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Señor
SUPERINTENDENTE DE INDUSTRIA Y COME
E. S.

Re: P1.-**GLAXOSMITHKLINE BIOLOGICALS S.A.**-Solicitud de Patente de Invención en Fase Nacional PCT para "**COMPOSICIÓN DE VACUNA PARA LA PREVENCIÓN DE INFECCIÓN POR Bordetella pertussis, Clostridium tetani, & Corynebacterium diphteriae**".-Clase **A61K 39/295**.-Expediente número **03-088.387A-**

1. Yo, **ALICIA LLOREDA RICAURTE**, mayor, vecina de esta ciudad, abogada titulada e identificada con la cédula de ciudadanía número 39.690.713 de Usaquén, Representante Legal en Bogotá, para todos los asuntos relacionados con la Propiedad Industrial de la sociedad **GLAXOSMITHKLINE BIOLOGICALS S.A.-** organizada y existente de conformidad con las leyes de Bélgica, domiciliada en Rixensart, Bélgica, atentamente manifiesto a usted que interpongo **RECURSO DE REPOSICIÓN** contra la Resolución Número **63149**, dictada por el señor Superintendente de Industria y Comercio el 17 de Noviembre de 2010 y notificada por Edicto fijado el 26 de Noviembre del mismo año, en cuanto hace a la negación del privilegio de patente de invención para la solicitud en referencia.

2. FUNDAMENTO MI RECURSO EN LOS SIGUIENTES HECHOS Y ARGUMENTOS TÉCNICOS Y JURÍDICOS

(1) Por medio de la Resolución Número **63149** se ha negado el privilegio de patente solicitado para la invención de la referencia, con base en una supuesta falta de Nivel Inventivo de la solicitud teniendo en cuenta la existencia de los documentos ***Pines, E et al Vaccine, vol. 17, 1650-1656, 1999 (D1)***, ***Choo S, et al Journal of infectious Diseases, vol. 182 (4); 1260-1263 (D2)***, ***Obaro, et al. Pediatric infectious Disease journal, vol. 19, 463-469*** y ***WO93/24148 (D4)***.

(2) De la lectura de la referida Resolución Número **63149** es evidente el análisis erróneo que hace ese Despacho del arte previo citado y la falta de consideración con respecto tanto del aporte que brinda la presente invención como de las características particulares que esta posee.

(3) Así mismo, de la lectura de la Resolución impugnada, es también claro que ese Despacho no repara en los argumentos esgrimidos en mi memorial de 16 de Septiembre de 2010, los cuales establecieron de forma incontrovertible el nivel inventivo que conlleva la solicitud denegada frente al estado del arte más próximo, y por lo tanto ese Despacho afirma que "*un técnico normalmente versado en la materia a partir de la*

información divulgada en D1 y D2 o D1 y D3 o D4 y D2 o D4 y D3, habría podido desarrollar la invención tal y como está planteada en la solicitud".

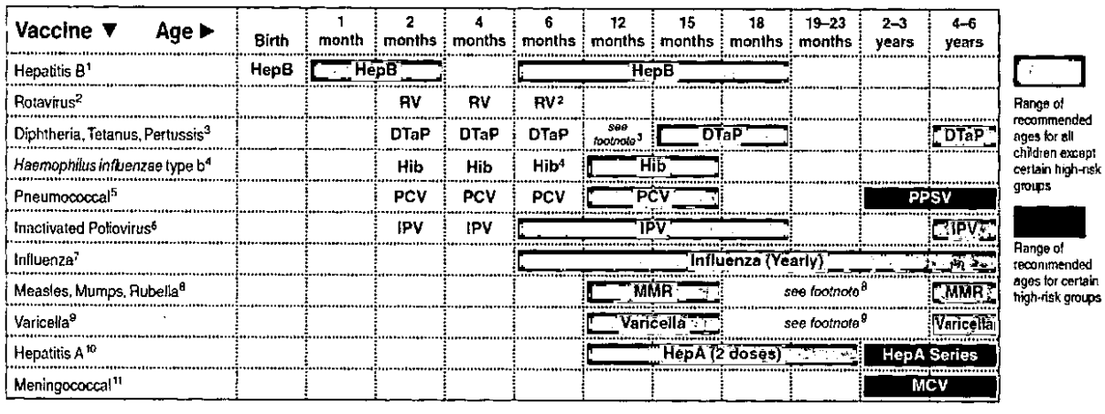
Sobre este particular, me permito dirigir la atención de ese Despacho a los siguientes argumentos técnicos, por medio de los cuales se puede afirmar sin lugar a dudas que la materia revelada en la invención denegada, es merecedora del privilegio de patente solicitado ya que, contrario a la posición de esa Oficina, dicha materia sí cumple con el requisito de nivel inventivo tal como es exigido en el Artículo 18 de la Decisión 486 de 2000, como me permito demostrar a continuación, siendo también dichos argumentos prueba de la violación del mencionado Artículo por parte de la SIC al negar el privilegio de patente solicitado.

(4) **La solicitud denegada brinda un aporte significativo al estado de la técnica que no es evidente a partir del arte previo.**

En primera instancia, me permito resaltar que la solicitud denegada proporciona una vacuna que comprende dos composiciones inmunogénicas multivalentes, la cual no es de ninguna manera revelada ni sugerida por el arte previo, y brinda una respuesta inmune satisfactoria para todos y cada uno de los antígenos presentes, evitando posibles interferencias entre dichos antígenos tanto intra como inter composiciones (es decir entre antígenos que se encuentran en la misma o en distintas composiciones). Así mismo, la cantidad particular del antígeno DT presente en la vacuna produce un aumento significativo en la respuesta inmunológica frente a todos los serotipos neumocócicos.

En este sentido, la invención reconoce por primera vez un problema que no había sido de ninguna manera planteado en el arte previo: **la posible existencia de interferencia entre antígenos de vacunas que son coadministradas**, en particular la interferencia de las vacunas neumocócicas con vacunas DTP-HepB.

Esto es de fundamental importancia en especial si se tiene en consideración que dentro de los primeros 18 meses de vida se requiere suministrar un gran número de antígenos al infante, y muchas veces los esquemas de vacunación incluyen la administración de estos antígenos en la misma etapa de crecimiento[1]:



[1] Recommended Immunization Schedule for Persons Aged 0 Through 6 Years—United Status, 2010, Department of Health and Human Services, Centers for Disease Control and Prevention, http://www.cdc.gov/vaccines/recs/schedules/downloads/child/2010/10_0-6yrs-schedule-pr.pdf

De esta forma, dado que no existen vacunas que incluyan en una sola composición todos los antígenos recomendados, es necesario administrar concomitantemente más de una vacuna para cubrir las necesidades de inmunización en cada etapa del crecimiento del infante, por lo tanto, es de vital importancia reconocer que puede existir interferencia cuando se co-administran dos vacunas, lo que conllevaría a que la respuesta inmune obtenida no será óptima.

Es por esto que la solución proporcionada por la presente solicitud, la cual revela dos composiciones multivalentes que, tal como han sido formuladas, permiten su administración concomitante produciendo una respuesta inmune satisfactoria para todos los antígenos presentes en ambas composiciones, se constituye en un aporte significativo al estado del arte, que no podría ser de ninguna manera deducido por el arte previo, toda vez que este de ninguna manera se había ocupado del problema de la interferencia entre vacunas.

Así, la invención denegada proporciona una vacuna que comprende dos composiciones separadas: la primera que incluye antígenos de pertussis, toxoide tetánico, toxoide diftérico, hepatitis B y virus de la polio, y la segunda que incluye conjugados de una proteína portadora y un poli u oligo sacárido de *Streptococcus pneumoniae*; en las cuales el contenido de DT en la primera composición es de 60-120 µg y en la segunda composición están presentes uno o más polisacáridos u oligosacáridos conjugados con DT y/o CRM197. Dichas composiciones, administradas concomitantemente elicitan una respuesta inmune satisfactoria para todos y cada uno de los antígenos presentes, brindando también un aumento significativo en la respuesta frente a los antígenos neumocócicos.

Sumado a lo anterior, el solicitante ha encontrado de manera sorprendente que la cantidad particular del antígeno DT que proporciona la vacuna de la presente invención, mejora los títulos de anticuerpo del polisacárido conjugado con DT, mientras que se reduce la reactogenicidad del DT usado como portador. Así mismo, de forma sorprendente se ha encontrado que si bien el contenido particular de DT es alto en el primer contenedor, no es lo suficientemente elevado como para inducir efectos de interferencia inmune de DT o supresión del portador, y por lo tanto se brinda una respuesta inmune satisfactoria.

Este aumento en la respuesta inmunológica elicitada por la vacuna de objeto de la solicitud denegada con respecto a aquellas del estado del arte que poseen un menor contenido de DT es evidente a partir de los resultados reportados en los documentos Olivier, et al, *Vaccine*, 26, 3142-3152, 2008[2] (A1) y Knuf, M. et al, *Vaccine*, 24, 4727-

[2] Olivier, et al, *"Immunogenicity, reactogenicity, and safety of a seven-valent pneumococcal conjugate vaccine (PCV7) concurrently administered with a fully liquid DTPa-IPV-Hib combination vaccine in health infants"*, Vaccine, 26, 3142-3152, **2008**.

4736, 2006[3] (A2), los cuales fueron discutidos en mis memoriales de Febrero 24 de 2010 y Septiembre 16 de 2010.

Dichos resultados muestran cómo la respuesta inmune frente a todos los serotipos neumocócicos es superior cuando se emplea una composición de vacuna que contiene una cantidad mayor de DT, como es el caso de la composición empleada en el documento Knuf, A2, mientras que dicha respuesta disminuye cuando esta cantidad de DT es menor como lo muestra la vacuna empleada por Olivier, A1.

Para comprobar este hecho me permito referirme a los resultados de porcentaje de individuos con valores de anticuerpos neumocócicos superiores o iguales a 0.15 µg/mL y 0.5 µg/mL para cada uno de dichos documentos:

Table 2 Percent of subjects (95% C.I.) achieving defined pneumococcal antibody levels of ≥0.15 µg/mL and ≥0.50 µg/mL post-dose 3 and post-dose 4 (test group, PCV7 + DTPa–IPV–HBV–Hib, ITT)

Serotypes (g/µg mL)	Post-dose 3 $n = 125$	Post-dose 4 $n = 116$
4		
≥0.15	99.2 (97.64–100.76)	98.28 (95.91–100.0)
≥0.50	96.8 (93.71–99.89)	94.83 (90.8–98.86)
6B		
≥0.15	92.8 (88.27–97.33)	98.28 (95.91–100.0)
≥0.50	79.2 (72.08–86.32)	95.69 (91.99–99.93)
9V		
≥0.15	99.2 (97.64–100.76)	100.0 (100.0–100.0)
≥0.50	95.2 (91.45–98.95)	93.7 (89.63–98.30)
14		
≥0.15	100 (100–100)	99.14 (97.46–100.0)
≥0.50	98.4 (96.2–100.6)	99.14 (97.46–100.0)
18C		
≥0.15	100 (100–100)	99.14 (97.46–100.0)
≥0.50	95.2 (91.45–98.95)	97.97 (89.63–98.3)
18C		
≥0.15	99.2 (97.64–100.76)	98.28 (95.91–100.0)
≥0.50	96.8 (93.71–99.89)	94.83 (90.8–98.86)
23F		
≥0.15	98.8 (88.27–97.33)	93.97 (89.63–98.30)
≥0.50	82.4 (75.72–89.08)	89.66 (84.11–95.20)

Table 4
Proportion of subjects achieving pneumococcal antibody concentrations of ≥0.15 and ≥0.50 µg/mL (%; 95% CI) after dose 3 and after booster dose (dose 4) of pneumococcal conjugate vaccine (PCV7 group)

Pneumococcal serotype	Post-dose 3 ($N = 115$)		Post-booster dose ($N = 106$)	
	≥0.15 µg/mL	≥0.50 µg/mL	≥0.15 µg/mL	≥0.50 µg/mL
4	100.0 (100.0–100.0)	99.13 (97.43–100.83)	100.0 (100.0–100.0)	100.0 (100.0–100.0)
6B	100.0 (100.0–100.0)	96.52 (93.17–99.87)	100.0 (100.0–100.0)	98.11 (95.52–100.0)
9V	98.25 (95.84–100.66)	97.37 (94.43–100.31)	100.0 (100.0–100.0)	98.11 (95.52–100.0)
14	100.0 (100.0–100.0)	97.39 (94.48–100.3)	100.0 (100.0–100.0)	99.06 (97.22–100.0)
18C	100.0 (100.0–100.0)	97.35 (94.38–100.31)	100.0 (100.0–100.0)	99.06 (97.22–100.0)
19F	100.0 (100.0–100.0)	98.26 (95.87–100.65)	100.0 (100.0–100.0)	98.11 (95.52–100.0)
23F	98.25 (95.84–100.66)	92.11 (87.16–97.06)	97.17 (94.01–100.0)	96.23 (92.6–99.85)

A partir de estos resultados es evidente la respuesta mejorada de la composición de vacuna empleada en el documento de Knuf, A2, la cual posee una cantidad de DT que está cubierta por el alcance de la presente invención.

En relación con lo anterior me permito señalar que **no existe ninguna indicación en el arte previo que permita suponer que si se formulan específicamente estos antígenos en ciertas cantidades particulares, y se administran**

[3] Knuf, M.et al *"Immunogenicity, reactogenicity, and safety of a 7-valent pneumococcal conjugate vaccine (PCV7) concurrently administered with a DTPa-HBV-IPV/Hib combination vaccine in healthy infants"*, Vaccine, 24, 4727-4736, **2006**.

2

concomitantemente con una composición que tiene poli u oligo sacáridos conjugados con DT y/o CRM197 se tendría una respuesta superior frente a cualquier serotipo neumocócico, por lo que el aporte realizado por la presente invención no es de ninguna manera evidente a la luz de dicho arte previo.

(5) **Los documentos citados no indican o sugieren el problema abordado por la presente invención**.

De otra parte, los documentos citados en la Resolución impugnada como arte previo relevante para el nivel inventivo de la solicitud denegada, si bien se refieren a vacunas multivalentes, son totalmente silenciosos respecto a una posible existencia de interferencia entre antígenos de diferentes vacunas que han sido co-administradas.

Así, el documento D1 hace referencia a vacunas combinadas que contienen antígenos acelulares de pertussis, resaltando que es un reto obtener formulaciones apropiadas de varios antígenos, toda vez que se requiere evaluar las reacciones potenciales aditivas o adversas inesperadas, al igual que la posible interferencia entre antígenos y el impacto negativo que pueden tener los demás componentes, **pero sin evaluar de ninguna manera las posibles interacciones entre antígenos que no hacen parte de la misma vacuna y que son administrados concomitantemente al paciente**.

Ahora bien, contrario a lo afirmado por la Resolución Número **63149**, las revelaciones de D1 no conducirían a la persona versada en la materia a la presente invención, sino que al contrario la alejarían de esta, dado que dicho documento claramente revela composiciones con cantidades de DT menores a las cobijadas por el alcance de la presente invención y no hace referencia a la administración concomitante de vacunas DTPa-HbIPV con una composición de poli u oligo sacáridos meningocócicos en la cual estos estén conjugados a DT y/o CMR 197.

Es decir, las enseñanzas de D1 no permiten deducir qué comportamiento se esperaría al realizar dicha coadministración particular de vacunas, ni sugieren que una cantidad relativamente alta de DT sería deseable para obtener una respuesta inmunológica superior.

Así mismo, el documento D2 no hace de ninguna manera referencia a la cantidad de DT requerida en vacunas de DTPaHBIPV acelular para coadministración con vacunas de conjugados de neumococo que se requeriría para de mejorar la respuesta inmune contra los antígenos de polisacárido neumocócico, sino que revela vacunas completamente diferentes (coadministrando en su lugar vacunas de DTPw de pertussis de célula completa muerta).

Sumado a lo anterior, el mencionado documento D2 es completamente silencioso respecto de la cantidad de DT empleada en las formulaciones y no proporciona ninguna

indicación que conduzca hacia el diseño de una vacuna de DTPaHBIPV con un alto contenido de DT con el propósito de tener respuestas de anticuerpo a polisacárido neumocócico mejoradas.

Por su parte, el documento D3 también se encuentra dirigido a la coadministración de una vacuna de Pw de célula completa muerta (DTPw) con una vacuna de neumococo, por lo tanto, es evidente que las enseñanzas de este documento no sugerirían a una persona versada en la materia que el tener una dosis relativamente alta de DT en este tipo de vacunas mejoraría la respuesta inmune elicitada contra los antígenos meningocócicos.

Finalmente, el documento D4 no se refiere de ninguna manera a la posibilidad de que exista una interferencia inter-vacunas, sino que está encaminado a encontrar la mejor manera de formular composiciones que contengan un antígeno de superficie de hepatitis B, y para ello señala como efectivo el uso del fosfato de aluminio como adyuvante.

En relación con lo anterior, la Resolución impugnada señala que "*se informa al solicitante que cuando se evalúa el nivel inventivo, por el método problema-solución, por ejemplo, en una de sus etapas se define el problema técnico a solucionar con base al estado de la técnica más cercano. En otras palabras, se valora el aporte que hace la invención para solucionar un problema, pero no para identificar el mismo, puesto que esto no corresponde a un avance per se del estado de la técnica*".

A este respecto me permito señalar que si bien el sólo hecho de identificar un problema técnico no puede ser considerado como un aporte brindado por una invención, la identificación de este problema si ayuda a establecer el estado del arte más próximo. Es decir, es necesario situarse en el momento en el cual se desarrolló la invención y evaluar qué revelaciones del arte previo podrían conducir a una persona versada en la materia a resolver un problema particular de una forma y no de otra.

Sin embargo, **si el arte previo ni siquiera resuelve el mismo problema que la persona versada en la materia está interesada en solucionar, por qué tomaría como punto de partida estas enseñanzas?, por qué no buscaría dentro del campo técnico alguna revelación que le brinde una expectativa razonable de éxito si decide realizar una modificación particular de lo ya existente?**

En este sentido es pertinente resaltar que dentro del ámbito de las patentes es un hecho aceptado que la evaluación de nivel inventivo de una solicitud no está dirigida a que **podría** haber hecho la persona versada en la materia, sino a lo que **realmente habría hecho** partiendo del arte previo. De esta forma, dicha persona versada en la materia debería encontrar dentro del estado del arte una indicación clara que la condujera a pensar que al realizar una cierta modificación a lo ya revelado se tendría realmente una

3

solución mejorada al problema que se desea solucionar, para que dicho arte previo restara altura inventiva al nuevo desarrollo.

Ahora bien, para el caso que nos compete, me permito resaltar que el solicitante de ninguna manera ha señalado que el aporte realizado por la presente invención sea únicamente la identificación del problema de la interferencia inter-vacuna, sino que este aporte se constituye en que dicho problema es resulto de manera sorprendente gracias a la vacuna objeto de la presente invención. Es decir, **el aporte realizado por la invención se constituye en una nueva composición de vacuna que de manera sorprendente elicita respuestas inmunológicas superiores comparadas con aquellas producidas por las composiciones ya existentes.**

En conexión con lo anterior la Resolución Número **63149** señala que dentro del estado de la técnica *"no hay evidencia de la interferencia que se puede dar entre los antígenos arriba mencionados tal y como se observa en el documento D2, por ejemplo, en el que se combinan composiciones DTP+7VPnC/Hib sin evidenciar interferencia. Incluso, los mismos documentos (A1 y A2) aportados por el solicitante en su respuesta al Oficio No. 11617, muestran que las vacunas comerciales DTPa-HBV-IPV/Hib, tales como Infanrix (GlaxoSmithKline) o Hexavac (Aventis Pasteur(, pueden ser coadministradas con la vacuna 7-valente combinada con Streptococcus pneumoniae conocida como Prevnar (Wyeth) y obtener protección inmunológica adecuada para los diferentes antígenos (ver resumen de A1 y A2)."*

Sobre este particular me permito señalar que si bien el documento D2 muestra la administración concomitante de dos composiciones de vacuna estas no contienen exactamente los mismos antígenos que la revelada por la presente invención, toda vez que emplea antígenos de pertussis de célula completa "wP" y no componentes acelulares como los que hacen parte de la vacuna de la presente solicitud.

Así mismo, con esta afirmación la Resolución impugnada está ratificando lo argumentado por el solicitante: que el arte previo no indica ni sugiere el problema que resuelve la presente invención. En este sentido me permito resaltar **que aún cuando el documento D2 no señala una posible interferencia, no significa que esta no se presente ni que no se obtenga una respuesta inmunológica superior si se emplea la vacuna objeto de la solicitud denegada.**

De esta forma, contrario a lo señalado por la Resolución Número **63149**, el documento de Knuf, A2, es una clara prueba de la respuesta inmunológica mejorada, con respecto a la obtenida en el documento de Olivier, A1, que es obtenida al emplear específicamente las cantidades de antígenos presentes en la vacuna de la invención, por lo que es evidente la ventaja técnica que posee dicha vacuna de la solicitud denegada.

En este sentido la Resolución impugnada afirma que los resultados obtenidos en el documento de Knuf, A2, no podrían ser extrapolados a la presente invención, dado que dentro de la descripción de la solicitud el ejemplo 5 muestra respuestas inmunológicas diferentes.

En conexión con lo anterior me permito llamar la atención a ese Despacho a que **los resultados del mencionado ejemplo 5 de la página 31 de la descripción de la solicitud no pueden ser comparados directamente con aquellos obtenidos por Knuf, A2, toda vez que no corresponden exactamente a las mismas composiciones**.

De esta forma, un análisis detallado de dicho ejemplo 5 permite evidenciar que este hace pruebas clínicas de **la vacuna del ejemplo 4**, es decir, una vacuna 11 valente neumocócica conjugada en PD, en la cual se reconstituyó un polisacárido capsular de H. *influenzae* tipo B, es decir, dicha vacuna **no contiene un segundo contenedor que comprenda un poli u oligo sacárido neumocócico conjugado específicamente con DT y/o CRM197**.

Consecuentemente, si bien muchos de los antígenos presentes en esta composición de vacuna son similares a los empleados en el documento A2, las dos composiciones no son idénticas porque no contienen la misma cantidad de DT y la composición del ejemplo 5 no comprende un poli u oligo sacárido neumocócido conjugado con DT y/o CRM 197.

En vista de lo anterior, los resultados reportados por Knuf en A2 tienen una conexión directa con la materia revelada en la descripción, y muestran de forma contundente la respuesta inmunológica mejorada que es elicitada por la vacuna de la solicitud denegada.

Así mismo, dichos resultados son sorprendentes toda vez que ninguno de los documentos citados sugiere que la administración concomitante de dos composiciones que contengan específicamente los antígenos y las cantidades de estos presentes en la vacuna de la invención podría llegar a elicitar una respuesta inmune superior.

(6) **La combinación de los documentos citados no conduce de forma evidente a la vacuna reclamada por la solicitud denegada**.

Ahora bien, aún cuando los documentos señalados no se ocupan de ninguna manera del problema resuelto por la presente invención, y por lo tanto no sería evidente para la persona versada en la materia tomar como punto de partida sus enseñanzas, si dicha persona versada en la materia decidiera realizar la combinación de las revelaciones de estos documentos no obtendría de forma obvia la solicitud denegada.

Es así como si se combinara D1 y D2 la persona versada en la materia de ninguna manera podría fijar el valor particular de DT presente en la primera composición de la vacuna de la invención, toda vez que el documento D1 revela composiciones con valores bajos de este antígeno y D2 no se refiere a la cantidad de DT empleada.

De manera similar, al combinar D1 y D3 la mencionada persona versada en la materia no podría fijar el contenido específico de DT, ni mucho menos predecir que aún cuando este sea relativamente alto no presentará reacciones adversas.

Así mismo, la combinación de D4 con cualquier otro documento no conduciría indefectiblemente a la vacuna reivindicada, toda vez que dicho documento está específicamente dirigido a buscar una formulación mejorada del antígeno de superficie de hepatitis B, empleando fosfato de aluminio y no se refiere de ninguna manera a una cantidad específica de antígeno DT y mucho menos a una posible ventaja de tener una cantidad relativamente alta de dicho antígeno.

Consecuentemente, la combinación de los documentos citados de ninguna manera permite la obtención de la composición de vacuna particular que es objeto de la invención denegada.

(7) **Dado que la combinación de los documentos citados no produce de forma evidente la invención denegada, esta se ajusta totalmente a lo preceptuado por el Artículo 18 de la Decisión Andina 486 de 2000**.

En virtud de los hechos discutidos anteriormente, se ha establecido que la persona versada en la materia partiendo de las enseñanzas de D1, D2, D3 y D4 por sí mismos o en cualquiera de sus combinaciones, no podría de ninguna manera obtener específicamente una vacuna que comprenda dos composiciones multivalentes, una DTPaHepB-virus de la polio y otra neumocócica, específicamente donde la cantidad de DT en la primera composición sea 60-120 µg, y el en la segunda composición comprenda poli u oligo sacáridos neumocócicos conjugados con DT y/o CMR197, buscando obtener una respuesta inmune satisfactoria para todos los antígenos suministrados.

Dentro de este contexto me permito referirme al señalamiento que hace el Tribunal de Justicia de la Comunidad Andina con respecto a las definiciones de "obvio" y "evidente" en la evaluación del Nivel Inventivo de una solicitud de patente[4]:

"Resulta pertinente, a los efectos del artículo 4 de la Decisión 344, definir qué se entiende por "obvio" y por "evidente" a fin de determinar qué se debe entender por nivel inventivo.

[4] Tribunal de Justicia de la Comunidad Andina, PROCESO 192-IP-2005

Lo obvio es aquello que salta a la vista de manera clara y directa; lo "que se encuentra o pone delante de los ojos, muy claro o que no tiene dificultad". Mientras que lo evidente consiste en la "certeza clara y manifiesta de la que no se puede dudar; cierto, claro, patente y sin la menor duda". De forma que algo que resulte obvio no es necesariamente evidente; empero lo que es evidente, es también obvio.

En este orden de ideas, se puede concluir que una invención goza de nivel inventivo cuando a los ojos de un experto medio en el asunto de que se trate, se necesita algo más que la simple aplicación de los conocimientos técnicos en la materia para llegar a ella, es decir, que de conformidad con el estado de la técnica el invento no sea consecuencia clara y directa de dicho estado."

De este señalamiento es claro que dentro de la legislación Andina, para que exista afectación del requisito de nivel inventivo de una solicitud de patente es necesario que la invención allí revelada sea derivable de forma clara y directa del arte previo, y que las enseñanzas de este arte previo sin lugar a dudas conduzcan a dicha invención.

Ahora bien, para el caso que nos compete se ha demostrado que a partir de las enseñanzas de D1, D2, D3 y D4 solos o en cualquiera de sus posibles combinaciones no "salta a la vista de manera clara y directa" una vacuna que comprenda dos composiciones multivalentes, una DTPaHepB-virus de la polio y otra neumocócica, donde la cantidad de DT en la primera composición sea 60-120 μg, y el en la segunda composición comprenda poli u oligo sacáridos neumocócicos conjugados con DT y/o CMR197; así como también se ha establecido que a partir de dichos documentos no se tiene "certeza clara y manifiesta" de que dicha vacuna elicitaría una respuesta inmune satisfactoria para todos los antígenos presentados, aumentando significativamente la presencia de anticuerpos para todos los serotipos neumocócicos.

En vista de lo anterior, es claro que la solicitud denegada se ajusta totalmente a los lineamientos establecidos por el Artículo 18 de la Decisión Andina 486 de 2000, y por lo tanto la Resolución Número **63149** incurriría en una contravención a dicho Artículo al negar el privilegio de patente a una solicitud que si conlleva Nivel Inventivo.

En conexión con este hecho me permito señalar que en su análisis de Nivel Inventivo de la solicitud denegada ese Despacho incurre en lo que dentro del ámbito de las patentes se conoce como "hindsight", o análisis "Ex post facto", toda vez que conociendo de antemano la invención se están seleccionado partes especificas de las enseñanzas contenidas dentro de los documentos citados, agrupándolas de una manera particular y modificándolas de manera que permitan construir la materia reclamada por la presente solicitud, pasos que de ninguna manera podrían ser evidentes para la persona versada en la materia en el momento en el cual se desarrolló la invención.

5

En este sentido vale la pena resaltar que al tener conocimiento de la invención es de esperarse que dichas selección, agrupación y modificaciones particulares de las enseñanzas de D1, D2, D3 y D4 parezcan obvias, toda vez que ya se sabe de antemano que efectivamente estos elementos en combinación resuelven el problema planteado; sin embargo, esta información no era conocida por el inventor en el momento de desarrollar la invención, por lo que no podría tener certeza de la utilidad de realizar estos pasos.

En vista de lo anterior, dado que la única motivación que tendría la persona versada en la materia de reunir estas características particulares reveladas en D1, D2, D3 y D4 sería la misma invención como ha sido revelada, es evidente el Nivel Inventivo que conlleva dicha solicitud.

3. CONCLUSIÓN

Por último, me permito señalar a ese Despacho que los hechos discutidos anteriormente en este escrito han establecido de forma incontrovertible que la presente solicitud sí cumple con el requisito **Nivel Inventivo** de acuerdo con lo establecido por el Artículo 18 de la Decisión 486, dado que a partir de las enseñanzas de los citados documentos D1, D2, D3 y D4 no sería posible obtener de manera evidente las características particulares que distinguen la vacuna reclamada por la solicitud denegada.

De esta forma, se demostró que ninguno de los documentos citados se ocupa del problema planteado por la presente solicitud: la existencia de interferencias entre antígenos que hacen parte de dos composiciones distintas.

Por otro lado, se estableció que el documento D1 hace referencia a la existencia de interferencias, pero estas son consideradas únicamente entre antígenos incluidos en la misma composición y dentro de esta se emplean cantidades de DT menores a las presentes en la vacuna de la invención.

Así mismo, se comprobó que el documento D2 emplea antígenos de pertussis de célula completa, que no están incluidos en la composición de la invención denegada, y no se refiere de ninguna manera a la cantidad de antígeno DT presente.

De forma similar, se comprobó que el documento D3 incluye antígenos de pertusis de célula completa y no indica la utilidad de tener un antígeno DT en cantidades relativamente altas.

Igualmente, se demostró que el documento D4 se refiere principalmente a la manera de formular antígenos de superficie de hepatitis B en vacunas multivalentes, pero no se refiere de manera particular a composiciones como las presentes en la vacuna de la invención.

Sumado a todo lo anterior, se demostró que el documento de Knuf, A2, es una prueba clara de la ventaja técnica que posee la invención, al compararlo con lo obtenido por Olivier, A1, ventaja que resulta sorprendente y que de ninguna manera sería evidente a partir de los documentos citados.

Por lo tanto, se ha comprobado claramente el Nivel Inventivo que conlleva la solicitud denegada frente al estado del arte más próximo.

4. SOLICITUD

En vista de todo lo anterior, ruego a usted proceder a estudiar nuevamente la solicitud a la luz de las argumentaciones presentadas y revocar la Resolución impugnada para conceder el privilegio de patente solicitado.

5. PODER

En este punto me permito mencionar ante este Despacho que el documento que acredita mi calidad de Representante Legal en Bogotá para asuntos relativos a la Propiedad Industrial de la sociedad peticionaria fue presentado en la solicitud original No. 03-088.387 con memorial de 21 de Octubre de 2004.

6. ANEXOS

Anexo me permito presentar:

i) Copia del documento: Olivier, et al, *"Immunogenicity, reactogenicity, and safety of a seven-valent pneumococcal conjugate vaccine (PCV7) concurrently administered with a fully liquid DTPa-IPV-HBV-Hib combination vaccine in health infants"*, Vaccine, 26, 3142-3152, 2008.; y

ii) Copia del documento: Knuf, M.et al "Immunogenicity, reactogenicity, and safety of a 7-valent pneumococcal conjugate vaccine (PCV7) concurrently administered with a DTPa-HBV-IPV/Hib combination vaccine in healthy infants", Vaccine, 24, 4727-4736, 2006.

7. DOMICILIO

Mi dirección es Calle 72 No. 5-83, Piso 6º., Bogotá D.C., Colombia.

Del Señor Superintendente,

ALICIA LLOREDA RICAURTE
T. P. 53.215

NOTARIA 41 DEL CÍRCULO DE BOGOTÁ D.C.
DEPARTAMENTO DE CUNDINAMARCA
REPÚBLICA DE COLOMBIA

El Notario 41 del círculo de Bogotá, da fe de que compareció personalmente: Alicia Lloreda Ricaurte identificada (o) con: 39690713 y tarjeta profesional de abogado No. 53215 del Consejo Superior de la Judicatura, quien autentica la firma puesta por ella (el) mismo en este escrito.
(Art. 84 C. P. C. Decreto. 2282/89 mod. 36)

Bogotá 09 DIC. 2010
ALICIA LLOREDA RICAURTE
C.C. No. 39690713 de Usaquén CLIENTE

NOTARIO 41 EN PROPIEDAD
Alvaro Vivanco Calvo

GlaxoSmithKline Biologicals S.A.
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REVIEW

Immunogenicity, reactogenicity, and safety of a seven-valent pneumococcal conjugate vaccine (PCV7) concurrently administered with a fully liquid DTPa–IPV–HBV–Hib combination vaccine in healthy infants☆

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KEYWORDS
Seven-valent pneumococcal conjugate vaccine; Hexavalent diphtheria–tetanus–acellular pertussis–hepatitis B-inactivated polio virus–*Haemophilus influenzae* type b vaccine; Concomitant administration

Abstract

Aim of the study: To evaluate the immunogenicity, safety and reactogenicity of a seven-valent pneumococcal conjugate vaccine (PCV7) when given concomitantly with a fully liquid DTaP–IPV–HBV–Hib combination vaccine.

Methods: Two hundred and sixty-six healthy infants in France ($n = 136$) and Germany ($n = 130$) were randomized to receive DTaP–IPV–HBV–Hib and PCV7 (test group) at the age of 2, 3 and 4 months (primary series) and 12–15 months (booster dose), or to receive DTaP–IPV–HBV–Hib at the same time points but PCV7 at the ages of 5, 6, 7 and 13–16 months (control group). Antibody levels to all vaccine antigens were measured before dose 1, 1 month after dose 3, at the time of booster, and 1 month later. Safety data were collected after each vaccine dose.

Results: Two hundred and fifty-seven infants (test group, 131; control group, 126) completed the primary immunization series and two hundred and forty-five received the booster dose (test group, 125; control group, 120).

Depending on the serotype, 92.8–100% of subjects in the test group achieved antibody levels ≥0.15 μg/mL for PCV7 antigens at 5 months of age, and 89.7–99.1% of them antibody levels ≥0.50 μg/mL 1 month after booster.

☆ Note, in September 2005, the European Medicines Agency suspended the marketing authorization for the fully liquid DTaP–IPV–HBV–Hib vaccine due to concerns about the long-term protection against hepatitis B following identification of a decreased immunogenicity of the hepatitis B component.

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For DTaP–IPV–HBV–Hib, there was no statistically significant difference between the two groups in the proportion of infants that achieved pre-defined seroprotective levels for each antigen at 5 months and 1 month after booster.
Frequency of local and systemic reactions was similar in both groups except for fever above 38.0 °C, which was more frequent in the test group after dose 1, 2 or 4. Fever >39.0 °C was only reported from three children in each group.
Conclusion: The PCV7 vaccine was highly immunogenic, well tolerated, and safe when coadministered with the DTPa–IPV–HBV–Hib vaccine at 2, 3, and 4 months of age and a booster dose at 12–15 months. In this study, PCV7 did not show any relevant influence on the immunogenicity and safety of the concurrently administered DTPa–IPV–HBV–Hib vaccine.

Contents

1. Introduction

Streptococcus pneumoniae is an important cause of morbidity and mortality in persons of all ages worldwide. In children, it is the most common bacterial cause of pneumonia, bacteremia, and otitis media [1]. Moreover, with the development and widespread use of *Haemophilus influenzae* type b (Hib) conjugate vaccines and the resulting near eradication of invasive Hib disease in immunized children [2], in Europe the pneumococcus has become the leading cause of bacterial meningitis in children less than 2 years of age [3–20]. An analysis of bacterial meningitis in children has shown that pneumococcal meningitis leads to substantially greater mortality or neurological sequelae than meningitis due to either *H. influenzae* type b or *Neisseria meningitides* [21]. In children between 6 and 24 months of age, the reported invasive pneumococcal disease (IPD) incidence ranges from 10 to 40 cases per 100,000 patient years in countries such as Denmark, Finland, France, Germany, and the UK. By contrast, it is in the range of 50–170 cases per 100,000 patient years among Spanish children [3].

The effectiveness of the seven-valent pneumococcal conjugate vaccine (PCV7) against IPD varies by country epidemiology, clinical disease and the age of the children. Protection provided by vaccination is most advantageous for blood culture-positive infections (i.e., sepsis, bacteremic pneumonia, bacteremia, and meningitis) in children less than 2 years of age. For children <2 years in Europe, PCV7 vaccine serotypes represent about 70% of all IPD. Given any cross protection of vaccine serogroups, the vaccine coverage could be estimated at about 75% for invasive pneumococcal infections in European children [7], as evaluated in the pivotal study performed at the Northern California Kaiser Permanente trial [22] and observed after 2 years of surveillance after introduction of the PCV7 in the USA [23].

Beyond the age of 2 years, there is some increase in the diversity of pneumococcal serotypes causing IPD. Likewise, there is a decrease in the vaccine coverage provided against pneumococcal meningitis, which in Europe seems to be due to a rise in the number of cases due to serotype 1 [5,17–20].

The seven-valent pneumococcal conjugate vaccine received its European registration in February 2001, and

it is used throughout Europe in childhood vaccination programs. The paediatric hexavalent combination vaccines received market authorization in October 2000 and entered the national vaccination calendars of selected countries. The concomitant administration of the seven-valent pneumococcal conjugate vaccine with the fully liquid DTaP–IPV–HBV–Hib vaccine (Hexavac®, Aventis Pasteur MDS) was evaluated in accordance with commonly used immunization schedules in Europe at 2, 3, 4 and 12–15 months of age. In September 2005, the European Medicines Agency suspended the marketing authorization for Hexavac® due to concerns about the long-term protection against hepatitis B following identification of a decreased immunogenicity of the hepatitis B component of the DTaP–IPV–HBV–Hib vaccine itself [45].

2. Population and methods

2.1. Methods

This was an open-labelled randomized multicentre study conducted in France and Germany. The study was conducted in accordance with the principles of good clinical practices (GCP), the national laws and the declaration of Helsinki (revised Edinburgh, Scotland, October 2000). Approval was obtained from the respective ethics review committee of the participating study sites.

2.2. Study objectives

This study aimed to investigate the concurrent administration of a seven-valent pneumococcal conjugate vaccine and a DTPa–IPV–HBV–Hib (PRP-T) combination vaccine at the age of 2, 3, 4 and 12–15 months with regard to immunogenicity, safety and tolerance, and whether the immunogenicity of the DTPa–IPV–HBV–Hib vaccine is influenced.

2.3. Subjects

Two hundred sixty-six healthy, 2-month-old infants (57 days to 112 days of age) were recruited in 18 study centres in France ($n = 136$) and 12 study centres in Germany ($n = 130$).

Healthy infants, 57–112 days old, were eligible for inclusion and written informed consent was obtained from parents/guardians before enrolment in the study.

Infants were not included in case of known or suspected history of diphtheria, pertussis, tetanus, polio, Hib or hepatitis B disease or of invasive pneumococcal diseases; a prior history of immunization against these diseases; a previous anaphylactic reaction or serious vaccine-associated adverse reaction; a known hypersensitivity to any component of the study vaccines; a birth weight of <2500 g and/or less than 37 gestational weeks; a vaccination of the mother with a hepatitis B virus vaccine in the previous 12 months; a known or suspected impairment of the immune system, a treatment with immunosuppressive agents; a major congenital, developmental or serious chronic disorder; a confirmed or suspected underlying evolving neurological disorder or history of encephalopathy and/or seizures, sequelae of perinatal hypoxia; a family history suggesting that the child is at increased risk of an evolving genetic neurological disorder; or a known bleeding disorder for which intramuscular administration of vaccine is contraindicated because of the risk of bleeding.

2.4. Vaccines

Each 0.5 mL dose of the PCV7 vaccine (Prevenar®, Wyeth Vaccines) was composed of 2 μg of each pneumococcal saccharide of serotypes 4, 9V, 14, 18C, 19F, and 23F and 4 μg of 6B each independently coupled to the CRM_{197} carrier protein, and 0.5 mg of aluminium phosphate adjuvant.

A 0.5 mL dose of the DTPa–IPV–HBV–Hib vaccine (Hexavac®, Aventis Pasteur MSD) contained 30 LF diphtheria toxoid, 10 LF of tetanus toxoid, 25 μg pertussis toxoid, 25 μg filamentous hemagglutinin, 40 D-antigen units of type 1 (Mahoney), 8 D-antigen units of type 2 (MEF-1) and 32 D-antigen units of type 3 (Saukett) of the polio viruses, 5 μg of hepatitis B surface antigen and 0.3 mg of aluminium salts.

2.5. Study design

Eligible subjects were randomized into one of two groups. The test group received an intramuscular injection of PCV7 in the left thigh and of DTaP–IPV–HBV–Hib in the right thigh at 2, 3, and 4 months of age (primary doses) and at 12–15 months of age (booster doses). The control group received an intramuscular injection of DTaP–IPV–HBV–Hib at 2, 3, and 4 months of age (primary series) and at 12–15 months of age (booster dose) into the right thigh.

After completing primary immunization with DTPa–IPV–HBV–Hib at 2, 3 and 4 months of age, the control group subjects were offered three doses of PCV7, to be administered at 5, 6, and 7 months of age, and the booster dose to be administered 4–6 weeks after the booster dose of the hexavalent vaccination. PCV7 immunogenicity results from the control group will not be presented here.

2.6. Immunogenicity evaluation

To determine the serum antibody responses, blood samples were drawn immediately before administration of the first dose of vaccine (pre-dose 1), 1 month (28–42 days) following administration of the third dose (post-dose 3), immediately before administration of the booster (pre-dose 4), and 1 month (28–42 days) following administration of the booster dose (post-dose 4).

Sera were analyzed for IgG antibodies to all seven pneumococcal serotypes by a validated ELISA method [24–26], and the antibody concentration of 0.15 μg/mL was used as well as the 0.50 μg/mL limit, which was considered as a conservative upper limit, likely to be higher than the minimum protective level [22].

Concentration of antibodies for diphtheria, tetanus, Hib (PRP-T), hepatitis B, and pertussis (PT and FHA) were measured by standard ELISA techniques [27–30] and antibodies to poliovirus 1, 2, and 3 by a neutralization

test [31]. Generally accepted protective levels for diphtheria antitoxin (≥0.01 IU/mL and ≥0.10 IU/mL), tetanus antitoxin (≥0.01 IU/mL and ≥0.10 IU/mL), PRP antibody (≥0.15 μg/mL and ≥1.00 μg/mL), neutralizing antibody for poliovirus types 1, 2 and 3 (≥1:8), and anti-HBS antibody (≥10 mIU/mL) were used in the calculation of protective immune responses [32–34]. In the absence of well-established serological markers for protection against pertussis, antibody levels between ≥2- and ≥4-fold rises from baseline were set for PT and FHA.

2.7. Safety evaluation

Following each immunization, the investigator or study nurse observed the infant for 20 min (France) or 30 min (Germany) for signs or symptoms due to any immediate reaction. Parents/guardians were provided with standard thermometers and callipers. They monitored any local reactions and systemic events on the day of immunization and for 3 days after each immunization. They took rectal temperatures each day at bedtime and at any time when the child felt warmer than usual. Parents/guardians also noted the presence and size of any erythema, induration, swelling, or tenderness at the respective injection site. All measurements and information regarding local and systemic reactions were recorded on diary cards by parents/guardians. Any illnesses or events that required a physician's intervention or the use of a prescription medication within 7 days of immunization, any hospitalization and other serious adverse events at any time during the course of the study were reported immediately to the investigator.

2.8. Statistical analysis

Demographic characteristics and time interval between each dose were analyzed.

Geometric mean concentration (GMC) values for antibody titres for each vaccine antigen were calculated for pre-dose 1 and post-dose 3, as well as for pre-dose and post-dose 4, in both groups. Post-dose 3 and 4 GMC values were compared between groups by ANOVA using pre-dose concentration as a covariate. If significant differences between groups for age, time intervals, or gender were found, these were also included in the model. The lower and upper 95% confidence limits of the mean of the GMC values and the mean differences in GMC values were also calculated. The percentage of subjects with antibody titres above defined post-dose 3 and post-dose 4 levels was calculated for each group. The differences in the percentages between groups were analyzed using Fisher's Exact test.

To assess the difference between injection site reactions in the test group, local reactions at the PCV7 site were compared to those at the DTaP–IPV–HBV–Hib site using Fisher's Exact test. Severe local reactions, defined as a diameter >2.4 cm were analyzed for erythema, induration or swelling, as well as tenderness interfering with limb movement.

All adverse events (e.g., physician visits, use of prescription medications within 7 days, or hospitalizations) were compared between groups using Fisher's Exact test. Febrile reactions were classified into two groups: temperature ≥38.0 °C or >39.0 °C.

3. Results

3.1. Study population

Two hundred sixty-six infants were randomized (136 in France, 130 in Germany) into the two study groups, 135 infants in the test group (PCV7 plus DTPa–IPV–HBV–Hib), and 131 infants in the control group (DTaP–IPV–HBV–Hib alone). One subject was randomized to receive PCV7, but was withdrawn by the parents before the first dose of vaccine.

The groups ($n = 265$) did not differ significantly for any of the demographic (gender distribution, mean age) and baseline characteristics (time intervals between doses or post-dose 3 and post-dose 4 bleed).

Two hundred and sixty-five infants received at least one dose during the primary immunization phase (i.e., intent to treat), but eight of these infants were excluded from the intent to treat (ITT) analysis due to missing blood samples during the primary series ($n = 257$). Two hundred and forty-five children received the booster dose but fourteen were excluded for the same reason during the booster phase ($n = 231$).

Sixty-two subjects (primary series) and fifty-six (booster phase) of the ITT population were excluded from the per-protocol (PP) analysis. Most common reason for exclusion from the PP analysis was time window violations between primary series doses or between vaccination and bleed (i.e. <27 days or >43 days), which occurred in 59 (33 in the test group, 26 in the control group) and 46 subjects (21 in the test group, 26 in the control group), respectively, in primary series and booster phase. Other reasons for exclusion from the PP analysis were: age not between 57 and 112 days at inclusion (1 in each group), over-dosage ($n = 1$, test group), age not between 12 and 15 months for booster ($n = 15$ in the test group, $n = 7$ in the control group) and vaccination error ($n = 1$, measles–mumps–rubella vaccine dose instead of PCV7 booster dose).

3.2. Immunogenicity results

For primary immunization phase, the ITT data included all subjects enrolled in the study who received the 2-, 3- and 4-month vaccinations (doses 1, 2, and 3) and had both blood samples drawn (pre-dose 1 as well as post-dose 3).

For booster immunization phase, the ITT data included all subjects enrolled in the study that received the booster dose (dose 4) and had both blood samples drawn (pre-dose 4 as well as post-dose 4).

Among the 266 randomized infants, 195 (73.3%) were included in the PP analysis for the primary series evaluation and 175 (65.8%) for the booster dose evaluation; 257 (96.6%) were included in the ITT analysis for the primary series evaluation and 231 (86.8%) for the booster dose evaluation (245 children actually received the booster dose, but 14 of them had missing pre-booster or post-booster dose blood samples). According to the protocol, ITT analysis was performed because, as planned in the protocol, the difference in number of subjects between the ITT population and the PP population was greater than 10% (i.e., 24% difference).

Table 1 Pre-dose 1 and post-dose 3, pre-dose 4 and post-dose 4 pneumococcal antibody GMCs [μg/mL] (95% C.I.) (test group, PCV7 + DTPa–IPV–HBV–Hib, ITT)

Serotype	Pre-dose 1	Post-dose 3	Pre-dose 4	Post-dose 4
4	0.13 (0.11–0.16)	3.03 (2.6–3.52)	0.45 (0.38–0.53)	4.19 (3.35–5.24)
6B	0.42 (0.33–0.53)	1.3 (1.03–1.64)	0.75 (0.6–0.95)	7.57 (5.95–9.64)
9V	0.34 (0.29–0.40)	2.06 (1.79–2.37)	0.53 (0.47–0.61)	2.74 (2.33–3.24)
14	0.65 (0.52–0.81)	6.74 (5.72–7.95)	2.79 (2.34–3.33)	10.97 (9.2–13.08)
18C	0.22 (0.18–0.27)	2.08 (1.82–2.38)	0.33 (0.28–0.39)	2.12 (1.76–2.55)
19F	0.58 (0.48–0.71)	3.63 (3.11–4.23)	0.91 (0.69–1.18)	3.91 (3.10–4.95)
23F	0.23 (0.18–0.29)	1.46 (1.17–1.83)	0.32 (0.26–0.41)	4.20 (3.19–5.54)

As immunogenicity results from the ITT and the PP analysis were similar (data not shown), this publication will focus on the results of the ITT analysis.

3.2.1. Antibody response to pneumococcal antigens

3.2.1.1. Primary phase results. A statistically significant increase in GMC values was noticed for all seven serotypes 1 month after dose 3 for the test group (PCV7 plus DTPa–IPV–HBV–Hib) (Table 1).

After dose 3, between 92.8% and 100% of PCV7 recipients achieved pneumococcal antibody levels ≥0.15 μg/mL (Table 2). Between 79.2% and 98.4% of infants of the test group achieved pneumococcal antibody levels ≥0.50 μg/mL.

3.2.1.2. Booster series results. After boosting with the PCV7 vaccine, pneumococcal GMC values in the test group ranged from 2.12 μg/mL to 10.97 μg/mL (Table 1).

After the booster dose, 94% (serotype 23F) to 100% (serotype 9V) of infants of the test group achieved pneumococcal antibody levels of ≥0.15 μg/mL, depending on the serotype (Table 2). Between 89.7% (serotype 23F) and 99.1% (serotype 14) of PCV7 recipients achieved pneumococcal antibody levels ≥0.50 μg/mL (Table 2).

3.3. Antibody response to DTaP–IPV–HBV–Hib vaccine antigens

3.3.1. Primary phase results

Statistically significant increases from baseline (pre-dose 1) to post-dose 3 were observed for all antigens within each of the study groups except for diphtheria antigen, which increased in the test group but decreased in the control group (Table 3).

Table 2 Percent of subjects (95% C.I.) achieving defined pneumococcal antibody levels of ≥0.15 μg/mL and ≥0.50 μg/mL post-dose 3 and post-dose 4 (test group, PCV7 + DTPa–IPV–HBV–Hib, ITT)

Serotypes (g/μg mL)	Post-dose 3 $n = 125$	Post-dose 4 $n = 116$
4		
≥0.15	99.2 (97.64–100.76)	98.28 (95.91–100.0)
≥0.50	96.8 (93.71–99.89)	94.83 (90.8–98.86)
6B		
≥0.15	92.8 (88.27–97.33)	98.28 (95.91–100.0)
≥0.50	79.2 (72.08–86.32)	95.69 (91.99–99.93)
9V		
≥0.15	99.2 (97.64–100.76)	100.0 (100.0–100.0)
≥0.50	95.2 (91.45–98.95)	93.7 (89.63–98.30)
14		
≥0.15	100 (100–100)	99.14 (97.46–100.0)
≥0.50	98.4 (96.2–100.6)	99.14 (97.46–100.0)
18C		
≥0.15	100 (100–100)	99.14 (97.46–100.0)
≥0.50	95.2 (91.45–98.95)	97.97 (89.63–98.3)
18C		
≥0.15	99.2 (97.64–100.76)	98.28 (95.91–100.0)
≥0.50	96.8 (93.71–99.89)	94.83 (90.8–98.86)
23F		
≥0.15	98.8 (88.27–97.33)	93.97 (89.63–98.30)
≥0.50	82.4 (75.72–89.08)	89.66 (84.11–95.20)

Table 3 Pre-dose 1, post-dose 3, pre-dose 4 and post-dose 4, DTaP, PRP-T, IPV and HepB antibody GMCs[a] (95% C.I.) (test group, PCV7 + DTPa–IPV–HBV–Hib, and control group, DTPa–IPV–HBV–Hib, ITT)

Antigen	Test group				Control group			
	$n = 122$–125[b]		$n = 116$[b]		$n = 119$–122[b]		$n = 112$[b]	
	Pre-dose 1	Post-dose 3	Pre-dose 4	Post-dose 4	Pre-dose 1	Post-dose 3	Pre-dose 4	Post-dose 4
Diphtheria	0.14 (0.09–0.21)	0.18 (0.15–0.22)	0.11 (0.09–0.14)	5.95 (5.03–7.05)	0.15 (0.1–0.23)	0.09 (0.07–0.11)	0.13 (0.1–0.16)	3.81 (3.05–4.75
Tetanus	0.65 (0.52–0.80)	1.72 (1.45–2.04	0.28 (0.23–0.34)	6.41 (5.32–7.71)	0.76 (0.62–0.92)	2.05 (1.72–2.44)	0.31 (0.25–0.37)	7.2 (5.9–8.79)
Pertussis FHA	9.27 (7.72–11.13)	149.74 (133.61–167.83)	31.97 (37.31–37.43)	250.82 (217.16–289.71)	10.28 (8.62–12.24)	191.51 (175.21–209.33)	41.22 (35.1–48.4)	343.37 (295.6–398.85)
Pertussis PT	1.84 (1.48–2.28)	53.88 (47.97–60.53)	6.46 (5.63–7.42)	54.82 (49.17–61.12)	1.73 (1.42–2.11)	63.39 (57.73–69.61)	8.18 (7.05–9.48)	63.3 (55.2–72.59)
Poliovirus Type 1	9.1 (6.47–12.8)	55.65 (42.13–73.52)	14.89 (10.59–20.94)	573.56 (449.30–732.18)	10.59 (7.55–14.87)	64.74 (48.04–87.24)	14.83 (10.23–21.51)	719.53 (515.37–1004.6)
Poliovirus Type 2	13.04 (9.29–18.3)	95.26 (70.21–129.25)	27.23 (19.02–39.00)	1308.3 (1049.2–1631.3)	15.36 (10.91–21.64)	106.85 (79.95–142.81)	24.87 (17.19–35.99)	1359.72 (999.81–1849.2
Poliovirus Type 3	6.33 (4.44–9.02)	123.78 (92.34–165.92)	29.08 (20.08–42.13)	1080.6 (802.52–1455.0)	6.01 (4.21–8.57)	147.71 (114.92–189.86)	33.87 (23.73–48.34)	1466.5 (1041.2–2065.7)
PRP-T (Hib)	0.16 (0.13–0.19)	1.57 (1.22–2.03)	0.47 (0.39–0.57)	15.63 (12.52–19.51)	0.14 (0.12–0.18)	1.49 (1.14–1.94)	0.46 (0.37–0.56	12.18 (9.31–15.94)
Hepatitis B	10.5 (6.06–18.2)	79.36 (56.34–111.77)	10.59 7.13–15.75)	318.44 (208.74–485.79)	10.68 (6.05–18.84)	138.91 (99.69–193.56)	18.06 (12.24–28.27)	603.92 (377.86–965.25)

[a] GMCs are expressed as μg/mL for Hib (PRP), IU/mL for diphtheria and tetanus, and mIU/mL for hepatitis, μ/mL pertussis antigens, and as neutralizing antibody/mL for poliovirus types 1, 2, and 3 (GMTs).

[b] Number varies with antigen.

There were no significant differences between groups regarding the percentage of subjects in whom defined antibody levels were achieved post-dose 3 for all vaccine antigens with the exception of diphtheria (Table 4). The percentage of subjects in the test group (71.2%) having achieved a diphtheria antibody level of ≥0.10 IU/mL was significantly higher ($p < 0.0001$).

3.3.2. Booster phase

The percentage of subjects in whom defined antibody levels were achieved post-dose 4 did not differ significantly between the study groups for any of the antigens contained in the hexavalent vaccine (Table 4).

In both groups, statistically significant increases ($p < 0.0001$) from pre-dose 4 to post-dose 4 were observed for all DTaP, IPV, polio types 1, 2 and 3, Hib and HBV antibody GMC values (Table 3).

When comparing the post-dose 4 GMC values, no statistically significant differences were found for tetanus toxoid, pertussis toxoid, polio types 1, 2 and 3, Hib and Hepatitis B post-dose 4 antibody GMC values between both groups.

The post-dose 4 GMC for diphtheria antigen was statistically significantly greater for the test group compared to the control group, while post-dose 4 GMC values for pertussis FHA were statistically significantly reduced.

Based on the test group to control group ratio of post-booster dose GMC values (GMRs), notably higher diphtheria antitoxin GMC values, but notably lower hepatitis B and pertussis FHA antibody GMC values were found in the test group.

3.4. Safety

All infants who received at least one dose of vaccine were included in the safety evaluation. The primary immunization population comprised 265 infants (test group, 134; control group, 131). The booster phase safety population included 245 children (test group, 125; control group, 120).

3.4.1. Local reactions

Local reactions of the test group subjects were comparable between the PCV7 and the DTaP–IPV–HBV–Hib injection sites after each dose (Table 5). Frequency of local reactions did not increase from dose 1 to dose 3, but was notably greater after the booster dose in both groups. The percentage of subjects with severe erythema, induration, swelling or tenderness within 3 days of dose 1, 2 or 3 were less than or equal to 10%, 12%, 6.5% and 2.4%, respectively, in both groups. After the booster dose, significant erythema was seen in 17% of PCV7 recipients and 26.1% of infants in the control group ($p = 0.11$). Percentages of subjects with significant induration, swelling or tenderness within 3 days of booster dose were 12.6% (test) and 14.7% (control), 12.5% (test) and 14.8% (control), 3.6% (test) and 3.5% (control), respectively.

3.4.2. Systemic reactogenicity

The percentage of infants with any systemic reaction (except fever) decreased with subsequent doses (Table 6).

After dose 1, the percentage of subjects with any systemic reaction was significantly higher in the test group

Table 4 Percent (95% C.I.) of subjects with defined diphtheria, tetanus, pertussis, Hib, poliovirus and hepatitis B antibody levels post-dose 3 and post-dose 4 (test group, PCV7 + DTPa–IPV–HBV–Hib, and control group, DTPa–IPV–HBV–Hib, ITT)

Antigen	Post-dose 3			Post-dose 4		
	Test group $n = 122–125$[a]	Control group $n = 119–122$[a]	p-Value[b]	Test group $n = 116$[a]	Control group $n = 112$[a]	p-Value[b]
Diphtheria						
≥0.01 IU/mL	99.2 (97.64–100.76)	99.18 (97.58–100.78	1.0000	100.0 (100.0–100.0)	100.0 (100.0–100.0)	N/A
≥0.10 IU/mL	71.2 (63.26–79.14)	36.89 (28.32–45.45)	<0.0001	100.0 (100.0–100.0)	99.11 (97.36–100.0)	0.4934
Tetanus						
≥0.01 IU/mL	100.00 (100–100)	100.00 (100.0–100.0)	N/A	100.0 (100.0–100.0)	100.0 (100.0– 100.0)	N/A
≥0.10 IU/mL	99.19 (97.62–100.77)	100.0 (100.0–100.0)	1.000	99.14 (97.46–100.0)	99.10 (97.34–100.0)	1.000
Pertussis FHA						
≥4-fold rise	84.68 (78.34–91.02)	90.91 (85.79–96.03)	0.1728	81.9 (74.89–88.9)	80.18 (72.76–87.6)	0.8656
Pertussis toxin						
≥4-fold rise	89.52 (84.12–94.91)	93.33 (88.87–97.8)	0.3631	81.03 (73.9–88.17)	81.08 (73.79–88.37)	1.000
Poliovirus Type 1						
≥8	96.77 (96.66–99.88	95.04 91.17–98.91	0.5361	98.28 (95.91–100.0)	97.27 (94.23–100.0)	0.6766
Poliovirus Type 2						
≥8	95.08 (91.24–98.92)	99.16 (97.52–100.8)	0.1197	100.0 (100.0–100.0)	98.18 (95.68–100.0)	0.2358
Poliovirus Type 3						
≥8	96.77 (93.66–99.88)	100.0 (100–100)	0.1220	98.28 (95.91–100.0)	97.27 (94.23–100.0)	0.6766
PRP-T (Hib)						
≥0.15 μg/mL	94.4 (90.37–98.43)	93.39 (88.96–97.82)	0.7947	100.0 (100.0–100.0)	100.0 (100.0–100.0)	N/A
≥1.00 μg/mL	63.2 (54.75–71.65)	64.46 (55.93–72.99)	0.8947	98.28 (95.91–100.0)	94.64 (90.47–98.81)	0.1656
Hepatitis B						
≥10 mIU/mL	89.43 (84.0–94.86)	93.33 (88.87–97.8)	0.3624	92.98 (88.29–97.67)	92.73 (81.81–97.58)	1.000

[a] Number varies with antigen.
[b] p-Value assesses the difference between treatment groups using Fisher's Exact test.

Table 5 Number of subjects with a local reaction (% of total number of subjects) within 3 days of doses 1–4 in the test group (PCV7 + DTPa–IPV–HBV–Hib) or control group (DTPa–IPV–HBV–Hib)

	Dose 1			Dose 2			Dose 3			Dose 4		
	Test left thigh	Control right thigh	p-Value[a]	Test left thigh	Control right thigh	p-Value[a]	Test left thigh	Control right thigh	p-Value[a]	Test left thigh	Control right thigh	p-Value[a]
N[b]	122–126	121–126		122–127	125–130		119–124	117–125		112	112	
Erythema												
Any	41 (33.3)	42 (33.6)	1.00	57 (44.9)	65 (50.0)	0.46	48 (39.0)	54 (44.0)	0.44	62 (55.4)	66 (57.4)	0.79
Any significant[c]	4 (3.3)	5 (4.0)	1.00	10 (7.9)	13 (10.0)	0.66	9 (7.3)	10 (8.2)	1.00	19 (17.0)	30 (26.1)	0.11
Induration												
Any	52 (41.3)	54 (42.9)	0.90	59 (47.6)	61 (48.0)	1.00	54 (43.5)	54 (43.5)	1.00	62 (55.9)	72 (62.1)	0.35
Any significant[c]	9 (7.1)	15 (11.9)	0.28	12 (9.7)	13 (10.2)	1.00	7 (5.6)	8 (6.5)	1.00	14 (12.6)	17 (14.7)	0.70
Swelling												
Any	26 (20.8)	24 (19.5)	0.87	34 (26.8)	35 (29.6)	1.00	25 (20.5)	22 (18.0)	0.75	47 (42.0)	49 (42.6)	1.00
Any significant[c]	6 (4.8)	8 (6.5)	0.59	7 (5.5)	6 (4.6)	0.78	6 (4.9)	3 (2.5)	0.50	14 (12.5)	17 (14.8)	0.71
Tenderness												
Any	19 (15.6)	28 (23.0)	0.19	24 (19.7)	21 (16.8)	0.62	15 (12.4)	15 (12.4)	1.00	44 (39.6)	45 (39.5)	1.00
Any significant[c]	3 (2.4)	2 (1.7)	1.00	0 (0.0)	0 (0.0)	N/A	0 (0.0)	0.00	N/A	4 (3.6)	4 (3.5)	1.00

[a] p-Value assess the difference between treatment groups using Fisher's Exact test.
[b] Number varies with reaction.
[c] Defined as >2.4 cm.
[d] Interfers with leg movement.

(82%) than in the control group (66%). This was mainly due to a significantly higher percentage of subjects showing sleepiness after dose 1 in the test group (53.2% vs. 39.7% in the control group). After dose 4, decreased appetite occurred significantly more frequently in the test group.

Body temperature greater than 38.0 °C was seen significantly more often in the test group compared to the control group after dose 1, dose 2 and the booster dose (24.8%, 26.9%, and 46.5%, respectively in the test group vs. 10.1%, 15.4% and 30.8% in the control group). Fever ≥39.1 °C was seen in nine cases, six in the test group and three in the control group. Intake of antipyretic medication was comparable in both groups after each dose of the primary series, but was reported more frequently after the booster dose in the test group.

No deaths were reported during the course of the study.

Ten serious adverse events were reported (five in each group) and none of them was considered by the investigators to be related to immunization (test group: one acute pyelonephritis, one restlessness, one systemic viral infection, one inguinal hernia and one scald at the chin and right arm; control group: one laryngitis, two head injury, one angina and one gastroenteritis).

Globally, there was no difference in the incidence of serious adverse events (neither for all events nor for related adverse events) between the test and the control group.

4. Discussion

The seven-valent pneumococcal conjugate vaccine (PCV7) and the DTaP–IPV–HBV–Hib hexavalent combination vaccines were introduced in France and Germany in 2001. Since 2002, French infants were covered by broad recommendations for the use of the seven-valent pneumococcal conjugate vaccine, which includes children at higher risk of infection (i.e., comorbidities or collective care settings, multiple siblings, limited breast feeding, etc.). By contrast the German recommendations focused on comorbidities and perinatal status. However, the recommendations for PCV7 have been extended to all children less than 2 years old both in France [41] and Germany [42] in July 2006. With respect to the hexavalent combination vaccines, up to September 2005 for Hexavac®, these are widely used in Germany and much less in France. In both countries as well as in several other European countries, primary immunization is recommended at 2, 3 and 4 months to provide an early protection against pneumococcal meningitis, pertussis and Haemophilus influenzae type b cases that could occur in early infancy.

This study aimed to evaluate the immunogenicity and reactogenicity of the seven-valent pneumococcal vaccine (Prevenar®, Wyeth Vaccines), when given along with the DTaP–IPV–HBV–Hib vaccine (Hexavac®, Aventis Pasteur MSD) for primary and for booster immunization according to the recommended immunization schedules for Prevenar in both France and Germany, and for Hexavac in Germany (3 Hexavac® doses in primary vaccination were not recommended in France: two doses were recommended at 2 and 4 months with a DTaP–IPV–Hib pentavalent combination vaccine in between at 3 month of age). A similar study has been conducted in Germany to evaluate the seven-valent

Table 6 Percentage of total number of subjects with a systemic reaction and use of antipyretic within 3 days of doses 1–4 in the test group (PCV7 + DTPa–IPV–HBV–Hib) or control group (DTPa–IPV–HBV–Hib)

	Dose 1			Dose 2			Dose 3			Dose 4		
	Test	Control	*p*-Value[a]	Test	Control	*p*-Value[a]	Test	Control	*p*-Value[a]	Test	Control[b]	*p*-Value[a]
N[c]	124–129	121–124		126–130	121–122		119–122	118–121		112–116	101–106	
Fever ≥38 °C	24.8	10.1	0.004	26.9	15.4	0.031	20.8	12.2	0.082	46.5	30.8	0.019
Fever ≥39.1 °C	0.8	0.8	1.000	0.0	0.8	0.486	1.7	0.9	1.000	2.6	0.0	0.247
Antipyretics	38.3	43.9	0.434	40.0	32.2	0.233	34.5	32.2	0.782	42.0	28.7	0.048
Fussiness	58.3	48.4	0.130	38.6	40.5	0.796	33.9	35.0	0.893	39.6	31.5	0.259
Sleepiness	53.2	39.7	0.040	36.2	34.4	0.793	33.6	26.9	0.323	21.6	23.4	0.871
Decreased appetite	31.7	25.4	0.325	24.6	23.1	0.881	20.7	24.6	0.537	30.6	14.8	0.006
Any systemic event[d]	82.2	66.1	0.004	63.1	56.6	0.306	56.6	51.2	0.441	54.5	46.3	0.281

[a] *p*-Value assess the difference between treatment groups using Fisher's Exact test.
[b] Control/catch-up group received primary series of PCV7 at 5, 6, 7 and booster dose about 1 month after the DtaP–IPV–HBV/Hib booster dose.
[c] Number varies with reaction.
[d] Does not include fever.

pneumococcal vaccine concurrently administered with the other DTaP–IPV–HBV/Hib vaccine (Infanrix hexa®, GSK) and results have recently been published [33].

In our study, the PCV7 vaccine was highly immunogenic in early infancy. In infants who received the primary immunization at 2, 3 and 4 months of age with the pneumococcal vaccine, antibody concentrations for all seven serotypes increased substantially. GMC values for all seven serotypes after dose 3 are similar to those reported in four previous studies conducted in Germany and/or France, where PCV7 was administered with the same schedule concomitantly to other recommended paediatric vaccines [32,33,43,44], except for serotype 6B for which GMC values were higher in 3 of these studies (2.5–3.2 μg/mL vs. 1.3) [32,43,44]. In our study, about 92% of subjects achieved serotype 6B antibody levels ≥0.15% and 80% antibody levels ≥0.50 μg/mL after the third dose of the primary series. However, the PCV7 booster elicited a sixfold increase in GMC of antibodies against 6B which reached 7.57 μg/mL, and about 96% of subjects were ≥0.50 μg/mL

During the pivotal study performed at Northern California Kaiser Permanente (NCKP) [22] a vaccine efficacy of 97% was measured. Furthermore, in a subset of children enrolled in the NCKP trial in whom immunogenicity was assessed, at least 97% of the PCV7 recipients achieved pneumococcal antibody levels ≥0.15 μg/mL for all serotypes after the primary series (at age 2, 4 and 6 months), and between 82% (serotype 23F) and 97% (serotype 14) of vaccinees achieved antibody concentrations ≥0.50 μg/mL. These results correlate with the levels of antibodies following the primary series in the test group in our study [22].

In the test group, pneumococcal antibody concentrations declined between the third dose and the booster dose under the level of 0.50 μg/mL for serotypes 4, 18C and 23F. However, the booster dose between 12 and 15 months of age induced an antibody response that was at least comparable to that after the third dose of the primary course for serotypes 4, 9V, 18C and 19F or even led to a further increase of specific antibodies for serotypes 6B, 14 and 23F.

The proportion of toddlers with pneumococcal antibody levels ≥0.50 μg/mL after booster immunization with DTaP–IPV–HBV–Hib coadministered with PCV7 (test group) ranged between about 90% (serotype 23F) and 99% (serotype 14). As with the primary series, these results are very similar to the immunogenicity results that were observed in the Northern California Kaiser Permanente efficacy trial or in other studies with the concomitant use of generally recommended paediatric vaccines, including the DTaP–IPV–Hib combination vaccine [22,32,33,35,44].

One objective of the study was to confirm that the DTPa–IPV–HBV–Hib vaccine was immunogenic in both the test group and the control group. Post-dose 3 diphtheria antibody levels were two times higher in the test group than in the control group, whereas antibody levels for hepatitis B were 1.75 times higher in the control group. The percentage of infants achieving defined antibody levels post-dose 3 for all antigens contained in the DTaP–IPV–HBV–Hib vaccine was not statistically different, except for diphtheria antibody levels, which were higher in the test group. In other studies with CRM197 based conjugated vaccine higher diphtheria antitoxin response has been consistently reported suggesting carrier protein mediated enhancement

phenomenon [36–38]. However, such effect is unlikely to be clinically relevant since in both groups the same high level of children achieving seroprotective titre criteria for diphtheria is achieved.

Post-dose 4 antibody GMC values were comparable in both groups for most of the antigens contained in the DTaP–IPV–HBV–Hib vaccine, except for diphtheria toxoid having a significantly greater GMC value in the test group, and the pertussis FHA antibody having a significantly lower GMC value in the test group.

There were no significant differences between the study groups based on GM ratio of post-booster GMC values of the control group/test group, except for diphtheria toxoid indicating higher GMC values in the test group and for pertussis FHA antibody and hepatitis B surface antibody indicating lower GMC values in the test group.

The percentage of children with pre-defined protective antibody levels 1 month after dose 4 was comparable, without any significant differences between groups for each of the antigens contained in the DTPa–IPV–HBV–Hib vaccine. The observed interference in the antibody response to pertussis FHA will likely have no consequence on clinical protection against pertussis since the decrease in FHA antibody GMC is of limited magnitude; furthermore, an identical proportion of children in both study groups (approximately 80%) achieved a fourfold rise in FHA antibody titre values after the booster dose.

Despite two times higher antibody levels for hepatitis B in the control group after the booster dose, a similar proportion (93%) of children in both study groups achieved the classical threshold level of $\geq$10 mIU/mL, which has been considered to indicate immunological priming and long-term protection [39,40]. This percentage appears slightly lower that what would be expected based on the Hexavac® phase III studies (34) where between 94.9% and 98.2% of infants achieved seroprotective titres ($\geq$10 mIU/mL) and where GMT values for HBsAg were found to have been higher than in our study. However, our data are in accordance with the decision of the European Medicines Agency in September 2005 to suspend the marketing authorization for Hexavac® due to concerns about the long-term protection against hepatitis B following identification of a decreased immunogenicity of the hepatitis B component [45].

For both groups, local reactions at the PCV7 and DTPa–IPV–HBV–Hib injection sites were comparable in overall frequency and severity after each dose. When the PCV7 vaccine was administered with the DTaP–IPV–HBV–Hib vaccine, the rate of febrile reactions was higher than when the DTaP–IPV–HBV–Hib vaccine was administered alone. These febrile reactions were low-grade (38–39 °C) and transient, and were well controlled by prophylactic antipyretic treatment given according to local medical practice. Fever >39 °C was seen only in a few subjects, with no statistical difference between the groups, and was not associated with clinically significant complications (no febrile seizures). There were very few adverse events reported during the study, and none of them were attributed to the study vaccines.

Overall, the pneumococcal conjugate vaccine concomitantly administered with the DTaP–IPV–HBV–Hib vaccine was well tolerated. The great majority of adverse events were mild and self-limited.

5. Conclusion

The PCV7 vaccine was highly immunogenic, well tolerated, and safe when concurrently administered with the DTPa–IPV–HBV–Hib vaccine (Hexavac®) at a schedule of 2, 3 and 4 months of age with a booster dose at 12–15 months. In this study, PCV7 did not show any relevant influence on the immunogenicity and safety of the concurrently administered vaccine DTPa–IPV–HBV–Hib.

References

[1] Klein JO. The epidemiology of pneumococcal disease in infants and children. Rev Infect Dis 1981;3(2):246–53.

[2] Adam WG, Deaver KA, Cochi SL, Plikaytis BD, Zell ER, Broome CV, et al. Haemophilus influenzae Study Group. Decline of childhood Haemophilus influenzae type b (Haemophilus) disease in the Haemophilus vaccine era. JAMA 1993;269(2): 221–6.

[3] Diez-Domingo J, Pereiro I, Morant A, Gimeno C, Lerma M, Oyaguez I, et al. Epidemiology of invasive *Streptococcus pneumoniae* infections in children in Spain, 1996–1998. J Infect 2002;45(3):139–43.

[4] Dominguez A, Salleras L, Cardenosa N, Ciruela P, Carmona G, Martinez A, et al. The epidemiology of invasive Streptococcus pneumoniae disease in Catalonia (Spain). A hospital-based study. Vaccine 2002;20(23/24):2989–94.

[5] Von Kries R, Hermann M, Hachmeister A, Siedler A, Schmitt HJ, Al-Lahham A, et al. Prediction of the potential benefit of different pneumococcal conjugate vaccines on invasive pneumococcal disease in German children. Pediatr Infect Dis J 2002;21(11):1017–23.

[6] Von Kries R, Hermer-Lang D, Reinert RR. A nationwide study of the incidence of systemic pneumococcal infections in infancy in Germany. In: International symposium on pneumococci and pneumoccal disease abstract book. 1998. p. 39.

[7] Black S, Shinefield H, Cohen R, Floret D, Gaudelus J, Olivier C, et al. Efficacité du vaccin pneumococcique conjugué (Prevenar) contre les infections invasives à pneumocoque: bénéfice attendu pour les enfants en France. Arch Pediatr 2004; 11(7):843–53.

[8] Gaudelus J, Cohen R, Reinert P. Epidemiology of pneumococcal infections in French children. Acta Paediatr Suppl 2000;89(435):27–9.

[9] Olivier C, Bégué P, Cohen R, Floret D. Méningites à pneumocoque de l'enfant. Résultat d'une enquête nationale (1993–1995). Bulletin Epidémiologique Hebdomadaire n 16/ 2000; 18 *avril* 2000:67–9.

[10] Sleeman K, Knox K, George R, Miller E, Waight P, Griffiths D, et al. Invasive pneumococcal disease in England and Wales: vaccination implications. J Infect Dis 2001;183(2):239–46.

[11] Eriksson M, Henriques B, Ekdahl K. Epidemiology of pneumococcal infections in Swedish children. Acta Paediatr Suppl 2000;89(435):35–9.

[12] Kaltoft ZN, Konradsen HB. Epidemiology of invasive pneumococcal infections in children aged 0–6 years in Denmark: a 19-year nationwide surveillance study. Acta Paediatr Suppl 2000;89(435):3–10.

[13] Miller E, Waight P, Efstratiou A, Brisson M, Johnson A, George R. Epidemiology of invasive and other pneumococcal disease in children in England and Wales 1996–1998. Acta Paediatr Suppl 2000;89(435):11–6.

[14] Syriopoulou V, Daikos GL, Soulis K, Michos A, Alexandrou H, Pavlopoulou I, et al. Epidemiology of invasive childhood pneumococcal infections in Greece. Acta Paediatr Suppl 2000; 89(435):30–4.

[15] Venetz I, Schopfer K, Muhlemann K. Paediatric, invasive pneumococcal disease in Switzerland. 1985–1994. Swiss Pneumococcal Study Group. Int J Epidemiol 1998;27(6):1101–4.
[16] Eskola J, Takala AK, Kela E, Pekkanen E, Kalliokoski R, Leinonen M. Epidemiology of invasive pneumococcal infections in children in Finland. JAMA 1992;268(23):3323–7.
[17] Hausdorff WP, Bryant J, Kloek C, Paradiso PR, Siber GR. The contribution of specific pneumococcal serogroups to different disease manifestations: implications for conjugate vaccine formulation and use, part II. Clin Infect Dis 2000;30(1): 122–40.
[18] Hausdorff WP, Bryant J, Paradiso PR, Siber GR. Which pneumococcal serogroups cause the most invasive disease: implications for conjugate vaccine formulation and use, part I. Clin Infect Dis 2000;30(1):100–21.
[19] McIntosh ED, Booy R. Invasive pneumococcal disease in England and Wales: What is the true burden and what is the potential for prevention using 7 valent pneumococcal conjugate vaccine? Arch Dis Child 2002;86(6):403–6.
[20] Hausdorff WP. Invasive pneumococcal disease in children: geographic and temporal variations in incidence and serotype distribution. Eur J Pediatr 2002;161(12 Suppl 2):S135–9.
[21] Baraff LJ, Lee SI, Schriger DL. Outcomes of bacterial meningitis in children: a meta-analysis. Pediatr Infect Dis J 1993; 12(5):389–94.
[22] Black S, Shinefield H, Fireman B, Lewis E, Ray P, Hansen, et al. Efficacy, safety and immunogenicity of heptavalent pneumococcal conjugate vaccine in children. Northern California Kaiser Permanente Vaccine Study Center Group. Pediatr Infect Dis J 2000;19(3):187–95.
[23] Whitney CG, Farley MM, Hadler J, Harrison LH, Bennett NM, Lynfield R, et al. Decline in invasive pneumococcal disease after the introduction of protein–polysaccharide conjugate vaccine. N Engl J Med 2003;34(18):1737–46.
[24] Quataert SA, Kirsh C, Wiedl L, Phipps D, Strohmeyer S, Cimino C, et al. Assignment of weight based antibody units to human pneumococcal standard reference serum. Clin Diagn Lab Immunol 1995;2(5):590–7.
[25] Wernette CM, Frash CE, Madore D, Carlone G, Goldblatt D, et al. Enzyme linked immunosorbent assay for quantitation of human antibodies to pneumococcal polysaccharides. Clin Diagn Lab Immunol 2003;10:514–9.
[26] Rubin LG, Mardy GV, Pais L, Carlone G. Human anticapsular antibody concentration required for protection against experimental pneumococcal bacteremia (Abstract G-47). In: Proceedings of the 35th interscience conference of antimicrobialagents and chemotherapy. 1995.
[27] Paradiso PR, Hogerman DA, Madore DV, Keyserling H, King J, ReisinerKS, et al. Safety and immunogenicity of a combined diphtheria, tetanus, pertussis and haemophilus influenza type b in young infants. Pediatrics 1993;92(6):827–32.
[28] Phipps DC, West J, Eby R, Koster M, Madore DV, Quataert SQ. An ELISA employing an haemophilus influenza type b oligosaccharide–human serum albumin conjugate correlates with the radioantigen binding assay. J Immunol Methods 1990; 135(1/2):121–8.
[29] Manclarke CR, Meade BD, Bursyn DG. Serological response to *Bordetella pertussis*. In: Rose NR, Friedman H, Fahey JL, editors. Manual of clinical laboratory immunology. 3rd ed. Washington, DC; 1986. p. 388–94.
[30] Pitichero ME, Passador S. Administration of combined diphteria and tetanus toxoids and pertussis vaccine, hepatitis B vaccine and Haemophilus influenzae type b (Hib) vaccine to infants and response to a booster dose of Hib conjugate vaccine. Clin Infect Dis 1997;25:1378–84.
[31] Grenier B, Hamza B, Biron G, Xueref C, Viarme F, Roumianteff M. Seroimmunity following vaccination in infants by an inactivated poliovirus preparation on Vero cells. Rev Infect Dis 1984;6(Suppl 2):545–7.
[32] Schmidt HJ, Faber J, Lorenz I, Schmöle-Thoma B, Ahlers N. The safety, reactogenicity and immunogenicity of a 7-valent pneumococcal conjugate vaccine (PCV7) concurrently administred with a combination DTaP–IPV–Hib vaccine. Vaccine 2003;21(25/26):3653–62.
[33] Tichmann-Schumann I, Soemantri P, Behre U, et al. Immunogenicity and reactogenicity of four doses of diptheria–tetanus–three-component acellular pertussis–hepatitis B-inactivated plio virus–haemophilus influenzae type b vaccine coadminitered with 7-valent pneumococcal conjugate vaccine. Pediatr Infect Dis J 2005;24(1):70–7.
[34] Mallet E, Belohradsky BH, Lagos R, et al. A liquid hexavalent combination against diphtheria, tetanus, pertussis, poliomyelitis. Haemophilus influenzae type and hepatitis B: review of immunogenicity and safety. Vaccine 2004;22(11/12):1343–57.
[35] Rennels MB, Edwards KM, Keyserling HL, et al. Safety and immunogenicity of heptavalent pneumococcal vaccine conjugated to CRM197 in United States infants. Pediatrics 1998; 101(4):604–11.
[36] Choo S, Seymour L, Morris R, Quataert S, et al. Immunogenicity and reactogenicity of a pneumococcal conjugate vaccine administered combined with a *Haemophilus influenzae* type b conjugate vaccine in United Kingdom infants. Pediatr Infect Dis J 2000;19(9):854–62.
[37] Miller MA, Meschievitz CK, Ballanco GA, Daum RS. Safety and immunogenicity of PRP-T combined with DTP: excretion of capsular polysaccharide and antibody response in the immediate post-vaccination period. Pediatrics 1995;95(4):522–7.
[38] Daum RS, Hogerman D, Rennels MB, et al. Infant immunization with pneumococcal CRM197 vaccines: effect of saccharide size on immunogenicity and interactions with simultaneous administered vaccines. J Infect Dis 1997;176(2):445–55.
[39] Banatvala J, Van damme PV, Oehen S. Lifelong protection against hepatitis B: the role of vaccine immunogenicity in immunememory. Vaccine 2001;19(7/8):877–85.
[40] European Consensus Group on Hepatitis B immunity. Are booster immunizations needed for lifelong hepatitis B immunity? Lancet 2000;355:561–5.
[41] Calendrier Vaccinal 2006. Avis du Conseil Supérieur d'Hygiène Publique de France, 19 Mai 2006. Bulletin Epidémiologique Hebdomadaire no. 29–30/2006;18 Juillet 2006:211–226.
[42] Epidemiologishes Bulletin. Robert Koch Institut. 28. Juli 2006/Nr. 30.
[43] Reinert P, Guy M, Girier B, et al. The safety and immunogenicity of an heptavalent pneumococcal polysaccharide conjugate vaccine (Prevenar) administered in association with a whole-cell pertussis-based paediatric combination vaccine (DTP–IPV/PRP-T) to French infants with a two-, three-, and four-month schedule. Arch Pediatr 2003;10(12):1048–55.
[44] Knuf M, Habermehl P, Cimino C, Petersen G, Schmitt HJ, et al. Immunigenicity, reactogenicity and safety of a 7-valent pneumococcal conjugate vaccine (PCV7) concurrently administred with a DTPa–IPV–HBV/Hib combination vaccine in healthy infants. Vaccine 2006;24(22):4727–36.
[45] European Medicines Agency. Press release 20 September 2005 (Doc Ref. EMEA/297369/2005). http://www.emea.europa.eu/pdfs/human/press/pr/29736905en.pdf.

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Immunogenicity, reactogenicity and safety of a 7-valent pneumococcal conjugate vaccine (PCV7) concurrently administered with a DTPa-HBV-IPV/Hib combination vaccine in healthy infants

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Abstract

Background: To evaluate immunogenicity, reactogenicity, and safety of a hexavalent combination vaccine diphtheria-tetanus-acellular pertussis-hepatitis B-inactivated polio virus-*Haemophilus influenzae* type b (DTPa-HBV-IPV/Hib) when coadministered with a 7-valent pneumococcal conjugate vaccine (PCV7).

Methods: Infants received either a hexavalent diphtheria-tetanus-acellular pertussis-hepatitis B-inactivated polio virus-*H. influenzae* type b vaccine concomitantly with PCV7 or DTPa-HBV-IPV/Hib alone infants were vaccinated at 2, 3 and 4 months (primary immunization) and 12–15 months of age (booster dose). Local and systemic reactions and adverse events were monitored following each dose and compared between groups. Blood was obtained prior to dose 1, one month after dose 3, immediately prior to and 1 month following the booster dose to measure antibody responses to each of the antigens.

Results: Two hundred and fifty-three subjects (PCV7, 127; Control, 126) were enrolled. Antibody responses were compared in 226 subjects for the primary immunization and 212 for the booster dose (per-protocol (PP) population). Although there were some differences in geometric mean concentrations (GMCs) to the DTPa-HBV-IPV/Hib antigens after the primary series, GMCs for all antigens after the booster dose were similar in both groups, except for diphtheria which was significantly higher in the PCV7 group (PCV7, 7.41 IU/mL; Control, 5.78 IU/mL). Reactogenicity and safety data were compared in 252 infants receiving primary immunization and 235 children receiving the booster dose. Site reactions were similar in both groups. Fever ≥38.0 °C following each vaccination was reported more frequently in the PCV7 group (28.3–50.0%) than in the Control group (15.6–33.6%) whereas fever >39.0 °C occurred only in a few cases and to the same extent in both groups (PCV7, 0.8–2.7%; Control, 1.6–4.1%). Only one reported serious adverse event was characterized as being related to the study vaccines: control subject was hospitalized with a fever.

Conclusion: DTPa-HBV-IPV/Hib and PCV7 were highly immunogenic, well-tolerated and safe when coadministered at 2, 3 and 4 months of age with a booster dose at 12–15 months of age. These results support the coadministration of PVC7 with DTPa-HBV-IPV/Hib as part of the routine immunization schedule for infants and children.

Keywords: 7-valent pneumococcal conjugate vaccine; Hexavalent diphtheria-tetanus-acellular pertussis-hepatitis B-inactivated polio virus-*Haemophilus influenzae* type b vaccine; Concomitant administration

1. Introduction

Streptococcus pneumoniae is a significant cause of morbidity and mortality worldwide in persons of all ages, and it is the most common bacterial cause of otitis media, pneumonia and bacteremia in children [1]. Moreover, with the

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widespread use of *Haemophilus influenzae* type b (Hib) conjugate vaccines and the resulting virtual eradication of invasive Hib disease in immunized children [2], pneumococcus has become one of the leading causes of childhood bacterial meningitis. Analysis of bacterial meningitis in children has shown that the disease caused by pneumococci has substantially greater mortality and neurologic sequelae than meningitis caused by Hib or *Neisseria meningitides* [3].

In children between 6 and 24 months of age, the invasive pneumococcal disease (IPD) incidence ranges from 10 to 40 cases per 100,000 patient years in countries such as Denmark, Finland, France, Germany, and the UK [4,5]. By contrast, it is reported to be in the range of 50–170 cases per 100,000 patient years among Spanish children [6].

The continued prevalence and severity of invasive pneumococcal infections, together with the emergence of pneumococcal strains that are resistant to routinely used antibiotic regimens [7,8], have emphasized the need for an improved vaccine for the prevention of pneumococcal infections in children.

Licensure of 7-valent pneumococcal conjugate vaccine (PCV7) in the USA followed a randomized control study in which PCV7 administered to 38,000 infants at 2, 4, 6 and 12–15 months of age was shown to be 97.4% (82.7–99.9%) efficacious in preventing invasive pneumococcal disease due to vaccine serotypes [9]. The benefits of PCV7, however, go far beyond the prevention of IPD in vaccinated children [10]. In addition, the induction of herdimmunity, especially in non-vaccinated elderly people, and a substantial influence on the reduction of antibiotic-resistence profiles are other major advantages of PCV7 [10]. In February 2001 PCV7 was licensed in the European Union.

Two hexavalent combination vaccines containing diphtheria, tetanus, acellular pertussis, inactivated poliovirus, hepatitis B and *H. influenzae* type b antigens were already licensed in Europe (October 2000) and routinely used for childhood vaccination in several European countries. In Germany the hexavalent vaccines (GSK and Aventis Pasteur) were currently administered in more than 90% of routine childhood vaccinations.

In September 2005, the European Medicines Agency (EMEA) recommends as a precautionary measure the suspension of the marketing authorisation for Hexavac™ due to concerns about the long-term protection against hepatitis B.

This study was designed to assess the immunogenicity, reactogenicity, and safety of DTPa-HBV-IPV/Hib vaccine (Infanrix hexa, GSK Biologicals) when coadministered with PCV7 (Prevenar, Wyeth) following the Germany immunization schedule at 2, 3, 4 and 12–15 months of age.

2. Methods

This was an open-labeled randomized study conducted in 27 pediatric offices across Germany. Approval was obtained from the respective ethics review committee of each of the participating study sites.

2.1. Study objectives

The main objective of the study was to assess the immunogenicity, reactogenicity, and safety of DTPa-HBV-IPV/Hib vaccine when coadministered with PCV7 according to the schedule recommended by the German Advisory Committee of Immunization Practices (Ständige Impfkommission, STIKO) at 2, 3, 4 and 12–15 months of age.

2.2. Subjects

Healthy infants 2 months of age (57–112 days of age at enrollment) who were available for the entire study period and with written consent were eligible to participate. The relative wide range of the age at enrollment represents the vaccination practice in Germany and should be without any impact on the results and conclusions. Excluded were children with a known or suspected history of diphtheria, pertussis, tetanus, polio, Hib or hepatitis B-related disease or invasive pneumococcal-related diseases; a prior history of immunization against these diseases; a previous anaphylactic reaction or serious vaccine-associated adverse reaction; a known hypersensitivity to any component of the study vaccines; a birth weight of <2500 g and/or less than 37 gestational weeks; a vaccination of the mother with a hepatitis B virus vaccine in the previous 12 months; a known or suspected impairment of the immune system or a treatment with immunosuppressive agents; a major congenital, developmental or serious chronic disorder; a confirmed or suspected underlying evolving neurological disorder or history of encephalopathy and/or seizures, sequelae of perinatal hypoxia; a family history suggesting that the child is at increased risk of an evolving genetic neurological disorder; or a known bleeding disorder for which intramuscular administration of vaccine is contraindicated. Additionally, children participating in another investigational study within 30 days before study inclusion were excluded.

2.3. Study design

Subjects were randomly assigned in a 1:1 ratio into one of two groups in balanced blocks specified for each site: PCV7 group receiving PCV7 in the left thigh and DTPa-HBV-IPV/Hib in the right thigh or Control group receiving DTPa-HBV-IPV/Hib alone. Assignments were linked to a subject number serially assigned at enrollment. Subjects received vaccines at 2, 3 and 4 months of age and again at 12–15 months of age. After completing primary immunization with DTPa-HBV-IPV/Hib, the Control group subjects were offered three doses of PCV7, to be administered at 5, 6 and 7 months of age and after completing booster immunization were offered a booster dose of PCV7 to be administered

at 12–16 months of age. These results will not be presented here.

2.4. Vaccines

Commercial lots of vaccines (0.5 mL dose) were injected intramuscularly at separate sites. A 0.5 mL dose of the DTPa-HBV-IPV/Hib vaccine (GlaxoSmithKline Biologicals, Rixensart Belgium) was prepared by reconstituting the lyophilized Hib component with the liquid DTaP-HBV-IPV. Each vaccine dose contains ≥30 IU diphtheria toxoid, ≥40 IU tetanus toxoid; 3 acellular pertussis components: 25 μg of pertussis toxoid (PT), 25 μg of filamentous hemagglutinin (FHA), 8 μg of pertactin (PRN); 10 μg of recombinant hepatitis B surface antigen (HBsAg); 40 D-antigen units of type 1, 8 D-antigen units of type 2 and 32 D-antigen units of type 3 polio virus; 10 μg of the Hib polysaccharide polyribosylribitol phosphate (PRP) conjugated to 20–40 μg of tetanus toxoid and 0.82 mg of aluminum as salts. Two lots of vaccine were used.

Each 0.5 mL dose of Prevenar (Wyeth Pharmaceuticals Inc., Philadelphia, PA) contains seven capsular antigens of *S. pneumoniae* individually conjugate to diphtheria CRM_{197}: 2 μg of each serotypes 4, 9V, 14, 18C, 19F, and 23F and 4 μg of serotype 6B, (16 μg total saccharide); approximately 20 μg of CRM_{197} carrier protein; and 0.125 mg of aluminum per dose as aluminum phosphate. One lot of vaccine was used in this study.

2.5. Immunogenicity evaluation

Venous blood samples were obtained immediately prior to administration of dose 1, one month following administration of dose 3 (21–42 days), immediately prior to administration of the booster dose and 1 month following administration of the booster dose (21–42 days), Standardized assays were performed to measure antibody responses to the vaccines by laboratory staff blinded to the subject's assigned groups [11–18].

Concentrations of antibodies to all seven pneumococcal serotypes were determined using standardized ELISA and serological concentration of 0.15 and 0.5 μg/mL. Concentration of antibodies against tetanus, Hib (PRP-T), hepatitis B, and pertussis (FHA, PT, PRN) were measured by standard ELISA techniques [14–16], antibodies to poliovirus 1, 2 and 3 by a using a neutralization assay [17], and diphtheria antibodies by Vero Cell Microneutralization Assay [18]. Usually accepted protective levels for diphtheria antitoxin (≥0.01 and ≥0.10 IU/mL), tetanus antitoxin (≥0.01 and ≥0.10 IU/mL), PRP antibody (≥0.15 and ≥1.00 μg/mL), neutralizing antibody for poliovirus types 1, 2 and 3 (≥1:8), and anti-HBS antibody (≥10 mIU/mL) were used in the calculation of protective immune responses [19–21]. In the absence of well-established serological markers for protection against pertussis, antibody levels ≥2-fold and ≥4-fold rises from baseline were set for pertussis FHA, pertussis toxin and pertactin.

2.6. Safety evaluation

Following each immunization the infant was observed for 30 min for any immediate reaction. For the PCV7 group parents were unaware of which vaccine was injected in the thigh. Parents/guardians of the subjects were asked to monitor and record on diary cards local injection site reactions (erythema, induration, swelling or tenderness), fever (daily rectal temperatures) and other systemic events (fussiness, sleepiness, and decreased appetite) on the day of immunization and for 3 days after each immunization. The use of antipyretic treatment was also recorded. Diary cards were returned to the study site at the next visit. At least one telephone interview with parents was conducted after the primary and prior to the booster immunizations to obtain information regarding subjects' clinical status for both groups. Illnesses or events that required physician intervention or the use of a prescription medication within 7 days of immunization were recorded. Hospitalization or other serious adverse events were reported at any time during the course of the study.

2.7. Statistical analysis

Distribution of gender, mean age at first dose, weight and height were summarized.

The response of primary interest was the comparability of immune responses to DTaP-IPV-HBV/Hib antigens when DTaP-IPV-HBV/Hib is given concurrently with PCV7 at two separate injection sites or given alone as measured by geometric mean concentrations (GMCs) and antibody response rates at predefined levels generally indicative of protection. Post-dose GMCs were compared between treatment groups by ANCOVA, using pre-dose concentrations as a covariate. The lower and upper 95% confidence limits on the mean of the GMCs were calculated. The percentage of subjects with defined antibody concentrations was calculated for each treatment group after dose 3 and after the booster dose. The differences in the percentages between treatment groups were analyzed using Fisher's exact test.

Rates of local reactions and systemic events over the 3 days post-vaccination were calculated for each treatment group and dose. Rates were compared between treatment groups using Fisher's exact test (two-sided).

3. Results

3.1. Study population

A total of 252 infants were enrolled and received vaccine (126 per group) and 235 children (93.3%: PCV7, 116; Control, 119) completed the study. Of the 17 prematurely terminated subjects most were due to parental request or loss to follow up. One subject (Control group) inadvertently received two different hexavalent combination vaccines following the primary phase and was withdrawn from further study.

Per-protocol (PP) immunogenicity results are presented. The PP primary immunization cohort was defined as vaccinated subjects who: (1) met all eligibility criteria; (2) complied with protocol defined procedures (i.e., immunized at appropriate age for primary series); (3) had follow up blood drawn 28–42 days [±1 day] post-vaccination; and (4) had available post immunization data for at least one pneumococcal serotype or for at least one of the DTPa-HBV-IPV/Hib antigens. The PP booster immunization cohort were subjects that completed primary immunizations on schedule, were immunized at appropriate age for the booster immunization, blood sampling was on schedule (timing between vaccination and blood withdraw 28–42 days [±1 day]; pre-booster blood work was done ≤7 days before administration of booster and had available post immunization for at least one pneumococcal serotype or for at least one of the DTPa-HBV-IPV/Hib antigens. A total of 226 (PCV7, 115; Control, 111) and 212 (PCV7, 106; Control, 106) subjects were included in the analysis of primary and booster cohorts, respectively.

At enrollment both groups were similar with respect to gender (male: PCV7, 50.8%; Control, 52.4%), mean age (days) at first vaccine dose (PCV7, 80.7 days; Control, 78.9) days, mean weight (PCV7, 5780 g; Control, 5794 g) and height (PCV7, 59.9 cm; Control, 60.0 cm).

3.2. Immunogenicity results

3.2.1. Antibody response to DTaP-IPV-HBV/Hib antigens

Statistically significant increases from baseline (pre-dose 1) to post-dose 3 were observed for all antigens of the DTaP-IPV-HBV/Hib vaccine. Following primary series GMCs for diphtheria (PCV7, 0.15 IU/mL, Control, 0.09 IU/mL/$p = 0.001$), tetanus (PCV7, 2.70 U/mL; Control, 3.57 U/mL/$p = 0.017$), pertussis FHA (PCV7, 75.63 U/mL; Control, 86.62 U/mL/$p = 0.001$), pertussis PRN (PCV7, 193.80 U/mL; Control, 244.57 U/mL/$p = 0.006$) and poliovirus type 1 (PCV7, 198.31 U/mL; Control, 299.25 U/mL/$p = 0.033$) were statistically different between the groups. No statistically significant differences were found between the groups for post-booster GMCs except for diphtheria (PCV7, 7.41 IU/mL; Control, 5.78 IU/mL/$p = 0.008$), which was significantly higher in the PCV7 group. GMCs for all antigens in both groups declined at 12 months but rose sharply after the booster immunization (Table 1).

No statistically significant differences were seen between treatment groups regarding the percentage of subjects achieving predefined post-dose 3 antibody levels for all vaccine antigens with the exception of diphtheria (Table 2). The percentage of subjects in the PCV7 group having achieved a diphtheria antibody level of ≥0.10 IU/mL was significantly higher compared to the Control group (PCV7, 59.1%; Control, 39.64%/$p = 0.004$). Post-dose 4, no significant difference between the treatment groups was detected for any of the antigens contained in the hexavalent vaccine (Table 2).

Table 1
Antibody geometric mean concentration (GMC; 95% CI) to DTaP-IPV-HB/Hib vaccine post-dose 3 and pre/post-booster dose

Antigens	PCV7 group GMC[a] (95% CI)			Control group GMC[a] (95% CI)		
	Post-dose 3	Pre-booster dose	Post-booster dose	Post-dose 3	Pre-booster dose	Post-booster dose
	$N = 115$	$N = 106$		$N = 111$	$N = 106$	
Diphtheria	0.15[b] (0.12–0.19)	0.19 (0.16–0.24)	7.41[b] (6.37–8.63)	0.09[b] (0.08–0.11)	0.22 (0.17–0.28)	5.78[b] (4.88–6.85)
Tetanus	2.70[b] (2.32–3.15)	0.48 (0.40–0.58)	8.09 (6.89–9.51)	3.57[b] (2.92–4.35)	0.61 (0.50–0.74)	8.97 (7.60–10.59)
Pertussis FHA	75.632 (68.40–83.62)	20.26 (17.20–23.85)	165.6 (148.03–185.17)	86.62[b] (78.44–95.64)	25.32 (21.07–30.42)	171.4 (148.59–197.62)
Pertussis toxin	46.54 (41.89–51.70)	9.64 (8.32–11.17)	71.3 (63.69–79.77)	52.92 (46.98–59.61)	10.66 (8.01–12.75)	77.9 (66.39–91.48)
Pertactin	193.80[b] (168.33–223.11)	33.35 (27.31–40.73)	542.3 (444.28–661.98)	244.57[b] (211.75–282.48)	42.43 (35.09–51.30)	663.8 (554.21–795.02)
Hib (PRP-T)	1.16 (0.92–1.47)	0.32 (0.27–0.39)	11.43 (8.85–14.75)	1.16 (0.89–1.51)	0.39 (0.31–0.48)	13.15 (9.86–17.55)
Poliovirus type 1	198.31[b] (152.70–257.53)	26.13 (18.95–36.03)	869.57 (710.91–1063.62)	299.25[b] (218.86–409.18)	41.40 (29.03–59.04)	1153.2 (917.92–1448.77)
Poliovirus type 2	206.54 (152.85–279.09)	39.19 (28.58–53.75)	1438.72 (1156.70–1789.49)	324.56 (243.59–432.45)	57.21 (39.60–82.64)	1782.89 (1454.95–2184.74)
Poliovirus type 3	781.78 (623.61–980.07)	76.86 (57.87–102.08)	2200.74 (1815.77–2667.34)	926.63 (741.95–1157.28)	92.02 (65.66–128.95)	2276.15 (1852.18–2797.17)
Hepatitis B	404.52 (295.65–553.48)	192 (137.47–269.89)	4523.2 (3223.68–6346.64)	438.79 (329.59–584.19)	272.54 (211.12–351.83)	6539.2 (4979.0–8588.23)

[a] GMCs are expressed as μg/mL for Hib (PRP), IU/mL for diphtheria and tetanus, IU/mL for pertussis antigens and mIU/mL for hepatitis B and as neutralizing antibody/mL for poliovirus type 1, 2 and 3.
[b] p-value ($p < 0.05$) assesses the difference between treatment groups (geometric mean ratios) using Fisher's exact test.

Table 2
Percentage of subjects (%; 95% CI) with defined diphtheria, tetanus, pertussis, Hib, poliovirus and hepatitis B antibody concentrations post-dose 3 and pre/post-booster dose (dose 4)

Antigen		PCV7 group			Control group		
		Post-dose 3	Pre-booster dose	Post-booster dose	Post-dose 3	Pre-booster dose	Post-booster dose
		$N = 115$	$N = 106$		$N = 111$	$N = 106$	
Diphtheria	≥0.01 IU/mL	100.0 (100.0–100.0)	99.05 (97.19–100.0)	100.0 (100.0–100.0)	99.1 (97.34–100.86)	98.11 (95.52–100.0)	100.0 (100.0–100.0)
	≥0.10 IU/mL	59.13[a] (50.15–68.12)	69.52 (60.72–78.33)	100.0 (100.0–100.0)	39.64[a] (30.54–48.74)	73.58 (65.19–81.98)	100.0 (100.0–100.0)
Tetanus	≥0.01 IU/mL	100.0 (100.0–100.0)	100.0 (100.0–100.0)	100.0 (100.0–100.0)	100.0 (100.0–100.0)	100.0 (100.0–100.0)	100.0 (100.0–100.0)
	≥0.10 IU/mL	100.0 (100.0–100.0)	90.38 (84.72–96.05)	100.0 (100.0–100.0)	100.0 (100.0–100.0)	98.1 (95.48–100.0)	100.0 (100.0–100.0)
Hib (PRP-T)	≥0.15 μg/ml	93.91 (89.54–98.28)	78.10 (70.18–86.01)	100.0 (100.0–100.0)	90.99 (85.66–96.32)	81.13 (73.68–88.58)	100.0 (100.0–100.0)
	≥1.00 μg/ml	58.26 (49.25–67.27)	13.33 (6.83–19.84)	95.24 (91.16–99.31)	60.36 (51.26–69.46)	18.87 (11.42–26.32)	94.34 (89.94–98.74)
Pertactin	≥2-fold rise	95.58 (91.78–99.37)	–	–	95.45 (91.56–99.35)	–	–
	≥4-fold rise	86.73 (80.47–92.98)	n.a.	94.29 (89.85–98.73)	91.82 (86.70–96.94)	n.a.	95.24 (91.16–99.31)
Pertussis FHA	≥2-fold rise	85.09 (78.55–91.63)	–	–	89.19 (83.41–94.97)	–	–
	≥4-fold rise	71.93 (63.68–80.18)	n.a.	84.76 (77.89–91.64)	74.77 (66.70–82.85)	n.a.	77.88 (69.91–85.86)
Pertussis PT	≥2-fold rise	98.25[a] (95.84–100.66)	–	–	91.89[a] (86.81–96.97)	–	–
	≥4-fold rise	92.11 (87.16–97.06)	n.a.	78.64 (70.73–86.56)	89.19 (83.41–94.97)	n.a.	78.85 (71.0–86.7)
Poliovirus type 1	≥8	100.0 (100.0–100.0)	85.85 (79.21–92.48)	100.0 (100.0–100.0)	98.2 (95.72–100.67)	86.67 (80.16–93.17)	99.05 (97.19–100.0)
Poliovirus type 2	≥8	99.12 (97.39–100.84)	90.57 (85.00–96.13)	100.0 (100.0–100.0)	100.0 (100.0–100.0)	88.57 (82.49–94.66)	100.0 (100.0–100.0)
Poliovirus type 3	≥8	100.0 (100.0–100.0)	95.28 (91.25–99.32)	100.0 (100.0–100.0)	100.0 (100.0–100.0)	93.33 (88.56–98.10)	100.0 (100.0–100.0)
Hepatitis B	≥10 mIU/ml	96.4 (92.93–99.86)	94.23 (89.75–98.71)	99.04 (97.16–100.0)	99.05 (97.19–100.91)	100.0 (100.0–100.0)	100.0 (100.0–100.0)

[a] p-value ($p < 0.05$) assesses the difference between treatment groups using Fisher's exact test.

Table 3
Antibody geometric mean concentrations (μg/ml; 95% CI) to pneumococcal vaccine serotypes pre/post primary and booster immunization (PCV7 group)

Pneumococcal serotype	Primary immunization		Booster immunization	
	Pre-dose 1	Post-dose 3	Pre-booster dose	Post-booster dose
PCV7 group	$N = 115$		$N = 106$	
4	0.09 (0.07–0.12)	5.19 (4.57–5.89)	0.55 (0.47–0.63)	4.91 (4.25–5.68)
6B	0.42 (0.33–0.54)	3.20 (2.68–3.83)	0.61 (0.50–0.75)	9.27 (7.60–11.30)
9V	0.25 (0.21–0.31)	2.60 (2.25–3.01)	0.51 (0.44–0.59)	2.87 (2.48–3.32)
14	0.38 (0.28–0.51)	6.88 (5.75–8.24)	2.33 (1.93–2.82)	9.34 (7.94–10.99)
18C	0.21 (0.17–0.25)	2.38 (2.08–2.71)	0.30 (0.26–0.34)	2.37 (2.03–2.78)
19F	0.49 (0.39–0.61)	4.44 (3.93–5.03)	0.61 (0.52–0.72)	4.50 (3.84–5.28)
23F	0.18 (0.14–0.22)	2.00 (1.67–2.40)	0.35 (0.29–0.43)	6.14 (4.77–7.91)

3.2.2. Antibody response to pneumococcal antigens

A statistically significant increase in GMCs was seen for all seven serotypes 1 month after dose 3 for the PCV7 group (Table 3). After dose 3, 98.3% (serotypes 9V and 23F) to 100% (serotypes 4, 6B, 14, 18C, and 19F) of subjects achieved pneumococcal antibody concentrations ≥0.15 μg/mL and 92.1% (serotype 23F) to 99.1% 1 (serotype 4) of subjects achieved concentrations ≥0.50 μg/mL (Table 4).

After boosting with PCV7, pneumococcal GMCs in the PCV group ranged from 2.87 to 9.34 μg/mL (Table 3). 97.2% (serotype 23F) to 100% (serotypes 4, 6B, 9V, 4, 18C, and 19F) of subjects achieved pneumococcal antibody concentrations of ≥0.15 μg/mL and 96.2% (serotype 23F) to100.0% (serotype 4) of subjects achieved concentrations ≥0.50 μg/mL after the booster dose (Table 4).

3.3. Safety assessment

All infants who had received at least one dose of vaccine were included in the safety analysis. The safety population comprised of 252 infants (126 per group) for primary immunization and 240 children (PCV7, 116; Control, 124) for the booster immunization.

3.3.1. Local reactogenicity

There were no statistically significant differences after administration of PCV7 in the left thigh and DTPa-HBV-IPV/Hib in the right thigh with local reactions. Frequency of local reactions did not increase from dose 1 to dose 3, but was slightly higher after the booster dose in both groups. Significant local reactions were seen in 0.8–8.5% regarding the different doses and the type of reaction (Table 5). Further, there were no statistically significant differences between the two groups regarding local reactions at the DTPa-IPV-HBV/Hib injection site.

3.3.2. Systemic reactogenicity

The percentage of infants with any systemic reaction (except fever) decreased from dose 1 to 3 in both treatment groups but increased slightly with dose 4 (Table 6). After dose 1, the percentage of subjects with any systemic reaction was significantly higher in the PCV7 group (64%) than in the control group (48%) mainly due to a higher rate of sleepiness (PCV7, 43.2%; Control, 27.4%/$p = 0.011$). No statistically significant difference was seen after doses 2 and 3 except decreased appetite after dose 3, which was significantly higher in the PCV7 group (PCV7, 21.8%; Control, 7.6%/$p = 0.003$). After dose 4, sleepiness, fussiness and also presence of any event occurred more frequent in the PCV7 group.

Fever greater than 38.0 °C was seen significantly more often in the PCV7 group compared to the Control group after dose 1 (PCV7, 32.8%; Control, 15.6%/$p = 0.002$), dose 2 (PCV7, 42.3%; Control, 32.1%/$p = 0.002$) and the booster dose (PCV7, 50.0%; Control, 33.6%/$p = 0.015$). Fever >39.0 °C was seen in 19 cases, 7 in the PCV7 group (dose 1, 1; dose 2, 1; dose 3, 2; dose 4, 3) and 12 in the Control group (dose 1, 2; dose 2, 5; dose 3, 2; dose 4, 3). Intake of antipyretic medication was reported with a frequency

Table 4
Proportion of subjects achieving pneumococcal antibody concentrations of ≥0.15 and ≥0.50 μg/mL (%; 95% CI) after dose 3 and after booster dose (dose 4) of pneumococcal conjugate vaccine (PCV7 group)

Pneumococcal serotype	Post-dose 3 ($N = 115$)		Post-booster dose ($N = 106$)	
	≥0.15 μg/mL	≥0.50 μg/mL	≥0.15 μg/mL	≥0.50 μg/mL
4	100.0 (100.0–100.0)	99.13 (97.43–100.83)	100.0 (100.0–100.0)	100.0 (100.0–100.0)
6B	100.0 (100.0–100.0)	96.52 (93.17–99.87)	100.0 (100.0–100.0)	98.11 (95.52–100.0)
9V	98.25 (95.84–100.66)	97.37 (94.43–100.31)	100.0 (100.0–100.0)	98.11 (95.52–100.0)
14	100.0 (100.0–100.0)	97.39 (94.48–100.3)	100.0 (100.0–100.0)	99.06 (97.22–100.0)
18C	100.0 (100.0–100.0)	97.35 (94.38–100.31)	100.0 (100.0–100.0)	99.06 (97.22–100.0)
19F	100.0 (100.0–100.0)	98.26 (95.87–100.65)	100.0 (100.0–100.0)	98.11 (95.52–100.0)
23F	98.25 (95.84–100.66)	92.11 (87.16–97.06)	97.17 (94.01–100.0)	96.23 (92.6–99.85)

Table 5
Frequency of local reactions over four doses at the injection site of 7-valent pneumococcal conjugate vaccine (PCV7) and hexavalent combination vaccine (Hexa) for PCV7 group

Reaction	Dose 1 (2 months)			Dose 2 (3 months)			Dose 3 (4 months)			Dose 4 (12–15 months)		
	PCV7 (left site)	Hexa (right site)	p-value[a]	PCV7 (left site)	Hexa (right site)	p-value[a]	PCV7 (left site)	Hexa (right site)	p-value[a]	PCV7 (left site)	Hexa (right site)	p-value[a]
N[b]	118–119	119–122		119–120	118–120		114–117	117–118		108–111	108–110	
Erythema												
Any	21.8	21.3	1.0000	29.2	32.5	0.6751	23.9	29.7	0.3774	32.1	37.6	0.4774
Any significant[c]	0.8	0.8	1.0000	3.3	1.7	0.6835	3.4	4.2	1.0000	7.3	10.1	0.6321
Induration												
Any	24.4	24.8	1.0000	28.6	36.7	0.2147	21.6	25.4	0.5388	36.9	43.6	0.3386
Any significant[c]	2.5	5.8	0.3335	5.0	7.5	0.5953	5.2	8.5	0.4385	8.1	9.1	0.8153
Swelling												
Any	5.9	7.4	0.7970	7.5	11.7	0.3808	8.6	11.9	0.5192	11.8	17.6	0.2549
Any significant[c]	1.7	3.3	0.6837	0.8	1.7	1.000	1.7	1.7	1.0000	0.9	3.7	0.2102
Tenderness												
Any	17.6	19.0	0.8679	15.0	16.1	0.8593	12.9	14.4	0.8496	26.4	23.9	0.7557
Any significant[d]	1.7	1.7	1.0000	0.8	1.7	0.6218	0.9	0.9	1.0000	1.9	1.09	1.000

[a] p-value assesses the difference of reactions between injection sites and was calculated using Mc Nemar's test.
[b] Number varies due to missing values.
[c] Defined as >2.4 cm.
[d] Interferes with leg movement.

between 6.0 and 18.9% and significantly higher in the PCV7 group after dose 2 (PCV7, 16.8%; Control, 7.5%/$p = 0.031$) and dose 4 (PCV7, 18.9%; Control, 6.1%/$p = 0.006$).

The overall frequency of subjects with adverse events was similar between treatment groups during primary immunization (50.0% in the PCV7 group versus 42.9% in the Control group). The most frequent adverse events reported were infection, pyrexia, and skin disorders. Few events (17.5% of subjects in the PCV7 group versus 11.1% in the Control group) were characterized by the investigator as possibly, probably or definitely related to the study vaccines. Following booster dose administration adverse events occurred in 35.3% (PCV7group) versus 25.5% (Control group) of subjects; 13.8% versus 9.2% of events were considered to be possibly, probably or definitely related to the study vaccines. Fever was the most frequently reported adverse event in both treatment groups (12.1% in PCV7 group, 7.6% in Control group).

Eight serious adverse events in six children (three in each group) were observed during primary immunization. Only one out of the events was characterized as probably related to the vaccination (hospitalization due to fever after dose 2 in the Control group). Serious adverse events after booster dose were reported in four children (two in each group). None of these events was considered by the investigators to be related to administration of study vaccines.

Following the primary phase, one subject (Control group) received two different hexavalent combination vaccines and was withdrawn from further study. The event was reported as

Table 6
Frequency of systemic reactions over four doses of 7-valent pneumococcal conjugate vaccine co-administered with a DTaP-IPV-HB/Hib vaccine (PCV7 group) or DTaP-IPV-HB/Hib vaccine alone (Control group)

Reaction	Dose 1 (2 months)			Dose 2 (3 months)			Dose 3 (4 months)			Dose 4 (12–15 months)		
	PCV7 group	Control group	p-value[a]	PCV7 group	Control group	p-value[a]	PCV7 group	Control group	p-value[a]	PCV7 group	Control group	p-value[a]
N[b]	122–125	120–124		122–123	121–124		119–120	118–120		107–112	114–115	
Fever ≥38 °C	32.8	15.6	0.0018	42.3	32.1	0.0017	28.3	18.3	0.0927	50.0	33.6	0.0148
Fever ≥39.1 °C	0.8	1.6	0.6189	0.8	4.1	0.1181	1.7	1.7	1.0000	2.7	2.7	1.0000
Decreased appetite	24.4	15.8	0.1109	20.5	15.6	0.4053	21.8	7.6	0.0030	18.5	16.7	0.7280
Sleepiness	43.2	27.4	0.0117	27.9	25.8	0.7741	20.8	15.0	0.3125	35.5	10.5	<0.0001
Fussiness	39.3	30.1	0.1411	36.9	25.4	0.0720	26.1	25.8	1.0000	47.7	19.1	<0.0001
Any event[c]	64.0	48.4	0.0153	52.8	44.4	0.2039	45.0	32.5	0.0633	60.7	27.8	<0.0001
Antipyretic use	13.2	10.0	0.5472	16.8	7.5	0.0305	12.1	6.0	0.1686	18.9	6.1	0.0066

[a] p-value assesses the difference between treatment groups and is calculated using Fisher's exact test.
[b] Number varies with reaction.
[c] Does not include fever.

an overdose and except for safety follow up, the subject was withdrawn from further participation.

No deaths were reported during the entire study period.

4. Discussion

Hexavalent combination vaccines are widely used in Germany and some other European countries for routine childhood vaccination. PCV7 marketed in Germany since April 2001, is administered only to infants and children up to the age of 5 years with an increased risk for pneumococcal infections with focus on comorbidities and perinatal status following STIKO recommendations (German Advisory Committee of Immunization Practice). In contrast, a broad vaccination program with PCV7 has been implemented in France since 2002, which includes children at higher risk for pneumococcal infection (i.e. comorbidities or day care settings, multiple siblings, limited breast feeding, etc.) and in some regions of Italy.

This study was designed to assess the safety, immunogenicity and reactogenicity of DTPa-HBV-IPV/Hib (GSK) when concomitantly administrated with PCV7 according to the recommended immunization schedules in Germany and several other European countries [22]. In the PCV7 group, PCV7 was administered concurrently with DTPa-HBV-IPV/Hib according to the German primary immunization schedule at 2, 3 and 4 months of age and a booster between 12 and 15 months of age. The Control group received DTPa-HBV-IPV/Hib alone at 2, 3 and 4 months, with a booster given at 12–15 months. Vaccination with PCV7 was offered to the Control group at 5, 6 and 7 months of age with booster given 1 month after the booster dose of DTPa-HBV-IPV/Hib. A similar study was conducted in Germany and France to evaluate the 7-valent pneumococcal vaccine concurrently administered with the other DTaP-IPV-HBV-Hib vaccine (Hexavac®, Aventis Pasteur) [23,24].

This study demonstrated that coadministration of PCV7 did not diminish responses to DTPa-HBV-IPV/Hib. GMCs for all antigens contained in the DTPa-HBV-IPV/Hib vaccine increased statistically significant from baseline to post-dose 3 and also from pre-booster to post-booster dose. Diphtheria antibody concentrations were significantly higher in the PCV7 group than in the Control group which corresponds to results from other studies with the PCV7concomitantly administered with a pentavalent combination DTaP-IPV-Hib vaccine [9]. Although statistically significant differences occurred in the current study between the groups regarding GMC concentrations for tetanus, pertussis FHA, and PRN, as well as for poliovirus type 1 after primary immunization, no difference was seen after booster dose. Regarding the percentage of subjects achieving the predefined antibody levels of the DTPa-HBV-IPV/Hib vaccine no difference between the groups exist except for diphtheria which was significantly higher in the PCV7 group after dose 3. In other studies with CRM197 based conjugated vaccine higher diphtheria antitoxin response has been consistently reported suggesting carrier protein mediated enhancement phenomenon [26–28]. However, such an effect is unlikely to be relevant since in both groups the same high level of children achieving seroprotective titer criteria for diphtheria neutralizing toxoids was achieved.

In this study, PCV7 was highly immunogenic for all serotypes contained in the vaccine. After primary immunization, GMCs for all seven serotypes had increased significantly from baseline for the PCV7 group, and decreased, as expected, from baseline in the Control group. GMCs declined between the third dose and the booster dose. However, the booster dose between 12 and 15 months of age induced an antibody response at least comparable to that after the third dose of the primary series for serotypes 4, 9V, 18C and 19F or even led to a further increase of specific antibodies for serotypes 6B, 14 and 23F. Because control infants were administered PCV7 at 5, 6 and 7 months of age no analysis was done on this group. The results in this study were similar compared to a published study with a similar design except that PCV7 was administered to control infants after the completion of the booster phase. Tichman et al. reported similar PCV7 responses post primary and post-booster except for a significantly lower immune response to pneumococcal serotype 6B (primary: 3.20 μg/mL versus 0.91 μg/mL/booster: 9.27 μg/mL versus 3.92 μg/mL) and higher response to serotype 9V after booster dose (2.87 μg/mL versus 6.77 μg/mL) [20]. The pneumococcal assays were performed at GlaxoSmithKline Biologicals and assay variability from lab to lab may explain the differences noted above.

In this study more than 98% (serotypes 9V and 23F) of subjects and more than 92% (serotype 23F) of subjects achieved antibody concentrations ≥015 and ≥0.50 μg/mL, respectively, following three doses of PCV7. Following the booster immunization more than 97% (serotype 23F) of subjects were ≥015 μg/mL and more than 96% (serotype 23F) of subjects were ≥0.50 μg/mL. Oliver et al. reported similar GMCs and slightly lower percentage of subjects with serotype 23F antibody concentrations ≥0.50 μg/mL (82.4% after dose 3 and 89.7% after dose 4) with subjects that were administered PCV7 with DTaP-IPV-HBV-Hib produced by Sanofi Pasteur MSD [23]. Pneumococcal assays was performed in the same laboratory as in this study. The results in this study are also comparable to the immunogenicity results that were observed in the PCV7 efficacy trial conducted in the United States where PCV7 was administered at 2, 4 and 6 months of age and 97.7% efficacy was measured [9,19,20,25]. Black et al. reported 97% of the PCV7 recipients achieved pneumococcal antibody concentrations ≥0.15 μg/mL for all serotypes after the primary series and between 82% (for serotype 23F) and 97% (for serotype 14) achieved antibody levels ≥0.50 μg/mL. He proposed that the minimum antibody associated with protection following three doses was in the range of 0.15–0.50 μg/mL.

For both treatment groups, local reactions at the PCV7and DTPa-HBV-IPV/Hib injection sites were comparable in overall frequency and severity after each dose. When PCV7 was administered with the DTPa-HBV-IPV/Hib, the rate of febrile reactions was higher than when the DTPa-HBV-IPV/Hib vaccine was administered alone. These reactions were low-grade (38–39 °C) and transient. Fever reactions >39.0 °C occurred only in a few subjects with no statistical difference between the groups and with no clinically significant complications like febrile seizures. A low proportion of subjects (less than 20%) were treated with antipyretics according to local medical practice with a higher rate in the PCV7 group (12.1–18.9%) compared to the Control group (6.0–10.0%). These results were similar to a published study where PCV7 was administered to subjects with the pentavalent DTaP-IPV-Hib vaccine [19].

The frequency of other systemic events was comparable between treatment groups after dose 1–3 and was more frequent in the PCV7 group compared to the Control group following the booster dose. Unsolicited adverse events reported by the investigators were similar for both treatment groups; few of them were attributed to the study vaccine. Only one serious adverse event (hospitalization due to fever after vaccination in a subject of the Control group) was attributed to the study vaccines. No deaths occurred during this study.

Overall, both study vaccines PCV7 and DTaP-PV-HBV/Hib were highly immunogenic, well tolerated and safe when concurrently administered at 2, 3, 4 and 12–15 months of age. In conclusion, PCV7 did not show any clinically relevant influence on the immunogenicity and safety of the concurrently administered vaccine DTaP-IPV-HBV/Hib, demonstrating that it is compatible with the DTaP-IPV-HBV/Hib vaccine as part of the routine immunization schedule for infants and children.

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References

[1] Klein JO. The epidemiology of pneumococcal disease in infants and children. Rev Infect Dis 1981;3:246–53.

[2] Adam WG, Deaver KA, Cochi SL, et al., Haemophilus influenzae Study Group. Decline of childhood *Haemophilus influenzae* type b (Haemophilus) disease in the Haemophilus vaccine era. JAMA 1993;269:221–6.

[3] Baraff LJ, Lee SI, Schriger DL. Outcomes of bacterial meningitis in children: a meta-analysis. Pediatr Infect Dis J 1993;12:389–94.

[4] Hausdorff WP. Invasive pneumococcal disease in children: geographic and temporal variations in incidence and serotype distribution. Eur J Pediatr 2002;161(Suppl. 2):S135–9.

[5] Von Kries R, Siedler A, Schmitt HJ, Reinert RR. Proportion of invasive pneumococcal infections in German children preventable by pneumococcal conjugate vaccines. Clin Infect Dis 2000;31(2):482–7.

[6] Diez-Domingo J, Pereiro I, Morant A, Gimeno C, Lerma M, Oyaguez I, et al. Epidemiology of invasive *Streptococcus pneumoniae* infections in children in Spain, 1996–1998. J Infect 2002;45:139–43.

[7] Magnus T, Andersen BM. Serotypes and resistance patterns of *Streptococcus pneumoniae* causing systemic disease in northern Norway. Eur J Clin Microbiol Infect Dis 1995;14:229–34.

[8] Appelbaum PC. Antimicrobial resistance in *Streptococcus pneumonias*: an overview. Clin Infect Dis 1992;15:77–83.

[9] Black S, Shinefield H, Fireman B, et al. Efficacy, safety and immunogenicity of heptavalent pneumococcal conjugate vaccine in children. Pediatr Infect Dis J 2000;19:187–95.

[10] CDC. Direct and indirect effects of routine vaccination of children with 7-valent pneumococcal conjugate vaccine on incidence of invasive pneumococcal disease—United States, 1998–2003. MMWR 2005;54:893–916.

[11] Koskela M. Serum antibodies to pneumococcal C-polysaccharide in children: response to acute pneumococcal *Otitis Media* or to vaccination. Pediatr Infect Dis J 1987;6:519–26.

[12] Siber GR, Priehs C, Madore D. Standardization of antibody assays for measuring the response to pneumococcal infection and immunization. Pediatric Res 1989;8(1):84–91.

[13] Quataert SA, Kirch C, Wiedl L, Phipps D, Strohmeyer S, Cimino C, et al. Assignment of weight based antibody units to human pneumococcal standard reference serum. Clin Diagn Lab Immunol 1995;2(5):590–7.

[14] Paradiso PR, Hogerman DA, Madore DV, Keyserling H, King J, ReisinerKS, et al. Safety and immunogenicity of a combined diphtheria, tetanus, pertussis and *Haemophilus influenza* type b in young infants. Pediatrics 1993;92:827–32.

[15] Philips DC, West J, Eby R, Koster M, Madore DV, Quataert SQ. An ELISA employing an *Haemophilus influenza* type b oligosaccharide-human serum albumin conjugate correlates with the radioantigen binding assay. J Immunol Methods 1990;13:121–8.

[16] Rubin LG, Mardy GV, Pais L, Carlone G. Human anticapsular antibody concentration required for protection against experimental pneumococcal bacteremia (Abstract G-47). In: Proceedings of the 35th Interscience Conference of AntimicrobialAgents and Chemotherapy, San Franscisco, CA, 17–20 September 1995. Washington (DC): American Society for Microbiology; 1995.

[17] Saladino RA, Stack AM, Malley R, Thompson CP, Siver CR, Fleiser GO. Bacterial polysaccharide immune globulin (BPIG) protects infants rats with *S. pneumoniae* (SP) pneumonia from bacteremia, meningitis and death. Pediatr Res 1996;36:184A.

[18] Miyamura K, Nishio S, Ito A, Murata R, Kono R. Microcell culture method for the determination of diphtheria toxin and anti-toxin titers using VERO cells: I. Studies on factors affecting the toxin and antitoxin titration. J Biol Stand 1974;2:189.

[19] Schmitt HJ, Faber J, Lorenz I, Schmöle-Thoma B, Ahlers N. The safety, reactogenicity and immunogenicity of a 7-valent pneumococcal conjugate vaccine (7VPnC) concurrently administered with a combination DTaP-IPV-Hib vaccine. Vaccine 2003;21:3653–62.

[20] Tichmann-Schumann I, Soemantri P, Behre U, et al. Immunogenicity and reactogenicity of four doses of diphteria-tetanus-three-component acellular pertussis-hepatitis B-inactivated plio virus-*Haemophilus influenzae* type b vaccine coadministered with 7-valent pneumococcal conjugate vaccine. Pediatr Infect Dis J 2005;24:70–7.

[21] Mallet E, Belohradsky BH, Lagos R, et al. A liquid hexavalent combination against diphtheria, tetanus, pertussis, poliomyelitis, *Haemophilus influenzae* type and hepatitis B: review of immunogenicity and safety. Vaccine 2004;22:1343–57.

[22] Schmitt H-J, Booy R, Weil-Olivier C. Child vaccination policies in Europe: a report from the Summits of Independent European Vaccination Experts. Lancet ID 2003;3:103–8.

[23] Olivier C, Liese J, Stojanov R, Tetelboum R, Cottard M, Fritzell B, et al. Immunogenicity and safety of a fourth (booster) dose of a 7-valent pneumococcal conjugate vaccine (7VPnC-Prevenar) coad-

ministered with a hexavalent pediatric combination vaccine (DTaP-IPV-HBV/Hib; Hexavac). Paper presented at: 22nd Annual Meeting of the European Society for Paediatric Infectious Diseases; Tampere Finland; 26–28 May 2004. Poster 288.

[24] Olivier C, Liese J, Stojanov R, Tetelboum R, Cottard M, Fritzell B, et al. Immunogenicity and safety of the 7-valent pneumococcal conjugate vaccine (7VPnC-Prevenar) coadministered with a hexavalent DTaP-IPV-HBV/Hib vaccine (Hexavac). In: Proceedings of the 42nd Interscience Conference of Antimicrobial Agents and Chemotherapy, San Diego, CA; 27–30 September 2002. Washington, DC: American Society for Microbiology; 2002. Poster G-836;19: 187–95.

[25] Rennel MB, Edwards KM, Keyserling HL, et al. Safety and immunogenicity of heptavalent pneumococcal vaccine conjugated to CRM197 in United States infants. Pediatrics 1998;101:604–11.

[26] Choo S, Seymour L, Morris R, Quataert S, et al. Immunogenicity and reactogenicity of a pneumococcal conjugate vaccine administered combined with a *Haemophilus influenzae* type b conjugate vaccine in United Kingdom infants. Pediatr Infect Dis J 2000;19:854–62.

[27] Miller MA, Meschievitz CK, Ballanco GA, Daum RS. Safety and immunogenicity of PRP-T combined with DTP: excretion of capsular polysaccharide and antibody response in the immediate postvaccination period. Pediatrics 1995;95:522–7.

[28] Daum RS, Hogerman D, Rennels MB, et al. Infant immunization with pneumococcal CRM197 vaccines: effect of saccharide size on immunogenicity and interactions with simultaneous administered vaccines. J Infect Dis 1997;176:445–55.

18