

# Immunogenicity and safety of a combined DTaP–IPV vaccine compared with separate DTaP and IPV vaccines when administered as pre-school booster doses with a second dose of MMR vaccine to healthy children aged 4–6 years

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## Abstract

Combination vaccines represent one solution to the problem of increased numbers of injections during single clinic visits. A combined DTaP–IPV (*Infanrix*<sup>®</sup>-IPV) vaccine has been developed for use as a pre-school booster. Four hundred healthy children aged 4–6 years previously primed with 4 doses of DTaP vaccine (*Infanrix*<sup>®</sup>), 3 doses of poliovirus vaccine and 1 dose of MMR vaccine were randomized to receive single doses of either the combined DTaP–IPV vaccine or separate DTaP and IPV vaccines in a Phase II trial (DTaP–IPV-047). All children also received a second dose of MMR vaccine. Immunogenicity was assessed in serum samples taken before and 1 month after booster administration. Safety was actively assessed for 42 days post-vaccination. Non-inferiority of the DTaP–IPV vaccine to separate DTaP and IPV vaccines was demonstrated for all DTaP antigen booster response rates and poliovirus geometric mean titers of antibody ratios. Post-vaccination,  $\geq 99.4\%$  of children in both groups had seroprotective levels of anti-diphtheria and anti-tetanus antibodies ( $\geq 0.1$  IU/mL) and seroprotective anti-poliovirus antibody titers ( $\geq 1:8$ ). All children in both groups were seropositive for measles, mumps and rubella antibodies, with similar post-vaccination geometric mean concentrations/titers. No significant differences were observed in the incidence of solicited local or general symptoms, unsolicited symptoms and serious adverse events between the two groups. This combined DTaP–IPV appeared safe and immunogenic when given as a booster dose at 4–6 years of age. The DTaP–IPV vaccine had no negative effect on the response to co-administered MMR vaccine, making it well-suited for use as a pre-school booster.

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## 1. Introduction

Vaccination of infants against diphtheria, tetanus, pertussis and polio is well-established in industrialized countries. Pediatric vaccination schedules vary between countries. In Europe, for primary immunization, infants typically receive 2–3 doses of diphtheria-tetanus-acellular pertussis (DTaP)

vaccine and inactivated poliovirus (IPV) within the first 6 months of life, followed by a booster dose at 11 to 18–24 months of age [1]. In the United States (US), for primary immunization, infants are primed with 3 doses of DTaP at 2, 4 and 6 months of age, with a fourth dose administered during the second year of life. IPV is given at 2 and 4 months of age, with a third dose administered between 6 and 18 months of age [2]. While primary vaccination of infants against pertussis has been effective in reducing the incidence of disease in young children, peak incidence has shifted to

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older age groups, particularly adolescents and adults in whom vaccine-induced immunity has waned [3–6]. Provision of a fifth pertussis-containing vaccine prior to school entry, and a sixth pertussis-containing vaccine in adolescence, is therefore now included in many national immunization schedules to provide protection to older children and improve herd immunity [3,4,7–10]. In the US, a fifth dose of DTaP vaccine and a fourth dose of IPV vaccine is administered to children 4–6 years of age [2], and a sixth dose of pertussis-containing vaccine in the form of reduced antigen content tetanus-diphtheria-acellular pertussis vaccine (Tdap) is administered to adolescents [10].

Use of combination vaccines to deliver multiple antigens in a single injection is an established approach for simplifying pediatric immunization schedules [11–15]. Combination vaccines represent one solution to the problem of increased numbers of injections during single clinic visits. Administration of DTaP and IPV in a single injection would be expected to promote compliance with the vaccination schedule and increase vaccine coverage rates, while increasing convenience for both the vaccinee and the immunization provider. In particular, use of this combination vaccine as a pre-school booster would reduce the number of injections required for US children at 4–6 years from 3 (DTaP, IPV and measles, mumps, rubella [MMR]) to 2 (DTaP-IPV and MMR). This study was undertaken to compare the immunogenicity and safety of a combined DTaP-IPV vaccine with separate administration of DTaP and IPV vaccines when co-administered as booster vaccines with a second dose of MMR vaccine at 4–6 years of age.

## 2. Methods

### 2.1. Study design and subjects

This open, randomized, phase II, non-inferiority study with two parallel groups with 1:1 randomization was conducted at 14 centers in the US (DTPa-IPV-047). Healthy children (as established by medical history and physical examination) aged between 4 and 6 years who had previously received 4 doses of DTaP vaccine [(*Infanrix*<sup>®</sup>) primary vaccination at 2, 4 and 6 months with booster dose during the second year of life], 3 doses of poliovirus-containing vaccine (either the sequential poliovirus vaccine schedule [2 doses of IPV followed by 1 dose of oral poliovirus; OPV] or IPV exclusively) during the first 2 years of life, and a single dose of MMR vaccine during the second year of life were eligible for study entry. Exclusion criteria included receipt of any investigational or non-registered drug or any vaccine other than the study vaccines within 30 days of study entry, a history of hypersensitivity to any components of the vaccines, history of previous diphtheria, tetanus, pertussis, polio, measles, mumps or rubella disease, or DTP, IPV or MMR vaccine contraindication. The study was approved by the appropriate ethics committees and was conducted in accordance with Good Clinical Practice guidelines and the Declaration of

Helsinki. Written informed consent was obtained from the parents/guardians of all children prior to study entry.

### 2.2. Study vaccines and administration

Children received either a single dose of DTaP-IPV vaccine (*Infanrix*<sup>®</sup>-IPV, GlaxoSmithKline Biologicals, Rixensart, Belgium) or separate injections of DTaP (*Infanrix*<sup>®</sup>, GlaxoSmithKline Biologicals, Rixensart, Belgium) and IPV vaccine (IPOL<sup>™</sup>, Aventis Pasteur, Lyon, France). A dose of MMR vaccine (MMR<sup>™</sup><sub>II</sub>, Merck & Co., Inc., Whitehouse Station, US) was co-administered to all children.

Each dose of DTaP-IPV and DTaP vaccine contained 25 Lf diphtheria toxoid, 10 Lf tetanus toxoid, 25 µg pertussis toxin (PT), 25 µg filamentous hemagglutinin (FHA) and 8 µg pertactin (PRN). Each dose of DTaP-IPV and IPV vaccine contained 40 D-Ag units (DU) poliovirus type 1, 8 DU poliovirus type 2, and 32 DU poliovirus type 3. The MMR vaccine contained  $\geq 1000$  tissue culture infectious doses (TCID<sub>50</sub>) measles virus, 20,000 TCID<sub>50</sub> mumps virus, and 1000 TCID<sub>50</sub> rubella virus. The DTaP-IPV and DTaP vaccines were administered as deep intramuscular injections into the left deltoid region. The IPV and MMR vaccines were administered as subcutaneous injections in the lower right and upper right deltoid regions, respectively.

### 2.3. Assessment of immunogenicity

Serum samples were obtained immediately prior to and 1 month after vaccination and stored at  $-20^{\circ}\text{C}$  until analysis. Assays for antibodies to diphtheria, tetanus, PT, FHA, PRN and poliovirus types 1, 2 and 3 were performed in the laboratories of Dr. Michael Pichichero, MEP Laboratories, University of Rochester (Rochester, US). Assays for antibodies to measles, mumps and rubella were carried out at GlaxoSmithKline Biologicals (Rixensart, Belgium). Standardized enzyme-linked immunosorbent assays (ELISA) were used to assess antibody concentrations to diphtheria and tetanus toxoids, PT, FHA, PRN, measles and rubella. Neutralization assays were used to assess antibody titers to the 3 poliovirus types and mumps.

Antibody concentrations for both diphtheria and tetanus  $\geq 0.1$  IU/mL, and for the 3 poliovirus types antibody titers  $\geq 1:8$ , were pre-specified to indicate seroprotection. Antibody concentrations/titers to each pertussis antigen of  $\geq 5$  ELISA units (EL.U)/mL, to measles  $\geq 150$  mIU/mL, to mumps  $\geq 1:28$ , and to rubella  $\geq 4$  IU/mL were pre-specified to indicate seropositivity. Antibody concentrations to rubella  $\geq 10$  IU/mL were pre-specified to indicate seroprotection. Booster response for diphtheria and tetanus was defined as a post-vaccination antibody concentration  $\geq 0.4$  IU/mL in initially seronegative children (antibody concentrations  $< 0.1$  IU/mL) or a post-vaccination increase of at least four times the pre-vaccination antibody concentration for initially seropositive children. Booster response for PT, FHA, and PRN was defined as an antibody concentration

$\geq 20$  EL.U/mL in children who were seronegative (antibody concentrations  $< 5.0$  EL.U/mL) before vaccination or at least a 4-fold increased antibody concentration in children with pre-vaccination antibody concentrations  $\geq 5.0$  and  $< 20$  EL.U/mL or at least a 2-fold increased antibody concentration in children with pre-vaccination antibody concentrations  $\geq 20$  EL.U/mL. Booster response for the 3 poliovirus types was defined as a post-vaccination antibody titer  $\geq 1:32$  in initially seronegative children (antibody titers  $< 1:8$ ) or a post-vaccination increase of at least four times the pre-vaccination antibody titer for initially seropositive children.

#### 2.4. Assessment of reactogenicity

Data on solicited local and general adverse events were collected by the parents/guardians using standardized diary cards for 15 consecutive days following vaccination. Local adverse events included, pain, redness and swelling at all injection sites, and measurement of mid upper-arm circumference. Mid upper-arm circumference was measured using a provided measuring gauge and performed at the mid-point of the upper arm, at mid distance between the acromion and the tip of the elbow, while the arm was held parallel to the trunk and the elbow flexed in front at  $90^\circ$ . General adverse events included fever [oral temperature  $\geq 37.5^\circ\text{C}$  ( $\geq 99.5^\circ\text{F}$ )], drowsiness and loss of appetite. Intensity of symptoms was graded on a scale of 0–3. Clinically relevant (Grade 3) symptoms were defined as follows: for local pain, as crying when the limb was moved or a spontaneously painful limb; for local redness and swelling, as diameter  $\geq 50$  mm; for fever, a temperature  $> 39.0^\circ\text{C}$  ( $> 102.2^\circ\text{F}$ ); for loss of appetite, as not eating at all; and for all other adverse events (including functional impairment), as preventing normal daily activities.

The children were observed for 30 min after vaccination and parents/guardians were instructed to contact study personnel immediately and to visit the investigator's site for an evaluation if their child experienced a large injection site swelling reaction (swelling with a diameter of  $> 50$  mm at any injection site, an increase in mid-upper arm circumference  $> 30$  mm, or diffuse swelling that interfered with or prevented normal daily activities).

Solicited general symptoms specific to MMR vaccine (for example, rash/exanthema, parotid/salivary gland swelling) were recorded over a period of 43 days following vaccination (Day 0–42). Unsolicited adverse events occurring within 31 days of vaccination were recorded. The children were monitored for an additional 5-month period for non-routine medical visits, visits to an emergency room, onset of new chronic illness, and serious adverse events (SAEs).

#### 2.5. Statistics

The primary cohort for assessment of immunogenicity was the according to protocol (ATP) cohort. Safety analyses were performed on the total cohort.

The primary objective of the study was to show non-inferiority of the combined DTaP–IPV vaccine compared with separate administration of DTaP and IPV vaccines in terms of diphtheria, tetanus, PT, FHA and PRN booster responses and poliovirus geometric mean titers (GMTs) of antibodies 1 month after vaccination. Non-inferiority was defined as being reached if the upper limits of the standardized asymptotic two-sided 95% CIs [16] calculated for the differences in booster response rates for diphtheria, tetanus, PT, FHA and PRN were  $\leq 10\%$ , and if the upper limit of the two-sided 95% CI for the GMT ratio for each of the 3 poliovirus types between groups was  $\leq 2$ . For each antigen 1 month after vaccination, 95% CIs of the geometric mean concentrations/titers (GMC/GMT) of antibody ratios (DTaP + IPV + MMR divided by DTaP–IPV + MMR) were computed using an ANCOVA model on the logarithm<sub>10</sub> transformation of the concentrations/titers. The ANCOVA model included the vaccine group as fixed effect and the log-transformed pre-vaccination concentration/titer as regressor. All safety and reactogenicity analyses were descriptive, with incidences of solicited and unsolicited symptoms and corresponding 95% CIs calculated per group. A two-sided Fisher's exact test *P*-value below 0.05 was considered significant for the exploratory between group comparisons performed for incidence of solicited symptoms.

Statistical analyses were performed using SAS Software 8.02 and Proc StatXact 5.0 on Windows NT 4.0. The study was designed, and data collected and analyzed, by Glaxo-SmithKline in coordination with the lead author who had access to the data and attests to the accuracy of the data and data analysis.

### 3. Results

#### 3.1. Study population

A total of 400 children were enrolled, randomized and vaccinated; 200 were randomized to DTaP–IPV + MMR (combination group) and 200 to DTaP + IPV + MMR (separate group). A total of 368 children were eligible for inclusion in the ATP analysis for immunogenicity, 181 of whom received the combined DTaP–IPV vaccine and 187 separate DTaP and IPV vaccines (Fig. 1). There were no significant differences between groups with respect to age, gender, race, and time period since the children received their last doses of DTaP and poliovirus vaccines. For the total cohort, the mean age was  $4.1 \pm 0.3$  years, 51.5% were female, 54.8% were White/Caucasian, 16.8% were American Hispanic and 11% were Black (race was self reported by the parents/guardians), and the mean time since last DTaP and IPV vaccination was 35.7 and 39.4 months, respectively. For the children in the ATP cohort for immunogenicity, 91.7% in the DTaP–IPV + MMR group, and 89.3% in the DTaP + IPV + MMR group had previously received all 3 doses of polio vaccine as IPV. None of the parents/guardians

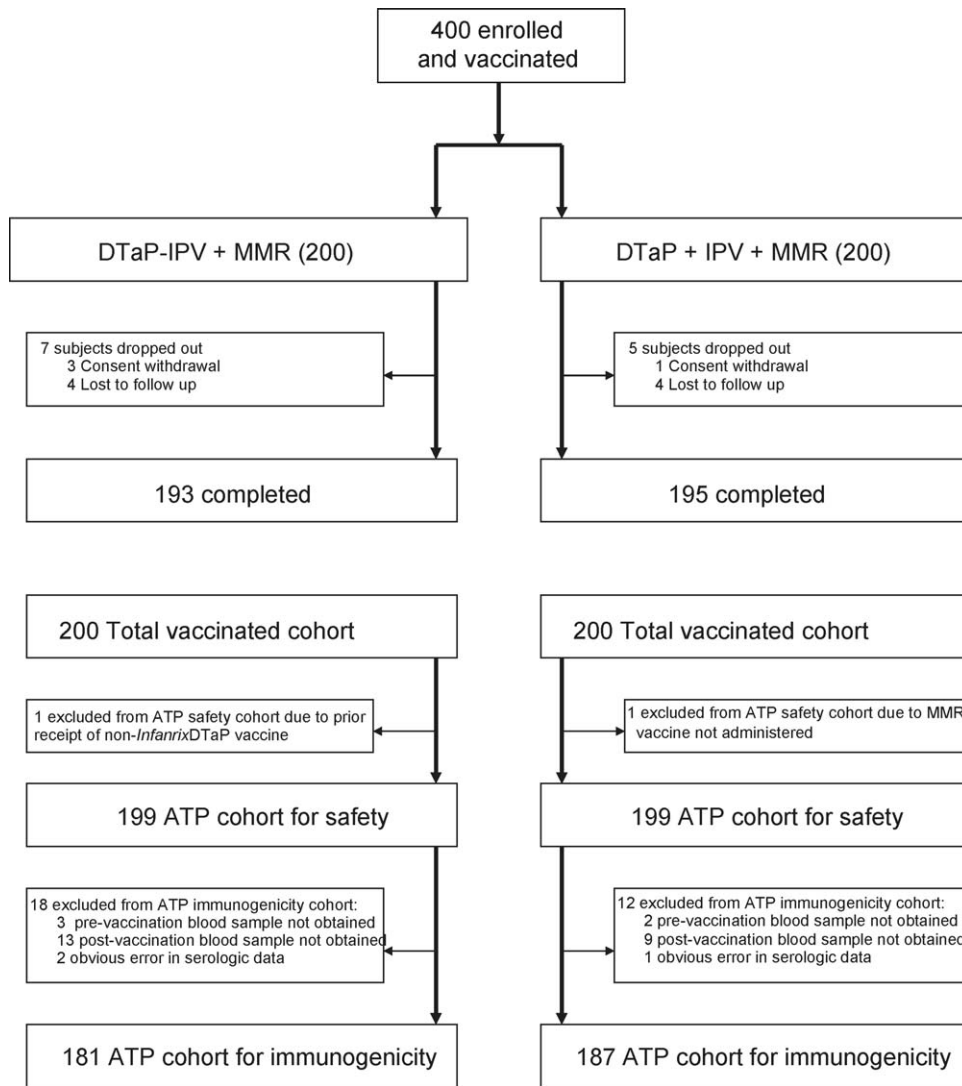


Fig. 1. Subject disposition.

self-reported that their child experienced a severe local reaction after administration of the fourth dose of DTaP vaccine. Ninety-seven percent of the children/parents/guardians complied with study instructions.

### 3.2. Immunogenicity

Pre-booster seroprotection/seropositivity rates were similar in the two study groups and were  $\geq 90.5\%$  for all antigens with the exception of diphtheria ( $\geq 79.3\%$ ) and PT ( $\geq 42.3\%$ ) (Table 1). Post-booster seroprotection/seropositivity rates and GMC/GMT values in the two study groups for all antigens are shown in Table 1. One month after booster vaccination, all but one child in the DTaP-IPV + MMR group had seroprotective levels of anti-diphtheria antibodies and all children in both groups had seroprotective levels of anti-tetanus antibodies. One month after booster dosing, all children in both groups were seropositive for anti-FHA and anti-PRN antibodies, and all but three children (one in the

DTaP-IPV + MMR group and two in the DTaP + IPV + MMR group) were seropositive for anti-PT. All children in both groups had seroprotective levels of anti-poliovirus types 1, 2 and 3 antibodies. All children in both groups were seropositive for measles, mumps and rubella antibodies and had seroprotective levels of anti-rubella antibodies following administration of the booster dose of MMR vaccine. Both groups exhibited a robust boost in GMC/GMT of antibodies for all antigens following booster vaccination, with similar GMC/GMT values seen between the two study groups (Table 1).

Booster response rates were comparable in the two study groups (Table 2). At least 91.5% of children in both groups met pre-specified criteria for a booster response to diphtheria, tetanus and the three pertussis antigens. Non-inferiority of the DTaP-IPV vaccine versus separate administration of DTaP and IPV vaccines was demonstrated since the upper limit of the standardized asymptotic two-sided 95% CIs for the difference between the two groups was below the pre-specified

Table 1

Antibody responses to diphtheria and tetanus toxoids, and pertussis, poliovirus, measles, mumps, and rubella antigens before and 1 month following vaccination with DTaP-IPV + MMR or DTaP + IPV + MMR (ATP cohort for immunogenicity)

Antibody (and response)	Timing	DTaP-IPV + MMR Group					DTaP + IPV + MMR Group				
		Seroprotected/seropositive			GMC/GMT		Seroprotected/seropositive			GMC/GMT	
		N	%	95% CI	Value	95% CI	N	%	95% CI	Value	95% CI
Anti-diphtheria ( $\geq 0.1$ IU/mL)	Pre-booster	179	79.3	72.7, 85.0	0.221	0.190, 0.257	185	84.3	78.3, 89.2	0.230	0.201, 0.263
	Post-booster	178	99.4	96.9, 100	7.821	6.674, 9.165	187	100	98.0, 100	7.702	6.798, 8.726
Anti-tetanus ( $\geq 0.1$ IU/mL)	Pre-booster	179	90.5	85.2, 94.4	0.326	0.283, 0.375	185	93.0	88.3, 96.2	0.348	0.204, 0.399
	Post-booster	178	100	97.9, 100	8.040	7.095, 9.111	187	100	98.0, 100	7.440	6.573, 8.421
Anti-PT ( $\geq 5$ EL.U/mL)	Pre-booster	168	42.3	34.7, 50.1	4.7	4.1, 5.4	170	45.9	38.2, 53.7	4.8	4.3, 5.4
	Post-booster	177	99.4	96.9, 100	102.2	88.3, 118.4	185	98.9	96.1, 99.9	91.6	80.2, 104.6
Anti-FHA ( $\geq 5$ EL.U/mL)	Pre-booster	175	96.6	92.7, 98.7	36.1	30.6, 42.5	183	97.3	93.7, 99.1	30.5	26.4, 35.3
	Post-booster	176	100	97.9, 100	378.8	336.6, 426.4	183	100	98.0, 100	384.8	343.0, 431.6
Anti-PRN ( $\geq 5$ EL.U/mL)	Pre-booster	179	94.4	90.0, 97.3	38.5	32.4, 45.8	185	95.7	91.7, 98.1	30.5	26.0, 35.7
	Post-booster	178	100	97.9, 100	657.5	566.7, 762.9	187	100	98.0, 100	507.5	441.8, 583.0
Anti-poliovirus (type 1 $\geq 1:8$ )	Pre-booster	170	97.1	93.3, 99.0	74.1	60.9, 90.3	169	97.0	93.2, 99.0	70.8	59.8, 83.9
	Post-booster	178	100	97.9, 100	1337.0	1172.9, 1524.1	187	100	98.0, 100	1337.2	1173.3, 1524.1
Anti-poliovirus (type 2 $\geq 1:8$ )	Pre-booster	169	95.3	90.9, 97.9	80.7	66.0, 98.7	167	98.2	94.8, 99.6	84.5	70.5, 101.2
	Post-booster	178	100	97.9, 100	1217.7	1074.2, 1380.3	187	100	98.0, 100	1241.6	1083.3, 1423.1
Anti-poliovirus (type 3 $\geq 1:8$ )	Pre-booster	159	91.2	85.7, 95.1	83.9	66.0, 106.5	163	92.0	86.7, 95.7	74.6	59.7, 93.2
	Post-booster	172	100	97.9, 100	2089.9	1776.6, 2458.5	184	100	98.0, 100	2314.7	1947.4, 2751.3
Anti-measles ( $\geq 150$ mIU/mL)	Pre-booster	181	98.9	96.1, 99.9	4236.9	3694.1, 4859.5	187	100	98.0, 100	4684.9	4155.3, 5282.0
	Post-booster	181	100	98.0, 100	5729.4	5227.9, 6279.0	187	100	98.0, 100	5344.0	4818.2, 5927.2
Anti-mumps ( $\geq 1:28$ )	Pre-booster	175	98.9	95.9, 99.9	258.7	223.3, 299.6	181	100	98.0, 100	264.2	230.3, 303.1
	Post-booster	170	100	97.9, 100	443.7	387.0, 508.6	171	100	97.9, 100	404.0	358.4, 455.4
Anti-rubella ( $\geq 4$ IU/mL)	Pre-booster	181	99.4	97.0, 100	70.5	61.7, 80.6	187	99.5	97.1, 100	69.2	60.4, 79.2
	Post-booster	181	100	98.0, 100	152.2	139.3, 166.2	187	100	98.0, 100	157.5	144.0, 172.3
Anti-rubella ( $\geq 10$ IU/mL)	Pre-booster	181	98.9	96.1, 99.9	70.5	61.7, 80.6	187	97.9	94.6, 99.4	69.2	60.4, 79.2
	Post-booster	181	100	98.0, 100	152.2	139.3, 166.2	187	100	98.0, 100	157.5	144.0, 172.3

Seroprotection:

- for anti-diphtheria and anti-tetanus = antibody concentration  $\geq 0.1$  IU/mL by ELISA;
- for anti-poliovirus types 1, 2 and 3 = antibody titer  $\geq 1:8$  by neutralization;
- for anti-rubella = antibody concentration  $\geq 10$  IU/mL by ELISA.

Seropositivity:

- for anti-PT, anti-FHA and anti-PRN = antibody concentration  $\geq 5$  EL.U/mL by ELISA;
- for anti-measles = antibody concentration  $\geq 150$  mIU/mL by ELISA;
- for anti-mumps = antibody titer  $\geq 1:28$  by neutralization;
- for anti-rubella = antibody concentration  $\geq 4$  IU/mL by ELISA.

GMC/GMT, geometric mean antibody concentration/geometric mean antibody titer; N, number of children with available results; %, percentage of children with antibody concentrations/titers above specified cut-off; pre-booster, pre-vaccination blood sample at Day 0; post-booster, post-vaccination blood sample 1 month after vaccination; 95% CI, 95% confidence interval.

Table 2

Differences in percentage of children with booster response<sup>a</sup> rates between the DTaP–IPV + MMR and DTP + IPV + MMR groups 1 month after vaccination (ATP cohort for immunogenicity)

Antibody	DTaP–IPV + MMR Group		DTaP + IPV + MMR Group		Difference in booster response rate (DTaP + IPV minus DTaP–IPV)	
	<i>N</i>	%	<i>N</i>	%	%	95% CI
Anti-diphtheria <sup>b</sup>	176	96.6	185	98.9	2.33	–0.87, 6.29 <sup>c</sup>
Anti-tetanus <sup>b</sup>	176	97.2	185	97.3	0.14	–3.70, 4.11 <sup>c</sup>
Anti-PT <sup>b</sup>	165	91.5	168	94.6	3.13	–2.46, 9.03 <sup>c</sup>
Anti-FHA <sup>b</sup>	170	96.5	179	96.1	–0.38	–4.79, 4.06 <sup>c</sup>
Anti-PRN <sup>b</sup>	176	96.0	185	97.3	1.27	–2.72, 5.61 <sup>c</sup>
Anti-poliovirus type 1	167	88.0	169	87.0	–1.04	–8.27, 6.18 <sup>d</sup>
Anti-poliovirus type 2	166	86.7	167	79.0	–7.71	–15.87, 0.39 <sup>d</sup>
Anti-poliovirus type 3	152	85.5	162	85.8	0.28	–7.56, 8.23 <sup>d</sup>

*N*, number of children with both pre- and post-vaccination results; %, percentage of children with a booster response 1 month after vaccination; 95% CI, standardized asymptotic 95% confidence interval for difference.

<sup>a</sup> Booster response for diphtheria and tetanus denotes post-vaccination antibody concentration  $\geq 0.4$  IU/mL in initially seronegative children (antibody concentrations  $< 0.1$  IU/mL) or post-vaccination increase of at least 4 times pre-vaccination antibody concentration for initially seropositive children. Booster response for PT, FHA, and PRN denotes antibody concentration  $\geq 20$  EL.U/mL in children seronegative (antibody concentrations  $< 5.0$  EL.U/mL) before vaccination or at least 4-fold increase pre-vaccination antibody concentrations  $\geq 5.0$  and  $< 20$  EL.U/mL or at least 2-fold increase in children with pre-vaccination antibody concentrations  $\geq 20$  EL.U/mL. Booster response for the 3 poliovirus types denotes post-vaccination antibody titer  $\geq 1:32$  in initially seronegative children (antibody titers  $< 1:8$ ) or post-vaccination increase of at least 4 times the pre-vaccination antibody titer in initially seropositive children.

<sup>b</sup> Primary endpoint.

<sup>c</sup> Booster response to DTaP–IPV was non-inferior to DTaP + IPV (upper limit of the two-sided 95% CI on the difference DTaP + IPV minus DTaP–IPV was  $\leq 10\%$ ).

<sup>d</sup> Non-inferiority criteria not pre-specified for this endpoint.

clinical limit of 10% for diphtheria, tetanus, and the 3 pertussis antigens. At least 85.5% of children in the DTaP–IPV group and 79.0% of those in the DTaP + IPV group showed a booster response to each of the three poliovirus types. The upper limits of the two-sided 95% CIs for the GMT ratios between the two groups was below the pre-specified clinical limit for non-inferiority of 2 for all 3 poliovirus types (Table 3), confirming the non-inferiority of the DTaP–IPV vaccine versus DTaP and IPV vaccines administered separately. Analysis restricted to the approximately 90% of children primed with 3 doses of IPV also showed the DTaP–IPV vaccine to be non-inferior to separate administration of DTaP and IPV vaccines in terms of anti-poliovirus antibody response (upper limits of the two-sided 95% CIs for the GMT

ratios between the two groups was below the pre-specified clinical limit of 2) for all 3 poliovirus types (data not shown).

### 3.3. Reactogenicity

No statistically significant differences in the incidence of solicited local or general symptoms were seen between the combination and separate vaccine groups during the 4- and 15-day post-vaccination periods (Tables 4 and 5). Local injection site reactions were the most commonly reported adverse event in both study groups. The incidence of Grade 3 general solicited symptoms was  $\leq 3.1\%$  in both study groups. The incidence of general solicited symptoms specific to MMR vaccination was  $\leq 3.6\%$  in both study groups

Table 3

Adjusted GMC/GMT ratios between the DTaP–IPV + MMR and DTP + IPV + MMR groups 1 month after vaccination (ATP cohort for immunogenicity)

Antibody	DTaP–IPV + MMR Group		DTaP + IPV + MMR Group		Adjusted ratio ([DTaP + IPV]/[DTaP–IPV])	
	<i>N</i>	Adjusted GMC/GMT	<i>N</i>	Adjusted GMC/GMT	Value	95% CI
Anti-diphtheria	176	7.840	185	7.579	0.967	0.815, 1.146 <sup>a</sup>
Anti-tetanus	176	8.157	185	7.325	0.898	0.774, 1.042 <sup>a</sup>
Anti-PT	165	101.6	168	92.7	0.913	0.760, 1.096 <sup>a</sup>
Anti-FHA	170	374.1	179	398.0	1.064	0.918, 1.233 <sup>a</sup>
Anti-PRN	176	620.4	185	533.8	0.860	0.728, 1.017 <sup>a</sup>
Anti-poliovirus type 1 <sup>b</sup>	167	1336.0	169	1320.0	0.988	0.812, 1.203 <sup>c</sup>
Anti-poliovirus type 2 <sup>b</sup>	166	1226.5	167	1180.5	0.962	0.793, 1.168 <sup>c</sup>
Anti-poliovirus type 3 <sup>b</sup>	152	2108.7	162	2227.3	1.056	0.827, 1.348 <sup>c</sup>

*N*, number of children with both pre- and post-vaccination results; adjusted GMC/GMT, geometric mean antibody concentration/titer adjusted for baseline concentration; 95% CI, 95% confidence interval limit for the ratio (ANCOVA model: adjustment for baseline).

<sup>a</sup> Non-inferiority criteria not pre-specified for this endpoint.

<sup>b</sup> Primary endpoint.

<sup>c</sup> GMT for DTaP–IPV was non-inferior to DTaP + IPV (upper limit of the two-sided 95% CI for the adjusted GMT ratio for each of the 3 poliovirus types DTaP + IPV/DTaP–IPV was  $\leq 2$ ).

Table 4

Incidence of solicited local symptoms at the DTaP–IPV or DTaP injection site during the 4- and 15-day follow-up periods after booster vaccination with DTaP–IPV or DTaP + IPV co-administered with MMR vaccine (total vaccinated cohort)

Symptom	Injection site	Onset (days)	Intensity	DTaP–IPV + MMR (N=196)			DTaP + IPV + MMR (N=195)			P-value
				n	%	95% CI	n	%	95% CI	
Pain	DTaP–IPV/DTaP	4	Any	118	60.2	53.0–67.1	112	57.4	50.2–64.5	0.608
			Grade 3	5	2.6	0.8–5.9	8	4.1	1.8–7.9	0.415
		15	Any	118	60.2	53.0–67.1	112	57.4	50.2–64.5	0.608
			Grade 3	5	2.6	0.8–5.9	8	4.1	1.8–7.9	0.415
Redness	DTaP–IPV/DTaP	4	Any	112	57.1	49.9–64.2	99	50.8	43.5–58.0	0.224
			≥50 mm	55	28.1	21.9–34.9	45	23.1	17.4–29.6	0.297
		15	Any	112	57.1	49.9–64.2	99	50.8	43.5–58.0	0.224
			≥50 mm	56	28.6	22.4–35.4	46	23.6	17.8–30.2	0.300
Swelling	DTaP–IPV/DTaP	4	Any	78	39.8	32.9–47.0	80	41.0	34.0–48.3	0.837
			≥50 mm	30	15.3	10.6–21.1	27	13.8	9.3–19.5	0.775
		15	Any	78	39.8	32.9–47.0	80	41.0	34.0–48.3	0.837
			≥50 mm	30	15.3	10.6–21.1	27	13.8	9.3–19.5	0.775

One subject in the DTaP + IPV + MMR group did not receive the MMR vaccination. 4-day follow-up period, Day 0–3; 15-day follow-up period, Day 0–14; N, number of children with diary cards completed; n (%), number (percentage) of children reporting specified adverse event; Grade 3 pain, cried when limb was moved; 95% CI, exact 95% confidence interval; P-value, two-sided Fisher's exact test.

(data not shown). Rash was reported by 3.6% of children in each group, with a single report considered by the investigators to be causally related to MMR vaccination in each group. Parotid gland swelling occurred in a single child in each group.

Large injection site reactions at the DTaP–IPV vaccine or DTaP vaccine injection site were reported by 26 children (13.0%) in the combination vaccine group and 29 children (14.5%) in the separate vaccine group. Thirty-seven of these 55 children (67%) presented to their investigator for a swelling evaluation; swelling information on the remaining 18 children was collected through review of diary cards, and telephone or direct interviews by the investigators with the

parents/guardians. An additional child in the separate vaccine group reported large injection site swelling at the MMR injection site. All large injection site swellings occurred within 48 h of booster vaccine administration, with the exception of one child in the separate vaccine group in whom onset occurred on Day 3. Functional impairment was not associated with the large injection site swelling in 83% of these 55 children, and severe (Grade 3) functional impairment was reported by only a single child in each group. Only 3 of these 55 children (2 in the combination vaccine group and 1 in the separate vaccine group), representing 0.7% of the 400 vaccinated children, reported swelling that involved an adjacent joint, in all cases the elbow. For all large swellings for which

Table 5

Incidence of solicited general symptoms during the 4- and 15-day follow-up periods after booster vaccination with DTaP–IPV or DTaP + IPV co-administered with MMR vaccine (total vaccinated cohort)

Symptoms	Onset (days)	Intensity	DTaP–IPV + MMR (N=196)			DTaP + IPV + MMR (N=194)			P-value
			n	%	95% CI	n	%	95% CI	
Fever (oral)	4	≥37.5 °C	37	18.9	13.7–25.1	42	21.6	16.1–28.1	0.530
		>39.0 °C	1	0.5	0.0–2.8	2	1.0	0.1–3.7	0.622
	15	≥37.5 °C	50	25.5	19.6–32.2	51	26.3	20.2–33.1	0.908
		>39.0 °C	5	2.6	0.8–5.9	4	2.1	0.6–5.2	1.000
Drowsiness	4	Any	48	24.5	18.6–31.1	43	22.2	16.5–28.7	0.633
		Grade 3	4	2.0	0.6–5.1	1	0.5	0.0–2.8	0.372
	15	Any	54	27.6	21.4–34.4	49	25.3	19.3–32.0	0.647
		Grade 3	6	3.1	1.1–6.5	3	1.5	0.3–4.5	0.503
Loss of appetite	4	Any	41	20.9	15.4–27.3	34	17.5	12.5–23.6	0.441
		Grade 3	3	1.5	0.3–4.4	3	1.5	0.3–4.5	1.000
	15	Any	45	23.0	17.3–29.5	42	21.6	16.1–28.1	0.808
		Grade 3	5	2.6	0.8–5.9	3	1.5	0.3–4.5	0.724

4-day follow-up period, Day 0–3; 15-day follow-up period, Day 0–14; N, number of children with diary cards completed; n (%), number (percentage) of children reporting specified adverse event; Grade 3 drowsiness, drowsiness that prevented normal daily activities; Grade 3 loss of appetite, not eating at all; 95% CI, exact 95% confidence interval; P-value, two-sided Fisher's exact test.

outcome data were available, the episodes resolved without sequelae.

Grade 3 unsolicited symptoms were reported by 4.5% of children in the combination vaccine group and 4.0% of children in the separate vaccine group during the 31-day follow-up period after vaccination. Two SAEs were reported by 2 children in the separate vaccine group during the 31-day period following vaccination. Both of these SAEs were considered unrelated to vaccination by the investigators.

At least 97% of the children completed the additional 5-month safety follow-up evaluation. During the extended safety follow-up period, the percentages of children reporting a SAE, new onset of a chronic illness, or an adverse event that led to an emergency room or non-routine office visit were similar in both groups, and below 1.5, 1, 3.6 and 4.1%, respectively. Four SAEs were reported by 3 children in the combination vaccine group during the extended safety follow-up period. All of these SAEs were considered unrelated to vaccination by the investigators, and none were of potential autoimmune or new onset chronic in nature. No child withdrew from this study due to adverse events.

#### 4. Discussion

This study was undertaken to compare the immunogenicity and safety of a combined DTaP–IPV vaccine with separate administration of DTaP and IPV vaccines when co-administered as a fifth dose of acellular pertussis vaccine with a second dose of MMR vaccine in children aged 4–6 years. The relatively low pre-booster seroprotection rates for diphtheria and seropositivity rates for PT seen in the children participating in this study support the need for a booster dose of DTaP in children at this age to boost waning immunity and provide long-term protection against these vaccine preventable diseases. Results showed the combined DTaP–IPV vaccine to be non-inferior to separate DTaP and IPV vaccines in terms of booster response rates to diphtheria, tetanus, PT, FHA, PRN and GMTs for antibodies against all 3 poliovirus types, regardless of an all inactivated or a mixed sequence of OPV/IPV vaccine priming history. One month after booster vaccination,  $\geq 98.9\%$  of children in both study groups were seroprotected/seropositive for antibodies against all vaccine antigens. Importantly, co-administration of the combined DTaP–IPV vaccine did not interfere with immune response to the second dose of MMR vaccine, with all children seropositive for measles, mumps and rubella antibodies and all seroprotected for rubella 1 month after vaccination. Post-vaccination GMCs/GMTs for antibodies against all co-administered antigens were similar in both groups. The immune response seen to booster vaccination with the DTaP–IPV vaccine in this study is in keeping with that reported in other studies where this combined vaccine was administered as a fourth or fifth dose of DTaP in children aged 4–6 years, either alone or together with a booster dose of MMR vaccine [17,18].

Booster vaccination with the combined DTaP–IPV vaccine was well-tolerated, with reactogenicity comparable to that seen in children receiving the separate DTaP and IPV vaccines. No significant differences in the incidence of solicited and unsolicited adverse events were seen between study groups. Local injection site reactions were the most common adverse events reported and the incidence of Grade 3 general solicited symptoms was low ( $\leq 3.1\%$ ) in both study groups.

Large local injection site reactions have been documented with booster doses of acellular pertussis vaccines with the highest rates reported after the fourth and fifth doses [19–23]. The pathogenesis of these reactions is unknown, likely multifactorial and perhaps a cumulative response to several vaccine component antigens. Studies have inconsistently reported the following to be associated with large injection site swelling: pertussis toxin, diphtheria toxoid, and aluminum content of the vaccine, high pre-vaccination antibody titers to diphtheria, tetanus, or pertussis toxin, subcutaneous vaccine administration, and a Th2 orientation of cytokine production [20,24,25]. The methodology used in this study to capture cases of large DTaP injection site swelling was very sensitive in that it involved daily measurement of both injection site swelling and mid-upper arm circumference. Large swelling at the DTaP-based injection site was reported by 13.0% of children who received the DTaP–IPV vaccine and 14.5% of children who received a separate DTaP vaccine. The majority of these large injection site reactions did not involve an adjacent joint, more than two-thirds were not associated with functional impairment and all cases in which outcome data were available resolved without sequelae. This is consistent with the results of previous studies with this combined DTaP–IPV vaccine [17,18,26], and is also in keeping with the reported range for other DTaP-containing vaccines [19–22,24,27–31].

The use of combination vaccines to provide simultaneous protection against multiple infectious diseases in a single injection is an established approach for simplifying pediatric vaccination schedules [11,12,14,15]. By reducing the number of injections, combination vaccines offer benefits in terms of increased compliance and convenience of immunization practice potentially leading to better disease control and a reduction in overall immunization costs [12,14]. However, it is essential that the immunogenicity and safety of any new combination vaccine is comparable to that of the individual component vaccines [32]. Results of this study show booster vaccination with combined DTaP–IPV vaccine to be as immunogenic and well-tolerated as separate administration of currently licensed DTaP and IPV vaccines when co-administered with a second dose of MMR vaccine in healthy children aged 4–6 years. Taken together with the results of other studies [17,18], available data suggest that this DTaP–IPV vaccine is likely to be effective, safe, and useful as a pre-school booster in a wide variety of vaccination schedules. It would offer one solution to the problem of increased numbers of injections during single clinic visits.

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## References

- [1] Schmitt HJ, Booy R, Weil-Olivier C, Van Damme P, Cohen R, Peltola H. Child vaccination policies in Europe: a report from the Summits of independent European vaccination experts. *Lancet Infect Dis* 2003;3:103–8.
- [2] Centers for Disease Control and Prevention. General recommendations on immunization. Recommendations of the Advisory Committee on Immunization Practices (ACIP) and the American Academy of Family Physicians (AAFP). *MMWR* 2002;51(RR-2):1–35.
- [3] Therre H, Baron S. Pertussis immunisation in Europe—the situation in late 1999. *Euro Surveill* 2000;5:6–10.
- [4] Centers for Disease Control and Prevention. Pertussis, United States, 1997–2000. *MMWR* 2002;51:73–6.
- [5] von König CH, Halperin S, Riffelmann M, Guiso N. Pertussis of adults and infants. *Lancet Infect Dis* 2002;2:744–50.
- [6] Tan T, Trindade E, Skowronski D. Epidemiology of pertussis. *Pediatr Infect Dis J* 2005;24(Suppl.):S10–8.
- [7] Impfempfehlungen der ständigen impfkommision (STIKO) am Robert-Koch-Institut. Stand: January 2000. *Dt Ärzteblatt* 2000; 16(Suppl.):3–18.
- [8] Gibbons V. Addition of acellular pertussis to preschool booster in the United Kingdom. <http://www.eurosurveillance.org/ew/2001/011115.asp>; 2005 [accessed July 28, 2005].
- [9] Andersen P. Danish childhood vaccination programmer modified to include pertussis and polio boosters at 5 y of age. <http://www.eurosurveillance.org/ew/2003/030828.asp>.
- [10] Advisory Committee on Immunization Practices. ACIP recommends adolescent vaccination for tetanus, diphtheria and pertussis vaccine. Available at: [http://www.cdc.gov/nip/pr/pr\\_idap\\_jun2005.htm](http://www.cdc.gov/nip/pr/pr_idap_jun2005.htm); 2005 [accessed July 28, 2005].
- [11] Andre FE. Development and clinical application of new polyvalent combined paediatric vaccines. *Vaccine* 1999;17:1620–7.
- [12] Bogaerts H. The future of childhood immunizations: examining the European experience. *Am J Manag Care* 2003;9(Suppl.):S30–6.
- [13] Centers for Disease Control and Prevention. Combination vaccines for childhood immunization. Recommendations of the Advisory Committee on Immunization Practices (ACIP) the American Academy of Pediatrics (AAP), and the American Academy of Family Physicians (AAFP). *MMWR* 1999;48(RR-5):1–15.
- [14] Dodd D. Benefits of combination vaccines: effective vaccination on a simplified schedule. *Am J Manag Care* 2003;9(Suppl.):S6–S12.
- [15] Rennels MB. Combination vaccines. *Pediatr Infect Dis J* 2002;21:255–7.
- [16] Miettinen O, Nurminen M. Comparative analysis of two rates. *Stat Med* 1985;4:213–26.
- [17] Marshall H, Nolan T, Robertson D, et al. A comparison of booster immunisation with a combination DTaP–IPV vaccine or DTaP plus IPV in separate injections when co-administered with MMR at age 4–6 years. *Vaccine* 2006;24:6120–8.
- [18] Nilsson L, Faldella G, Jacquet JM, Storsaeter J, Silfverdal SA, Ekholm L. A fourth dose of DTPa–IPV vaccine given to 4–6 year old children in Italy and Sweden following primary vaccination at 3, 5 and 11–12 months of age. *Scand J Infect Dis* 2005;37:221–9.
- [19] Pichichero M, Edwards K, Anderson E, et al. Safety and immunogenicity of six acellular pertussis vaccines and one whole-cell pertussis vaccine given as a fifth dose in four to six year old children. *Pediatrics* 2000;105(1). Available at: <http://www.pediatrics.org/cgi/content/full/105/1/e11>.
- [20] Rennels MB. Extensive swelling reactions occurring after booster doses of diphtheria-tetanus-acellular pertussis vaccines. *Semin Pediatr Infect Dis* 2003;14:196–8.
- [21] Rennels MB, Deloria M, Pichichero M, et al. Extensive swelling after booster doses of acellular pertussis-tetanus-diphtheria vaccines. *Pediatrics* 2000;105(1). Available at: <http://www.pediatrics.org/cgi/content/full/105/1/e12>.
- [22] Skowronski D, Remple V, Macnabb J, et al. Injection-site reactions to booster doses of acellular pertussis vaccine: rate, severity and anticipated impact. *Pediatrics* 2003;112:e453. Available at: <http://www.pediatrics.org/cgi/content/full/112/6/e453>.
- [23] Woo E, Burwen D, Gatumu S, Ball R, the VAERS working group. Extensive limb swelling after immunization: reports to the vaccine adverse event reporting system. *Clin Infect Dis* 2003;37:351–8.
- [24] Centers for Disease Control. Use of diphtheria toxoid-tetanus toxoid-acellular pertussis vaccine as a five-dose series: Supplemental Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR* 2000;49(RR-13):1–8.
- [25] Ryan EJ, Nilsson L, Kjellman N, Gothefors L, Mills KH. Booster immunization of children with an acellular pertussis vaccine enhances Th2 cytokine production and serum IgE responses against pertussis toxin but not against common allergens. *Clin Exp Immunol* 2000;121:193–200.
- [26] Jacquet JM, Begue P, Grimprel E, et al. Safety and immunogenicity of a combined DTPa–IPV vaccine administered as a booster from 4 years of age: a review. *Vaccine* 2006;24(13):2440–8.
- [27] GlaxoSmithKline Biologicals. Rixensart, Belgium, *Infanrix*® package insert, August 2003.
- [28] Halperin S, Scheifele D, Barreto L, et al. Comparison of a fifth dose of a five-component acellular or a whole cell pertussis vaccine in children four to six years of age. *Pediatr Infect Dis J* 1999;18:772–9.
- [29] Halperin S, Scheifele D, Mills E, et al. Nature, evolution, and appraisal of adverse events and antibody response associate with the fifth consecutive dose of a five-component acellular pertussis-based combination vaccine. *Vaccine* 2003;21:2298–306.
- [30] Scheifele DW, Halperin SA, Ferguson AC. Assessment of injection site reactions to an acellular pertussis-based combination vaccine, including novel use of skin tests with vaccine antigens. *Vaccine* 2001;19:4720–6.
- [31] Tozzi AE, Anemona A, Stefanelli P, et al. Reactogenicity and immunogenicity at preschool age of a booster dose of two three-component diphtheria-tetanus-acellular pertussis vaccines in children primed in infancy with acellular vaccines. *Pediatrics* 2001;107(2). Available at: <http://www.pediatrics.org/cgi/content/full/107/2/e25>.
- [32] Yeh SH, Ward JI. Strategies for development of combination vaccines. *Pediatr Infect Dis J* 2001;20:S5–9.