Immunogenicity and safety of combined adsorbed low-dose diphtheria, tetanus and inactivated poliovirus vaccine (REVAXIS®) versus combined diphtheria, tetanus and inactivated poliovirus vaccine (DT Polio®) given as a booster dose at 6 years of age

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Key words: REVAXIS®, DT polio®, diphtheria, tetanus, poliomyelitis, immunogenicity, safety, booster, children

Abbreviations: AFSSaPS, Agence Française de Sécurité Sanitaire des Produits de Santé; CI, confidence interval; CPP, Comité de Protection des Personnes; Td-IPV, diphtheria (low dose), tetanus and inactivated poliovirus vaccine (adsorbed); DT-IPV, combined, standard dose diphtheria toxoid, tetanus and inactivated poliovirus vaccine; EIA, enzyme immunoassay; GMT, geometric mean titer; GMTR, geometric mean of individual post-dose to pre-dose titer ratio; IPV, inactivated poliovirus; SN, seroneutralization; SOC, system organ class; SPC, summary of product characteristics; WHO, World Health Organization

This randomized, comparative, phase-IIIb study conducted in France aimed to demonstrate whether seroprotection against diphtheria, tetanus and poliomyelitis 1 month after a single dose of REVAXIS (low-dose diphtheria) is non-inferior to seroprotection 1 month after a single dose of DT Polio (standard-dose diphtheria), both vaccines being given as a second booster to healthy children at 6 years of age. Children were randomly assigned to receive a single intramuscular dose of REVAXIS or DT Polio. Primary endpoints were the 1-month post-booster seroprotection rates for diphtheria, tetanus and poliovirus type-1, -2 and -3 antigens. Secondary endpoints were immunogenicity and safety observations. Of 788 children screened, 760 were randomized: REVAXIS group, 384 children; DT Polio group, 376 children. No relevant difference in demographic characteristics at baseline was observed between REVAXIS and DT Polio groups. Non-inferiority of REVAXIS compared with DT Polio for seroprotection was demonstrated against diphtheria (respectively 98.6% and 99.3%), tetanus (respectively 99.6% and 100%) and poliovirus antigens (100% for each types in both groups). No allergic reactions to REVAXIS were reported. A benefit/risk ratio in favor of REVAXIS was suggested by the trend towards a better tolerability of REVAXIS compared with DT Polio regarding the rate of severe solicited injection-site reactions. The results support the use of REVAXIS as a booster at 6 years of age in infants who previously received a three-dose primary series within the first 6 months of life and a first booster including diphtheria, tetanus and poliovirus vaccine(s) given before 2 years of age.

Introduction

Maintenance of life-long immunity and the corresponding improvement in population-based immunization coverage against diphtheria, tetanus and poliomyelitis is an important public health goal. Immunity acquired by childhood vaccination, however, decreases over time and requires regular booster vaccinations.¹⁻⁵

Following primary vaccination within the first 6 months of life, and a first booster during the second year of life, most

European children receive further booster vaccinations for diphtheria and tetanus, together with pertussis and/or poliomyelitis, at 4–6 years of age (i.e., before school entry). These booster vaccinations contain a standard (≥ 30 IU) or low (≥ 2 IU) dose of diphtheria toxoid and different doses of tetanus toxoid (≥ 20 IU or ≥ 40 IU). A booster vaccination containing the low-dose diphtheria toxoid is also given 5–10 years later, and may be repeated at further 10-year intervals.⁶

In France, vaccination using the standard-dose diphtheria-tetanus combination with inactivated poliovirus types 1, 2 and

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Table 1. Composition of the vaccines Td-IPV (REVAXIS) and DT-IPV (DT Polio) used for intramuscular (deltoid) booster vaccination against diphtheria, tetanus and poliomyelitis in children at 6 years of age.

REVAXIS

Dose: 0.5-mL suspension for injection in a pre-filled syringe

Active ingredients:

Purified diphtheria toxoid	≥2 IU (5 Lf)
Purified tetanus toxoid	≥20 IU (10 Lf)
Inactivated poliovirus type 1	40 DU
Inactivated poliovirus type 2	8 DU
Inactivated poliovirus type 3	32 DU

Adsorbant:

Aluminum hydroxide.	0.35 mg (as aluminum)
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DT Polio

Dose: 0.5-mL suspension for injection in a pre-filled syringe:

Active ingredients:

Purified diphtheria toxoid	≥30 IU
Purified tetanus toxoid	≥40 IU
Inactivated poliovirus type 1	40 DU
Inactivated poliovirus type 2	4 DU
Inactivated poliovirus type 3	8 DU

DU: Antigen unit D or equivalent quantity of antigen determined according to an appropriate immunochemical method

Lf: limit of flocculation

3 is recommended for a three-dose primary series (given within the first 6 months of life) followed by two booster doses at 16–18 months and 6 years of age.^{7,8} The second booster includes purified tetanus toxoid, purified diphtheria toxoid and inactivated poliovirus types 1, 2 and 3 (DT Polio®, Sanofi Pasteur MSD).⁹

REVAXIS® (Td-IPV, Sanofi Pasteur MSD),¹⁰ is a combination vaccine consisting of an adsorbed purified tetanus toxoid, purified low-dose diphtheria toxoid and inactivated poliovirus types 1, 2 and 3. A comparison of the composition of REVAXIS and DT Polio is given in Table 1. The immunogenicity and safety profile of REVAXIS as a booster vaccination has been demonstrated in adults of all ages¹¹ and in children between 6 years and 9 years of age.¹² In France, this vaccine has been primarily indicated as a booster for adults. In many European countries (Austria, Belgium, Germany, Greece, Spain, Ireland, Italy, Luxembourg, The Netherlands, Portugal and the UK) it is also indicated in children (from 6 years of age) and adolescents after primary active immunization.

In 2004, during a temporary interruption of production of DT Polio in France, the use of REVAXIS as a booster in children from 6 years of age was authorized by the Agence Française de Sécurité Sanitaire des Produits de Santé (AFSSaPS). To support this indication⁸ on a permanent basis, AFSSaPS required comparative data on seroprotection and antibody titers 1 month after administration of a booster vaccine of REVAXIS or DT Polio.

The primary objective of this randomized, comparative, phase-IIIb study in France was to ascertain if seroprotection against diphtheria, tetanus and poliomyelitis 1 month after a single dose

Figure 1. Children participating in the study.

of REVAXIS is non-inferior to seroprotection 1 month after a single dose of DT Polio, both vaccines being given as a second booster to healthy children at 6 years of age.

Results

Study population. From 788 children screened over 11 months in 2007, 760 were randomized to the study (REVAXIS group, 384 children; DT Polio group, 376 children) (Fig. 1). Two children, one in each group, withdrew from the study after randomization and were not vaccinated, and one child who was randomized to the DT Polio group received REVAXIS. Therefore, the safety set comprised 758 children: 384 in the REVAXIS group and 374 in the DT Polio group.

In addition to the two subjects who withdrew from the study, three subjects did not have post-vaccination immunogenicity data, so five subjects were excluded from the full analysis set (755 subjects; 382 in the REVAXIS group and 373 in the DT Polio group). Of the 760 children randomized, 193 were excluded from the per-protocol analysis, mainly due to non-adherence with selection criteria [i.e., 123 children were not previously vaccinated with three doses of a diphtheria, tetanus and poliomyelitis vaccine within the first 6 months of life and a booster vaccination for diphtheria, tetanus and poliomyelitis at 16–18 months of life (± 2 months)]. The per-protocol set therefore comprised 567 children (284 in the REVAXIS group and 283 in the DT Polio group). This set was used for the primary analysis and was defined as all randomized children excluding those with at least one protocol deviation that may have interfered with the immunogenicity evaluation.

There was no relevant difference between the REVAXIS and DT Polio groups with respect to demographic characteristics at baseline. The male/female ratio was 1.1 and the mean age ($\pm$ SD) was 6.4 ± 0.3 years in each group.

Primary endpoint. Immunogenicity. In the per-protocol analysis, non-inferiority in seroprotection rates between the REVAXIS and DT Polio groups was demonstrated for the five antigens (diphtheria, tetanus and poliomyelitis types 1, 2 and 3) (Table 2). One month after vaccination, 100% of children in the per-protocol set reached the pre-determined seroprotection level of 8 (1/dilution) for seroneutralizing antibodies for the three serotypes of poliovirus. The seroprotective threshold of 0.1 IU/mL

Table 2. Post-vaccination diphtheria, tetanus and poliovirus types 1, 2 and 3 seroprotection rates 1 month after booster injections of Td-IPV (REVAXIS) or DT-IPV (DT Polio) in children at 6 years of age: non-inferiority analysis (per-protocol set)

	REVAXIS (N = 284)	DT Polio (N = 283)	Difference (REVAXIS- DT Polio)	Non-inferiority*
Diphtheria, seroprotection rate (SN) ≥0.1 IU/mL				
n (%) [95% CI]	280 (98.6) [96.4; 99.6]	281 (99.3) [97.5; 99.9]	-0.7 [-2.9; 1.3]	Yes
Tetanus, seroprotection rate (EIA) ≥0.1 IU/mL				
n (%) [95% CI]	283 (99.6) [98.1; 100.0]	283 (100) [98.7; 100.0]	-0.4 [-2.0; 1.0]	Yes
Poliovirus type 1, seroprotection rate (SN) ≥8 (1/dilution)				
n (%) [95% CI]	284 (100) [98.7; 100.0]	283 (100) [98.7; 100.0]	0 [-1.3; 1.3]	Yes
Poliovirus type 2, seroprotection rate (SN) ≥8 (1/dilution)				
n (%) [95% CI]	284 (100) [98.7; 100.0]	283 (100) [98.7; 100.0]	0 [-1.3; 1.3]	Yes
Poliovirus type 3, seroprotection rate (SN) ≥8 (1/dilution)				
n (%) [95% CI]	284 (100) [98.7; 100.0]	282 (100) [98.7; 100.0]	0 [-1.3; 1.3]	Yes

*Non-inferiority is demonstrated if the lower 95% confidence interval (CI) of the difference is higher than -5% (i.e., the non-inferiority margin). SN, seroneutralization; EIA, enzyme immunoassay.

was reached by 98.6% and 99.3% of children for diphtheria [as measured by seroneutralization (SN)] and by 99.6% and 100% of children for tetanus [as measured by enzyme immunoassay (EIA)] in the REVAXIS and DT Polio groups, respectively.

Similar results were generated in the full analysis set, with seroprotection rates for all antigens ranging from 99% to 100% in both groups (data not shown).

Secondary endpoints. Immunogenicity. According to the secondary endpoints, the percentages of children reaching seroresponse rates against tetanus (threshold of 1.0 IU/mL as measured by EIA) were similar in the REVAXIS and DT Polio groups. Based on non-overlapping confidence intervals (CIs), the seroresponse rates for diphtheria (threshold of 1.0 IU/mL as measured by SN) were higher in the DT Polio group than in the REVAXIS group (Table 3).

The post-vaccination geometric mean titers (GMT) and the geometric mean of individual post-dose to pre-dose titer ratios (GMTR) are shown in Table 3. Based on non-overlapping CIs, the GMT and the GMTR were higher in the DT Polio group than in the REVAXIS group for diphtheria, tetanus and poliovirus type 1, and higher for poliovirus type 3 in the REVAXIS group than in the DT Polio group. Diphtheria responses in terms of GMTs were 3.7 IU/mL and 23.3 IU/mL in the REVAXIS and DT Polio groups, respectively.

Safety. From day 0 to day 28, the rates of children reporting at least one adverse event were comparable in both groups (83.1% and 85.6% in the REVAXIS and DT Polio groups, respectively). Adverse events occurred mainly between day 0 and day 7. No vaccine-related serious adverse events were reported during the study period, and no adverse event led to withdrawal from the study (Table 4).

In both groups, all injection-site reactions occurred between day 0 and day 7. However, fewer children in the REVAXIS

group reported severe solicited injection-site reactions (erythema, 7.0%; pain, 2.9%; and swelling, 3.9%) compared with the DT Polio group (erythema, 17.6%; pain, 4.8%; and swelling, 10.4%) (Table 4).

The rates of children with systemic adverse events were comparable in the REVAXIS and DT Polio groups from day 0 to day 7 (42.4% and 40.9%, respectively) as well as from day 8 to day 28 (10.7% and 11.0%, respectively). From day 0 to day 7, most of the systemic adverse events were solicited (headache, myalgia, pyrexia and malaise) and considered by investigators to be vaccine-related in 31.3% and 32.9% of children in the REVAXIS and DT Polio groups, respectively (Table 5).

No systemic allergic reactions relating to vaccination were reported in children receiving REVAXIS or DT Polio.

Discussion

The present study demonstrated the non-inferiority of REVAXIS compared with DT Polio for seroprotection against diphtheria, tetanus and poliovirus antigens when given as a second booster to 6-year-old children.

The GMTs for diphtheria and tetanus were higher after a booster dose with DT Polio than after REVAXIS. This reflects the higher levels of toxoids in DT Polio (≥ 30 IU and ≥ 40 IU, for diphtheria and tetanus, respectively) than in REVAXIS (≥ 2 IU and ≥ 20 IU for diphtheria and tetanus, respectively). Non-inferiority of seroprotection rates (threshold of 0.1 IU/mL) was demonstrated, however, with a strong booster response observed after both vaccines. Given the high seroprotection rates in both groups (diphtheria 98.6% for REVAXIS and 99.3% for DT Polio; tetanus, 99.6% for REVAXIS and 100% for DT Polio), differences in GMT and at the 1.0 IU/mL threshold are unlikely to be clinically relevant.

Table 3. Post-vaccination geometric mean titer (GMT), geometric mean of individual post-dose to pre-dose titer ratio (GMTR) and seroresponse rates (titer ≥ 1.0 IU/mL) for diphtheria and tetanus and GMT and GMTR for poliovirus types 1, 2 and 3, 1 month after booster injections of Td-IPV (REVAXIS) or DT-IPV (DT Polio) in children at 6 years of age (per-protocol set)

	REVAXIS (N = 284)	DT Polio (N = 283)
Diphtheria antibody titer (IU/mL-SN)		
GMT [95% CI]	3.7 [3.1; 4.4]	23.3 [19.5; 27.9]
GMTR [95% CI]	58.6 [47.6; 72.2]	307.6 [247.5; 382.3]
n (%) ≥ 1.0 IU/mL [95% CI]	244 (85.9) [81.3; 89.7]	279 (98.6) [96.4; 99.6]
Tetanus antibody titer (IU/mL-EIA)		
GMT [95% CI]	9.4 [8.3; 10.6]	13.9 [12.2; 15.8]
GMTR [95% CI]	38.7 [33.4; 44.9]	58.9 [51.6; 67.3]
n (%) ≥ 1.0 IU/mL [95% CI]	280 (98.6) [96.4; 99.6]	276 (97.5) [95.0; 99.0]
Poliovirus type 1 antibody titer (1/dilution-SN)		
GMT [95% CI]	4776.8 [4093.4; 5574.2]	7705.4 [6681.9; 8885.7]
GMTR [95% CI] ^a	63.5 [49.1; 82.2]	122.0 [93.6; 159.0]
Poliovirus type 2 antibody titer (1/dilution-SN)		
GMT [95% CI]	5715.4 [4919.4; 6640.2]	4534.2 [3932.0; 5228.7]
GMTR [95% CI]	56.9 [44.3; 73.2]	54.1 [42.0; 69.7]
Poliovirus type 3 antibody titer (1/dilution-SN)		
GMT [95% CI] ^b	6016.0 [5138.4; 7043.4]	2248.5 [1906.6; 2651.7]
GMTR [95% CI] ^b	51.5 [40.3; 65.9]	23.7 [18.9; 29.7]

SN, seroneutralization; EIA, enzyme immunoassay.

Among the 760 children randomized to the study groups, 123 (16%) were excluded from the per-protocol analysis because they were not previously vaccinated with three doses of a diphtheria, tetanus and poliomyelitis vaccine within the first 6 months of life and a booster vaccination for diphtheria, tetanus and poliomyelitis at 16–18 (± 2) months of life (non-adherence with selection criteria). This observation is relevant to clinical practice and indicates that recommendations are not always respected in a ‘real life’ setting. However, the analysis of all children with a post-booster immunogenicity evaluation, regardless of their adherence to the recommended primary series schedule (the full analysis set), demonstrated high seroprotection rates in both groups for all antigens, similar to those observed in the per protocol set.

The diphtheria responses observed in the REVAXIS group were consistent with those observed in a study undertaken in Germany in 301 children between 6 years and 9 years of age which compared REVAXIS with a standard tetanus-low-dose diphtheria booster.¹² The 1-month post-REVAXIS diphtheria GMT (using the SN assay) in the present study was lower (3.7 IU/mL) than that observed in the German study (9.7 IU/mL).¹² However, a stronger booster immune response was observed in the present study (GMTR >58 compared with 36.7 in the study carried out in Germany [unpublished data]). GMTR provides a measure of the magnitude of increase in antibodies as a result of vaccination. A high GMTR represents a large post-dose increase in antibodies, indicating the presence of immune memory and protection against future exposure to the pathogen. A similar trend was observed with

the tetanus antigen. Cross-study comparisons have well-known limitations. In particular, the difference in the ages of the children included in the two studies (6 years old in the present study compared with 6–9 years old in the German study) is a limitation to this comparison. Nevertheless, the results from both studies are clearly of the same order of magnitude.

A mathematical model for predicting the long-term duration of antibodies demonstrates that differences observed in antibody levels 1 month after booster vaccination at 4–6 years of age are unlikely to be of clinical relevance at the time of the adolescent booster.¹³ Based on observed and predicted long-term persistence of vaccine-induced antibodies, similar conclusions have been reached from studies undertaken with other vaccines.^{14–23} These data confirm the hypothesis that the half-life of antibody levels is a function of time: it is short after the initial vaccination and increases with subsequent booster doses.

A model constructed from data obtained in recent clinical trials conducted with one low-dose diphtheria-tetanus-acellular pertussis vaccine predicted that 98.6% of children were likely to remain seroprotected against diphtheria 10 years after vaccination compared with 99.6% vaccinated with the standard pediatric formulation diphtheria-tetanus-acellular pertussis vaccine.¹³ Such modeling suggests that children will maintain their protection against diphtheria between their booster dose with REVAXIS at 6 years of age and the next one (which is recommended at 11–13 years of age in France). Nevertheless, ensuring that a continuing high level of protection is maintained

Table 4. Adverse events from day 0 to day 28 in 758 children given a booster vaccination against diphtheria, tetanus and poliovirus types 1, 2 and 3 with Td-IPV (REVAXIS) or DT-IPV (DT Polio) at 6 years of age (safety set)

	REVAXIS (N = 384) n (%)	DT Polio (N = 374) n (%)
Adverse events from day 0 to day 28	319 (83.1)	320 (85.6)
Adverse events from day 0 to day 7 ^a	317 (82.6)	315 (84.2)
Injection-site reactions	292 (76.0)	294 (78.6)
Solicited	292 (76.0)	294 (78.6)
Erythema	157 (40.9)	176 (47.1)
≥5.0 cm	27 (7.0)	66 (17.6)
Pain	268 (69.8)	261 (69.8)
Severe	11 (2.9)	18 (4.8)
Swelling	124 (32.3)	140 (37.4)
≥5.0 cm	15 (3.9)	39 (10.4)
Unsolicited ^b	13 (3.4)	21 (5.6)
Systemic adverse events (see Table 5)	163 (42.4)	153 (40.9)
Adverse events from day 8 to day 28	41 (10.7)	41 (11.0)
Injection-site reactions	0 (0)	0 (0)
Systemic adverse events (see Table 5)	41 (10.7)	41 (11.0)
Withdrawal due to an adverse event from day 0 to visit 2	0 (0)	0 (0)
Serious adverse events from day 0 to visit 2	0 (0)	1 (0.3) (c)
Vaccine-related	0 (0)	0 (0)

n (%): number and percentage (calculated within the vaccinated children) of children presenting with an adverse event at least once. ^aIntensity scale (1) for measurable injection-site adverse reaction (mild: diameter <2.5 cm; moderate: diameter ≥2.5 to <5 cm; severe: diameter ≥5 cm) (2) for non-measurable (mild: easily tolerated/noticeable but does not interfere with daily activities; moderate: sufficiently discomforting to interfere with normal behavior or activities/interferes with daily activities; severe: incapacitating, unable to carry out usual activities/prevents daily activities). ^bno severe unsolicited injection-site reaction. ^cNose fracture, not related to vaccine administration.

in adolescents and adults is of importance. Consequently, longer-term follow-up of vaccinated children will be important in determining the long-term protection provided by low-dose booster vaccinations.

Application of the model proposed by Gottlieb et al. to the pre- and 1-month post-REVAXIS individual titers observed in the current study showed that 100% of children who received REVAXIS would be likely to maintain their diphtheria toxoid antibody titer ≥0.01 IU/mL 5 years after the booster vaccination. A titer of 0.01 IU/mL when measured by an SN assay is accepted as evidence of protection. This level of protection reduces the risk of infection and complications,²⁵ although a titer of 0.1 IU/mL may confer a better probability of long-term protection.²⁶

Patients and Methods

Study participants and ethics. The study was conducted in accordance with applicable national and local requirements. Before the study commenced, the protocol was approved by the French Ethical Research Committee, “Comité de Protection des Personnes” (CPP) of Ile de France XI, and the AFSSaPs, and was in accordance with the Helsinki Declaration.²⁷ Written informed consent was obtained from parents and/or legal guardians.

Healthy children who were 6 years of age on the day of vaccination were eligible for inclusion in the study. Children were

recruited from 63 centers in France (pediatricians in private practice and general practitioners).

For inclusion, children must have been previously vaccinated with three doses of diphtheria, tetanus and poliovirus vaccine(s) (alone or in combination) given within the first 6 months of life, and a booster dose of diphtheria, tetanus and poliovirus vaccine(s) (alone or in combination) at 16–18 months of age (±2 months) according to the recommended vaccination calendar in France. Children were excluded if they had been vaccinated in the previous 30 days, had severe hypersensitivity after earlier immunization against diphtheria and/or tetanus, or had chronic severe disease or any other medical condition that, in the opinion of the investigator, could interfere with the immune response and/or evaluation of the study objectives.

Study design and vaccines. In this open-label study, children were randomly assigned (1:1 ratio) to one of two groups to receive a booster at 6 years of age with a single intramuscular (deltoid) dose of REVAXIS (batch numbers Z6574-1 and A5662-1) or a single intramuscular (deltoid) dose of DT Polio (batch numbers A5183-1 and A6208-1) (**Table 1**).

Schedule of immunogenicity and safety assessments. Two 4.0-mL samples of blood were obtained, one prior to vaccination and a second between day 28 and day 35 after vaccination. Immune responses were measured in pre- and post-vaccination blood samples for all antigens. All children were kept under

Table 5. Summary of systemic adverse events from day 0 to day 28 with a prevalence^a of $\geq 1\%$ in 758 children given a booster vaccination against diphtheria, tetanus and poliovirus types 1, 2 and 3 with Td-IPV (REVAXIS) or DT-IPV (DT Polio) at 6 years of age (safety set)

	REVAXIS (N = 384)		DT Polio (N = 374)	
	All	Related	All	Related
	n (%)	n (%)	n (%)	n (%)
Systemic adverse event from day 0 to day 7	163 (42.4)	128 (33.3)	153 (40.9)	127 (34.0)
Solicited systemic adverse event from day 0 to day 7^b	136 (35.4)	120 (31.3)	136 (36.4)	123 (32.9)
Headache	58 (15.1)	47 (12.2)	72 (19.3)	62 (16.6)
Myalgia	82 (21.4)	74 (19.3)	67 (17.9)	64 (17.1)
Pyrexia	43 (11.2)	37 (9.6)	54 (14.4)	42 (11.2)
Unsolicited systemic adverse event from day 0 to day 7	47 (12.2)	12 (3.1)	46 (12.3)	11 (2.9)
Abdominal pain	4 (1.0)	3 (0.8)	8 (2.1)	2 (0.5)
Vomiting	4 (1.0)	3 (0.8)	4 (1.1)	2 (0.5)
Rhinitis	7 (1.8)	1 (0.3)	5 (1.3)	0 (0)
Tonsillitis	4 (1.0)	0 (0)	1 (0.3)	0 (0)
Cough	8 (2.1)	1 (0.3)	6 (1.6)	0 (0)
Systemic adverse event from day 8 to day 28	41 (10.7)	5 (1.3)	41 (11.0)	2 (0.5)
Pyrexia	6 (1.6)	1 (0.3)	4 (1.1)	0 (0)
Ear infection	3 (0.8)	0 (0)	7 (1.9)	0 (0)
Gastroenteritis	1 (0.3)	0 (0)	4 (1.1)	0 (0)
Nasopharyngitis	5 (1.3)	0 (0)	0 (0)	0 (0)
Pharyngitis	5 (1.3)	0 (0)	2 (0.5)	0 (0)
Tonsillitis	4 (1.0)	0 (0)	2 (0.5)	0 (0)
Headache	5 (1.3)	4 (1.0)	2 (0.5)	0 (0)
Cough	6 (1.6)	0 (0)	3 (0.8)	0 (0)

^aOnly adverse events (AEs) with an prevalence $\geq 1\%$ are presented. n (%), number and percentage (calculated within the vaccinated subjects) of subjects presenting at least once the considered event. ^bThe prevalence of the solicited AE malaise was $<1\%$.

medical surveillance for 20 minutes post-vaccination to observe all immediate adverse events and were followed for safety throughout the study.

Immunogenicity measurements. Immunogenicity tests were conducted centrally by the Global Clinical Immunology department in sanofi pasteur. Individuals conducting the tests were blinded with respect to the bleed interval (i.e., before or after vaccination) and the vaccination group. Standard methods²⁸⁻³¹ were used for measuring antibodies to diphtheria (SN test in Vero cells), poliovirus (SN using a micrometabolic inhibition test) and tetanus [immunoglobulin G (Ig) EIA]. In case of insufficient volume of serum, priority in terms of antibody titration was: diphtheria SN, tetanus EIA, poliovirus type-1 SN, poliovirus type-2 SN, poliovirus type-3 SN. In addition, anti-diphtheria antibody titers were measured by EIA (data not shown).

Primary endpoints. The primary endpoints were the 1-month (28–35 days) post-booster seroprotection rates for diphtheria and tetanus (≥ 0.1 IU/mL) and for poliovirus type-1, -2 and -3 antigens [≥ 8 (1/dilution)].

Secondary endpoints. *Immunogenicity.* The secondary endpoints for immunogenicity were the antibody titer (GMT, GMTR) for diphtheria, tetanus and poliovirus type-1, -2 and -3 antigens and the 1-month post-booster rates at the threshold of 1.0 IU/mL for diphtheria and tetanus.

Safety. Safety endpoints were solicited injection-site reactions (erythema, pain and swelling) and solicited systemic adverse events (headache, myalgia, pyrexia and malaise), between day

0 and day 7, unsolicited (spontaneously reported) injection-site reactions and systemic adverse events between day 0 and day 28 and serious adverse events throughout the study.

Statistical analyses. Study power was calculated using the Farrington and Manning sample size formulae.³² It was assumed that there would be 330 evaluable children in each group, that the true diphtheria seroprotection rate was 98%, the true tetanus seroprotection was 99%, the true poliovirus type-1 and -3 seroprotection rates were 98%, and that there would be no difference between groups. Immunogenicity was evaluated in the per protocol set, which included all infants without protocol deviations which might have interfered with the immunogenicity evaluation (main analysis), and on the full analysis set, which included all infants with any post-vaccination immunogenicity evaluation (supportive analysis). Estimates of the difference between the seroprotection rates between the groups (REVAXIS and DT Polio) were calculated together with their two-sided 95% CI based on the two-sample Wilson score method without continuity correction as reported by Newcombe.³³ If the lower bounds of the 95% CI of the difference was higher than -5% (i.e., the non-inferiority margin) it was concluded that REVAXIS was non-inferior to DT Polio. Descriptive summaries were provided by groups for all safety data.

Conclusions

The percentage of children reaching seroprotection rates according to the primary study endpoints was similar in the REVAXIS

and DT Polio groups. No allergic reactions to REVAXIS were reported in the present study, and the trend towards a better tolerability of REVAXIS compared with DT Polio suggests a benefit/risk ratio in favor of REVAXIS.

These data support the use of REVAXIS as a booster at 6 years of age in infants who previously received a three-dose primary series within the first 6 months of life and a first booster including diphtheria, tetanus and poliovirus vaccine(s) given before 2 years of age.

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Conflict of Interest

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Trial Registration

The trial is registered with ClinicalTrials.gov; National Clinical Trials Identifier (NCT ID) NCT00447525.

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