

Supplemental digital content 1 - Supplementary methods

Study vaccines and procedures

Each dose (0.5 mL) of 10-valent pneumococcal non-typeable *H. influenzae* protein D conjugate vaccine (PHiD-CV, *Synflorix*TM) contained 1 µg of each capsular polysaccharide of the pneumococcal serotypes 1, 5, 6B, 7F, 9V, 14 and 23F, and 3 µg of serotype 4 capsular polysaccharide, conjugated individually to protein D; 3 µg of serotype 18C capsular polysaccharide conjugated to tetanus toxoid; and 3 µg of serotype 19F capsular polysaccharide conjugated to diphtheria toxoid.

Each dose (0.135 mL) of oral poliovirus vaccine (OPV, *Polio Sabin*TM) contained polioviruses type 1 (LS-c, 2ab, $\geq 10^6$ TCID₅₀), type 2 (P712, Ch, 2ab, $\geq 10^5$ TCID₅₀) and type 3 (Leon 12a1b, $\geq 10^{5.5}$ TCID₅₀).

For diphtheria-tetanus-whole cell pertussis-hepatitis B virus and *H. influenzae* type b vaccine¹ (DTPw-HBV/Hib, *Tritanrix*TM *HepB Hib*), a vial containing 10 µg of Hib capsular polyribosylribitol phosphate polysaccharide conjugated to 20–40 µg tetanus toxoid was reconstituted with one vial of DTPw-HBV (containing ≥ 30 international units [IU] diphtheria toxoid, ≥ 60 IU tetanus toxoid, ≥ 4 IU inactivated *B. pertussis*, and 10 µg Hepatitis B surface antigen).

PHiD-CV was administered intramuscularly in the right thigh or deltoid (in children aged ≥ 12 months if the size of the deltoid muscle was adequate). The DTPw-HBV/Hib vaccine was administered intramuscularly in the left thigh and OPV was given orally. All vaccines were manufactured by GSK Vaccines, Rixensart, Belgium.

Treatment allocation

The treatment allocation according to age group was performed at the investigator site using a treatment allocation system accessed through the internet (SBIR, GSK

Vaccines). After having checked the eligibility of a child, the study staff in charge of the vaccination obtained the treatment number through internet based on the age and the identification number of the child.

Immunogenicity assessment

Blood samples were collected at pre-vaccination and at pre-determined timepoints, as presented in Figure 1.

Antibody concentrations against vaccine and vaccine-related (6A and 19A) pneumococcal serotypes were determined by GSK's 22F-inhibition enzyme-linked immunosorbent assay (ELISA).² Anti-protein D antibodies were quantified using an ELISA developed by GSK Vaccines. Anti-diphtheria, anti-tetanus and anti-inactivated *B. pertussis* antibody concentrations were also determined by ELISA.

S. pneumoniae opsonophagocytic activity (OPA) against vaccine and vaccine-related (6A and 19A) pneumococcal serotypes was measured by a killing-assay using an HL-60 cell line.³ Of note, OPA titers were not measured at pre-vaccination in children from the <6S and <6NS groups. The cut-off of the assay was an opsonic titer of 8.

Safety assessment

Solicited local (pain, redness, and swelling at the injection site) or general (drowsiness, irritability, loss of appetite, and fever) adverse events (AEs) were recorded by a field worker during 4 days post-vaccination on the child's diary card. Field workers were nurses who visited the infants for physical examination (including body temperature measurement) and interviewed their parents or legally acceptable representatives each morning during the 4-day follow-up period. The parents/legally acceptable representatives did not record anything between the visits, but the information they

mentioned to the field workers was reported on the diary card. Fever was defined as rectal temperature $\geq 38.0^{\circ}\text{C}$. Grade 3 symptoms were defined as follows: redness or swelling with a diameter >30 mm; fever of rectal temperature $>40^{\circ}\text{C}$; pain at the injection site if the child cried when its limb was moved or if its limb was spontaneously painful; irritability/fussiness and drowsiness preventing normal activity; loss of appetite if the child was not eating at all. For children 9 months of age or above, large post-vaccination swelling reactions (i.e., swelling with a diameter >50 mm, noticeable diffuse swelling, or noticeable increase of limb circumference) were recorded. Unsolicited AEs were recorded during one month post-vaccination, and serious AEs (SAEs) throughout the study. Antipyretic(s)/medication(s) taken by the children at any time during 4 days post-vaccination were also collected.

References

1. Prymula R, Plisek S. Clinical experience with DTPw-HBV and DTPw-HBV/Hib combination vaccines. *Expert Opin Biol Ther.* 2008;8:503-513.
2. Concepcion NF, Frasch CE. Pneumococcal type 22f polysaccharide absorption improves the specificity of a pneumococcal-polysaccharide enzyme-linked immunosorbent assay. *Clin Diagn Lab Immunol.* 2001;8:266-272.
3. Romero-Steiner S, Libutti D, Pais LB, et al. Standardization of an opsonophagocytic assay for the measurement of functional antibody activity against *Streptococcus pneumoniae* using differentiated HL-60 cells. *Clin Diagn Lab Immunol.* 1997;4:415-422.