Randomized trial to assess the immunogenicity, safety and antibody persistence up to three years after a single dose of a tetravalent meningococcal serogroups A, C, W-135 and Y tetanus toxoid conjugate vaccine in toddlers

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Effective vaccines offering broad protection to toddlers, who are at high risk for invasive meningococcal disease, are needed. Here, the immunogenicity, safety and antibody persistence of the tetravalent meningococcal ACWY tetanus toxoid conjugate vaccine (MenACWY-TT) were evaluated in toddlers. Healthy participants aged 12 to 23 months (n = 304) were randomized (3:1) to receive one dose of MenACWY-TT or a monovalent meningococcal serogroup C conjugate vaccine (MenC-CRM_{197}). Serum bactericidal activity was evaluated with assays using rabbit (rSBA) and human (hSBA) complement up to three years post-vaccination. MenACWY-TT was demonstrated to be non-inferior to MenC-CRM_{197} in terms of immunogenicity to serogroup C, and the pre-specified immunogenicity criteria for serogroups A, W-135 and Y were met. Exploratory analyses suggested that rSBA geometric mean titers (GMTs), hSBA GMTs and proportions of toddlers with rSBA titers ≥ 1:128 and hSBA titers ≥ 1:4 and ≥ 1:8 were higher for all serogroups at one month post-vaccination with MenACWY-TT compared with MenC-CRM_{197}. At three years post-vaccination, at least 90.8% and 73.6% of MenACWY-TT recipients retained rSBA titers ≥ 1:8 for all serogroups and hSBA titers ≥ 1:4 for serogroups C, W-135 and Y, respectively, but the percentages of toddlers with hSBA titers ≥ 1:4 for serogroup A decreased to 21.8%. In both groups, grade 3 adverse events were infrequently reported and no serious adverse events were considered causally related to vaccination. These results suggest that one single dose of MenACWY-TT induces a robust and persistent immune response and has an acceptable safety profile in toddlers. This study has been registered at www.clinicaltrials.gov NCT00427908.

Introduction

The incidence of invasive diseases caused by *Neisseria meningitidis*, such as meningitis and septicaemia, is generally highest in children less than five years of age with a second peak during adolescence, and a third peak in people older than 65 y of age as observed in the United States.[1-4] Meningococcal diseases are associated with high levels of morbidity and mortality, and patients surviving the disease are often left with long-term sequelae, including limb or hearing loss and neurological disability.[2,5-7] Meningococcal invasive disease may be endemic but the highest incidence of the disease is reported during epidemics occurring mostly in the meningitis belt of sub-Saharan Africa.[5] Currently, five meningococcal serogroups (A, B, C, W-135 and Y) cause the majority of the disease cases across the world, although serogroup X has been identified as an additional cause of epidemics in Africa in the last decade.[5,8-11]

In Europe, the overall notification rate (annual rate of cases notified to national control or surveillance programs) of invasive meningococcal disease was estimated to be 0.89 cases per 100,000 population (4,495 cases) in 2009, with the highest number of cases in young children (7.37 cases per 100,000 children under five years of age).[12] Serogroups B and C are the most common meningococcal serogroups reported in Europe, although disease

cases caused by serogroups A and Y have also been reported in some countries.[3,12-18] Moreover, the incidence of meningococcal diseases caused by serogroup W-135 has increased in the last decade; this serogroup was imported in European countries by pilgrims returning from the Hajj pilgrimage after the outbreak in 2000–2001.[15,19] In the United Kingdom, the reported incidence of serogroup W-135 meningococcal disease in Hajj pilgrims reached 30 cases per 100,000 pilgrims.[20] In addition, there is some evidence of emerging serogroup W-135 disease independent of the Hajj outbreak.[21]

Vaccination remains the best strategy to prevent meningococcal diseases and the development of effective vaccines against *N. meningitidis* is a public health priority.[22] Plain polysaccharide vaccines against meningococcal serogroups A, C, W-135 and Y have been available since the 1980s in Europe.[23] However, these vaccines usually do not offer long-term protection, especially in people who are not repeatedly colonized, do not induce immune memory, and are poorly immunogenic in children younger than two years of age.[24] To overcome these limitations, meningococcal conjugate vaccines were developed, in which capsular polysaccharides are covalently coupled to carrier proteins. In 1999, the United Kingdom launched a mass vaccination program with monovalent meningococcal conjugate vaccines against serogroup C targeting the inhabitants between 2 mo and 25 y of age.[25,26] These vaccines were subsequently introduced into the immunization program for infants, which led to a substantial reduction in the incidence of meningococcal diseases caused by this serogroup and to the extension of monovalent meningococcal serogroup C conjugate vaccination as part of the routine immunization program for toddlers in many European countries.[27-29]

More recently, tetravalent meningococcal conjugate vaccines using various carrier proteins have been developed to offer broader protection against the most common disease-causing serogroups.[30-32] A tetravalent meningococcal serogroups A, C, W-135 and Y conjugate vaccine using tetanus toxoid (TT) as carrier protein [Nimenrix™ (GlaxoSmithKline Vaccines); MenACWY-TT] has been recently approved in the European Union (EU) for the active immunization of subjects ≥ 12 mo of age. MenACWY-TT vaccine has been shown to be immunogenic and well-tolerated in toddlers, children, adolescents and young adults.[33-40]

In this study, the immunogenicity, safety and antibody persistence of one dose of the MenACWY-TT vaccine were evaluated and compared with those of a licensed meningococcal vaccine: either a monovalent meningococcal serogroup C conjugate vaccine, using mutant diphtheria toxoid (CRM_{197}) as carrier protein (Meningitec™, Pfizer, formerly Wyeth; hereafter referred as MenC-CRM_{197}) in toddlers in the second year of life or a tetravalent plain polysaccharide vaccine in children between two and ten years of age. This manuscript discusses the results obtained in toddlers while those obtained in the children are the subject of a separate publication.

Results

Study participants. In this study, 304 healthy toddlers aged 12 to 23 mo were randomized into two parallel treatment groups:

toddlers in the ACWY-TT group received one dose of the MenACWY-TT vaccine (n = 229) and toddlers in the MenC-CRM group one dose of the MenC-CRM_{197} vaccine (n = 75). Of these, 299 toddlers completed the active phase of the vaccination stage at one month post-vaccination and 296 toddlers completed the extended safety follow-up phase at six months post-vaccination. Of these, 293, 261 and 223 toddlers were enrolled in the persistence follow-up stage of the study at one, two and three years after vaccination, respectively (**Fig. 1**). Among the 41 toddlers not eligible for the study, 12/13 toddlers in the ACWY-TT group and 28/28 toddlers in the MenC-CRM group were excluded because they had been re-vaccinated with a monovalent meningococcal serogroup C conjugate vaccine during the study period (**Fig. 1**). No toddlers were withdrawn due to an adverse event (AE) during any phase of the study.

The demographic characteristics of the toddlers included in the two groups were comparable (**Table 1**). Overall, the mean age of the toddlers was 19.1 mo at the time of vaccination, and the majority of the toddlers (98.0%) were of Caucasian/European heritage.

Immunogenicity. With the serum bactericidal activity assay using baby rabbit complement (rSBA) performed at GlaxoSmithKline Vaccines, the percentages of toddlers with rSBA titers ≥ 1:8 for serogroup C increased from 39.4% to 100% in the ACWY-TT group and from 31.1% to 98.5% in the MenC-CRM group between pre- and post-vaccination. At one month post-vaccination, 99.1% of the toddlers in the ACWY-TT group and 82.4% of the toddlers in the MenC-CRM group had rSBA titers ≥ 1:128 for serogroup C. The rSBA geometric mean titers (GMTs) for serogroup C reached 878.7 in the ACWY-TT group and 415.0 in the MenC-CRM group (**Table 2**). The lower limit (LL) of the standardized asymptotic 95% confidence interval (CI) for the difference between the ACWY-TT and the MenC-CRM groups regarding the percentages of toddlers with rSBA titers ≥ 1:8 for serogroup C was -0.27%. As a result, the non-inferiority of the immunogenicity to serogroup C of MenACWY-TT vs. MenC-CRM_{197} was shown, and the first co-primary objective was met (data not shown). Exploratory analyses suggested that the ACWY-TT group had a higher percentage of toddlers with rSBA titers ≥ 1:128 and higher adjusted rSBA GMTs for serogroup C compared with the MenC-CRM group (**Table 2**).

At one month post-vaccination, all the toddlers in the ACWY-TT group had rSBA titers ≥ 1:8 for serogroups A, W-135 and Y, and the percentage of toddlers with rSBA titers ≥ 1:128 for these serogroups ranged from 99.5% to 100% (**Table 2**). Since the LLs of the two-sided exact 95% CIs for the percentages of toddlers with rSBA titers ≥ 1:8 for serogroups A, W-135 and Y in the ACWY-TT group were above 80% (98.4% for each serogroup), the immunogenicity of the MenACWY-TT vaccine to these serogroups was demonstrated and the second co-primary objective was met. As expected, exploratory analyses suggested that the percentages of toddlers with rSBA titers ≥ 1:8 and ≥ 1:128 and the adjusted rSBA GMTs for serogroups A, W-135 and Y were higher in the ACWY-TT group than in the MenC-CRM group, in which toddlers had not been vaccinated against these serogroups (**Table 2**).
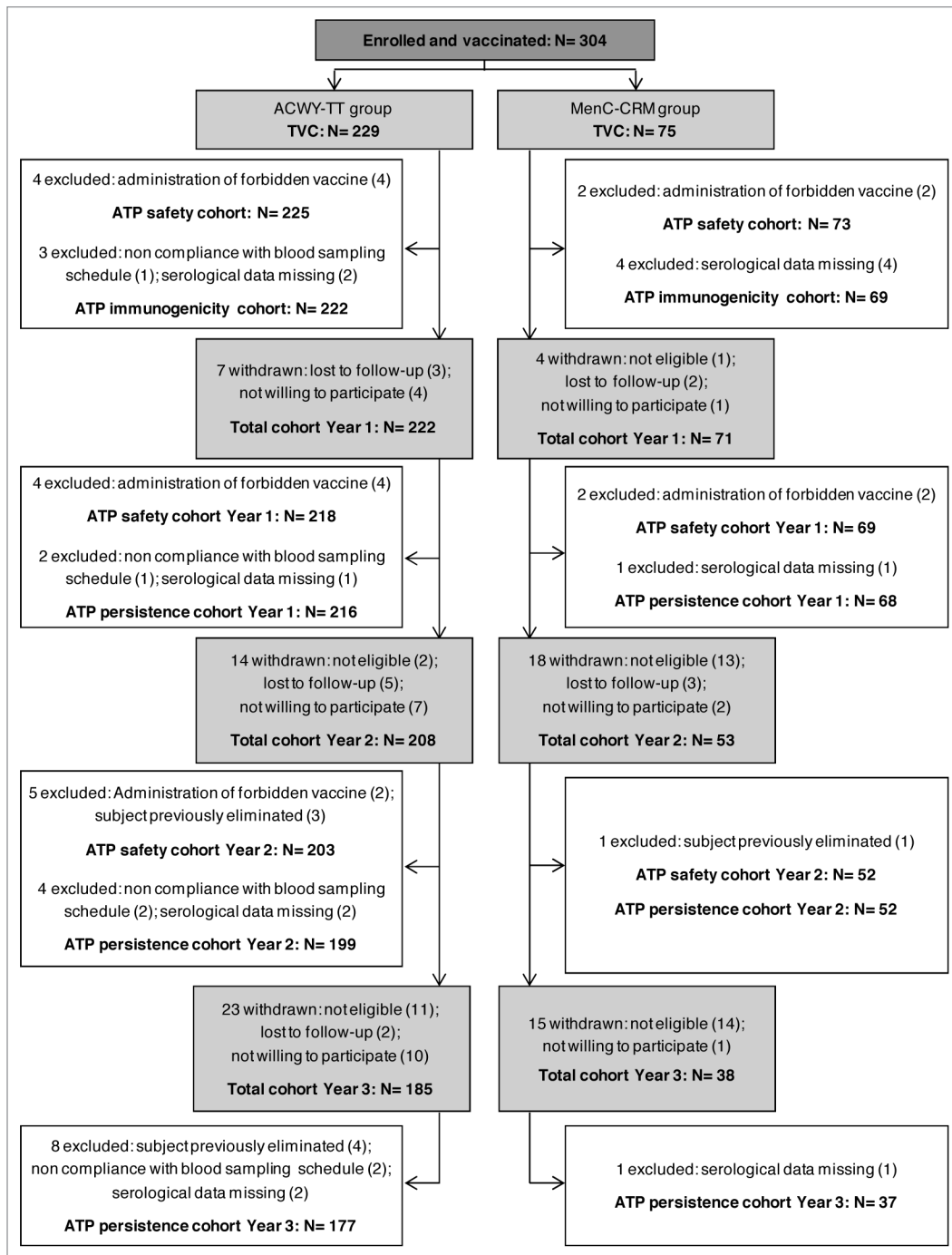
Figure 1. Participant flow diagram. ACWY-TT, toddlers vaccinated with one dose of MenACWY-TT; MenC-CRM, toddlers vaccinated with one dose of MenC-CRM$_{197}$; ATP, according to protocol; TVC, total vaccinated cohort; N, number of toddlers.

With the serum bactericidal activity assay using human complement (hSBA), 99.1% of the toddlers in the ACWY-TT group and 72.1% of the toddlers in the MenC-CRM group had hSBA titers ≥ 1:4 and ≥ 1:8 for serogroup C at one month post-vaccination (**Table 3**). The percentage of toddlers with hSBA titers ≥ 1:4 and ≥ 1:8 for serogroups A, W-135 and Y in the ACWY-TT group ranged from 66.7% to 93.5%. For the four serogroups, exploratory analyses suggested that the percentages of toddlers

with hSBA titers ≥ 1:4 and ≥ 1:8 and the adjusted hSBA GMTs were higher in the ACWY-TT group than in the MenC-CRM group (**Table 3**).

Persistence of the immunogenicity. In the ACWY-TT group, the percentage of toddlers with rSBA titers ≥ 1:8 for the four serogroups remained high (at least 98.1% at one year, 93.9% at two years and 90.8% at three years post-vaccination). However, the percentages of toddlers with rSBA titers ≥ 1:128 were lower

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

Characteristic		ACWY-TT[a]	MenC-CRM[b]
N[c]		229	75
Age (months)	Mean (SD)	19.1 (2.96)	19.3 (3.07)
	Range	12–23	13–23
Gender	Female n (%)	116 (50.7)	39 (52.0)
	Male n (%)	113 (49.3)	36 (48.0)
Race	White–Caucasian / European heritage n (%)	224 (97.8)	74 (98.7)
	White-Arabic / North African heritage n (%)	2 (0.9)	0 (0.0)
	Other (%)	3 (1.3)	1 (1.3)

[a]ACWY-TT, toddlers vaccinated with one dose of MenACWY-TT; [b]MenC-CRM, toddlers vaccinated with one dose of MenC-CRM$_{197}$; [c]Abbreviations: N, number of toddlers; SD, standard deviation; n (%), number (percentage) of toddlers with the specified characteristic.

at one year (69.1% to 98.1%), two years (55.8% to 93.7%), and three years post-vaccination (50.0% to 94.7%) compared with those observed at one month post-vaccination (99.1% to 100%) (**Table 2**).

For serogroups C, W-135 and Y, the percentages of toddlers in the ACWY-TT group with hSBA titers ≥ 1:4 were at least 93.5% at one year, 90.8% at two years and 73.6% at three years post-vaccination (**Table 3**). The percentage of toddlers with hSBA titers ≥ 1:4 and hSBA GMTs for serogroups W-135 and Y increased between one month and one year post-vaccination, but then decreased between one and three years post-vaccination. The percentages of toddlers with hSBA titers ≥ 1:4 for serogroup A decreased to 23.4% at one year post-vaccination, then reached 40.6% at two years post-vaccination and decreased again to 21.8% at three years post-vaccination.

For serogroup C, exploratory analyses suggested that the rSBA GMTs, hSBA GMTs and proportions of toddlers with rSBA titers ≥ 1:8 and ≥ 1:128 and hSBA titers ≥ 1:4 and ≥ 1:8 were higher in the ACWY-TT group than in the MenC-CRM group at one year and two years after vaccination (**Tables 2 and 3**). In contrast, no difference between the two groups was observed at three years post-vaccination.

As expected, exploratory analyses suggested that the percentages of toddlers with rSBA titers ≥ 1:8 and ≥ 1:128 and hSBA titers ≥ 1:4 and the adjusted rSBA and hSBA GMTs for serogroups A, W-135 and Y remained higher in the ACWY-TT group than in the MenC-CRM group at one, two and three years post-vaccination (**Tables 2 and 3**).

Evaluation of safety and reactogenicity. The MenACWY-TT conjugate vaccine induced an overall reactogenicity profile that was similar to that of the MenC-CRM$_{197}$ vaccine during the four-day post-vaccination follow-up period (**Fig. 2**). Injection site redness was the most frequently reported solicited local symptom in both groups, and was reported in 84/228 (36.8%) toddlers in

the ACWY-TT group and 24/73 (32.9%) toddlers in the MenC-CRM group. The most common solicited general symptom was irritability in both groups, which was reported in 88/228 (38.6%) toddlers in the ACWY-TT group and 29/73 (39.7%) toddlers in the MenC-CRM group. Grade 3 solicited symptoms were each reported in no more than 3.5% of the toddlers in each group.

During the 31-d post-vaccination period, unsolicited AEs were reported in 121/229 (52.8%) toddlers in the ACWY-TT group, of which 26 unsolicited AEs were of grade 3 intensity, and in 38/75 (50.7%) toddlers in the MenC-CRM group, of which 11 unsolicited AEs were of grade 3 intensity. The most frequently reported unsolicited symptom was rhinitis in the ACWY-TT group (reported in 19 toddlers; 8.3%) and pyrexia in the MenC-CRM group (reported in 10 toddlers; 13.3%) (data not shown).

During the six-month safety follow-up period of the vaccination stage, serious adverse events (SAEs) were reported in 12 toddlers; 5 toddlers in the ACWY-TT group and 7 toddlers in the MenC-CRM group. No SAEs considered causally related to vaccination and no deaths were reported throughout the study. During the six-month safety follow-up period of the vaccination stage, one toddler in the ACWY-TT group reported a new onset chronic illness (NOCI; rheumatoid arthritis), which was not considered causally related to vaccination (data not shown).

Discussion

Monovalent meningococcal conjugate vaccines against serogroup C are widely used to prevent diseases caused by *N. meningitidis* in young children, an age group at increased risk for meningococcal infections. However, effective vaccines offering broader serogroup coverage in this vulnerable age group are needed. In this study, the immunogenicity, including the antibody persistence, and the safety of the EU-licensed MenACWY-TT vaccine were compared with those of a licensed monovalent serogroup C conjugate vaccine (MenC-CRM$_{197}$) in toddlers in the second year of life.

The MenACWY-TT vaccine was shown to induce higher levels of functional antibodies against meningococcal serogroup C in toddlers than the MenC-CRM$_{197}$ vaccine. The non-inferiority of the MenACWY-TT vaccine vs. the MenC-CRM$_{197}$ vaccine was demonstrated in terms of immunogenicity to this serogroup, which indicates that MenACWY-TT could be used to fulfill national requirements for the routine meningococcal serogroup C vaccination of infants and toddlers. These observations are consistent with those of previous studies, in which the observed rSBA antibody titers for serogroup C induced by the MenACWY-TT vaccine were higher than those induced by the MenC-CRM$_{197}$ vaccine.[35,38]

Furthermore, the immunogenicity of the MenACWY-TT vaccine to meningococcal serogroups A, W-135 and Y was demonstrated in toddlers. As expected, the functional antibody titers measured for these serogroups in the toddlers vaccinated with MenC-CRM$_{197}$, which reflect the development of natural immunity, were lower than those measured in the toddlers who received the MenACWY-TT vaccine. The expansion of

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

Antibody	Group	Timing	N[c]	% ≥ 1:8 [95% CI]	% ≥ 1:128 [95% CI]	GMT [95% CI]
rSBA-MenA	ACWY-TT[a]	PRE	191	42.4 [35.3 - 49.8]	30.9 [24.4 - 38.0]	21.6 [16.1 - 29.0]
		M1	222	100 [98.4 - 100]*	100 [98.4 - 100]*	3706.5 [3327.2 - 4128.9]*
		Y1	212	99.1 [96.6 - 99.9]*	98.1 [95.2 - 99.5]*	967.0 [843.0 - 1109.3]*
		Y2	190	98.9 [96.2 - 99.9]*	93.7 [89.2 - 96.7]*	567.7 [489.8 - 658.0]*
		Y3	170	98.8 [95.8 - 99.9]*	94.7 [90.2 - 97.6]*	518.6 [447.6 - 600.8]*
	MenC-CRM[b]	PRE	65	40.0 [28.0 - 52.9]	30.8 [19.9 - 43.4]	20.2 [12.1 - 33.8]
		M1	63	34.9 [23.3 - 48.0]	25.4 [15.3 - 37.9]	17.1 [10.1 - 29.0]
		Y1	49	32.7 [19.9 - 47.5]	28.6 [16.6 - 43.3]	18.3 [9.5 - 35.1]
		Y2	45	66.7 [51.0 - 80.0]	55.6 [40.0 - 70.4]	51.3 [29.0 - 90.8]
		Y3	32	84.4 [67.2 - 94.7]	65.6 [46.8 - 81.4]	117.1 [65.0 - 211.2]
rSBA-MenC	ACWY-TT	PRE	203	39.4 [32.6 - 46.5]	14.3 [9.8 - 19.9]	13.9 [11.0 - 17.5]
		M1	220	100 [98.3 - 100]	99.1 [96.8 - 99.9]*	878.7 [779.4 - 990.7]*
		Y1	207	98.1 [95.1 - 99.5]*	69.1 [62.3 - 75.3]*	195.3 [166.3 - 229.3]*
		Y2	197	93.9 [89.6 - 96.8]*	55.8 [48.6 - 62.9]*	117.4 [97.1 - 141.9]*
		Y3	174	90.8 [85.5 - 94.7]	50.0 [42.3 - 57.7]	125.1 [96.7 - 162.0]
	MenC-CRM	PRE	61	31.1 [19.9 - 44.3]	6.6 [1.8 - 15.9]	9.7 [6.8 - 13.9]
		M1	68	98.5 [92.1 - 100]	82.4 [71.2 - 90.5]	415.0 [296.9 - 580.0]
		Y1	63	79.4 [67.3 - 88.5]	49.2 [36.4 - 62.1]	77.1 [49.1 - 121.1]
		Y2	52	73.1 [59.0 - 84.4]	32.7 [20.3 - 47.1]	57.7 [33.5 - 99.3]
		Y3	37	97.3 [85.8 - 99.9]	59.5 [42.1 - 75.2]	185.7 [118.3 - 291.5]
rSBA-MenW-135	ACWY-TT	PRE	208	28.4 [22.3 - 35.0]	18.3 [13.3 - 24.2]	11.3 [8.9 - 14.3]
		M1	222	100 [98.4 - 100]*	100 [98.4 - 100]*	5394.6 [4869.9 - 5975.7]*
		Y1	216	100 [98.3 - 100]*	98.1 [95.3 - 99.5]*	855.1 [757.1 - 965.9]*
		Y2	199	99.5 [97.2 - 100]*	89.9 [84.9 - 93.8]*	415.9 [362.4 - 477.3]*
		Y3	174	98.9 [95.9 - 99.9]*	86.8 [80.8 - 91.4]*	439.8 [370.5 - 522.0]*
	MenC-CRM	PRE	62	38.7 [26.6 - 51.9]	25.8 [15.5 - 38.5]	16.5 [10.2 - 26.7]
		M1	63	42.9 [30.5 - 56.0]	28.6 [17.9 - 41.3]	20.3 [12.5 - 33.2]
		Y1	64	59.4 [46.4 - 71.5]	37.5 [25.7 - 50.5]	36.7 [22.6 - 59.7]
		Y2	44	54.5 [38.8 - 69.6]	25.0 [13.2 - 40.3]	27.5 [15.7 - 48.1]
		Y3	33	72.7 [54.5 - 86.7]	42.4 [25.5 - 60.8]	64.5 [33.5 - 124.3]
rSBA-MenY	ACWY-TT	PRE	208	55.3 [48.3 - 62.2]	37.0 [30.4 - 44.0]	33.8 [25.6 - 44.6]
		M1	222	100 [98.4 - 100]*	99.5 [97.5 - 100]*	2823.8 [2529.0 - 3153.1]*
		Y1	216	99.1 [96.7 - 99.9]*	94.9 [91.1 - 97.4]*	766.4 [661.0 - 888.5]*
		Y2	197	98.0 [94.9 - 99.4]*	88.8 [83.6 - 92.9]*	504.1 [421.0 - 603.6]*
		Y3	177	98.3 [95.1 - 99.6]*	87.6 [81.8 - 92.0]*	583.2 [479.0 - 709.9]*
	MenC-CRM	PRE	67	61.2 [48.5 - 72.9]	41.8 [29.8 - 54.5]	45.3 [27.0 - 75.8]
		M1	66	74.2 [62.0 - 84.2]	47.0 [34.6 - 59.7]	77.1 [46.3 - 128.2]
		Y1	66	63.6 [50.9 - 75.1]	48.5 [36.0 - 61.1]	65.1 [36.9 - 114.7]
		Y2	50	74.0 [59.7 - 85.4]	52.0 [37.4 - 66.3]	93.5 [51.8 - 168.7]
		Y3	36	91.7 [77.5 - 98.2]	58.3 [40.8 - 74.5]	176.0 [97.6 - 317.3]

[a]ACWY-TT, toddlers vaccinated with one dose of MenACWY-TT; [b]MenC-CRM, toddlers vaccinated with one dose of MenC-CRM$_{197}$; [c]Abbreviations: N, number of toddlers with available results; %, percentage of toddlers with titer within the specified range; 95% CI, 95% confidence interval; GMT, geometric mean antibody titer calculated on all toddlers; PRE, pre-vaccination; M1, Y1, Y2 and Y3, one month, one year, two years and three years post-vaccination. *Higher values compared with the control group (exploratory analysis).

Table 3. Percentage of toddlers with hSBA titers equal to or above cut-off values and hSBA GMTs at pre-vaccination, one month post-vaccination (ATP immunogenicity cohort), one year post-vaccination (ATP persistence cohort Year 1), two years post-vaccination (ATP persistence cohort Year 2) and three years post-vaccination (ATP persistence cohort Year 3)

Antibody	Group	Timing*	N[c]	% ≥ 1:4 [95% CI]	% ≥ 1:8 [95% CI]	GMT [95% CI]
hSBA-MenA	ACWY-TT[a]	PRE	215	1.4 [0.3 - 4.0]	0.0 [0.0 - 1.7]	2.0 [2.0 - 2.1]
		M1	217	93.5 [89.4 - 96.4]*	91.2 [86.7 - 94.6]*	59.0 [49.3 - 70.6]*
		Y1	201	23.4 [17.7 - 29.9]*	20.4 [15.1 - 26.6]*	3.6 [3.1 - 4.2]*
		Y2	180	40.6 [33.3 - 48.1]*	36.1 [29.1 - 43.6]*	5.1 [4.2 - 6.1]*
		Y3	170	21.8 [15.8 - 28.7]*	17.6 [12.2 - 24.2]	3.3 [2.8 - 3.8]*
	MenC-CRM[b]	PRE	68	4.4 [0.9 - 12.4]	4.4 [0.9 - 12.4]	2.2 [2.0 - 2.3]
		M1	65	6.2 [1.7 - 15.0]	4.6 [1.0 - 12.9]	2.3 [2.0 - 2.6]
		Y1	63	4.8 [1.0 - 13.3]	3.2 [0.4 - 11.0]	2.2 [2.0 - 2.4]
		Y2	50	12.0 [4.5 - 24.3]	4.0 [0.5 - 13.7]	2.3 [2.0 - 2.5]
		Y3	36	5.6 [0.7 - 18.7]	5.6 [0.7 - 18.7]	2.2 [1.9 - 2.6]
hSBA-MenC	ACWY-TT	PRE	214	1.4 [0.3 - 4.0]	1.4 [0.3 - 4.0]	2.1 [2.0 - 2.2]
		M1	221	99.1 [96.8 - 99.9]*	99.1 [96.8 - 99.9]*	190.0 [164.7 - 219.2]*
		Y1	200	96.0 [92.3 - 98.3]*	96.0 [92.3 - 98.3]*	88.7 [73.8 - 106.5]*
		Y2	191	93.7 [89.3 - 96.7]*	92.1 [87.4 - 95.5]*	55.6 [45.1 - 68.5]*
		Y3	166	88.6 [82.7 - 93.0]	88.6 [82.7 - 93.0]	65.1 [49.6 - 85.5]
	MenC-CRM	PRE	67	1.5 [0.0 - 8.0]	1.5 [0.0 - 8.0]	2.1 [1.9 - 2.3]
		M1	68	72.1 [59.9 - 82.3]	72.1 [59.9 - 82.3]	21.2 [13.9 - 32.3]
		Y1	64	53.1 [40.2 - 65.7]	53.1 [40.2 - 65.7]	12.2 [7.6 - 19.5]
		Y2	51	54.9 [40.3 - 68.9]	54.9 [40.3 - 68.9]	11.8 [6.8 - 20.6]
		Y3	33	75.8 [57.7 - 88.9]	75.8 [57.7 - 88.9]	32.5 [16.0 - 65.9]
hSBA-MenW-135	ACWY-TT	PRE	205	1.0 [0.1 - 3.5]	1.0 [0.1 - 3.5]	2.1 [2.0 - 2.1]
		M1	177	81.9 [75.4 - 87.3]*	79.7 [73.0 - 85.3]*	38.8 [29.7 - 50.6]*
		Y1	175	98.3 [95.1 - 99.6]*	98.3 [95.1 - 99.6]*	225.1 [184.5 - 274.7]*
		Y2	178	96.1 [92.1 - 98.4]*	96.1 [92.1 - 98.4]*	111.4 [90.9 - 136.5]*
		Y3	164	79.9 [72.9 - 85.7]*	79.9 [72.9 - 85.7]*	40.8 [31.1 - 53.6]*
	MenC-CRM	PRE	64	4.7 [1.0 - 13.1]	3.1 [0.4 - 10.8]	2.2 [2.0 - 2.4]
		M1	58	1.7 [0.0 - 9.2]	1.7 [0.0 - 9.2]	2.0 [2.0 - 2.1]
		Y1	62	4.8 [1.0 - 13.5]	4.8 [1.0 - 13.5]	2.4 [1.9 - 3.0]
		Y2	51	9.8 [3.3 - 21.4]	9.8 [3.3 - 21.4]	2.9 [2.1 - 4.0]
		Y3	35	8.6 [1.8 - 23.1]	8.6 [1.8 - 23.1]	3.0 [1.9 - 4.7]
hSBA-MenY	ACWY-TT	PRE	189	3.7 [1.5 - 7.5]	2.6 [0.9 - 6.1]	2.2 [2.0 - 2.3]
		M1	201	67.2 [60.2 - 73.6]*	66.7 [59.7 - 73.1]*	24.4 [18.6 - 32.1]*
		Y1	214	93.5 [89.3 - 96.4]*	93.5 [89.3 - 96.4]*	105.1 [85.2 - 129.7]*
		Y2	173	90.8 [85.4 - 94.6]*	90.8 [85.4 - 94.6]*	72.5 [57.0 - 92.4]*
		Y3	159	73.6 [66.0 - 80.3]*	73.6 [66.0 - 80.3]*	37.3 [27.2 - 51.2]*
	MenC-CRM	PRE	57	3.5 [0.4 - 12.1]	0.0 [0.0 - 6.3]	2.1 [2.0 - 2.2]
		M1	59	5.1 [1.1 - 14.1]	5.1 [1.1 - 14.1]	2.4 [1.9 - 3.1]
		Y1	68	11.8 [5.2 - 21.9]	11.8 [5.2 - 21.9]	3.2 [2.3 - 4.4]
		Y2	43	30.2 [17.2 - 46.1]	30.2 [17.2 - 46.1]	5.0 [3.1 - 7.9]
		Y3	33	27.3 [13.3 - 45.5]	27.3 [13.3 - 45.5]	6.0 [3.0 - 11.9]

[a]ACWY-TT, toddlers vaccinated with one dose of MenACWY-TT; [b]MenC-CRM, toddlers vaccinated with one dose of MenC-CRM$_{197}$; [c]Abbreviations: N, number of toddlers with available results; %, percentage of toddlers with titer within the specified range; 95% CI, 95% confidence interval; GMT, geometric mean antibody titer calculated on all toddlers; PRE, pre-vaccination; M1, Y1, Y2 and Y3, one month, one year, two years and three years post-vaccination. *Higher values compared with the control group (exploratory analysis).
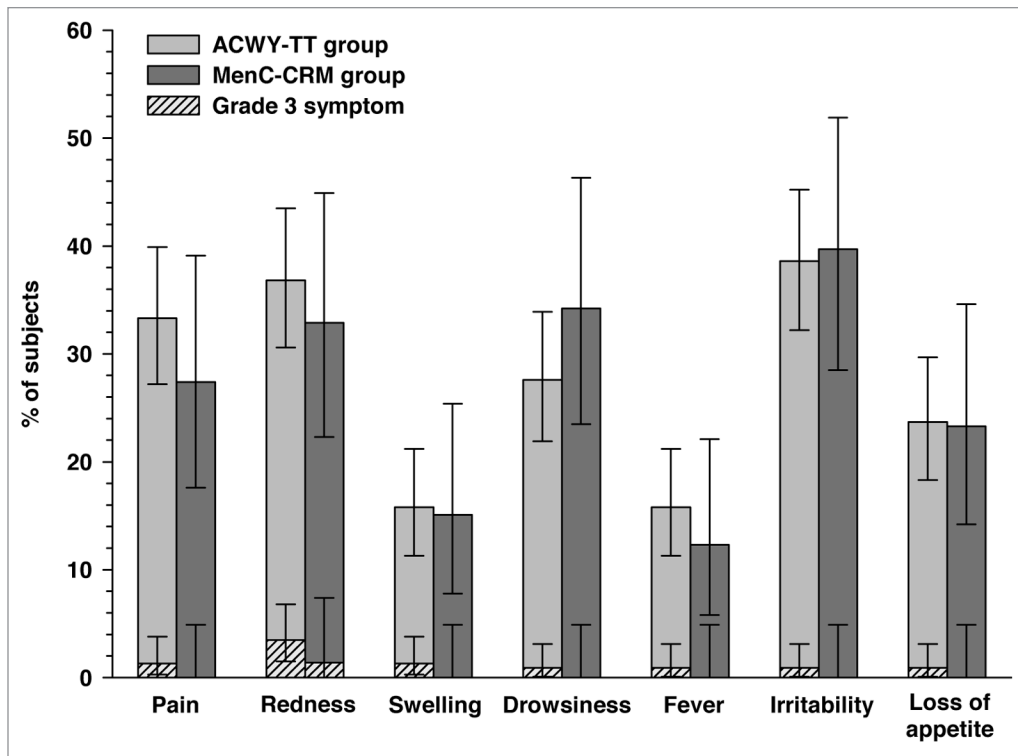
Figure 2. Incidence (with 95% CI) of solicited local and general symptoms occurring within four days after the first vaccination in toddlers (total vaccinated cohort). ACWY-TT, toddlers vaccinated with one dose of MenACWY-TT; MenC-CRM, toddlers vaccinated with one dose of MenC-CRM$_{197}$. Error bars represent 95% confidence interval.

the immunogenicity to serogroups A, W-135 and Y is a major benefit of the MenACWY-TT vaccine over the licensed MenC-CRM$_{197}$ vaccine. Moreover, the rSBA antibody titers for serogroups A, W-135 and Y observed here in toddlers vaccinated with MenACWY-TT were higher than those induced by a licensed plain polysaccharide vaccine in children enrolled in the same study (age group 2–10 y) or in a previous study (aged 3–5 y).[35] These results are in-line with other studies showing that other tetravalent meningococcal conjugate vaccines induce higher antibody titers than plain polysaccharide vaccines in children,[31,41] adolescents[42,43] and adults.[23]

In order to more completely characterize the immunogenicity of the MenACWY-TT vaccine, a secondary analysis using an hSBA assay was performed. Although a significant proportion of toddlers had existing rSBA antibody titers ≥ 1:8 prior to vaccination (between 28.4% and 61.2%), the percentage of toddlers with pre-vaccination hSBA antibody titers ≥ 1:4 was very low for all serogroups (between 1.0% and 4.7%). The high percentages of toddlers reaching the threshold of hSBA antibody titers ≥ 1:4 for each serogroup at one month after vaccination (between 67.2% and 99.1%) confirm the immunogenicity of a single dose of MenACWY-TT to the four meningococcal serogroups in this age group. In addition, the hSBA assay against serogroup C appeared to distinguish further the MenACWY-TT vaccine from the licensed MenC-CRM$_{197}$ vaccine, with 99.1% of MenACWY-TT recipients achieving post-vaccination hSBA titers ≥ 1:4 and ≥ 1:8, as compared with 72.1% of MenC-CRM$_{197}$

vaccinees. The difference between the rSBA and the hSBA pre-vaccination seropositivity rates could be explained by the fact that hSBA assays may not measure naturally-acquired antibodies with the same sensitivity as rSBA assays.[44-48] The lower hSBA titers measured may also be due to the inability of *N. meningitidis* to bind and inactivate non-human factor H resulting in an increased sensitivity to complement-mediated bacteriolysis by non-human complement.[49,50]

The persistence of rSBA and hSBA antibodies against serogroup C was observed to be higher after vaccination with MenACWY-TT compared with MenC-CRM$_{197}$ up to two years post-vaccination. This is in line with previous studies showing a better persistence of functional antibodies up to booster vaccination in the second year of life in infants primed with a monovalent serogroup C conjugate vaccine using TT as carrier protein (MenC-TT) compared with MenC-CRM$_{197}$ conjugate vaccines, suggesting that there may be increased immunogenicity and better persistence of the immune response when TT is used as carrier protein compared with CRM$_{197}$.[51-54] In contrast, exploratory analyses did not detect any differences between the two groups in terms of rSBA or hSBA antibody titers to serogroup C at three years post-vaccination. This is largely explained by the higher proportion of toddlers vaccinated with MenC-CRM$_{197}$ compared with MenACWY-TT [28 toddlers (37.3%) vs. 12 toddlers (5.2%)], who were excluded from the study at three years post-vaccination because they had previously been re-vaccinated with a monovalent meningococcal serogroup C conjugate vaccine

due to failure to retain seroprotective antibody titers against this serogroup. This phenomenon introduced a selection bias into the assessment at three years post-vaccination since the toddlers with poor antibody persistence were excluded from the follow-up after two years.

The rSBA antibody titers remained high for serogroups A, W-135, and Y up to three years after vaccination with MenACWY-TT and at least 73% of toddlers retained hSBA antibody titers ≥ 1:4 for serogroups W-135 and Y. The hSBA antibody titers for serogroups W-135 and Y were observed to increase between one month and one year post-vaccination and then to decrease between one and three years post-vaccination. The reasons for this increase are not clear, but are unlikely due to assay variability, since a concomitant increase in the hSBA antibody titers in the control group was not observed. One possible explanation could be a different pattern of antibody kinetics for serogroups W-135 and Y as compared with serogroups A and C. This finding deserves additional exploration, which could be performed by obtaining serum samples for persistence evaluation prior to one year post-vaccination in a future study.

For serogroup A, the percentage of toddlers who retained hSBA titers ≥ 1:4 at one, two and three years post-vaccination was much lower. The clinical relevance of the decline in hSBA antibody titers to serogroup A between one month and one year post-vaccination is yet to be determined, but this observation is in line with previous studies, in which the immunogenicity of a tetravalent meningococcal conjugate vaccine using CRM_{197} as carrier protein was assessed by an hSBA assay.[41,43,55] This suggests that the lower persistence of hSBA antibody titers to serogroup A was not specific to a given meningococcal conjugate vaccine, but may be characteristic of the hSBA assay against serogroup A polysaccharide.[56] To our knowledge, persistence in terms of hSBA antibody titers to serogroup A has not been evaluated for MenA-TT, the only currently-licensed meningococcal serogroup A conjugate vaccine using TT as the carrier protein.

The reactogenicity and safety profiles of the MenACWY-TT and the $MenC-CRM_{197}$ vaccines were comparable, despite the additional meningococcal polysaccharide and protein content in the EU-licensed MenACWY-TT vaccine, and both vaccines were well-tolerated in toddlers in the second year of life. Solicited local and general symptoms of grade 3 intensity were infrequently reported, and no SAEs considered causally related to the vaccination were reported throughout the study. These results are consistent with those obtained in previous studies, in which MenACWY-TT and $MenC-CRM_{197}$ were administered to toddlers.[35,38]

This study was limited by its open design because the vials containing the MenACWY-TT vaccine differed from the commercial vials containing the control vaccine. The open design would not have influenced the immunogenicity results, as personnel in the laboratories remained blinded to treatment group allocation, but the safety analyses may have been biased toward increased reporting of AEs in the toddlers who received the MenACWY-TT vaccine, since the parents were aware that their child was receiving a vaccine containing four instead of only one antigen. This study was also potentially limited by the numerous exploratory statistical comparisons performed without adjustment for multiplicity. Nonetheless, the study was powered to demonstrate the two co-primary endpoints, so the conclusions drawn from those statistical evaluations are reliable. Another limitation was that the two other tetravalent meningococcal serogroups A, C, W-135 and Y conjugate vaccines (MenACWY-DT and $MenACWY-CRM_{197}$) were not used as comparator vaccines, since these vaccines were not approved in Europe at the time of vaccination in the present study. The use of MenC-TT vaccine as a control is another potential direction for future studies, since the MenC-TT vaccine is known to have increased immunogenicity as compared with $MenC-CRM_{197}$ vaccines.[52,53]

In conclusion, the MenACWY-TT vaccine was shown to be immunogenic to meningococcal serogroups A, C, W-135 and Y in toddlers, and to induce an immune response that persisted up to three years after vaccination. The non-inferiority of the immunogenicity to serogroup C of the MenACWY-TT vaccine vs. the licensed $MenC-CRM_{197}$ vaccine was demonstrated. The MenACWY-TT vaccine had a clinically acceptable safety profile, which was comparable to that of the control vaccine. MenACWY-TT may represent an important new means of protecting toddlers by providing broad coverage against four of the most common disease-causing serogroups of *N. meningitidis*.

Material and Methods

Study design. This was a phase II, open, randomized, controlled study conducted in 11 centers in Finland between February 2007 and May 2010. The study was conducted in two stages: a six-month vaccination stage and a five-year persistence follow-up stage. At the time of the preparation of this manuscript, the interim results for up to three years after vaccination were available.

Healthy toddlers aged 12 to 23 mo were randomized (3:1) into the two parallel treatment groups: the ACWY-TT group and the MenC-CRM group. 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, web-based randomization system and a randomization blocking scheme was used to ensure that balance between treatments was maintained. The randomization algorithm used a minimization procedure accounting for center. The study was open in design because the vials containing the study vaccines differed in appearance.

The study was conducted in accordance with the guidelines for Good Clinical Practice, all applicable regulatory requirements and the Declaration of Helsinki. The protocol and associated documents were reviewed and approved by the Ethics Committee of the Pirkanmaa Hospital District. Written informed consent was obtained from the parents or guardians of the toddlers prior to the performance of any study specific procedures. This study has been registered at www.clinicaltrials.gov NCT00427908. A summary of the protocol is available at http://www.gsk-clinical-studyregister.com (GSK study ID 108658, 108660, 108661 and 108663).

Study objectives. The first co-primary objective of this study in the toddlers' age stratum was to evaluate the non-inferiority of the immunogenicity to serogroup C induced by the MenACWY-TT vaccine vs. the MenC-CRM$_{197}$ vaccine in terms of rSBA titers. Non-inferiority was demonstrated if the LL of the asymptotic standardized 95% CI for the difference between the ACWY-TT group and the MenC-CRM group in terms of percentage of toddlers with rSBA titers ≥ 1:8 for serogroup C was ≥ -15% at one month after vaccination. The second co-primary objective in the toddlers' age stratum was to evaluate the immunogenicity to serogroups A, W-135 and Y induced by the MenACWY-TT vaccine in terms of the percentage of toddlers with post-vaccination rSBA titers ≥ 1:8. Immunogenicity was shown if the LL of the two-sided exact 95% CI for the percentage of toddlers in the ACWY-TT group with rSBA titers ≥ 1:8 for each serogroup was ≥ 80% at one month after vaccination.

Secondary objectives of this study included: (1) the comparison of the immunogenicity and antibody persistence of the MenACWY-TT vaccine and the MenC-CRM$_{197}$ vaccine using rSBA and hSBA assays, (2) the evaluation of the reactogenicity, and (3) the assessment of the safety profiles of both vaccines.

Study participants. Study participants were healthy toddlers aged 12 to 23 mo, who previously completed routine childhood vaccination to the best of their parents/guardians knowledge. Toddlers were excluded from participation if they had received previous vaccination with a meningococcal vaccine since birth or with a tetanus toxoid-containing vaccine within the last 28 d; had a history of meningococcal disease due to serogroup A, C, W-135 or Y; had received other investigational products or had planned their use during the study period; had received immunoglobulin or blood products within the three months preceding the study; were immunosuppressed for any reason; had allergic disease or reactions likely to be exacerbated by any component of the vaccines; had a major congenital defect or a serious chronic illness; or had a history of any neurological disorders or seizures. During the long-term persistence stages of the study, toddlers who did not retain rSBA titers ≥ 1:8 for meningococcal serogroup C and were re-vaccinated against this serogroup were excluded from the analyses performed at subsequent timepoints.

Study vaccines. One dose of the MenACWY-TT vaccine (GlaxoSmithKline Vaccines) contained 5 μg of each meningococcal serogroup A, C, W-135 and Y polysaccharide conjugated to TT (approximately 44 μg). The lyophilized vaccine was reconstituted with saline for delivery of 0.5 mL. The licensed MenC-CRM$_{197}$ vaccine (Meningitec™, Pfizer, formerly Wyeth) comprised 10 μg of polysaccharide from serogroup C conjugated to 15 μg of CRM$_{197}$ absorbed onto aluminum. The vaccines were administered intramuscularly into the non-dominant deltoid or thigh as deemed appropriate for the age of the toddler.

Immunogenicity assessment. Blood samples were collected from all toddlers at pre-vaccination, one month, and one, two and three years post-vaccination and all assays were performed at GlaxoSmithKline Vaccines' laboratories.

The blood samples were analyzed to determine rSBA titers for serogroups A, C, W-135 and Y. The cut-off value was an rSBA titer of 1:8, which has been associated with seroprotection for

serogroups C and this threshold was extended to the other serogroups in this study.[57-59] The percentage of toddlers with rSBA titers ≥ 1:128, which is the more conservative threshold to define seroprotection, was also evaluated.[58,59]

Blood samples were also analyzed to determine hSBA titers for serogroups A, C, W-135 and Y. The cut-off of the assay was an hSBA titer of 1:4, which has been associated with protection against serogroup C disease and was extended by convention to the other serogroups. The percentages of toddlers with hSBA titers ≥ 1:8 were also evaluated.[60,61]

Safety and reactogenicity assessment. Solicited local (pain, redness and swelling) and general [drowsiness, irritability, loss of appetite and fever (rectal temperature > 38.0°C)] symptoms were recorded up to four days after vaccination and unsolicited AEs up to 30 d following vaccination. The intensity of each symptom was graded on a three-grade scale: pain at the injection site was considered to have a grade 3 intensity if the limb was spontaneously painful or if the child cried when it was moved, redness and swelling at the injection site had a grade 3 intensity if the diameter was > 30 mm, fever if rectal temperature was > 40.0°C, loss of appetite if the child did not eat at all and irritability if the child cried and could not be comforted. All other AEs were considered of grade 3 intensity if they prevented normal activity.

The occurrence of SAEs, NOCIs (e.g., autoimmune disorders, asthma, type diabetes and allergies), rashes and AEs leading to emergency room visits and physician office visits (except those that have to do with routine pediatric conditions) was recorded up to six months post-vaccination. In addition, SAEs considered related to vaccination were reported throughout the study. SAEs were defined as any untoward medical occurrence that resulted in death, was life-threatening, required hospitalization or prolongation of existing hospitalisation, resulted in disability or incapacity, or was an important medical event. While all solicited local (injection site) reactions were considered causally related to vaccination, causality of all other AEs was assessed by the investigator.

Statistical analyses. With a sample size of 272 toddlers evaluable for immunogenicity at one month post-vaccination (204 toddlers in the ACWY-TT group and 68 toddlers in the MenC-CRM group), the study had 98.7% power to meet the primary non-inferiority objective of MenACWY-TT vs. MenC-CRM$_{197}$ in terms of rSBA titers for serogroup C. The power to meet the immunogenicity criteria for MenACWY-TT in terms of rSBA titers for serogroups A, W-135 and Y was at least 99.8%.

The total vaccinated cohort, on which the safety analyses were performed, included all vaccinated toddlers. The according to protocol (ATP) immunogenicity cohort, on which the immunogenicity analyses were performed, included all vaccinated toddlers who had complied with protocol-defined procedures, did not receive immunosuppressing medications, blood products, or other vaccines and had results available for at least one immunogenicity endpoint at one month after vaccination. The ATP persistence cohorts for Year 1, Year 2 and Year 3, on which the antibody persistence analyses were performed, included all vaccinated toddlers who had complied with protocol-defined procedures, had not received a previous dose of meningococcal vaccine

other than the study vaccines during the vaccination stage, and had results available for at least one immunogenicity endpoint at one, two and three years post-vaccination.

GMTs were calculated for each antibody by taking the antilog of the mean of the $\log_{10}$ titer transformations. For each treatment group, rSBA and hSBA GMTs for the four meningococcal serogroups were tabulated with their standardized asymptotic 95% CIs. The percentages of toddlers with rSBA and hSBA titers above the previously-mentioned thresholds were calculated with their exact 95% CIs.

In exploratory analyses, potential differences between the ACWY-TT and the MenC-CRM groups were highlighted if the standardized asymptotic 95% CI for the group difference in the percentage of toddlers with titers above the specified cutoffs 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 using the pre-vaccination $\log_{10}$ transformation of the titers and the vaccine group as covariates. As the 95% CIs were not adjusted for multiplicity of endpoints, statistically significant findings must be interpreted with caution.

The incidence and intensity of each solicited local and general symptom were calculated with exact 95% CI for each group while SAEs and NOCIs were described in detail. The statistical analyses were performed using the SAS® software version 9.1 or version 9.2 (SAS Institute Inc.) and Proc StatXact 7.0 or 8.1. Meningitec is a trademark of Pfizer, formerly Wyeth. Nimenrix is a trademark of the GlaxoSmithKline Group of companies.

Disclosure of Potential Conflicts of Interest

T.V. received consulting fees as well as support for meetings, travel or accommodation expenses from GlaxoSmithKline

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