A randomized study to assess the immunogenicity, antibody persistence and safety of a tetravalent meningococcal serogroups A, C, W-135 and Y tetanus toxoid conjugate vaccine in children aged 2–10 years

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Incidence of meningococcal diseases is high in children, and effective vaccines are needed for this age group. In this phase II, open, controlled study, 309 children aged 2–10 years from Finland were randomized (3:1) into two parallel groups to receive one dose of meningococcal ACWY-tetanus toxoid conjugate vaccine (ACWY-TT group; n = 231) or a licensed meningococcal ACWY polysaccharide vaccine (Men-PS group; n = 78). Serum bactericidal activity using rabbit complement (rSBA) was evaluated up to three years post-vaccination. Exploratory comparisons suggested that rSBA vaccine response rates and geometric mean titers (GMTs) for each serogroup at one month post-vaccination and rSBA GMTs for serogroups A, W-135 and Y up to three years post-vaccination were higher in the ACWY-TT compared with Men-PS group, but did not detect any difference between groups in terms of rSBA-MenC GMTs at three years post-vaccination; this is explained by the higher proportion of children from the Men-PS group who were excluded because they were re-vaccinated with a monovalent meningococcal serogroup C vaccine due to loss of protective antibody levels against this serogroup. Although there was a higher incidence of local reactogenicity in the ACWY-TT group, general and unsolicited symptoms reporting rates were comparable in both groups. This study showed that MenACWY-TT was immunogenic with a clinically acceptable safety profile in children aged 2–10 years. MenACWY-TT induced higher functional antibody titers for all serogroups, which persisted longer for serogroups A, W-135 and Y, than the MenACWY polysaccharide vaccine. This study has been registered at www.clinicaltrials.gov NCT00427908.

Introduction

Neisseria meningitidis is responsible for invasive bacterial infections associated with high levels of mortality, especially in children and adolescents.[1,2] Although the current level of meningococcal disease is low in industrialized countries,[3] the number of confirmed meningococcal disease cases reported to the European Centre for Disease Prevention and Control in 2009 was 7.37 per 100,000 children under five years of age[4] and the case fatality ratio of meningococcal disease was estimated to be 8% in Europe in 2004.[5]

N. meningitidis is classified into serogroups based on differences in the capsular polysaccharides, and invasive meningococcal diseases are mostly caused by five serogroups (A, B, C, W-135 and Y).[1,2] In the European Union (EU), serogroup B was responsible for 71%, serogroup C for 13%, and serogroups Y for 4% of reported cases of invasive meningococcal disease in 2009.[6] The incidence of serogroup C has declined in Europe since the introduction of conjugate vaccines against this serogroup in 1999,[2] and an increase of meningococcal disease due to serogroup Y has recently been observed in Scandinavian countries and in the United Kingdom.[7-10] Of note, there may be substantial regional

variation in the relative distribution of each serogroup, and new serogroups may appear in some countries as a result of strain importation and evolution.[2,11]

Vaccination remains the best strategy to prevent meningococcal disease, and broadly effective vaccines are needed.[11] Plain capsular polysaccharide vaccines providing protection against meningococcal serogroups A, C, W-135 and Y have been widely used in Europe over the last few decades. However, plain polysaccharide vaccines have limitations: they have lower immunogenicity among young children, they usually do not elicit long-term protection, they afford no herd immunity and no immune memory and they induce immunological hyporesponsiveness and a T-cell independent immune response.[12,13]

To overcome these limitations, capsular polysaccharides were covalently coupled to carrier proteins in meningococcal conjugate vaccines.[12-16] The first meningococcal conjugate vaccines were monovalent vaccines against serogroup C using mutant diphtheria toxoid (CRM_{197}) or tetanus toxoid (TT) as carrier protein.[17] These vaccines were introduced in vaccination programs in Europe and were highly successful in reducing the incidence of meningococcal disease due to serogroup C, including in the youngest age groups.[12,14,16-21] Subsequently, two tetravalent meningococcal serogroups A, C, W-135 and Y conjugate vaccines using diphtheria toxoid (DT) or CRM_{197} as carrier protein were licensed for use in various countries,[22-25] and a monovalent meningococcal serogroup A conjugate vaccine using TT as carrier protein was designed specifically for Africa.[26-29] In addition, a new tetravalent meningococcal serogroups A, C, W-135 and Y conjugate vaccine using TT as carrier protein [*Nimenrix*^{TM} (GlaxoSmithKline Vaccines); MenACWY-TT] has been recently approved by the European Medicines Agency for the active immunization of subjects older than 12 mo of age. This vaccine has been shown to be immunogenic with a clinically acceptable safety profile in toddlers, children, adolescents and young adults.[30-36]

This study assessed the immunogenicity, antibody persistence, reactogenicity and safety of one dose of the EU-licensed MenACWY-TT vaccine compared with one dose of a licensed monovalent meningococcal serogroup C conjugate vaccine in toddlers, and with one dose of a licensed tetravalent meningococcal serogroups A, C, W-135 and Y plain polysaccharide vaccine (Men-PS) in children aged 2–10 y. This manuscript will discuss the results obtained in children while those obtained in toddlers are presented in a separate publication.

Results

Study participants. In this study, 309 Finnish children aged 2–10 y were enrolled and randomized (3:1) to receive either the MenACWY-TT vaccine (ACWY-TT group) or the MenACWY polysaccharide vaccine (Men-PS group) (**Fig. 1**). Of these, 306 and 300 children completed the primary stage at one month post-vaccination and the six month safety follow-up of the vaccination phase, respectively. One child in the ACWY-TT group was withdrawn from the primary stage of the study due to a non-serious adverse event (AE; grade 3 swelling for which medical attention was sought). In the persistence follow-up stage, 299 children were enrolled at one year, 276 children at two years and 239 children at three years post-vaccination. In **Figure 1**, 45 children were not included in the persistence stage at two or three years post-vaccination because they were not eligible; 4/8 ineligible children in the ACWY-TT and 37/37 ineligible children in the Men-PS group were withdrawn because they had been re-vaccinated with a monovalent meningococcal serogroup C conjugate vaccine as they did not retain rSBA titers ≥ 1:8 for serogroup C. The two treatment groups were comparable in terms of demographic characteristics (**Table 1**).

Immunogenicity. One month after vaccination, the vaccine response rates, measured with a serum bactericidal activity assay using rabbit complement (rSBA), were at least 94.3% in the ACWY-TT group and 81.2% in the Men-PS group. Since the lower limit (LL) of the asymptotic standardized 95% confidence interval (CI) for the difference between the ACWY-TT and the Men-PS groups in terms of vaccine response rates was above -15% for each antigen, the primary objective to demonstrate the non-inferiority of MenACWY-TT vs. the MenACWY polysaccharide vaccine was met (**Table 2**).

At one month post-vaccination, all children in both groups had rSBA titers ≥ 1:8 and at least 99.6% and 94.6% of them had rSBA titers ≥ 1:128 for each individual serogroup in the ACWY-TT and Men-PS groups, respectively (**Table 3**). For the four serogroups, exploratory analyses suggested that vaccine response rates and post-vaccination rSBA geometric mean titers (GMTs) adjusted for baseline titers were higher among MenACWY-TT recipients than among children who received the MenACWY polysaccharide vaccine. Moreover, exploratory analyses suggested that a higher percentage of children had rSBA titers ≥ 1:128 for serogroup C in the ACWY-TT group compared with the Men-PS group.

The rSBA GMTs decreased in both groups between one month and one year post-vaccination and between one and two years post-vaccination, with a smaller decrease observed in the ACWY-TT group than in the Men-PS group for all serogroups (**Table 3**). At three years after vaccination, at least 98.4% of children in the ACWY-TT group retained rSBA antibody titers ≥ 1:8 for each serogroup while in the Men-PS group, the percentage of children retaining rSBA antibody titers ≥ 1:8 had fallen to 81.1–91.2%. At one year, two years and three years after vaccination, exploratory analyses suggested that the percentages of children retaining rSBA antibody titers ≥ 1:8 and ≥ 1:128 and post-vaccination rSBA GMTs adjusted for pre-vaccination titers were higher in the ACWY-TT group than in the Men-PS group for all serogroups, apart from serogroup W-135 for which 100% of the children in both groups retained rSBA titers ≥ 1:8 at one year post-vaccination, and serogroup C for which no difference was observed for adjusted rSBA GMTs or percentages of children with rSBA titers ≥ 1:128 at three years post-vaccination (**Table 3**). Of note, a selection bias was introduced in the persistence stage since a higher proportion of children in the Men-PS [37 children (47.4%)] compared with the ACWY-TT group [4 children (1.7%)] were excluded from the analyses because they had been re-vaccinated with a monovalent meningococcal serogroup C
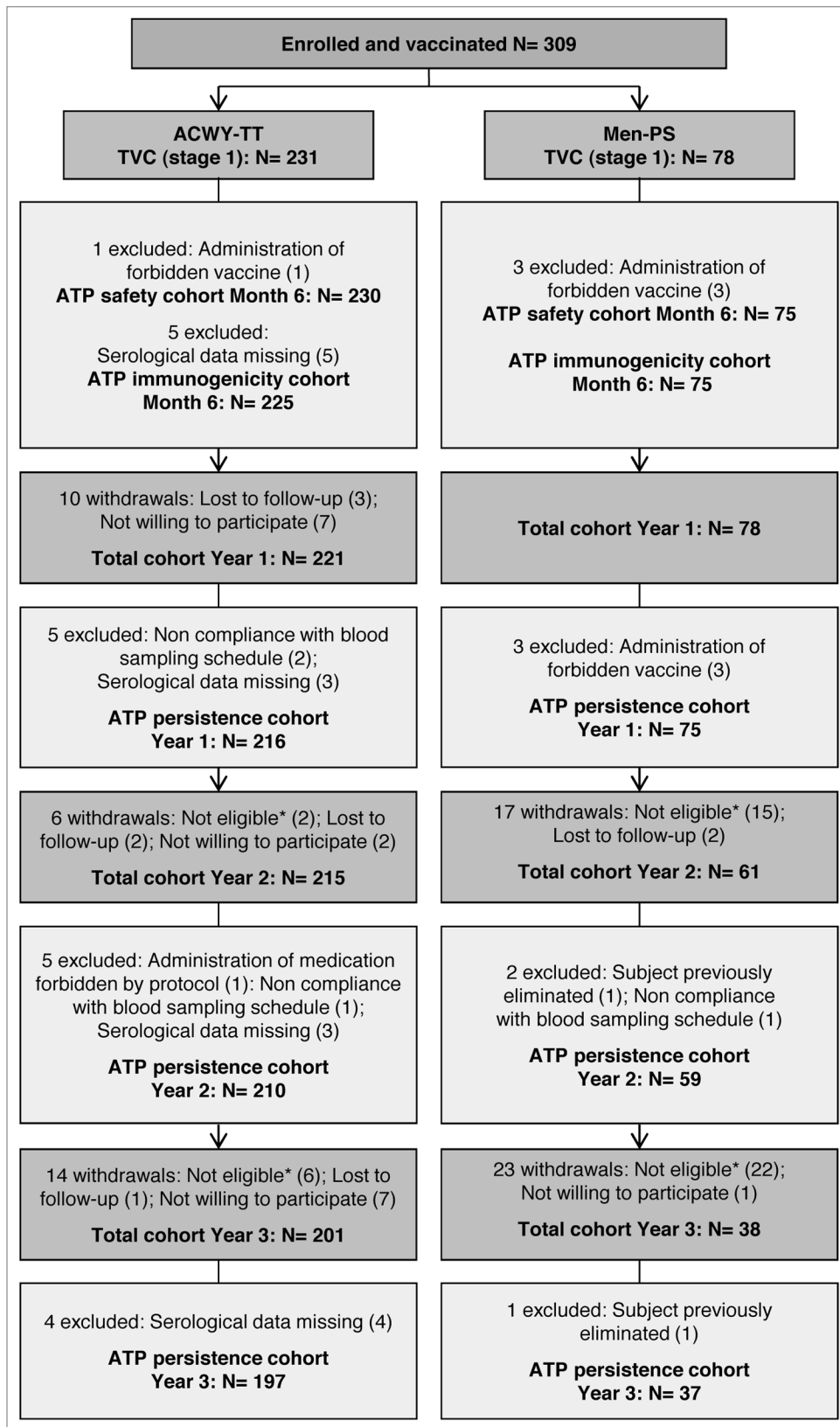
Figure 1. Participants' progression through the study. ACWY-TT group, group of children who received one dose of MenACWY-TT; Men-PS group, group of children who received one dose of the MenACWY polysaccharide vaccine; ATP, according to protocol; TVC, total vaccinated cohort; N, number of children; *Reasons for non-eligibility: suboptimal responder re-vaccinated with a monovalent meningococcal serogroup C vaccine (n = 41); suboptimal responder who did not come to the booster vaccination visit (n = 2); consent withdrawal (n = 1); and acute leukemia (n = 1).

Table 1. Demographic characteristics of enrolled and vaccinated children (total vaccinated cohort)

Characteristic		ACWY-TT group	Men-PS group
N		231	78
Age (months)	Mean (SD)	71.9 (31.19)	71.6 (30.22)
	Range	24–131	24–125
Gender	Female n (%)	115 (49.8)	37 (47.4)
	Male n (%)	116 (50.2)	41 (52.6)
Race	White - Caucasian / European heritage n (%)	225 (97.4)	76 (97.4)
	African heritage / African American n (%)	1 (0.4)	0 (0.0)
	Asian - East Asian heritage n (%)	1 (0.4)	0 (0.0)
	White - Arabic / North African heritage n (%)	1 (0.4)	0 (0.0)
	Other n (%)	3 (1.3)	2 (2.6)

ACWY-TT group, group of children who received one dose of MenACWY-TT; Men-PS group, group of children who received one dose of the MenACWY polysaccharide vaccine; N, number of children; n, number of children with the specified characteristic; %, percentage (n/N x 100) of children with the specified characteristic; SD, standard deviation.

conjugate vaccine due to loss of protective antibody levels against this serogroup.

Exploratory analyses at one month post-vaccination suggested that the percentage of children with anti-TT concentrations ≥ 1.0 IU/mL was higher in the ACWY-TT group [100%; 95% CI (89.1–100)] than in the Men-PS group [71.4%; 95% CI (29.0–96.3)] (data not shown).

Safety. Within four days following vaccination, pain was the most frequent solicited local symptom in children aged 2–5 y, which was reported in 45.1% [51/113] of children in the ACWY-TT group and 71.8% [28/39] of children in the Men-PS group (**Fig. 2A**). In this age stratum, drowsiness was the most common solicited general symptom in the ACWY-TT group [26.5% (30/113) of children] and irritability in the Men-PS group [28.2% (11/39) of children]. In children aged 6–10 y, pain was also the most frequently reported solicited local symptom in both groups [71.8% (84/117) of children in the ACWY-TT group and 82.1% (32/39) of children in the Men-PS group] and fatigue was the most common solicited general symptom reported within four days following vaccination [38.5% (45/117) and 20.5% (8/39) of children in the ACWY-TT and Men-PS groups, respectively] (**Fig. 2B**).

During the 31-d post-vaccination follow-up period, unsolicited symptoms were reported in 35.9% (83/231) and 30.8% (24/78) of children for any unsolicited symptom and 6.5% (15/231) and 5.1% (4/78) of children for grade 3 unsolicited symptoms in the ACWY-TT and Men-PS groups, respectively. The most frequently reported unsolicited AEs in the ACWY-TT group were pyrexia and otitis media [both reported in 5.2% (12/231) of children] and in the Men-PS group, upper respiratory tract infection

[reported in 5.1% (4/78) of children]. One grade 3 unsolicited symptom considered as related to vaccination (hematoma) was reported in a child from the ACWY-TT group.

During the six-month safety follow-up period of the primary stage, serious adverse events (SAEs) were reported in two children in the ACWY-TT group (laryngitis and hyperglycemia and pneumonia) and one child in the Men-PS group (testicular torsion). No SAEs were considered causally related to vaccination up to three years post-vaccination. A new onset of chronic illness (NOCI) was reported in six children [two children (0.9%) in the ACWY-TT group and four children (5.1%) in the Men-PS group] during the six-month safety follow-up period of the primary stage. The NOCIs were all allergic in nature, and none of the reported NOCIs were considered causally related to vaccination. No deaths were reported throughout the study.

Discussion

This study was designed to compare the immunogenicity, antibody persistence and safety profile of the EU-licensed MenACWY-TT vaccine and a licensed tetravalent meningococcal plain polysaccharide vaccine in children 2–10 y of age. The primary objective was reached as the non-inferiority of the MenACWY-TT vaccine vs. the MenACWY polysaccharide vaccine in terms of rSBA vaccine response to the four serogroups was demonstrated.

At one month post-vaccination, exploratory analyses detected higher rSBA vaccine response rates and GMTs for all serogroups in the children who received MenACWY-TT as compared with those who received the MenACWY polysaccharide vaccine. These results are consistent with other studies, in which MenACWY-TT was found to induce higher functional antibody titers than plain polysaccharide vaccines in children[32,33] or in adolescents and young adults.[31,34] This observation is also in line with previous studies comparing the post-vaccination functional antibody titers induced by tetravalent meningococcal serogroups A, C, W-135 and Y conjugate vaccines, using DT or CRM_{197} as carrier protein, and plain polysaccharide vaccines in children 2–10 y of age[24,25] or in adolescents and adults.[22,23,37]

Persistence of rSBA antibodies up to three years after vaccination was observed for both vaccines in at least 81.1% of children. Greater antibody persistence was observed in the children who received the MenACWY-TT vaccine than in those who received the MenACWY polysaccharide vaccine. This was expected as plain polysaccharide vaccines induce T-cell independent immune responses, which elicit shorter-term protection.[15,24] As expected, the observed rSBA GMTs for the four serogroups decreased between one month and one year post-vaccination, and a further decline was observed between one year and two years post-vaccination. In contrast, no significant decline in terms of rSBA GMTs was observed between two and three years post-vaccination in both treatment groups.

For serogroup C, exploratory analyses at three years post-vaccination did not detect any difference between the two groups in terms of rSBA GMTs. This is largely explained by the higher proportion of children vaccinated with the MenACWY

Table 2. rSBA vaccine response rates at one month after administration of MenACWY-TT or MenACWY polysaccharide vaccine (ATP immunogenicity cohort)

Antibody	ACWY-TT group		Men-PS group		Difference in vaccine response rate (ACWY-TT minus Men-PS)		
	N	% [95% CI]	N	% [95% CI]	%	95% CI	
						LL	UL
rSBA-MenA	185	98.4* [95.3 – 99.7]	62	91.9 [82.2 – 97.3]	6.44	1.15	16.04
rSBA-MenC	212	94.3* [90.3 – 97.0]	69	81.2 [69.9 – 89.6]	13.18	4.79	24.32
rSBA-MenW-135	199	100* [98.2 – 100]	68	95.6 [87.6 – 99.1]	4.41	1.51	12.21
rSBA-MenY	219	99.1* [96.7 – 99.9]	70	82.9 [72.0 – 90.8]	16.23	8.99	26.78

ACWY-TT group, group of children who received one dose of MenACWY-TT; Men-PS group, group of children who received one dose of the MenACWY polysaccharide vaccine; N, number of children with an rSBA result both pre- and post-vaccination; %, percentage of children with an rSBA vaccine response; 95% CI, 95% confidence interval; LL, lower limit; UL, upper limit; Vaccine response, for initially seronegative children (pre-vaccination rSBA titer < 1:8), post-vaccination rSBA titer ≥ 1:32; for initially seropositive children (pre-vaccination rSBA titer ≥ 1:8), post-vaccination rSBA titer ≥ 4-fold the pre-vaccination rSBA titer; *Indicates higher rSBA vaccine response in the MenACWY-TT group than in the Men-PS group (exploratory analysis).

polysaccharide vaccine [37 children (47.4%)] compared with MenACWY-TT [4 children (1.7%)] who were excluded from the persistence analyses because they had been re-vaccinated with a monovalent meningococcal serogroup C conjugate vaccine due to loss antibody against serogroup C. This introduced a selection bias in the persistence stage of the study since children who did not maintain rSBA titers ≥ 1:8 were excluded, leading to an over-estimation of the persistence as the study progressed.

A significant proportion of children in this study had rSBA antibodies against meningococcal serogroups A, C, W-135 and Y prior to vaccination, and the observed pre-vaccination seropositivity rates were comparable between the two treatment groups. As observed in a previous study, the percentage of seropositive children increased with age, suggesting a rising probability of acquiring functional antibodies against *N. meningitidis* through natural immunity mechanisms in older children.[38] Therefore, the vaccine response rate was an important primary endpoint as it assessed the ability of children to respond to the vaccine regardless of their serostatus at pre-vaccination. Here, significantly higher rSBA vaccine response rates for all four serogroups were observed in children who received the MenACWY-TT vaccine than in children who received the MenACWY polysaccharide vaccine.

As expected, the anti-TT antibody concentrations increased between pre- and post-vaccination in the children who received the MenACWY-TT vaccine. An increase in anti-TT antibody concentrations was also observed after vaccination with MenC-TT and MenA-TT monovalent conjugate vaccines.[29,39] However, functional anti-TT antibodies have not been assessed after administration of either vaccine.

In the present study, the MenACWY-TT vaccine induced a higher rate of injection site redness and swelling than the MenACWY polysaccharide vaccine, and this is likely due to the increased protein content in the conjugate vaccine. This was previously observed with the same vaccines in children,[32] adolescents and young adults[31,34] as well as with the two other licensed tetravalent meningococcal conjugate vaccines when compared with a plain polysaccharide vaccine.[22-25] However, this observation is in contrast with results of another study, in which MenACWY-TT

was compared with the MenACWY polysaccharide vaccine in children aged 2–10 y, where rates of injection site reactions were more comparable.[33] In the present study, the incidence of pain at the injection site was lower in the children aged 2–5 y who received MenACWY-TT compared with the MenACWY polysaccharide vaccine. This observation is consistent with results of the previous study comparing the same vaccines in children aged 2–10 y.[33]

A limitation of this study was its open design because the study vaccines differed both in appearance and route of administration (intramuscular for MenACWY-TT and subcutaneous for the MenACWY polysaccharide vaccine). The open design would not have influenced the immunogenicity results, but had the potential to bias the safety, although this bias would most likely be in favor of the control MenACWY polysaccharide vaccine vs. the MenACWY-TT vaccine. The multiple exploratory comparisons for both safety and immunogenicity endpoints could also have limited this study, which was only powered to address immunological non-inferiority of MenACWY-TT vs. the MenACWY polysaccharide vaccine in terms of rSBA vaccine response rates. Another limitation in the persistence stage of this study was the high number of children who were excluded because they failed to retain protective levels of rSBA antibodies against serogroup C and were therefore re-vaccinated with a monovalent meningococcal serogroup C conjugate vaccine. Re-vaccination was done to ensure that children remained protected against meningococcal serogroup C disease, but it means that observed titers at future persistence timepoints may be overestimated due to the differential drop-out of seronegative children in both groups.

In conclusion, this study showed that the MenACWY-TT vaccine induced an immune response against meningococcal serogroups A, C, W-135 and Y in children aged 2–10 y, which persisted up to three years post-vaccination. Exploratory analyses suggested higher rSBA vaccine response rates and GMTs for each serogroup at one month post-vaccination and higher rSBA GMTs for serogroups A, W-135 and Y up to three years post-vaccination in children vaccinated with MenACWY-TT compared with the MenACWY polysaccharide vaccine. Additional antibody persistence data will be generated at subsequent timepoints to see

Table 3. Percentage of children with rSBA titers equal to or above cut-off values, and rSBA GMTs before and one month after vaccination (ATP immunogenicity cohort), one year after vaccination (ATP persistence cohort Year 1), two years after vaccination (ATP persistence cohort Year 2) and three years after vaccination (ATP persistence cohort Year 3)

Antibody	Group	Timing	N	% ≥1:8 (95% CI)	% ≥ 1:128 (95% CI)	GMT (95% CI)
rSBA-MenA	ACWY-TT	Pre	185	67.0 (59.7 – 73.7)	51.9 (44.4 – 59.3)	57.9 (43.3 – 77.5)
		Month 1	225	100 (98.4 – 100)	99.6 (97.5 – 100)	7300.9* (6586.0 – 8093.4)
		Year 1	216	99.5* (97.4 – 100)	99.5* (97.4 – 100)	2448.1* (2149.6 – 2788.1)
		Year 2	208	100* (98.2 – 100)	99.0* (96.6 – 99.9)	1333.4* (1181.9 – 1504.2)
		Year 3	192	100* (98.1 – 100)	99.0* (96.3 – 99.9)	1184.2* (1054.2 – 1330.3)
	Men-PS	Pre	62	64.5 (51.3 – 76.3)	51.6 (38.6 – 64.5)	58.2 (33.8 – 100.1)
		Month 1	75	100 (95.2 – 100)	100 (95.2 – 100)	2033.4 (1667.1 – 2480.2)
		Year 1	71	90.1 (80.7 – 95.9)	80.3 (69.1 – 88.8)	358.5 (230.2 – 558.4)
		Year 2	56	91.1 (80.4 – 97.0)	75.0 (61.6 – 85.6)	202.5 (135.3 – 303.0)
		Year 3	34	91.2 (76.3 – 98.1)	79.4 (62.1 – 91.3)	218.8 (128.9 – 371.5)
rSBA-MenC	ACWY-TT	Pre	212	62.7 (55.8 – 69.3)	27.8 (21.9 – 34.4)	33.5 (26.0 – 43.1)
		Month 1	225	100 (98.4 – 100)	99.6* (97.5 – 100)	2435.3* (2105.8 – 2816.3)
		Year 1	215	99.5* (97.4 – 100)	89.3* (84.4 – 93.1)	489.5* (419.5 – 571.1)
		Year 2	210	98.6* (95.9 – 99.7)	75.7* (69.3 – 81.4)	256.0* (213.9 – 306.2)
		Year 3	192	98.4* (95.5 – 99.7)	72.9 (66.0 – 79.1)	244.3 (200.8 – 297.3)
	Men-PS	Pre	70	51.4 (39.2 – 63.6)	27.1 (17.2 – 39.1)	24.1 (15.2 – 38.2)
		Month 1	74	100 (95.1 – 100)	94.6 (86.7 – 98.5)	750.2 (555.2 – 1013.7)
		Year 1	65	80.0 (68.2 – 88.9)	58.5 (45.6 – 70.6)	113.5 (67.3 – 191.5)
		Year 2	59	66.1 (52.6 – 77.9)	45.8 (32.7 – 59.2)	59.9 (33.0 – 108.7)
		Year 3	37	83.8 (68.0 – 93.8)	67.6 (50.2 – 82.0)	163.5 (83.8 – 319.2)
rSBA-MenW-135	ACWY-TT	Pre	199	60.3 (53.1 – 67.2)	45.2 (38.2 – 52.4)	43.1 (32.4 – 57.4)
		Month 1	225	100 (98.4 – 100)	100 (98.4 – 100)	11777.0* (10666.2 – 13003.5)
		Year 1	216	100 (98.3 – 100)	99.1* (96.7 – 99.9)	2983.3* (2628.2 – 3386.3)
		Year 2	210	99.5* (97.4 – 100)	99.0* (96.6 – 99.9)	1298.0* (1135.5 – 1483.7)
		Year 3	196	100* (98.1 – 100)	98.0* (94.9 – 99.4)	1737.1* (1503.8 – 2006.7)
	Men-PS	Pre	68	57.4 (44.8 – 69.3)	35.3 (24.1 – 47.8)	40.1 (23.9 – 67.3)
		Month 1	75	100 (95.2 – 100)	100 (95.2 – 100)	2186.3 (1723.1 – 2773.9)
		Year 1	75	100 (95.2 – 100)	93.3 (85.1 – 97.8)	463.0 (367.4 – 583.5)
		Year 2	54	85.2 (72.9 – 93.4)	68.5 (54.4 – 80.5)	144.0 (90.1 – 230.2)
		Year 3	35	82.9 (66.4 – 93.4)	60.0 (42.1 – 76.1)	112.9 (59.9 – 212.6)
rSBA-MenY	ACWY-TT	Pre	219	67.1 (60.5 – 73.3)	44.7 (38.0 – 51.6)	57.3 (43.7 – 75.2)
		Month 1	225	100 (98.4 – 100)	99.6 (97.5 – 100)	6641.4* (6044.3 – 7297.4)
		Year 1	216	100* (98.3 – 100)	99.5* (97.4 – 100)	2172.1* (1939.6 – 2432.5)
		Year 2	210	100* (98.3 – 100)	99.5* (97.4 – 100)	1530.2* (1339.2 – 1748.4)
		Year 3	195	100* (98.1 – 100)	100* (98.1 – 100)	1551.6* (1381.2 – 1743.1)
	Men-PS	Pre	70	60.0 (47.6 – 71.5)	40.0 (28.5 – 52.4)	45.5 (26.8 – 77.0)
		Month 1	75	100 (95.2 – 100)	97.3 (90.7 – 99.7)	1409.9 (1085.9 – 1830.5)
		Year 1	71	90.1 (80.7 – 95.9)	78.9 (67.6 – 87.7)	332.4 (213.5 – 517.7)
		Year 2	55	74.5 (61.0 – 85.3)	54.5 (40.6 – 68.0)	96.9 (54.1 – 173.6)
		Year 3	37	81.1 (64.8 – 92.0)	51.4 (34.4 – 68.1)	103.8 (54.3 – 198.3)

ACWY-TT group, group of children who received one dose of MenACWY-TT; Men-PS group, group of children who received one dose of the MenACWY polysaccharide vaccine; GMT, geometric mean antibody titer calculated on all children; N, number of children with available results; %, percentage of children with titer within the specified range; 95% CI, 95% confidence interval; Pre, pre-vaccination; Month 1, Year 1, Year 2, Year 3, one month, one year, two years and three years after vaccination. *Indicates higher value in the ACWY-TT group compared with the Men-PS group (exploratory analyses). Differences between groups in terms of GMTs are based on values adjusted for pre-vaccination measurements.

if the higher functional antibody titers further translate into a longer duration of protection. The MenACWY-TT vaccine had a clinically acceptable safety profile, although it was followed by more local injection site reactions than the MenACWY polysaccharide vaccine. This study supports the administration of the MenACWY-TT vaccine in children aged 2–10 y to provide long-term protection against meningococcal diseases.

Methods

Study design. This phase II, open, randomized, controlled study was conducted in 11 centers in Finland between February 2007 and May 2010. The study was designed to evaluate the immunological non-inferiority, antibody persistence and safety profile of MenACWY-TT vs. a licensed tetravalent plain polysaccharide vaccine in children aged 2–10 y and a licensed monovalent meningococcal serogroup C conjugate vaccine in toddlers. The study was conducted in two stages: a six-month vaccination and safety follow-up primary stage, and a five-year persistence stage. In this manuscript, we present the results obtained in children 2–10 y of age up to three years after vaccination.

The children were stratified according to age (2–5 y and 6–10 y) to assess different profiles of solicited symptoms in the two age strata. Within each age stratum, children were randomized (3:1) into two parallel treatment groups: children in the ACWY-TT group received one dose of MenACWY-TT and children in the Men-PS group received one dose of the MenACWY polysaccharide vaccine. The randomization list was generated at GlaxoSmithKline, Rixensart, Belgium, using a standard SAS® program and was used to number the vaccines. Treatment allocation at the investigator site was performed using a central internet randomization system. The randomization algorithm used a minimization procedure (block size of 4) accounting for center in order to ensure a balanced distribution of the population in each group. The study was open in design because the study vaccines differed both in appearance and route of administration (intramuscular for MenACWY-TT and subcutaneous for the MenACWY polysaccharide vaccine).

The study was conducted in accordance with the Good Clinical Practice Guidelines and the Declaration of Helsinki. The protocol and associated documents were reviewed and approved by the Ethics Committee of Pirkanmaa Hospital District. Written informed consent was obtained from the parents/guardians of all the children prior to study entry. In addition, written assent was obtained from the children who were able to give assent to decisions about their participation in the study. This study has been registered at www.clinicaltrials.gov NCT00427908. A summary
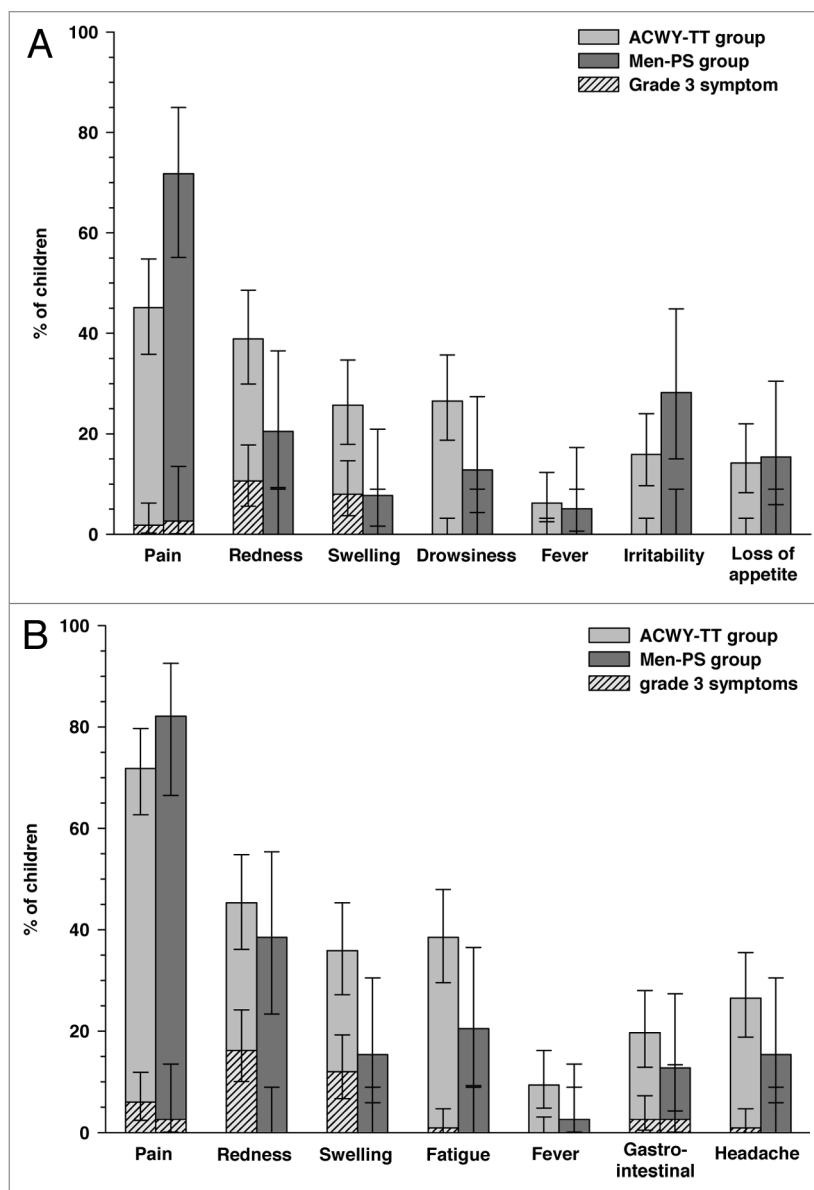

Figure 2. Incidence (with 95% CI) of solicited local and general symptoms occurring within 4 d after the first vaccination in children aged 2–5 y (**A**) or 6–10 y (**B**) (total vaccinated cohort). ACWY-TT group, group of children who received one dose of MenACWY-TT; Men-PS group, group of children who received one dose of the MenACWY polysaccharide vaccine. Error bars represent 95% confidence intervals.

of the protocol is available at www.gsk-clinicalstudyregister. com (GlaxoSmithKline study ID 108658, 108660, 108661 and 108663).

Study participants. Study participants were healthy children aged 2–10 y who previously completed routine childhood vaccinations. Children were not eligible to participate if they had used any investigational product other than the study vaccine within 30 d preceding the study or had planned use during the study period; if they were immunosuppressed from any cause; if they were planned to receive a vaccine not foreseen by the study protocol within one month post-vaccination; if they had previously received a meningococcal conjugate vaccine at any time, a

meningococcal polysaccharide vaccine within the last five years, or a vaccine containing TT within the last month; if they had history of meningococcal disease, neurologic disorders or seizures, allergic disease or reactions likely to be exacerbated by any component of the vaccine; if they had a major congenital defect or a serious chronic illness; if they had acute disease at the time of enrolment; or if they had received immunoglobulins or blood products within three months preceding the study or had planned administration during the study period. During the three-year persistence of the study, toddlers who did not retain rSBA titers ≥ 1:8 for meningococcal serogroup C and were re-vaccinated with a monovalent meningococcal serogroup C conjugate vaccine were excluded from the analyses performed at subsequent timepoints.

Vaccines. One dose of MenACWY-TT contained 5 μg of each of the meningococcal serogroups A, C, W-135 and Y polysaccharides, conjugated to TT (44 μg). The lyophilized vaccine was reconstituted with saline (0.5 mL) and administered intramuscularly into the non-dominant deltoid.

One dose of the MenACWY polysaccharide vaccine (*Mencevax™ACWY*, GlaxoSmithKline, Rixensart, Belgium) contained 50 μg of each of the meningococcal serogroups A, C, W-135 and Y polysaccharides. The lyophilized vaccine was reconstituted with saline (0.5 mL) and administered subcutaneously into the non-dominant upper arm.

Study objectives. The primary objective of this study was to evaluate the non-inferiority of the MenACWY-TT vaccine vs. the licensed MenACWY polysaccharide vaccine in terms of vaccine response rates to serogroups A, C, W-135 and Y in children aged 2–10 y. The vaccine response was defined as a post-vaccination rSBA titer ≥ 1:32 for initially seronegative children (rSBA titer < 1:8 at pre-vaccination) and as at least a 4-fold increase in rSBA titer from pre- to post-vaccination for initially seropositive children (rSBA titer ≥ 1:8 at pre-vaccination). Non-inferiority of MenACWY-TT compared with the MenACWY polysaccharide vaccine was demonstrated if the LL of the asymptotic standardized 95% CI for the difference between the ACWY-TT group and the Men-PS group in terms of percentage of children having a bactericidal vaccine response to serogroups A, C, W-135 and Y at one month after vaccination was ≥ -15%.

The secondary objectives included the comparison of the immunogenicity, antibody persistence and safety profile between MenACWY-TT and the MenACWY polysaccharide vaccine.

Immunogenicity assessment. Blood samples were collected from all the children before and one month, one year, two years and three years after vaccination. Functional antibody titers for meningococcal serogroups A, C, W-135 and Y were measured using a rSBA assay.[40] The presumed correlate of protection against meningococcal disease due to serogroup C was a rSBA titer ≥ 1:8[41] and this threshold had historically been extended to the other serogroups.[42] Moreover, the percentages of children with rSBA titers ≥ 1:128, which is the more conservative threshold for protection, were also evaluated.

Blood samples collected before and one month after vaccination were also analyzed to determine anti-TT antibody concentrations by enzyme-linked immunosorbent assay (ELISA) with a cut-off of 0.1 IU/mL.[43] All immunological assays were performed at GlaxoSmithKline laboratories.

Safety and reactogenicity assessment. The primary safety evaluation was performed separately on the 2–5 y and 6–10 y age strata, because the nature of the solicited general symptoms and the severity grading of the solicited local symptoms differed. In the 2–5 y age strata, solicited local symptoms (pain, redness and swelling) and general symptoms [drowsiness, irritability, loss of appetite and fever (rectal temperature ≥ 38.0°C)] were recorded using diary cards completed by the parent/legally-authorized representative up to four days after vaccination. Redness and swelling were of grade 3 intensity if their diameter was > 30 mm, pain if the child cried when the limb was moved or if the limb was spontaneously painful, loss of appetite if the child did not eat at all, fever if rectal temperature was > 40.0°C, and all other symptoms if they prevented normal activity.

In the 6–10 y age stratum, solicited local symptoms (pain, redness and swelling) and general symptoms (fatigue, gastrointestinal, headache and fever) were also recorded using diary cards completed by the parent/legally-authorized representative up to four days after vaccination. Redness and swelling were of grade 3 intensity if their diameter was > 50 mm, fever if rectal temperature was > 40°C, and all other symptoms if they prevented normal activity.

The occurrence of SAEs and NOCIs was recorded up to six months post-vaccination. SAEs were defined as any untoward medical occurrence that resulted in death, was life-threatening, required hospitalization or prolongation of existing hospitalization, resulted in disability or incapacity, or was an important medical event. NOCIs were identified by the investigator on the case report form, and confirmed by the GlaxoSmithKline Vaccines' physician responsible for the study at the time of data review. All solicited local (injection site) reactions were considered causally related to vaccination. Causality of all other AEs was assessed by the investigator. SAEs considered causally related to vaccination by the investigator were reported throughout the study.

Statistical analyses. With a sample size of 272 children aged 2–10 y evaluable for immunogenicity at one month post-vaccination (204 children in the ACWY-TT group and 68 children in the Men-PS group), the study had 76.5% power to meet the primary non-inferiority objective of MenACWY-TT vs. the MenACWY polysaccharide vaccine in terms of vaccine response to serogroups A, C, W-135 and Y.

The total vaccinated cohort, on which the safety analyses were performed, included all vaccinated children. The according to protocol (ATP) immunogenicity cohort, on which the primary immunogenicity analyses were performed, included all vaccinated children who had complied with protocol-defined procedures and had results available for at least one immunogenicity endpoint at one month after vaccination. The ATP persistence cohorts Year 1, Year 2 or Year 3 on which the antibody persistence analyses were performed included all vaccinated children who had complied with protocol-defined procedures, had not received

a previous dose of meningococcal vaccine other than the study vaccine during the study, and had results available for at least one immunogenicity endpoint at one year, two years or three years post-vaccination, respectively.

GMTs were calculated by taking the anti-log of the mean of the $\log_{10}$ titer transformations. For each treatment group and for each antibody assessed, rSBA GMTs were tabulated with their asymptotic 95% CIs, and the percentages of toddlers with rSBA titers ≥ 1:8 and ≥ 1:128, or anti-TT concentrations ≥ 0.1 IU/mL and ≥ 1.0 IU/mL were calculated with their exact 95% CIs.

As specified in the protocol, potential differences between the ACWY-TT and the Men-PS groups were highlighted in exploratory analyses if the standardized asymptotic 95% CI for the group difference in the percentage of toddlers with titers above the specified cut-offs did not include the value 0, or if the 95% CIs of the GMT ratio between the two groups did not include the value 1. The GMT ratios were calculated using an ANCOVA model on the $\log_{10}$ transformation of the titers or concentrations using the pre-vaccination $\log_{10}$ transformation of the titers or concentrations, the age strata and the vaccine group as covariates. The results of the exploratory group comparisons should be interpreted with caution considering that there was no adjustment for multiplicity of endpoints.

The incidence and intensity of each solicited and unsolicited local and general symptom was calculated with exact 95% CI per age strata for each group. SAEs and NOCIs were described in detail.

The statistical analyses were performed using the SAS® software version 9.1 (SAS Institute Inc.) and Proc StatXact 7.0.

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