Title:

**Safety and immunogenicity of the Cuban heptavalent pneumococcal conjugate vaccine in healthy infants. Results from a double-blind randomized control trial Phase I.**

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**Abstract**

**Background:** Cuba has a new pneumococcal conjugate vaccine candidate (PCV7-TT). This study evaluates the safety and immunogenicity in healthy infants using 2p+1 vaccination schedule.

**Methods:** A phase I, controlled, randomized and double blind clinical trial was designed. 30 unvaccinated healthy infants were included. 20 subjects were assigned to study group (PCV7-TT) and 10 to control group (Synflorix®) to receive the vaccines at 7, 8 month of age (primary series) and 11 months (booster dose). Blood samples were collected 30 days after second dose and post booster for antibodies measure analysis by ELISA and OPA. The statistics analysis included the frequency of occurrence for adverse events and the immune response. Non-parametric tests were used to compare the immune response. The clinical trial was published in the Cuban Public Register of Clinical Trials with code RPCEC00000173 available at http://registroclinico.sld.cu.

**Results:** Overall, the safety profile of PCV7-TT was similar to Synflorix®. Local reactions were predominantly and systemic events were mild in severity. Swelling and redness were frequently associated with PCV7-TT mainly after the first dose (50% and 40% respectively). 15% and 10% of subject reported severe swelling after first dose with PCV7-TT and after second dose with Synflorix®. Mild fever (≥38-≤39), vomiting and sleep disturb were the systemic events reported. 100% of infants achieved pneumococcal IgG antibody concentrations ≥0.35 µg/ml after booster dose for serotypes 1, 14, 18C and 19F in each vaccine group. For serotpes 5, 6B and 23F, more than 80% infants vaccinated with Synflorix® or PCV7-TT achieved protective IgG GMC above 0.35 µg/ml after booster dose. OPA proportion's responders to the seven common serotypes was 89.5 or more after the primary dose and 100% after booster dose in vaccinated with PCV7-TT.

**Conclusions:** The Cuban PCV7-TT is safe, well tolerated and immunogenic in healthy infants.

Keywords: Pneumococcal conjugate vaccine, immunogenicity, safety, infant, clinical trial, Cuban.

## Introduction

*Streptococcus pneumoniae* is responsible for pneumococcal invasive disease (IPD), causing significant morbidity and mortality worldwide (1). Prevention of pneumococcal infection through vaccination remains the best strategy to reduce the incidence of IPD. The pneumococcal conjugate vaccines (PCVs) have been shown to be highly immunogenic, safe, well tolerated and effective in reducing invasive and noninvasive pneumococcal disease in vaccinated children (2). Besides their direct effect in infants, PCVs have a substantial indirect effect, resulting in the reduction of disease burden among unvaccinated adults and toddlers (3,4). Available evidence from various countries have shown that PCVs can be administrated concurrently with other vaccines that are typically recommended during the first year of life (5,6).

To date Cuba and many other low-income countries have not yet introduced pneumococcal vaccination into their national immunization programs due to their elevated costs. Indeed, despite significant global efforts to facilitate vaccine procurement through different advantageous financial mechanisms, the introduction of these complex and relatively expensive vaccines has been lengthy worldwide (7). In 2007, Cuba initiated the development of a seven-valent tetanus toxoid conjugated PCV (PCV7-TT), the first in a subsequent series of more complex PCVs. PCV7-TT was developed bearing in mind the following criteria: a) to include seven serotypes, learning from Prevnar-7 that the impact of PCV could be high if the selected serotypes matched the current epidemiology well; b) to use tetanus toxoid conjugates to increase the immunogenicity induced against

the serogroups 19 and 6 through 19F and 6B conjugates; and c) to reduce the time of pharmaceutical development by reducing the complexity of the vaccine and making it available the earliest possible. The final composition of this vaccine consists of 2µg of PS from serotypes 1, 5, 14, 18C, 19F, 23F and 4µg of 6B, all conjugated to tetanus toxoid and adsorbed on aluminum phosphate (8). The vaccine is currently in late clinical development (8, 9) before the introduction in Cuba in 2018. We expect that the Cuban PCV7-TT may represent an appealing option for countries that have not yet introduced PCV considering its price for value. Indeed, the seven prevalent serotypes included in the vaccine account for over 70% of isolated serotypes worldwide (10), and we aim to demonstrate further cross protection of included serotypes against other circulating serotypes such as 19A and 6A. In the present article, we provide the first evidence of safety and immunogenicity of PCV7-TT in infants aged 7-11 months, who have not been previously vaccinated against pneumococcal disease.

## Methods

*Study design and ethical considerations*

A phase I, parallel, controlled, randomized and double blind clinical trial was designed with the primary objective to assess the safety of PCV7-TT in infants, using a two primary doses plus a booster (2p+1) schedule administrated at 7, 8 and 11 months of age. The secondary objective was to evaluate the immunogenicity of the PCV7-TT among the same study population.

The study protocol was reviewed and approved by the Ethics Committee of the Children University Hospital "Juan Manuel Márquez" in Havana, Cuba. The clinical trial was authorized by the Cuban National Regulatory Agency according the Good Clinical Practice (GCP) Cuban guidance (12), and published in the Cuban Public Register of Clinical Trials with the code RPCEC00000173 (a protocol summary is available at

http://registroclinico.sld.cu). It was conducted in accordance with the code of Ethic of the World Medical Association for experiments in human beings (13).

*Informed consent*

Written informed consent was obtained from the parents or legally acceptable representative of each infant before enrollment. Physicians explained in details the benefits and risk of the study, and parent's voluntary decided on the enrollment of their infants.

*Enrollment and selection criteria*

As PCV7-TT had not been previously evaluated in this population, 30 healthy infants from 38 eligible infants were randomized to the study and control groups. They were 7-months-old residents of Havana, who had not been previously vaccinated against pneumococcal disease and were enrolled upon parents' informed consent.

*Inclusion criteria*. We included healthy 7-months-old infants born at least at 36 weeks of gestation, weighing more than 2500g at birth, presenting adequate nutritional condition and having completed the vaccination schedule during their first semester of life.

*Exclusion criteria*

Infants were excluded if they had history of chronic diseases, immunomodulator treatment, any hypersensitivity reaction following a prior vaccination, prior vaccination against *S. pneumoniae*, or acute illness at the moment of vaccination. Infants were also excluded if another vaccine had been administered, or planned, from 30 days before and up to 30 days after the administration of a study vaccine dose.

*Vaccines, target groups and schedule*

Eligible participants were randomly assigned in a 2:1 ratio to receive PCV7-TT or Synflorix® at 7, 8 month of age (primary dose) and 11 months (booster dose), based on a random assignment schedule prepared by the sponsor. All participants, study staff,

and those assessing the outcomes were blinded to the group assignment. The intervals between consecutive primary vaccination doses were 30 days and 90 days between primary schedule and booster dose. We generated a random list using the R software for Statistical Computing and a randomization of 2:1 to assign 20 subjects to the group PCV7-TT and 10 subjects to the group Synflorix®. At enrollment, each child was assigned to the study or control group as specified inside a sealed envelope with a sequential number. Blinded statistical analysis was conducted.

PCV7-TT (Batch NEU.13.02, Manufacturer: Finlay Vaccine Institute, Cuba) contains 2µg of serotypes 1, 5, 14, 18C, 19F, 23F and 4µg of serotype 6B, each one conjugated to TT for 24.5 µg of total TT in the formulation, 125 µg of aluminum phosphate and 0.026 mg of thiomersal. The vaccine is a suspension for injection and is presented in single dose vial; one dose is contained in 0.5 ml. Its routine storage is between 2-8°C. Synflorix® vaccine (Batch ASPNA305AA, Manufacturer: GSK) was used as control. It is composed of the purified capsular polysaccharides from 10 serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F each one conjugated to a carrier protein, either protein D (PD), tetanus toxoid (TT) or diphtheria toxoid (DT). Synflorix® is a preservative-free liquid suspension, adsorbed on aluminum phosphate and presented as pre-filled glass syringes ready for intramuscular injection (14).

Both vaccines were administered intramuscularly with a 22–25 gauge needle (25 mm/long) into the right thigh of the infants. They received a 2-dose primary vaccination series (30 days' interval) followed by booster vaccination (90 days interval) (Figure 1), therefore, the schedule was at 7, 8 and 11 months of age. Blood specimen collection was scheduled at 30 days after the second and the booster dose.

In order to assure the double-blinding due to the different presentation of both vaccines, we used a double-nurse system where one nurse prepares the vaccine and the other vaccinates the child.

*Safety and reactogenicity evaluation*

The primary outcome of this study was the safety of the vaccine. Post-vaccination, children were followed up for 30 days to detect and assess any adverse events. Each subject was closely observed during three hours post-vaccination for identification of immediate adverse effects. Expected local reactions and systemic events were recorded actively during the 7-day post-vaccination period through medical visit. Unexpected adverse events information was collected during the 30 days post-vaccination period.

Diary cards were used to solicit the reporting of the occurrence of local symptoms (pain, redness swelling) and general symptoms (i.e. fever, gastrointestinal symptoms, sleep disturbance) for 7 days after each dose. The occurrence of other (unsolicited) adverse events was also collected using diary cards for 30 days after each dose. Serious adverse events (SAEs) defined as events that were life-threatening, required hospitalization or prolongation of hospitalization, or resulted in disability, incapacity or death, were recorded throughout the study. The investigators assessed the association between investigational products and the occurrence of each adverse event. Solicited local adverse events were all assumed to be associated with vaccine administration.

*Immunogenicity evaluation*

The secondary outcome of this study was to assess the vaccine's immunogenicity. Blood samples were collected 30 days after the second and the booster dose. Serum samples were stored at −20°C until ELISA and OPA analysis. Three hundred microliters of each serum samples were analyzed for ELISA and OPA at the World Health

Organization (WHO) Reference Laboratory for pneumococcal disease, at the Institute of Child Health in London, the United Kingdom.

Pneumococcal serotype-specific anti-capsular polysaccharide antibodies for the seven serotypes included in PCV7-TT and for the additional serotypes 19A and 6A (contained only in 13-valent PCVs) were measured by ELISA using double absorption with C and 22F polysaccharides, following the WHO recommended protocol (15). Functional opsonophagocytic antibodies were measured by a viable counting MOPA method using differentiated HL60 cells as effector cells. The OPA titer was expressed as the reciprocal of the serum dilution that caused a 50% reduction of the colony-forming units compared to a control that contained no human serum, following the WHO recommended protocol (16). Additionally, the antibody response to carrier protein TT was evaluated at the Finlay Vaccine Institute, using indirect ELISA assay for quantification of tetanus antitoxin in human serum, with the use of a previously calibrated standard (17).

*Statistics analysis*

The study corresponded to an exploratory phase I trial. Sample size was established by expert opinion and the analyses were performed on the Total Vaccinated Cohort.

*Safety*

We summarized the number and percentage of subjects with any solicited or general symptoms within the 7-day follow-up period per dose. We tabulated the number and percentage of subjects with unsolicited adverse events within the 30-day follow-up period according to the Medical Dictionary for Regulatory Activities (MedDRA) preferred terms. We tabulated SAEs occurring during the study period, including the long-term safety follow-up, according to MedDRA preferred terms. We used a non-

parametric test (Fisher's Exact Test for Count Data, with error α= 0.05 or 95% of safety) was used to compare incidence for each adverse event. We reported the frequency of occurrence for adverse events in both groups.

*Immunogenicity*

We tabulated the number and proportion of subjects achieving a serotype-specific IgG antibody concentration ≥0.35 μg/ml (responders) measured one month after the primary series and booster for seven common serotypes. We calculated the exact 2-sided 95% confidence intervals (CIs) on the proportions. We calculated the geometric mean antibody concentrations (GMCs) with their 95% CI for each serotype and at each applicable blood specimen collection time point for both group. We estimated two-sided 95% CIs by back transformation of the CIs for the mean of the logarithmically transformed assay results. We reported the functional antibody activity (OPA) as a geometric mean titer with 2-sided 95% confidence intervals. Non-parametric tests were used to compare after the primary series and after the booster dose between groups for each serotype and for TT antibodies (Mann Whitney U Test and Sign test with error α=0.05). We calculated the number of subjects with OPA titer ≥ 1:8 for each serotype in both groups.

**Results**

From 38 subject screened, a total of 30 infants were enrolled and randomly assigned to each group: 29 infants completed the primary vaccination series and booster dose. Only one loss to follow up occurred (Figure 2). Demographic characteristics of the two groups were similar with regards to gender, ethnicity and age.

*Pneumococcal vaccine safety*

Comparing infants in the two vaccine groups, in general, the safety profile of PCV7-TT was similar to that of Synflorix® (Table 1). Most of the local reactions and

systemic events reported were mild. Local reactions after any dose were the most frequently reported and their duration ranged between three and eleven days. Swelling and redness at the injection site were frequently associated with PCV7-TT mainly after the first dose (50% and 40% respectively). Fifteen percent and 10% of subjects respectively reported severe swelling at injection site following the first dose with PCV7-TT and after the second dose with Synflorix®. Mild fever (≥38-≤39°C), vomiting and sleep disturbance were the systemic solicited events reported. Fever was more frequent after the first dose of PCV7-TT (in 4/20 infants). The duration of systemic events did not last more than three days. The frequency of medication to treat symptoms was approximately 40% in each group after the first dose and ranged from 26% to 50% after the primary vaccination series and booster dose. Unsolicited events reported in infants vaccinated with PCV7-TT were diarrhea (5.15 % per total applied dose) and rhinorrhea (3.45%). In the Synflorix® vaccinated group, diarrhea and rash were reported in 3.3% (per total applied dose), and hyperemic conjunctival in 6.6%. All these events were more frequent after the first and second dose. No deaths were reported in this study.

*Pneumococcal vaccine immunogenicity*

Table 2 shows the percentage of subjects who achieved a pneumococcal IgG antibody concentration ≥0.35 µg/ml after the primary series and after the booster dose. All infants achieved a pneumococcal IgG antibody concentrations ≥0.35 µg/ml after the primary series for serotypes 1, 14 and 18C in each vaccine group. The percentages of infants achieving IgG antibody concentrations ≥0.35 µg/ml against serotype 19F were also high (94.74% in the PCV7-TT vaccinated group)). For 23F, 66.7% infants vaccinated with Synflorix® and 89.47% with PCV7-TT achieved protective IgG titers and more than 77% of vaccinated in both groups, for serotypes 5 and 6B. 100% of infants achieved pneumococcal IgG antibody concentrations ≥0.35 µg/ml after booster dose for serotypes

1, 14, 18C and 19F in each vaccine group. For serotpes 5, 6B and 23F, more than 80% infants vaccinated with Synflorix® or PCV7-TT achieved protective IgG GMC above 0.35 µg/ml after booster dose.

Among the vaccinated with PCV7-TT, the pneumococcal IgG GMCs shown after primary series were the lowest for serotype 5 (0.643 µg/ml) and 6B (0.991 µg/ml). After the booster dose, GMCs for the common seven serotypes ranges from 0.898 µg/ml to 8.082 µg/ml in PCV7-TT group and from 1.026 µg/ml to 10.196 µg/ml in the Synflorix® vaccinated group.

The comparison of IgG GMC between PCV7-TT and Synflorix® after the primary series for each of the common serotypes showed that IgG GMC ratios ranged was from 0.43 to 4.84 µg/ml. After the booster dose, the GMCs were higher in both groups for the common serotypes 6B, 14, 19F, 23F and also for serotypes 5 among the vaccinated with PCV7-TT.

Table 3 shows the OPA responders percentage (subjects who achieved OPA antibody titers ≥1:8) and OPA GMTs in both vaccine groups. The proportion of responders to the seven common serotypes was ≥89.5% after the primary series and 100% after the booster dose in those vaccinated with PCV7-TT. In the Synflorix® vaccinated group, the percentage of responders was ≥77.8% after the primary dose. After the booster dose, it exceeded 90%. OPA GMTs following the booster dose were higher than the levels observed after the primary dose for all serotypes in the control groups. In the study group results were similar, except for serotype 14.

For the serotypes 6A and 19A that are not included in the vaccines, we conducted additional analyzes that showed that the percentage, of subjects who achieved a pneumococcal IgG antibody concentration of ≥0.35 µg/ml after the primary series and

after the booster dose were lower in both vaccine groups (33.3% for serotype 6A and 55.6% for serotype 19A in the Synflorix® vaccinated following the primary series; 52.6% for serotype 6A and 23.6% for serotype 19A in the PCV7-TT vaccinated). The pneumococcal IgG GMCs in vaccinated subjects with PCV7-TT were 0.289 µg/ml for serotype 6A and 0.244 µg/ml for serotype 19A. In those vaccinated with Synflorix® it was similar with 0.218 µg/ml for serotype 6A and 0.352 µg/ml for 19 A serotype. The ratio of IgG GMC (PCV7-TT and Synflorix®) after the primary series was 0.69 for serotype 19A and 1.32 for serotype 6A. After the booster dose, in both groups we observed an increase in the GMC titers for serotypes 6A and 19A.

All subjects in both groups achieved protective titer levels against tetanus (0.1 UI/ml). The GMCs increased from 5.5 CI 95% 4.17-7.7 (after the primary series) to 5.9 CI 95% 4.72-7.31 (after the booster dose) in PCV7-TT vaccinated infants. This increase was not observed in the Synflorix® vaccinated group (GMCs of 3.5 and 3.3 respectively).

**Discussion**

We report the first results of the safety and immunogenicity of the Cuban-developed PCV7-TT among infants, compared to the widely used PCV-10 Synflorix. Among infants vaccinated with either vaccines, local reactions predominated and systemic events were mild. The new pneumococcal vaccine candidate PCV7-TT was immunogenic in the target infant population against the seven common serotypes included in the vaccine.

The trial was designed taking into account the schedule for the administration of pneumococcal vaccine comprising a two-dose primary series followed by a booster dose administrated at 11 months of age, as recommended by WHO (18). Because the carrier protein in PCV7-TT is tetanus toxoid, no interference with this antigen contained in other pediatric vaccine administrated in the first semester of life was explored (for example the

pentavalent vaccine against diphtheria, tetanus, pertussis, hepatitis B and *Haemophilus influenzae* type b).

The reactogenicity of PCV vaccines, including injection-site reactions and fever, has been evaluated in several randomized clinical trials and the findings of these trials are summarized in a systematic review (19). The safety of the new Cuban pneumococcal vaccine had been previous explored in other age groups including adults (7) and preschool children (8). The results among infants were comparable to those reported for other pneumococcal vaccines (20). The most frequently reported reactions were local, including tenderness, redness and swelling, and ranged from 26.7% to 53.25% in those vaccinated with Synflorix® and 27.8% to 58.2% in those vaccinated with PCV7-TT.

Our study did not identify any severe events associated with vaccination resulting in hospitalization, emergency or outpatient visits. The reported local and systemic reactions were typically mild and severe local reactions were uncommon and self-limited when they occurred. The consumption of antipyretic drugs occurred in approximately 40% of the vaccinated infants which is comparable to other studies using PCV7 vaccine (21).

The immunogenicity data in the current clinical trial is also consistent with previous studies of PCV7 administered in a simplified two-dose infant series followed by a booster (22). Both vaccines were immunogenic with two infant doses for the four common pneumococcal serotypes (all subjects were responders to serotypes 1, 14, and 18C). For the remaining vaccine serotypes, more than 66.7% of infants achieved IgG concentration higher than 0.35 µg/ml. In the case of PCV7-TT, the percentage of responders after booster dose were 100% for all serotypes except serotype 5 (95%), while for Synflorix® it was 90% for serotypes 5 and 6B of and 6B and 80% for serotype 23F.

Antibody functional induced for the new pneumococcal vaccine is one of the main finding of this study. Despite the variation between serotypes, more than 89% of subject vaccinated with PCV7-TT achieving OPA titer of 1:8 or above after primary series and 100% after booster dose respectively. An unintended result was the immune response of PCV7-TT to additional serotypes 6A and 19A. These serotypes are not included in the vaccine candidate; however, after booster dose 84.2% and 63.16% of subjects included in the study group were responders for 6A and 19A respectively, and the antibody functional activity was demonstrated. Because the clinical relevance of serotype 19A in Cuba identified through National Reference Laboratory Surveillance (23) and NP colonization studies (24), to explore in deep this finding through non inferiority immunogenicity studies is very important in our context. It may be effective in preventing disease caused by these serotypes in vaccinated population.

There is no other licensed PCV using only tetanus toxoid as carrier protein so, in one way the interference with other pediatric vaccines should be further studied, but in other side, the priming role of tetanus toxoid could be observed as reported studies with *Haemophilus vaccines* (25). This hypothesis will be tested in next studies planned in Cuba beginning in 2017 exploring the immunogenicity in a cohort of approximately 880 infants using different vaccination schedules (2p+1, and 3p+0).

The main limitation of the current study is the lower number of enrolled infants that limited the probability of detecting a significant difference among the vaccination groups in terms of immunogenicity. However, the result support further steps of clinical evaluation in this target group since the new vaccine is well tolerated in infants.

**Conclusions**

Our results showing an acceptable safety profile and immunological results for PCV7-TT administered in a two-dose primary series and a booster dose (2p+1) schedule

in healthy infants vaccine-naïve for pneumococcal vaccination. It supports further studies included in clinical evaluation strategy of PCV7-TT.

The immune response generated by PCV7-TT suggests that it could provide protection against pneumococcal disease. The comparable immunogenicity and safety profiles of PCV7-TT and Synflorix® suggest PCV7-TT could be a valuable addition to the national immunization schedule in Cuba and in other resource-limited settings.

**Declaration of interests:** Main authors of this paper: Nivaldo Linares-Pérez, PhD; Beatriz Paredes, BSc; Mayelín Mirabal, MSc; Dagmar García-Rivera, PhD; Yury Valdés Balbín, BSc and Vicente Verez-Bencomo, PhD, work at the manufacturing vaccine center "Finlay Vaccine Institute".

The rest of main authors: María E. Toledo-Romaní, PhD; Carlos P. Dotres, MD; Rinaldo Puga, MD; Yariset Ricardo, MD; and Carmen R. Broño, MD, working in the National Health System, and neither have contracts nor receive financing from the manufacturing center.

**Contributions of authors:** Nivaldo Linares-Pérez, PhD, María E. Toledo-Romaní, PhD and Mayelín Mirabal, MSc, analysis and interpretation of data and designed and wrote this paper. Carlos P. Dotres, MD; Beatriz Paredes, BSc; Rinaldo Puga, MD; Dagmar García-Rivera, PhD; Yariset Ricardo, MD; Carmen R. Broño, MD, participated the conception and design of the study and acquisition of data of this clinical trial. Laura M. Rodríguez, BsC and Amarilis Pérez, BsC, were in charge of the processing and evaluation of laboratory samples. All authors were involved as experts in the discussion, review and final approval of the version to be submitted.

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**References**

1. Simell B, et al. The fundamental link between pneumococcal carriage and disease. Expert Review of Vaccines 2012; 11: 841–855.
2. Pilishvili T, Lexau C, Farley MM, Hadler J, Harrison LH, Bennett NM, et al. Sustained reductions in invasive pneumococcal disease in the era of conjugate vaccine. J Infect Dis 2010;201:32–41.
3. Esposito S, Tansey S, Thompson A, Razmpour A, Liang J, Jones TR, et al. Safety and immunogenicity of a 13-valent pneumococcal conjugate vaccine compared to those of a 7-valent pneumococcal conjugate vaccine given as a three-dose series with routine vaccines in healthy infants and toddlers. Clin Vaccine Immunol 2010;17:1017–26.
4. Black, S. B., C. O. Cimino, J. Hansen, E. Lewis, P. Ray, B. Corsaro, J.Graepel, and D. Laufer. 2006. Immunogenicity and safety of measles mumps-rubella, varicella and

Haemophilus influenzae type b vaccines administered concurrently with a fourth dose of heptavalent pneumococcal conjugate vaccine compared with the vaccines administered without heptavalent pneumococcal conjugate vaccine. Pediatr. Infect. Dis. J. 25:306–311.

5. Knuf, M., P. Habermehl, C. Cimino, G. Petersen, and H. J. Schmitt. 2006. 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.
6. Schmitt, H. J., J. Faber, I. Lorenz, B. Schmo¨le-Thoma, and N. Ahlers. 2003. The safety, reactogenicity and immunogenicity of a 7-valent pneumococcal conjugate vaccine (7VPnC) concurrently administered with a combination DTaP-IPV-Hib vaccine. Vaccine 21:3653–3662.
7. Progress in Introduction of Pneumococcal Conjugate Vaccine — Worldwide, 2000–2012, MMWR, 2013 / 62(16);308-311
8. Dotres CP, Puga R, Ricardo Y, Brono CR, Paredes B, Echemendía V, et al. Safety and preliminary immunogenicity of Cuban pneumococcal conjugate vaccine candidate in healthy children: A randomized phase I clinical trial. Vaccine. 2014;32(41):5266-70.
9. González N, Paredes B, Pérez S, Mirabal M, Rivero I, González C, et al. Safety and immunogenicity of Cuban antipneumococcal conjugate vaccine PCV7-TTT in healthy adults. MEDICC Review. 2015;17(4):32-7.
10. Johnson HL, Deloria-Knoll M, Levine OS, Stoszek SK, Hance LF, Reithinger R, et al. Systematic evaluation of serotypes causing invasive pneumococcal disease among children under five: the pneumococcal global serotype project. PLoS medicine. 2010;7(10):e100034

11. Linares-Pérez N, Toledo-Romaní ME, Casanova MF, Paredes B, Váldes-Balbín Y, Santana-Mederos D, García-Rivera D, Toraño G, Dotres CP, Puga R y Verez-Bencomo V. La nueva vacuna cubana antineumocócica, de las evidencias científicas disponibles, a la estrategia de evaluación clínica y de impacto. Rev Cubana Pediatr. 2017;89(sup). Hallado en: URL:http://www.revpediatria.sld.cu/index.php/ped/issue/view/7
12. Linares-Pérez, N, Toledo-Romaní M, Santana-Mederos D, García-Fariñas A, García-Rivera D, Valdés-Balbín Y and Vérez-Bencomo V. From individual to herd protection of pneumococcal vaccines: The contribution of Cuban PCVs implementation strategy. IJID. En prensa, 2017. Available from: http://www.ijidonline.com/article/S1201-9712(17)30094-2/fulltext
13. Centro para el Control Estatal de Medicamentos, Equipos y Dispositivos Médicos, Requerimientos para la Certificación de Buenas Prácticas Clínicas La Habana, Cuba 2015 [citado de RESOLUCION No. 219]. 24]. Hallado en: http://www.cecmed.cu/sites/default/files/adjuntos/Reglamentacion/res.no_.219.regulacion.m_82-15.pdf
14. WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects. [Accessed 22 March 2013]. Available from: www.wma.net/en/30publications/10policies/b3/index.html
15. European Medicines Agency. Evaluation of Medicines for Human Use. ASSESSMENT REPORT FOR Synflorix. Pneumococcal polysaccharide conjugate vaccine (adsorbed) Procedure No. EMEA/H/C/000973. Available from: http://www.emea.europa.eu

16. Training manual for Enzyme linked immunosorbent assay for the quantitation of Streptococcus pneumoniae serotype specific IgG (Pn PS ELISA). (007sp Version). Available from: www.vaccine.uab.edu
17. Protocol for multiplexed opsonophagocytic killing assay (UAB-MOPA) for antibodies against Streptococcus pneumonia. Available from: www.vaccine.uab.edu
18. Ochoa R, Martínez JC, Fajardo EM, Alvarez E, Estrada E, García AM et al. Validación de un ELISA para la cuantificación de antitoxina tetánica en suero humano. VacciMonitor 2000;9:16-21
19. World Health Organization. 2007. Pneumococcal conjugate vaccine for childhood immunization—WHO position paper. Wkly. Epidemiol. Rec. 82:93–10
20. Thompson A1, Gurtman A, Patterson S, Juergens C, Laudat F, Emini EA, Gruber WC, Scott DA. Safety of 13-valent pneumococcal conjugate vaccine in infants and children: meta-analysis of 13 clinical trials in 9 countries. Vaccine. 2013 Oct 25;31(45):5289-95.
21. Martinón-Torres F, Czajka H, Center KJ, et al. 13-valent pneumococcal conjugate vaccine (PCV13) in preterm versus term infants. Pediatrics 2015;135:e876-e886
22. Esposito S & Principi N. Safety and tolerability of pneumococcal vaccines in children, Expert Opinion on Drug Safety, 2016. DOI:10.1517/14740338.2016.1160056
23. Esposito S, Tansey S, Thompson A, Razmpour A, Liang J, Jones TR, Ferrera G, Maida A, Bona G, Sabatini C, Pugni L, Emini EA, Gruber WC, Scott DA, Principi N. Safety and immunogenicity of a 13-valent pneumococcal conjugate vaccine compared to those of a 7-valent pneumococcal conjugate vaccine given as a three-dose series with routine vaccines in healthy infants and toddlers. Clin Vaccine Immunol. 2010 Jun;17(6):1017-26.

24. Toraño-Peraza G, Pías-Solis L, Abreu-Capote M, Rodríguez-Ortega M, Dickinson-Meneses F, Varcárcel-Sánchez M. Serotipos y resistencia antimicrobiana de aislamientos meníngeos de Streptococcus pneumoniae. Cuba, 2007-2012. Vaccimonitor. 2014;23(3):117-23.
25. Toledo ME, Casanova MF, Linares-Pérez N, García-Rivera D, Toraño Peraza G, Barcos Pina I, Montes de Oca M, Rodriguez-Noda LM, Mirabal M, Paredes B, Chávez Amaro DM, Santana Mederos D, Valdés-Balbín Y, Verez-Bencomo V, and Finlay Vaccine Institute Laboratory-Pneumococci Group and Cienfuegos Local Research Team Pneumococci Project Prevalence of Pneumococcal Nasopharyngeal Carriage Among Children 2-18 Months of Age: Baseline Study Pre Introduction of Pneumococcal Vaccination in Cuba. Pediatr Infect Dis J. 2017 Jan;36(1):e22-e28.1.
26. Carlsson RM1, Claesson BA, Iwarson S, Lagergård T, Käyhty Antibodies against Haemophilus influenzae type b and tetanus in infants after subcutaneous vaccination with PRP-T/diphtheria, or PRP-OMP/diphtheria-tetanus vaccines. Pediatr Infect Dis J. 1994 Jan;13(1):27-33.