHERIA TOXOID/CN
L5             2 SEA ABB=ON  PLU=ON  ("DIPHTHERIA TOXOID"/CN OR "DIPHTHERIA
                  TOXOID (CORYNEBACTERIUM DIPHTHERIAE)"/CN OR "DIPHTHERIA
                  TOXOIDS"/CN)
                  E DIPHTHERIA TOXIN/CN
L6             1 SEA ABB=ON  PLU=ON  "DIPHTHERIA TOXIN"/CN
                  E PERTUSSIS TOXOID/CN
L7             1 SEA ABB=ON  PLU=ON  ("PERTUSSIS TOXOID"/CN OR "PERTUSSIS
                  TOXOIDS"/CN)
                  E PERTUSSIS TOXIN/CN
L8             1 SEA ABB=ON  PLU=ON  "PERTUSSIS TOXIN"/CN
                  E CHOLERA TOXOID/CN
L9             2 SEA ABB=ON  PLU=ON  ("CHOLERA TOXOID (METHIONYL,41-TYROSINE
                  ) (VIBRIO CHOLERAE GENE CTXA SUBUNIT A FRAGMENT)"/CN OR
                  "CHOLERA TOXOID (METHIONYL,63-TYROSINE) (VIBRIO CHOLERAE
                  GENE CTXA SUBUNIT A FRAGMENT)"/CN)
                  E CHOLERA TOXIN/CN
L10            1 SEA ABB=ON  PLU=ON  "CHOLERA TOXIN"/CN
L11            5 SEA ABB=ON  PLU=ON  PNEUMOCOCCAL SURFACE PROTEIN A ?/CN
                  E PNEUMOCOCCAL PNEUMOLYSIN/CN
L12            3 SEA ABB=ON  PLU=ON  ("PNEUMOCOCCAL SURFACE ADHESIN A
                  (STREPTOCOCCUS PNEUMONIAE GENE PSAA FRAGMENT)"/CN OR
                  "PNEUMOCOCCAL SURFACE ADHESIN A (STREPTOCOCCUS PNEUMONIAE
                  GENE PSAA PRECURSOR)"/CN)
L13            7 SEA ABB=ON  PLU=ON  KEYHOLE LIMPET HEMOCYANIN?/CN
L14           19 SEA ABB=ON  PLU=ON  C5A PEPTIDASE ?/CN
L15            6 SEA ABB=ON  PLU=ON  TUBERCULIN ?/CN
L16           88 SEA ABB=ON  PLU=ON  (L1 OR L2 OR L3 OR L4 OR L5 OR L6 OR
                  L7 OR L8 OR L9 OR L10 OR L11 OR L12 OR L13 OR L14 OR L15)

     FILE 'HCAPLUS' ENTERED AT 07:59:27 ON 08 JAN 2021
L17       216410 SEA ABB=ON  PLU=ON  (?SACCHARIDE? OR CPS OR CP) AND
                  (PROTEIN OR PEPTIDE OR OLIGOPEPTIDE OR POLYPEPTIDE OR
                  POLYPROTEIN)
L18        42375 SEA ABB=ON  PLU=ON  L17 AND (CONJUGAT? OR ?LINK? OR
                  COUPL?)
L19         1513 SEA ABB=ON  PLU=ON  L18 AND ((STREPTOCOCC? OR S OR D OR
                  DIPLOCOCCI?)(W)PNEUMON? OR PNEUMOCOCC?)
L20            9 SEA ABB=ON  PLU=ON  L19 AND (23A OR 23B OR 35B)
L22        27442 SEA ABB=ON  PLU=ON  "STREPTOCOCCUS PNEUMONIAE"+OLD,PFT/CT
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L23        4159 SEA ABB=ON  PLU=ON  L22 AND (OLIGOPEPTIDES+OLD,PFT OR
                PEPTIDES OR PROTEINS)/CT
L24         945 SEA ABB=ON  PLU=ON  L23 AND (MONOSACCHARIDES+OLD,PFT OR
                OLIGOSACCHARIDES+OLD,PFT OR POLYSACCHARIDES+OLD,PFT OR
                CAPSULAR POLYSACCHARIDES+OLD,PFT OR SACCHARIDES OR
                CARBOHYDRATES)/CT
L25           7 SEA ABB=ON  PLU=ON  L24 AND (23A OR 23B OR 35B)
L26          10 SEA ABB=ON  PLU=ON  L20 OR L25

     FILE 'REGISTRY' ENTERED AT 08:09:35 ON 08 JAN 2021
L31           1 SEA ABB=ON  PLU=ON  100179-39-3/RN

     FILE 'HCAPLUS' ENTERED AT 08:10:33 ON 08 JAN 2021
L33       85765 SEA ABB=ON  PLU=ON  (?SACCHARIDE? OR CPS OR CP) (10A)(PROTE
                IN OR PEPTIDE OR OLIGOPEPTIDE OR POLYPEPTIDE OR POLYPROTEIN
                )
L34        6964 SEA ABB=ON  PLU=ON  L33(10A)(CONJUGAT? OR ?LINK? OR
                COUPL?)
L35         643 SEA ABB=ON  PLU=ON  L34(S)(L16 OR CRM197 OR (CROSS REACT?
                OR CRM)(2W)197 OR TT OR DT OR CT OR ?TOXOID?  OR PT OR
                ?TOXIN? OR PNEUMOCOCCAL PNEUMOLYSIN OR PNEUMOCOCC?(5W)(SPA
                OR PROTEIN A) OR PSPA OR (PSA OR PSP)(W)A OR PSSA OR
                C5A(S) PEPTIDASE OR NTHI OR INFLUENZA OR USPA OR USP A OR
                KLH)
L36         289 SEA ABB=ON  PLU=ON  L34(S)(L31 OR KEYHOLE LIMPET OR OMPC
                OR OUTER MEMBRANE? OR PPD OR TUBERCULIN OR PROTEIN D)
L40         285 SEA ABB=ON  PLU=ON  (L35 OR L36) AND  (?ADJUVANT? OR
                CHELAT? OR SURFACTANT OR SURFACE(2A)ACTIVE OR  EMULSIF? OR
                EMULSION OR BUFFER? OR PRESERVATIVE OR ANTIFUNG## OR
                FUNGICID? OR ANTI FUNG##)
L42          18 SEA ABB=ON  PLU=ON  L40 AND OPSONOPHAGOCY?
L43          96 SEA ABB=ON  PLU=ON  L19 AND OPSONOPHAGOCY?
L44          57 SEA ABB=ON  PLU=ON  L43 AND (L16 OR CRM197 OR (CROSS
                REACT? OR CRM)(2W)197 OR TT OR DT OR CT OR ?TOXOID?  OR PT
                OR ?TOXIN? OR PNEUMOCOCCAL PNEUMOLYSIN OR PNEUMOCOCC?(5W)(S
                PA OR PROTEIN A) OR PSPA OR (PSA OR PSP)(W)A OR PSSA OR
                C5A(S) PEPTIDASE OR NTHI OR INFLUENZA OR USPA OR USP A OR
                KLH)
L45          24 SEA ABB=ON  PLU=ON  L43 AND (L31 OR KEYHOLE LIMPET OR OMPC
                OR OUTER MEMBRANE? OR PPD OR TUBERCULIN OR PROTEIN D)
L46          28 SEA ABB=ON  PLU=ON  (L44 OR L45) AND  (?ADJUVANT? OR
                CHELAT? OR SURFACTANT OR SURFACE(2A)ACTIVE OR  EMULSIF? OR
                EMULSION OR BUFFER? OR PRESERVATIVE OR ANTIFUNG## OR
                FUNGICID? OR ANTI FUNG##)
                E OPSONOPHAGOCYSIS+ALL/CT
                E E4+ALL
L47        1186 SEA ABB=ON  PLU=ON  OPSONOPHAGOCYTOSIS+OLD,PFT/CT
L48          24 SEA ABB=ON  PLU=ON  L24 AND L47

     FILE 'MEDLINE, BIOSIS, EMBASE, WPIX, JAPIO, PQSCITECH' ENTERED AT
     08:22:02 ON 08 JAN 2021
L51          55 SEA ABB=ON  PLU=ON  L20

     FILE 'WPIX, PQSCITECH' ENTERED AT 08:22:33 ON 08 JAN 2021
L54          43 SEA ABB=ON  PLU=ON  L20
L55          25 SEA ABB=ON  PLU=ON  L54 AND PER/PHP
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     FILE 'HCAPLUS' ENTERED AT 08:25:23 ON 08 JAN 2021
                D QUE L20
                D QUE L25
                D L26 1-10

     FILE 'MEDLINE, BIOSIS, EMBASE, WPIX, JAPIO, PQSCITECH' ENTERED AT
     08:25:25 ON 08 JAN 2021
L56           48 DUP REM L55 L51 (32 DUPLICATES REMOVED)
                D L56 1-48

     FILE 'HCAPLUS' ENTERED AT 08:26:10 ON 08 JAN 2021
L57           44 SEA ABB=ON  PLU=ON  (L42 OR L46 OR L48) NOT L26
                D QUE L42
                D QUE L46
                D QUE L48
                D L57 1-44

     FILE 'MEDLINE, BIOSIS, EMBASE, WPIX, JAPIO, PQSCITECH' ENTERED AT
     08:27:33 ON 08 JAN 2021
L58           14 SEA ABB=ON  PLU=ON  L42
L59           26 SEA ABB=ON  PLU=ON  L46
L60           29 SEA ABB=ON  PLU=ON  (L58 OR L59) NOT L51

     FILE 'WPIX, PQSCITECH' ENTERED AT 08:31:23 ON 08 JAN 2021
L61           10 SEA ABB=ON  PLU=ON  L42
L62           10 SEA ABB=ON  PLU=ON  L46
L63            9 SEA ABB=ON  PLU=ON  (L61 OR L62) AND PER/PHP
L64            8 SEA ABB=ON  PLU=ON  L63 NOT L55

     FILE 'WPIX, MEDLINE, BIOSIS, EMBASE, PQSCITECH' ENTERED AT 08:32:42
     ON 08 JAN 2021
L65           23 DUP REM L64 L60 (14 DUPLICATES REMOVED)
                D L65 1-23

     FILE 'MEDLINE' ENTERED AT 08:33:20 ON 08 JAN 2021
L66        22959 SEA ABB=ON  PLU=ON  ("STREPTOCOCCUS PNEUMONIAE"/CT OR
                B3.353.750.737.872.550./CT OR B3.510.400.800.872.550./CT
                OR B3.510.550.737.872.550./CT)
L67          216 SEA ABB=ON  PLU=ON  L66 AND (23A OR 23B OR 35B)
L68       572648 SEA ABB=ON  PLU=ON  (POLYSACCHARIDES/CT OR D9.698./CT)
L69            8 SEA ABB=ON  PLU=ON  L67 AND (L68 OR D9./CT)
L70         3366 SEA ABB=ON  PLU=ON  L66 AND (L68 OR D9./CT)
L71          560 SEA ABB=ON  PLU=ON  L70 AND (CS OR BI)/CT
L72       300739 SEA ABB=ON  PLU=ON  (OLIGOPEPTIDES/CT OR D12.644.456./CT)
                E PEPTIDES+ALL/CT
L73      2752045 SEA ABB=ON  PLU=ON  (PEPTIDES/CT OR D12.644./CT)
L74          423 SEA ABB=ON  PLU=ON  L71 AND (D12.776./CT OR L72 OR L73)
L75          153 SEA ABB=ON  PLU=ON  L74 AND (CONJUGAT? OR ?LINK? OR
                COUPL?)
L76        50066 SEA ABB=ON  PLU=ON  (PHAGOCYTOSIS/CT OR G12.450.564.809./CT
                 OR G12.688./CT OR G4.417.350./CT OR G9.188.665./CT)
L77         4161 SEA ABB=ON  PLU=ON  ("OPSONIN PROTEINS"/CT OR D12.776.124.4
                86.485.114.767./CT OR D12.776.124.486.657./CT OR D12.776.12
                4.790.651.114.767./CT OR D12.776.377.715.548.114.767./CT)
L78            7 SEA ABB=ON  PLU=ON  L75 AND L76
L79            4 SEA ABB=ON  PLU=ON  L75 AND L77
L80           16 SEA ABB=ON  PLU=ON  L69 OR L78 OR L79
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                D QUE L69
                D QUE L78
                D QUE L79
                D L80 1-16

     FILE 'HCAPLUS, MEDLINE, BIOSIS, EMBASE, WPIX, JAPIO, PQSCITECH'
     ENTERED AT 08:42:16 ON 08 JAN 2021
L81          862 SEA ABB=ON  PLU=ON  "FORREST B"?/AU
L82         6550 SEA ABB=ON  PLU=ON  "LOVE J"?/AU
L83            4 SEA ABB=ON  PLU=ON  L81 AND L82
L84           11 SEA ABB=ON  PLU=ON  (L81 OR L82) AND L19
L85            2 SEA ABB=ON  PLU=ON  (L81 OR L82) AND L24
L86            5 SEA ABB=ON  PLU=ON  (L81 OR L82) AND L36
L87           11 SEA ABB=ON  PLU=ON  (L83 OR L84 OR L85 OR L86)
L88            4 DUP REM L87 (7 DUPLICATES REMOVED)
                D L88 1-4
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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

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /P.B/

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: /Padmavathi Baskar/	DATE CONSIDERED: 02/01/2021

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)				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	2	of	2	GROUP ART UNIT: 1645	EXAMINER: Not Yet Assigned

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /P.B/

		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:
/Padmavathi Baskar/	02/01/2021

[#] 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)	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 1	GROUP ART UNIT: 1645	EXAMINER: Padmavathi Baskar

U.S. PATENT DOCUMENTS

Examiner's Initials #	Cite No.	U.S. Patent Document		Name of Patentee or Applicant of Cited Document	Date of Publication or Issue of Cited Document MM-DD-YYYY
		Number	Kind Code		

FOREIGN PATENT DOCUMENTS

Examiner's Initials #	Cite No.	Foreign Patent Document			Name of Patentee or Applicant of Cited Document	Date of Publication of Cited Document MM-DD-YYYY	Translation (Y/N)
		Office/ Country	Number	Kind Code			

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)
		GUO et al., Genetic diversity of fluoroquinolone-nonsusceptible Streptococcus pneumoniae clinical isolates and the first identification of serotype 20B in China. Eur J Clin Microbiol Infect Dis. 2014 Mar;33(3):465-70. doi: 10.1007/s10096-013-1979-5. Epub 2013 Oct 4.	
		NUNES et al., The impact of private use of PCV7 in 2009 and 2010 on serotypes and antimicrobial resistance of Streptococcus pneumoniae carried by young children in Portugal: Comparison with data obtained since 1996 generating a 15-year study prior to PCV13 introduction. Vaccine. 2016 Mar 29;34(14):1648-56. doi: 10.1016/j.vaccine.2016.02.045. Epub 2016 Feb 23.	

[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.

Petitioner Merck Ex-1004, 1264

Electronic Patent Application Fee Transmittal

Application Number:	16089040
Filing Date:	27-Sep-2018
Title of Invention:	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF
First Named Inventor/Applicant Name:	Bruce D. FORREST
Filer:	Chelsea E. Witte-Garcia/Luke Brand
Attorney Docket Number:	P1143.70000US04

Filed as Small Entity

Filing Fees for U.S. National Stage under 35 USC 371

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
SUBMISSION- INFORMATION DISCLOSURE STMT	2806	1	130	130
		Total in USD ($)		**130**

Electronic Acknowledgement Receipt

EFS ID:	42727769
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:	23628
Filer:	Chelsea E. Witte-Garcia/Luke Brand
Filer Authorized By:	Chelsea E. Witte-Garcia
Attorney Docket Number:	P1143.70000US04
Receipt Date:	14-MAY-2021
Filing Date:	27-SEP-2018
Time Stamp:	15:24:03
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$130
RAM confirmation Number	E20215DF27170065
Deposit Account	232825
Authorized User	Luke Brand

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

37 CFR 1.17 (Patent application and reexamination processing fees)

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-CEW.pdf	26170 cde58c62d43e0956ff1a3899f43fdb4e5cb04883	no	1
Warnings:					
Information:					
2	Fee Worksheet (SB06)	P114370000US04-FEE-CEW.pdf	32711 1e1f0fe88d0ec81b3eee61fae9f16c71208f079d	no	1
Warnings:					
Information:					
3	Transmittal Letter	P114370000US04-IDSBODY-CEW.pdf	29549 ffe120f35b7ec19a4b879ba58ac1c27311c6a001	no	3
Warnings:					
Information:					
4	Non Patent Literature	Guo_et_al_Eur_J_Clin_Microbiol_Infec_Dis_2014_v33_p465.pdf	11162095 96d39f011d9f728bc00ac6ac869a6431226a112a	no	6
Warnings:					
Information:					
5	Non Patent Literature	Nunes_et_al_Vaccine_2016_v34_p1648.pdf	19399035 dede76ab14285ff6ad09ee58b2e93e655489edcf	no	9
Warnings:					
Information:					
6	Information Disclosure Statement (IDS) Form (SB08)	P114370000US04-IDS1449-CEW.pdf	76952 caca7b3baf2c09104dc6a9eaf7a7d6ab9a54669b	no	1
Warnings:					
Information:					

This is not an USPTO supplied IDS fillable form					
7	Fee Worksheet (SB06)	fee-info.pdf	30544 e0ea6766a88e39eb99ab559eb28a1b5659cd390b	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	30757056	

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.

DOCKET NO.: P1143.70000US04

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor:	Bruce D. Forrest
Application No.:	16/089,040
Confirmation No.:	8702
Filed:	September 27, 2018
For:	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF
Examiner:	Padmavathi Baskar
Art Unit:	1645

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: May 14, 2021 Electronic Signature for Luke B. Brand: /Luke B. Brand/

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

STATEMENT FILED PURSUANT TO THE DUTY OF DISCLOSURE UNDER 37 C.F.R. §§ 1.56, 1.97 AND 1.98

Commissioner:

Pursuant to the duty of disclosure under 37 C.F.R. §§ 1.56, 1.97 and 1.98, the undersigned requests consideration of this Information Disclosure Statement.

PART I: Compliance with 37 C.F.R. § 1.97

This Information Disclosure Statement has been filed after the mailing date of either a Final Action under 37 C.F.R. § 1.113 or a Notice of Allowance under 37 C.F.R. § 1.311 and is being filed on or before payment of an Issue Fee.

The undersigned hereby states, as specified in 37 C.F.R. § 1.97(e), that each item of information contained in this Information Disclosure Statement was first cited in any communication from a foreign patent office in a counterpart for the present Application not more than three months prior to the filing of this Statement. Therefore, please charge our credit card in the amount of $130.00 to cover the fee set forth in 37 C.F.R. § 1.17(p).

Petitioner Merck Ex-1004, 1270

PART II: Information Cited

The undersigned hereby makes of record in the present Application the information listed on the attached Form PTO-1449 (modified PTO/SB/08). The order of presentation of the references should not be construed as an indication of the importance of the references.

PART III: Remarks

Documents cited anywhere in the Information Disclosure Statement are enclosed unless otherwise indicated. It is respectfully requested that:

1. The Examiner consider completely the cited information, along with any other information, in reaching a determination concerning the patentability of the present claims;

2. The enclosed Form PTO-1449 (modified PTO/SB/08) be signed by the Examiner to evidence that the cited information has been fully considered by the United States Patent and Trademark Office during the examination of the present Application; and

3. The citations for the information be printed on any patent which issues from the present Application.

By submitting this Information Disclosure Statement, the undersigned makes no representation that a search has been performed, of the extent of any search performed, or that more relevant information does not exist.

By submitting this Information Disclosure Statement, the undersigned makes no representation that the information cited in the Statement is, or is considered to be, material to patentability as defined in 37 C.F.R. § 1.56(b).

By submitting this Information Disclosure Statement, the undersigned makes no representation that the information cited in the Statement is, or is considered to be, in fact, prior art as defined by 35 U.S.C. § 102.

Notwithstanding any statements by the undersigned, the Examiner is urged to form his or her own conclusion regarding the relevance of the cited information.

An early and favorable action is hereby requested.

The Director is hereby authorized to charge any deficiency in the fees due, or credit any overpayment, by the filing of this Information Disclosure Statement to our Deposit Account No. 23/2825, under Docket No. P1143.70000US04, from which the undersigned is authorized to draw.

Respectfully submitted,

Electronic signature: /Chelsea E. Witte-Garcia/
Chelsea E. Witte-Garcia, Ph.D.
Registration No.: 75,713
WOLF, GREENFIELD & SACKS, P.C.
600 Atlantic Avenue
Boston, Massachusetts 02210-2206
617.646.8000

Docket No.: P1143.70000US04
Date: May 14, 2021

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	1645
Examiner Name	Padmavathi Baskar
Attorney Docket Number	P1143.70000US04

ENCLOSURES (*Check all that apply*)

- [x] Fee Transmittal Form
  - [ ] Fee Attached
- [ ] Amendment/Reply
  - [ ] After Final
  - [ ] Affidavits/declaration(s)
- [ ] Extension of Time Request
- [ ] Express Abandonment Request
- [x] 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
- [ ] 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):

Form PTO-1449

Copies of cited references

Remarks

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	WOLF, GREENFIELD & SACKS, P.C.		
Signature	/Chelsea E. Witte-Garcia/		
Printed name	Chelsea E. Witte-Garcia, Ph.D.		
Date	May 14, 2021	Reg. No.	75,713

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: May 14, 2021 Electronic Signature for Luke B. Brand: /Luke B. Brand/

9238751.1

FEE TRANSMITTAL

Complete if known	
Application Number	16/089,040-Conf. #8702
Filing Date	September 27, 2018
First Named Inventor	Bruce D. Forrest
Examiner Name	Padmavathi Baskar
Art Unit	1645
Practitioner Docket No.	P1143.70000US04

[x] Applicant asserts small entity status. See 37 CFR 1.27.

[ ] Applicant certifies micro entity status. See 37 CFR 1.29. Form PTO/SB/15A or B or equivalent must either be enclosed or have been submitted previously.

TOTAL AMOUNT OF PAYMENT ($)130.00

METHOD OF PAYMENT (check all that apply)

[ ] Check [x] Credit Card [ ] Money Order [ ] None [ ] Other (please identify): _____

[x] Deposit Account Deposit Account Number: 23/2825 Deposit Account Name: Wolf, Greenfield & Sacks, P.C.

For the above-identified deposit account, the Director is hereby authorized to (check all that apply):

[ ] Charge fee(s) indicated below

[ ] Charge fee(s) indicated below, **except for the filing fee**

[x] Charge any additional fee(s) or underpayment of fee(s) under 37 CFR 1.16 and 1.17, **except for any excess claims fees or multiple dependent claim fee**

[ ] Charge any additional fee(s) or underpayment of fee(s) under 37 CFR 1.16 and 1.17

[x] Credit any overpayment of fee(s)

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

FEE CALCULATION

1. BASIC FILING, SEARCH, AND EXAMINATION FEES (U = undiscounted fee; S = small entity fee; M = micro entity fee)

	FILING FEES			SEARCH FEES			EXAMINATION FEES			
Application Type	**U ($)**	**S ($)**	**M ($)**	**U ($)**	**S ($)**	**M ($)**	**U ($)**	**S ($)**	**M ($)**	**Fees Paid ($)**
Utility	320	160*	80	700	350	175	800	400	200	
Design	220	110	55	160	80	40	640	320	160	
Plant	220	110	55	440	220	110	660	330	165	
Reissue	320	160	80	700	350	175	2,320	1,160	580	
Provisional	300	150	75	0	0	0	0	0	0	

* The $160 small entity status filing fee for a utility application is further reduced to $80 for a small entity status applicant who files the application via EFS-Web.

2. EXCESS CLAIM FEES

Fee Description	**Undiscounted Fee ($)**	**Small Entity Fee ($)**	**Micro Entity Fee ($)**
Each claim over 20 (including Reissues)	100	50	25
Each independent claim over 3 (including Reissues)	480	240	120
Multiple dependent claims	860	430	215

Total Claims	**Extra Claims**	**Fee ($)**	**Fee Paid ($)**
_____ - 20 or HP =	_____ x	_____ =	_____

HP = highest number of total claims paid for, if greater than 20.

Indep. Claims	**Extra Claims**	**Fee ($)**	**Fee Paid ($)**
_____ - 5 or HP =	_____ x	_____ =	_____

HP = highest number of independent claims paid for, if greater than 3.

Multiple Dependent Claims	
Fee ($)	**Fee Paid ($)**
_____	_____

3. APPLICATION SIZE FEE

If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is $420 ($210 for small entity) ($105 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).

Total Sheets	**Extra Sheets**	**Number of each additional 50 or fraction thereof**	**Fee ($)**	**Fee Paid ($)**
_____ - 100 =	_____ /50 =	_____ (round **up** to a whole number) x	_____ =	_____

4. OTHER FEE(S)

	Fees Paid ($)
Non-English specification, $130 fee (no small or micro entity discount)	
Non-electronic filing fee under 37 CFR 1.16(t) for a utility application, $400 fee ($200 small or micro entity)	
Other (e.g., late filing surcharge): 2806 Submission of an Information Disclosure Statement	130.00

SUBMITTED BY

Signature	/Chelsea E. Witte-Garcia/	Registration No. (Attorney/Agent)	75,713	Telephone	617.646.8000
Name (Print/Type)	Chelsea E. Witte-Garcia, Ph.D.			Date	May 14, 2021

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: May 14, 2021 Electronic Signature for Luke B. Brand: /Luke B. Brand/

9238750.1

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 NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/089,040	09/27/2018	Bruce D. FORREST	P1143.70000US04	8702

23628 7590 05/21/2021

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

EXAMINER
BASKAR, PADMAVATHI

ART UNIT	PAPER NUMBER
1645	

NOTIFICATION DATE	DELIVERY MODE
05/21/2021	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

Patents_eOfficeAction@WolfGreenfield.com
WGS_eOfficeAction@WolfGreenfield.com

Petitioner Merck Ex-1004, 1275

Notice of Allowability	Application No. 16/089,040	Applicant(s) FORREST et al.	
	Examiner Padmavathi Baskar	Art Unit 1645	AIA (FITF) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☑ This communication is responsive to 05/14/2021.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

3. ☑ The allowed claim(s) is/are See Continuation Sheet . As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see **http://www.uspto.gov/patents/init_events/pph/index.jsp** or send an inquiry to **PPHfeedback@uspto.gov.**

4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

   Certified copies:

   a) ☐ All b) ☐ Some *c) ☐ None of the:

   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____ .

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____ .

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☑ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date 05/14/2021.
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material _____.
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date. _____.
5. ☑ Examiner's Amendment/Comment
6. ☐ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

/Padmavathi Baskar/
Primary Examiner, Art Unit 1645

Petitioner Merck Ex-1004, 1276

Continuation of 3. The allowed claim(s) is/are: 67,74-75,78,81,97,106,109,112-114,154,256,297 and 299-303

DETAILED ACTION

Notice of Pre-AIA or AIA Status

1. The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

Information Disclosure Statement

2. The information disclosure statement (IDS) submitted on 05/14/2021 is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner and a signed copy of each is attached to this office action.

Conclusion

3. Claims 67, 74-75, 78, 81, 97, 106, 109, 297, 299-303, 112-114, 154 and 256 are allowed and are numbered as 1-19 respectively.

Correspondence

4. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Padmavathi Baskar whose telephone number is (571)272-0853. The examiner can normally be reached on Mon-Fri 9-5:30

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Nickol can be reached on 571-272-0835. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative

or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Respectfully,

/Padmavathi Baskar/
Primary Examiner, Art Unit 1645
Padmavathi Baskar, Ph.D. (Microbiology and Immunology)

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	16/089,040	FORREST et al.
	Examiner	**Art Unit**
	Padmavathi Baskar	1645

CPC						
Symbol					**Type**	**Version**
A61K	/	39	/	092	F	2013-01-01
A61K	/	47	/	646	I	2017-08-01
A61K	/	2039	/	55505	A	2013-01-01
A61K	/	2039	/	6037	A	2013-01-01
A61K	/	2039	/	70	A	2013-01-01

CPC Combination Sets							
Symbol					**Type**	**Set**	**Ranking**	**Version**
	/		/					

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	19	
/Padmavathi Baskar/ Primary Examiner, Art Unit 1645	18 May 2021	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	----------

U.S. Patent and Trademark Office — Part of Paper No.: 20210518

Petitioner Merck Ex-1004, 1280

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	16/089,040	FORREST et al.
	Examiner	**Art Unit**
	Padmavathi Baskar	1645

INTERNATIONAL CLASSIFICATION					
CLAIMED					
A61K39/09	/	39	/	09	
A61K47/64	/	47	/	64	

NON-CLAIMED					
A61K39/00	/	39	/	00	

US ORIGINAL CLASSIFICATION	
CLASS	**SUBCLASS**

CROSS REFERENCES(S)						
CLASS	**SUBCLASS (ONE SUBCLASS PER BLOCK)**					

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	19	
/Padmavathi Baskar/ Primary Examiner, Art Unit 1645	18 May 2021	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	----------

U.S. Patent and Trademark Office

Part of Paper No.: 20210518

Petitioner Merck Ex-1004, 1281

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	16/089,040	FORREST et al.
	Examiner	**Art Unit**
	Padmavathi Baskar	1645

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIMS

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
1	67	10	299	19	256										
2	74	11	300												
3	75	12	301												
4	78	13	302												
5	81	14	303												
6	97	15	112												
7	106	16	113												
8	109	17	114												
9	297	18	154												

NONE (Assistant Examiner)	(Date)	**Total Claims Allowed:** 19	
/Padmavathi Baskar/ Primary Examiner, Art Unit 1645 (Primary Examiner)	18 May 2021 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure ----------

U.S. Patent and Trademark Office

Part of Paper No.: 20210518

Petitioner Merck Ex-1004, 1282

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 1	GROUP ART UNIT: 1645	EXAMINER: Padmavathi Baskar

U.S. PATENT DOCUMENTS

Examiner's Initials #	Cite No.	U.S. Patent Document		Name of Patentee or Applicant of Cited Document	Date of Publication or Issue of Cited Document MM-DD-YYYY
		Number	Kind Code		

FOREIGN PATENT DOCUMENTS

Examiner's Initials #	Cite No.	Foreign Patent Document			Name of Patentee or Applicant of Cited Document	Date of Publication of Cited Document MM-DD-YYYY	Translation (Y/N)
		Office/ Country	Number	Kind Code			

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)
/P.B/		GUO et al., Genetic diversity of fluoroquinolone-nonsusceptible Streptococcus pneumoniae clinical isolates and the first identification of serotype 20B in China. Eur J Clin Microbiol Infect Dis. 2014 Mar;33(3):465-70. doi: 10.1007/s10096-013-1979-5. Epub 2013 Oct 4.	
/P.B/		NUNES et al., The impact of private use of PCV7 in 2009 and 2010 on serotypes and antimicrobial resistance of Streptococcus pneumoniae carried by young children in Portugal: Comparison with data obtained since 1996 generating a 15-year study prior to PCV13 introduction. Vaccine. 2016 Mar 29;34(14):1648-56. doi: 10.1016/j.vaccine.2016.02.045. Epub 2016 Feb 23.	

[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:
/Padmavathi Baskar/	05/18/2021

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.

Petitioner Merck Ex-1004, 1283

Docket No.: P1143.70000US04
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor:	Bruce D. Forrest
Application No.:	16/089,040
Confirmation No.:	8702
Filed:	September 27, 2018
For:	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF
Examiner:	Padmavathi Baskar
Art Unit:	1645

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: June 9, 2021 Electronic Signature for : /Trish McDonald/

AMENDMENT AFTER ALLOWANCE UNDER 37 C.F.R. § 1.312

Mail Stop Issue Fee
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Commissioner:

Prior to issuance of the patent from the above-referenced Application, Applicant respectfully requests entry of this Amendment under 37 C.F.R. § 1.312 and consideration of the following Remarks.

Amendments to the Specification begin on page 2 of this paper.

Amendments to the Claims are reflected in the Listing of Claims, which begins on page 3 of this paper.

Remarks begin on page 7 of this paper.

AMENDMENTS TO THE SPECIFICATION

Please replace paragraph **[0001]** with the following amended paragraph:

[0001] This application is a national stage filing under 35 U.S.C. § 371 of International PCT Application PCT/US2017/025621, filed March 31, 2017, which ~~is related to~~ claims priority under 35 U.S.C. § 119(e) to U.S. P[[p]]rovisional A[[a]]pplication ~~number~~, U.S.S.N. 62/316,555, filed on March 31, 2016,[[;]] which claims priority under 35 U.S.C. § 119(e) to U.S. P[[p]]rovisional A[[a]]pplication ~~number~~, U.S.S.N. 62/330,245, filed on May 2, 2016,[[;]] which claims priority under 35 U.S.C. § 119(e) to U.S. P[[p]]rovisional A[[a]]pplication ~~number~~, U.S.S.N. 62/372,263, filed on August 8, 2016,[[;]] which claims priority under 35 U.S.C. § 119(e) to U.S. P[[p]]rovisional A[[a]]pplication ~~number~~, U.S.S.N. 62/373,807, filed on August 11, 2016, each of which [[are]] is herein incorporated by reference ~~in their entirety~~.

Petitioner Merck Ex-1004, 1285

AMENDMENTS TO THE CLAIMS

This Listing of Claims will replace all prior versions, and listings, of claims in the Application:

Listing of Claims

1.-66. (Canceled)

67. (Previously Presented) A pharmaceutical composition comprising a plurality of at least two unique immunogenic saccharide-polypeptide conjugates, each comprising individually a capsular polysaccharides conjugated to a polypeptide, wherein each of the capsular polysaccharides is from a *Streptococcus pneumoniae* serotype selected from a group consisting of 23A, 23B, and 35B.

68.-73. (Canceled)

74. (Previously Presented) The pharmaceutical composition of claim 67, wherein at least one polypeptide comprises a mixture of polypeptides.

75. (Previously Presented) The pharmaceutical composition of claim 67, wherein at least one polypeptide of the plurality 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*, PspA, any fragment thereof, or any combination thereof.

76. - 77. (Canceled)

Petitioner Merck Ex-1004, 1286

78. (Previously Presented) The pharmaceutical composition of claim 67, further comprising an adjuvant; a chelating agent; a surfactant; an emulsifier; a buffering agent; a preservative; a salt; an anti-fungal compound; or a combination thereof.

79. - 80. (Canceled)

81. (Previously Presented) The pharmaceutical composition of claim 67, wherein the pharmaceutical composition is in the form of an intramuscularly injectable composition, intradermally injectable composition, subcutaneously injectable composition, or an intranasally administrable composition.

82. - 96. (Canceled)

97. (Currently Amended) The pharmaceutical composition of claim 67, wherein at least one of the ~~unique~~ immunogenic saccharide-polypeptide conjugates elicits an opsonophagocytic response.

98. - 105. (Canceled)

106. (Previously Presented) The pharmaceutical composition of claim 67, wherein the immunogenic saccharide-polypeptide conjugates are collectively present in an amount of at least 0.001%, by weight, based on the weight of the pharmaceutical composition.

107. - 108. (Canceled)

109. (Currently Amended) The pharmaceutical composition of claim 67, wherein each of the immunogenic saccharide-polypeptide conjugates comprise ~~comprises~~:

(i) the capsular polysaccharide at least partially embedded in the polypeptide,

(ii) the capsular polysaccharide chemically cross-linked to the polypeptide, and/or

Petitioner Merck Ex-1004, 1287

(iii) the capsular polysaccharide at least partially chemically cross-linked to the polypeptide.

110. - 111. (Canceled)

112. (Previously Presented) A method comprising administering to a subject a first composition, wherein the first composition is the pharmaceutical composition of claim 67.

113. (Previously Presented) The method of claim 112, 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 an immunogenic saccharide-polypeptide conjugate.

114. (Previously Presented) The method of claim 113, wherein the second composition comprises an immunogenic saccharide-polypeptide conjugate comprising a capsular polysaccharide from a serotype of *Streptococcus pneumoniae* conjugated to a second polypeptide.

115. - 153. (Canceled)

154. (Previously Presented) The method of claim 112, wherein the subject is a human.

155. - 255. (Canceled)

256. (Currently Amended) A method of making a composition comprising:

contacting the plurality of immunogenic saccharide-polypeptide conjugates of claim 67 with an excipient, an adjuvant, or any combination thereof.

257 - 296. (Canceled)

Petitioner Merck Ex-1004, 1288

297. (Previously Presented) The pharmaceutical composition of claim 67, wherein a toxin activity of at least one of the polypeptides of the plurality is at least partly mitigated.

298. (Canceled)

299. (Previously Presented) The pharmaceutical composition of claim 67, wherein each of the immunogenic saccharide-polypeptide conjugates is present in an amount of at least 0.001%, by weight, based on the weight of the pharmaceutical composition.

300. (Previously Presented) The method of claim 112, wherein the pharmaceutical composition is administered intramuscularly.

301. (Previously Presented) The pharmaceutical composition of claim 67, further comprising at least one additional immunogenic saccharide-polypeptide conjugate comprising a capsular polysaccharide from a unique *Streptococcus pneumoniae* serotype selected from the group consisting of 6C, 8, 9N, 10A, 11A, 12F, 15A, 15B, 15C, 16F, 20A, 20B, 22F, and 34.

302. (Currently Amended) The pharmaceutical composition of claim 67, wherein the plurality of unique immunogenic saccharide-polypeptide conjugate comprises individually capsular polysaccharides from a *Streptococcus pneumoniae* serotype comprising 6C, 9N, 15A, 15C, 16F, 23A, 23B, and 35B.

303. (Currently Amended) The pharmaceutical composition of claim 67, wherein the plurality of unique immunogenic saccharide-polypeptide comprises individually capsular polysaccharides from a *Streptococcus pneumoniae* serotype consisting of 6C, 9N, 15A, 15C, 16F, 23A, 23B, and 35B.

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REMARKS

Applicant requests entrance of this amendment to update the "Related Applications" section of this Application. Claims 97, 109, 256, 302, and 303 have been amended herewith for consistency. Applicant submits that no new matter is added to the Application by this amendment.

Applicant believes all fees due are being paid by credit card. However, if any additional fees are due, please charge them, or credit any overpayment, to our Deposit Account No. 23/2825, under Docket No. P1143.70000US04, from which the undersigned is authorized to draw.

Dated: June 9, 2021

Respectfully submitted,

By /Chelsea E. Witte-Garcia/
Chelsea E. Witte-Garcia, Ph.D.
Registration No.: 75,713
WOLF, GREENFIELD & SACKS, P.C.
600 Atlantic Avenue
Boston, Massachusetts 02210-2206
617.646.8000

Petitioner Merck Ex-1004, 1290

PART B–FEE(S) TRANSMITTAL

Complete and send this form, together with the applicable fee(s), by mail or fax, or via EFS-Web.

By mail, send to: Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

By fax, send to: (571) 273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

WOLF, GREENFIELD & SACKS, P.C.
600 Atlantic Avenue
Boston, Massachusetts 02210-2206

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of ~~Mailing or~~ Transmission

I hereby certify that this Fee(s) Transmittal is being ~~deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being~~ transmitted to the USPTO via EFS-Web ~~or by facsimile to (571) 273-2885,~~ on the date below.

Trish McDonald	(Typed or printed name)
/Trish McDonald/	(Signature)
VIA EFS-Web June 9, 2021	(Date)

APPLICATION NO	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO	CONFIRMATION NO
16/089,040	09/27/2018	Bruce D. Forrest	P1143.70000US04	8702

TITLE OF INVENTION: SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF

APPLN: TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	$600.00	$0.00	$0.00	$600.00	06/10/2021

EXAMINER	ART UNIT	CLASS-SUBCLASS
Baskar, P.	1645	424-244100

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363)

☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-09 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,

(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1. Wolf, Greenfield & Sacks, P.C.
2.
3.

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE: Pogona, LLC

(B) RESIDENCE: (CITY and STATE or COUNTRY): South Nyack, New York

Please check the appropriate assignee category or categories (will not be printed on the patent): ☐ Individual ☒ Corporation or other private group entity ☐ Government

4a. Fees Submitted: ☒ Issue Fee ☐ Publication Fee (if required) ☐ Advance Order - # of Copies _____

4b. Method of Payment (*Please first reapply any previously paid fee shown above*):

☒ Electronic Payment via EFS-Web ☐ Enclosed check ☐ Non-electronic payment by credit card (Attach form PTO-2038)

☒ The Director is hereby authorized to charge ~~the required fee(s),~~ any deficiency, or credit any overpayment to Deposit Account No. 23/2825

5. Change of Entity Status (from status indicated above)

☐ Applicant certifying micro entity status. See 37 CFR 1.29. NOTE: Absent a valid Certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

☐ Applicant asserting small entity status. See 37 CFR 1.27. NOTE: If the application was previously under micro entity status, checking this box will be taken as a notification of loss of entitlement to micro entity status.

☐ Applicant changing to regular undiscounted fee status. NOTE: Checking this box will be taken as a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature /Chelsea E. Witte-Garcia/ Date June 9, 2021

Typed or printed name Chelsea E. Witte-Garcia, Ph.D. Registration No. 75,713

9304049.1

Electronic Patent Application Fee Transmittal

Application Number:	16089040
Filing Date:	27-Sep-2018
Title of Invention:	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF
First Named Inventor/Applicant Name:	Bruce D. FORREST
Filer:	Chelsea E. Witte-Garcia/Patricia McDonald
Attorney Docket Number:	P1143.70000US04

Filed as Small Entity

Filing Fees for U.S. National Stage under 35 USC 371

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
UTILITY APPL ISSUE FEE	2501	1	600	600

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	**600**

Electronic Acknowledgement Receipt

EFS ID:	42945494
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:	23628
Filer:	Chelsea E. Witte-Garcia/Patricia McDonald
Filer Authorized By:	Chelsea E. Witte-Garcia
Attorney Docket Number:	P1143.70000US04
Receipt Date:	09-JUN-2021
Filing Date:	27-SEP-2018
Time Stamp:	17:52:34
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$600
RAM confirmation Number	E202169H52541000
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

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-CEW.pdf	26487 cb3c14e778aef2e0031f37ece698ab3724361983	no	1
Warnings:					
Information:					
2		P114370000US04-312AMN-CEW.pdf	45083 e4559d010b25b0877256487fcbef8a5990ea9f1f	yes	7

Multipart Description/PDF files in .zip description

Document Description	Start	End
Amendment after Notice of Allowance (Rule 312)	1	1
Specification	2	2
Claims	3	6
Applicant Arguments/Remarks Made in an Amendment	7	7

Warnings:					
Information:					
3	Issue Fee Payment (PTO-85B)	P114370000US04-ISSFEE-CEW.pdf	41320 72484211177716d6ec4cf856b1cbed33e5bbef09	no	1
Warnings:					
Information:					
4	Fee Worksheet (SB06)	fee-info.pdf	30752 e3dd8780f55867d7138242c2f2fc9104028ac31d	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	143642		

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.

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	1645
Examiner Name	Padmavathi Baskar
Attorney Docket Number	P1143.70000US04

ENCLOSURES (*Check all that apply*)

- [ ] Fee Transmittal Form
  - [ ] Fee Attached
- [x] 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
- [ ] 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):

  Fee Transmittal - Part B (PTOL-85)

Remarks

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	WOLF, GREENFIELD & SACKS, P.C.		
Signature	/Chelsea E. Witte-Garcia/		
Printed name	Chelsea E. Witte-Garcia, Ph.D.		
Date	June 9, 2021	Reg. No.	75,713

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: June 9, 2021 Electronic Signature for : /Trish McDonald/

9304052.1

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/089,040	09/27/2018	Bruce D. FORREST	P1143.70000US04	8702

23628 7590 06/17/2021

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

EXAMINER
BASKAR, PADMAVATHI

ART UNIT	PAPER NUMBER
1645	

NOTIFICATION DATE	DELIVERY MODE
06/17/2021	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

Patents_eOfficeAction@WolfGreenfield.com
WGS_eOfficeAction@WolfGreenfield.com

Petitioner Merck Ex-1004, 1298

Response to Rule 312 Communication	Application No.	Applicant(s)	
	16/089,040	FORREST et al.	
	Examiner	Art Unit	AIA (FITF) Status
	Padmavathi Baskar	1645	Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

1. ☑ The amendment filed on 09 June 2021 under 37 CFR 1.312 has been considered, and has been:
   a) ☑ entered.
   b) ☐ entered as directed to matters of form not affecting the scope of the invention.
   c) ☐ disapproved because the amendment was filed after the payment of the issue fee.
      Any amendment filed after the date the issue fee is paid must be accompanied by a petition under 37 CFR 1.313(c)(1) and the required fee to withdraw the application from issue.
   d) ☐ disapproved. See explanation below.
   e) ☐ entered in part. See explanation below.

/Padmavathi Baskar/
Primary Examiner, Art Unit 1645

Petitioner Merck Ex-1004, 1299

OK TO ENTER: /P.B/

Docket No.: P1143.70000US04
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor:	Bruce D. Forrest
Application No.:	16/089,040
Confirmation No.:	8702
Filed:	September 27, 2018
For:	SACCHARIDE-POLYPEPTIDE CONJUGATE COMPOSITIONS AND METHODS OF USE THEREOF
Examiner:	Padmavathi Baskar
Art Unit:	1645

Certificate of Electronic Filing under 37 C.F.R. § 1.8
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Dated: June 9, 2021 Electronic Signature for : /Trish McDonald/

AMENDMENT AFTER ALLOWANCE UNDER 37 C.F.R. § 1.312

Mail Stop Issue Fee
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Commissioner:

Prior to issuance of the patent from the above-referenced Application, Applicant respectfully requests entry of this Amendment under 37 C.F.R. § 1.312 and consideration of the following Remarks.

Amendments to the Specification begin on page 2 of this paper.

Amendments to the Claims are reflected in the Listing of Claims, which begins on page 3 of this paper.

Remarks begin on page 7 of this paper.

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	16/089,040	FORREST et al.
	Examiner	**Art Unit**
	Padmavathi Baskar	1645

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIMS

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
1	67	10	299	19	256										
2	74	~~11~~ 15	300												
3	75	12	301												
4	78	13	302												
5	81	14	303												
6	97	~~15~~ 11	112												
7	106	16	113												
8	109	17	114												
9	297	18	154												

Change(s) applied
to document,
/J.H./
3/23/2021

NONE (Assistant Examiner)	(Date)	**Total Claims Allowed:** 19	
/Padmavathi Baskar/ Primary Examiner, Art Unit 1645 (Primary Examiner)	04 March 2021 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure ----------

U.S. Patent and Trademark Office — Part of Paper No.: 20210304

Petitioner Merck Ex-1004, 1301

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APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/089,040	07/13/2021	11058757	P1143.70000US04	8702

23628 7590 06/23/2021

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

ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment is 104 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Data Management (ODM) at (571)-272-4200.

APPLICANT(s) (Please see PAIR WEB site http://pair.uspto.gov for additional applicants):

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

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Petitioner Merck Ex-1004, 1302