One or Two Doses of Quadrivalent Meningococcal Serogroups A, C, W-135 and Y Tetanus Toxoid Conjugate Vaccine Is Immunogenic in 9- to 12-Month-Old Children

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Background: The incidence of invasive meningococcal disease is highest in infants. A quadrivalent meningococcal serogroups A, C, W-135 and Y tetanus toxoid conjugate vaccine (MenACWY-TT) was evaluated in children 9–12 months of age.

Methods: We randomized infants (1:1) to receive 1 dose of MenACWY-TT at 12 months of age (ACWY-1 group) or 2 doses at 9 and 12 months (ACWY-2). We measured immunogenicity after each dose and 1 year after completing vaccination using human serum bactericidal antibody (hSBA) assays according to prespecified criteria of ≥1:8. Local and general symptoms were solicited for 8 days after vaccination. Adverse events were recorded for 6 months after the last dose.

Results: We enrolled and vaccinated 349 subjects, of whom 248 reenrolled at Year 1 for evaluation of antibody persistence. Percentages of subjects with postvaccination hSBA ≥1:8 in the ACWY-1 group were 79.5%, 94.6%, 50.8% and 56.1% and in the 2-dose group (ACWY-2) were 88.4%, 100%, 99.3% and 99.3% postdose 2 for serogroups A, C, W-135 and Y, respectively. At Year 1, 80.0–99.1% in each group had hSBA ≥1:8, except for serogroup A, for which 20.6% (ACWY-1) and 25.9% (ACWY-2) retained hSBA ≥1:8. Both schedules were well-tolerated, with no observed increase in reactogenicity after the second dose.

Conclusions: MenACWY-TT was immunogenic when administered as a single dose at 12 months of age, or as 2 doses at 9 and 12 months, and had a clinically acceptable safety profile. Good antibody persistence was observed through 12 months postvaccination after both treatment schedules for serogroups C, W-135, Y.

Key Words: quadrivalent meningococcal vaccine, conjugate vaccine, bactericidal activity, persistence, immunogenicity

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Neisseria meningitidis is an important global cause of invasive bacterial infection at all ages.[1,2] There are an approximately 1600 cases of invasive meningococcal disease (IMD) in the United States each year.[3] In the United States, the incidence of IMD is highest in infants. In US children younger than 1 year of age, the annual incidence of IMD was 5.38 cases per 100,000 population between 1998 and 2007, with a case fatality rate of 6.0%.[3,4] In 2010, the Active Bacterial Core Surveillance network, which represents a population of around 41 million in 10 US regions, recorded 11 meningococcal cases in US children younger than 1 year of age, resulting in an annual incidence of 2.26 cases per 100,000 population. In these children, IMD was caused by serogroups B (5/11 cases), Y (4/11) and C (2/11).[5] Serogroups A and W-135 are not common causes of IMD in the United States, but remain important causes of epidemic IMD in Africa and Asia.[1,2] Travelers to these regions are therefore at risk of infection with serogroups A and W-135 meningococcal disease, and these serogroups could also potentially be imported into naive populations.

Quadrivalent meningococcal conjugate vaccines containing polysaccharides from serogroups A, C, W-135 and Y (ACWY) conjugated to a carrier protein offer protection against 4 capsular serogroups that cause IMD. One meningococcal ACWY conjugate vaccine (MenACWY-DT; Menactra, Sanofi Pasteur, Swiftwater, PA) is currently licensed in the United States as a 2-dose vaccine for children 9 to 12 months of age, and is recommended for children at increased risk for IMD due to complement deficiency or exposure due to travel or residence in an endemic area.[6] A second meningococcal serogroup C and Y vaccine, combined with Hib (HibMenCY-TT, MenHibrix, GlaxoSmithKline, Rixensart, Belgium), was recently licensed in the United States for infants as young as 6 weeks of age.[7] In October 2012, the Advisory Committee on Immunization Practices recommended this vaccine also for children at increased risk for IMD.[8] Maintenance of detectable antibodies over time is considered necessary for ongoing protection against meningococcal disease.[9] However, few antibody persistence data after infant vaccination with multivalent meningococcal conjugate vaccines are currently available. Three years after vaccination of infants with 2 doses of MenACWY-DT at 9 and 12 months, 9 and 15 month or 12 and 15 months of age, the percentage of subjects with serum bactericidal antibody (human complement; hSBA) titers ≥1:8 was approximately 45% for serogroup A and around 20% or less for the other serogroups.[10] Five years after a 4-dose primary vaccination series with HibMenCY-TT, the percentage of children with persisting hSBA titers ≥1:8 was 82.9% for serogroup C and 69.5% for serogroup Y.[11]

To expand available options for vaccination of young children against IMD in the United States, GlaxoSmithKline has developed an ACWY vaccine with all serogroups conjugated to tetanus toxoid (MenACWY-TT, Nimenrix, GlaxoSmithKline, Rixensart, Belgium), which is licensed in Europe for individuals as of 1 year of age but remains investigational in the United States. Clinical trials conducted in Europe, Asia, the Middle East and the United

States have demonstrated that MenACWY-TT is immunogenic and well-tolerated in children from 12 months of age,[12–16] as well as in adolescents and adults.[17–21] Exploratory analyses in a study of US adolescents showed that a single dose of MenACWY-TT induced statistically significantly higher hSBA titers against all 4 serogroups and significantly higher percentages of subjects with hSBA titers ≥1:8 for serogroups A, W-135 and Y than the US-licensed MenACWY-DT vaccine.[18] Another study found that 15 months after vaccinating toddlers and children with MenACWY-TT, at least 92.3%, had persisting rSBA titers ≥1:8.[15] To date, no data regarding the use of MenACWY-TT in children less than 12 months of age are available.

This study evaluated (1) the immunogenicity and safety of 2 doses of MenACWY-TT administered to healthy infants at 9 and 12 months of age compared with a single dose administered at 12 months of age and (2) antibody persistence 1 year after vaccination.

METHODS

Study Design

We conducted this phase 2 randomized, open-label study in the United States between July 5, 2007, and November 26, 2008, and evaluated antibody persistence from October 24, 2008, through June 15, 2009. We conducted the vaccination phase (www.clinicaltrials.gov NCT00471081) in 16 medical centers, 10 of which were under the auspices of the Kaiser Permanente Vaccine Study Center within Kaiser Permanente Northern California. Kaiser Permanente Northern California conducted the 1-year immunogenicity follow-up phase in the 10 clinics, which participated in the original vaccination study (NCT00718666). The study protocol and associated documents were reviewed and approved by local ethics committees. The study was performed in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. We obtained informed consent from the parent/guardian of each child before performing any study-specific procedures.

In the primary vaccine study, subjects were randomized (1:1 ratio) to receive either 1 dose of MenACWY-TT at 12 months of age (ACWY-1 group) or 2 doses of MenACWY-TT administered at 9 and 12 months of age (ACWY-2 group). Subjects whose parents/guardians consented to participate in the 1-year serological follow-up phase retained their original study group assignment; subjects will be followed for up to 5 years after primary vaccination. Here we report results 1 month after vaccination and follow-up antibody persistence data until Year 1.

Study Subjects

Primary MenACWY-TT Study

Subjects were healthy 9-month-old infants who were required to have received all routine vaccinations according to the recommendations of the Advisory Committee on Immunization Practices at the time of enrollment. We excluded infants with a history of IMD or those who had previously received any meningococcal vaccine containing serogroups A, C, W-135 and/or Y. We also excluded children who had received any vaccine within 1 month of study enrolment (with the exception of influenza vaccine), were immunosuppressed from any cause including children on chronic (>14 days) immunosuppressants, had received blood products within 3 months of vaccination, had a history of allergic disease likely to be exacerbated by the study vaccine or had neurological disorders or seizures.

Long-term MenACWY-TT Immunogenicity Study

Only children who completed the primary MenACWY-TT study were eligible. Children were similarly excluded from the long-term persistence stage if, since the completion of the primary MenACWY-TT study, they had experienced IMD or had received any meningococcal vaccination outside of the primary vaccination phase, were immunosuppressed or had received blood products within 3 months of the Year 1 blood sampling, or had a bleeding disorder.

Study Vaccine

One 0.5 mL dose of MenACWY-TT contained 5 μg of each meningococcal serogroup A, C, W-135 and Y polysaccharide conjugated to TT (total TT content ~44 μg). The lyophilized vaccine was reconstituted with sterile saline for intramuscular injection into the left thigh. It was not possible to blind the study as the 2 vaccine groups received a different number of injections. Other vaccines that were part of the routinely recommended US vaccination schedule were given outside of a 30-day window before and after administration of study vaccines, with the exception of any vaccine containing tetanus toxoid, which could only be given after the final blood draw in the study. MenACWY-TT was administered alone at both 9 and 12 months of age.

Immunogenicity Assessment

We collected blood samples from all subjects 1 month after each vaccine dose (primary study) and again 12 months after the last vaccination (follow-up study). Samples were tested for hSBA and SBA using rabbit complement source (rSBA) for each serogroup based on the Centers for Disease Control and Prevention protocol,[22] using both positive and negative controls. The assay strains were strain 3125 for serogroup A (which expresses an L10 immunotype),[23] C11 for serogroup C, MP01240070 for serogroup W-135 and S-1975 for serogroup Y. Goldschneider et al demonstrated that hSBA-MenC titers ≥1:4 to correlate with seroprotection.[10,24] Although this cutoff has been extended to the other serogroups as well, we also assessed a more conservative threshold of hSBA titers ≥1:8. Similarly, an antibody titer ≥1:8 is considered indicative of seroprotection for rSBA-MenC, and we applied this cutoff as well as the more conservative threshold of 1:128 to all serogroups.[25–27]

Safety Assessment

We solicited specific local and general symptoms, which were recorded on diary cards for 8 days after vaccination, as were other (unsolicited) adverse events occurring through 31 days after each vaccination. We monitored for the occurrence of rash, new onset of chronic illness, adverse events resulting in an Emergency Room (ER) visit and any serious adverse events (SAEs) from the first vaccination up to 6 months after the last vaccination via scripted telephone call.

Statistical Analyses

The primary analysis of immunogenicity was performed on the according to protocol cohorts, which included subjects who had complied with all protocol-defined procedures and had data available for at least 1 immunogenicity endpoint. The according to protocol cohort for persistence Year 1 included all evaluable subjects who were eligible in the primary vaccine study, had received the primary vaccination with 1 or 2 doses of MenACWY-TT according to their treatment group and had available assay results for at least 1 tested antigen at Year 1. The primary analysis was conducted separately for the vaccination and Year 1 persistence phases. The primary analysis of safety was done on the total vaccinated

cohort, comprising all vaccinated children cohorts with safety data available.

The primary study prespecified that immunogenicity of 2 doses of MenACWY-TT was demonstrated if after dose 2, the lower limit of the 2-sided exact 95% confidence interval (CI) for the percentages of subjects with hSBA titers ≥1:8 was ≥90% for serogroup C and ≥80% for serogroups A, W-135 and Y.

One month after each vaccination and 1 year after completion of the vaccination schedule in both groups, the percentage of subjects with hSBA titers above the prespecified thresholds and the geometric mean antibody titers (GMT) were computed with 95% CIs. In an exploratory analysis, potential differences between the 1-dose and 2-dose schedules were highlighted if the asymptotic standardized 95% CI for the group difference in the percentage of subjects reaching specified immunological cutoffs did not contain the value "0," or if the standardized asymptotic 95% CI for the GMT ratio between groups did not contain the value "1."

Percentages of subjects with adverse events (solicited or unsolicited) were tabulated with exact 95% CIs for each group. Unsolicited symptoms were coded according to the Medical Dictionary for Regulatory Activities. The number of subjects who reported SAEs, other adverse events of interest and conditions prompting ER visits from the first vaccination until 6 months after the last vaccine dose was tabulated with exact 95% CI.

Enrolment of 600 subjects was initially planned, but due to challenges with enrolment of 9-month olds for a vaccination, the protocol was amended to terminate recruitment at approximately 376 subjects. With 144 subjects per group, the power to conclude on immunogenicity postdose 2 was ≥86.2%.

Statistical analyses were performed using SAS software version 9.2 (SAS Institute Inc., Cary, NC) and Proc StatXact 8.1 (Cytel Software, Cambridge, MA).

RESULTS

We enrolled 385 subjects, of whom 349 were vaccinated. The 36 subjects who were enrolled but not vaccinated were in the ACWY-1 group: the reason for the dropouts may have been due to the 3 month window between enrollment at 9 months and vaccination at 12 months. A total of 334 subjects (96% of those vaccinated) completed the active vaccination phase, and 248 (71% of those vaccinated) reenrolled at Year 1 (Fig. 1). The 2 treatment groups were similar with respect to demographic characteristics at enrolment and at Year 1 (Table 1). No subject withdrew from the study because of an adverse event (Fig. 1).

hSBA Responses

The lower limit of the exact 95% CI for the percentage of subjects with hSBA titers ≥1:8 in the ACWY-2 group postdose 2 were above the prespecified criteria, thus the study met the primary study objective (immunogenicity of 2 doses of MenACWY-TT) (Table 2).

The percentage of subjects in the ACWY-1 group with hSBA titers ≥1:8 was between 50.8 % and 94.6% for each serogroup (Table 2). In the ACWY-2 group, the percentage of subjects with hSBA titers ≥1:8 after a single dose of MenACWY-TT at 9 months of age ranged from 18.8% and 90.6% between vaccine serogroups, being lowest for serogroups W-135 and Y (18.8% and 37.4%, respectively). After the second MenACWY-TT dose, at least 88.4% of subjects had hSBA titers ≥1:8 for each serogroup; similarly, hSBA GMTs increased by 3.7-fold for serogroup A, 13.2-fold for serogroup C, 303.7-fold for serogroup W-135 and 67.9-fold for serogroup Y (Fig. 2).

Exploratory analyses showed that a single dose of MenACWY-TT at 12 months of age (ACWY-1 group) induced statistically significantly higher hSBA GMTs against all 4 serogroups, and a statistically significantly higher percentage of subjects who reached the 1:4 and 1:8 thresholds against serogroups A, W-135 and Y compared with a single dose of MenACWY-TT given at 9 months of age. Two doses of MenACWY-TT at 9 months and 12 months of age (ACWY-2 group) induced statistically significantly higher percentages of subjects who reached the 1:4 threshold against serogroups C, W-135 and Y, and a statistically significantly higher hSBA GMTs and percentages of subjects who reached the 1:8 threshold against all vaccine serogroups than did a single dose of MenACWY-TT administered at 12 months of age (exploratory analyses, see Table, Supplemental Digital Content 1, http://links.lww.com/INF/B467).

One year after the last vaccine dose, hSBA GMTs for serogroups A and C in the ACWY-1 group and all serogroups in the ACWY-2 group had decreased, with the largest decrease occurring for serogroup A. By contrast, GMTs for serogroups W-135 and Y increased in the ACWY-1 group (Fig. 2). The percentage of subjects retaining hSBA titers ≥1:8 at Year 1 were ≥80.0% for serogroups C, W-135 and Y in the ACWY-1 group and ≥91.2% in the ACWY-2 group. For serogroup A, the percentage with titers ≥1:8 was 20.6% in the ACWY-1 group and 25.9% in the ACWY-2 group (Table 2). In an exploratory analysis, the percentage of subjects with serogroups W-135 and Y hSBA titers ≥1:4 (data not shown) and ≥1:8, as well as serogroup W-135 hSBA GMTs, was statistically significantly higher in the ACWY-2 group than in the ACWY-1 group at Year 1 (see Table, Supplemental Digital Content 1, http://links.lww.com/INF/B467). No other significant differences were observed.

rSBA Responses

All except 1 subject achieved rSBA titers ≥1:8 after the first vaccine dose (see Table, Supplemental Digital Content 2, http://links.lww.com/INF/B468). After the second ACWY-TT dose in the ACWY-2 group, rSBA GMTs increased 6.5-fold for serogroup C, 2.1-fold for serogroup W-135 and 1.4-fold for serogroup Y. No increase in GMT for serogroup A was observed.

Exploratory analyses showed that the percentage of subjects with rSBA titers ≥1:128 was statistically significantly higher postdose 2 in the ACWY-2 group than in the ACWY-1 group only for serogroup C (data not shown). For all 4 serogroups, rSBA GMTs were statistically significantly higher after a single vaccine dose at 12 months of age than at 9 months of age. After 2 ACWY-TT doses, rSBA GMTs group were significantly higher than the ACWY-1 group for serogroup C, but were significantly lower than the ACWY-1 group for serogroups A and W-135. Exploratory analyses did not detect any difference between groups in percentages of subjects with rSBA titers ≥1:128 or in GMTs for any serogroup, although significantly more subjects in the ACWY-2 group had rSBA-MenW-135 and MenY titers ≥1:8 in an exploratory analysis performed at the 1-year time-point.

Reactogenicity and Safety

Both vaccination regimens were well-tolerated. Pain and irritability were the most common solicited symptoms reported after any dose. After any dose, symptoms categorized as grade 3 were reported by a maximum of 3.0% of subjects. Fever was reported by 3.3% of subjects vaccinated at 9 months of age and in 9.6% and 10.8% of subjects vaccinated at 12 months of age (ACWY-2 and ACWY-1 groups, respectively). No child reported fever >40°C. Only fever and loss of appetite were reported more often after the second dose than the first dose in the ACWY-2 group (Fig. 3).

From dose 1 until 6 months after the last vaccine dose (ie, 9 months of safety follow-up in the ACWY-1 group and 6 months of follow-up in the ACWY-2 group), 7 subjects (5 [3.1%] in the

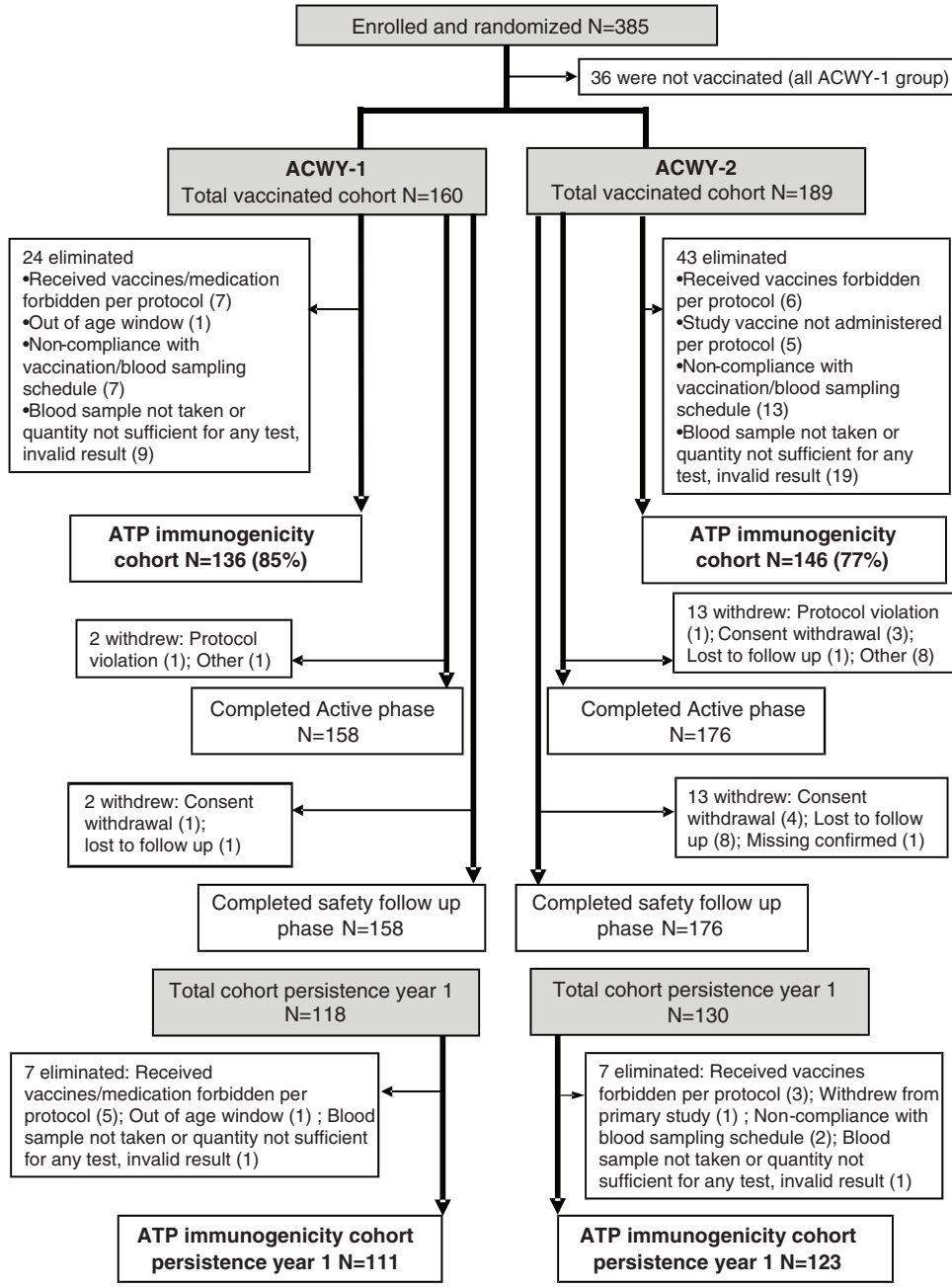

FIGURE 1. Subject flow through the study. Note that subjects who were withdrawn from the active phase were contacted for safety follow-up with a telephone call unless they had specifically withdrawn consent from the study. For example, the majority of subjects withdrawn from the active phase for "other" reasons had lost their health insurance associated with the study site. These subjects were still contacted for the safety follow-up. Therefore, the 15 subjects listed as withdrawn in each study phase are not necessarily the same individuals throughout the study. ACWY-1 indicates subjects vaccinated with 1 dose of MenACWY-TT at 12 months of age; ACWY-2, subjects vaccinated with 2 doses of MenACWY-TT administered at 9 and 12 months of age; ATP, according to protocol.

ACWY-1 groups and 2 [1.1%] in the ACWY-2 group) reported SAEs, none of which were considered by investigators as related to vaccination. New onset of chronic disease was reported in 19 subjects (11.9%) in the ACWY-1 group and by 38 subjects (20.1%) in the ACWY-2 group (Table 3). Asthma/bronchial hyperreactivity, eczema/atopic dermatitis, allergic rhinitis, food/milk allergy and drug eruptions/drug hypersensitivity were the most common new onset chronic diseases reported, and accounted for 86% of diagnoses. Rash was reported by 10.0% of subjects in the ACWY-1 group and 32.3% in the ACWY-2 group. The most frequently reported rashes were diaper rash (1.9% in the ACWY-1 group and 11.1% in the ACWY-2 group) and rash (unspecified) (3.1% in the ACWY-1 group and 10.1% in the ACWY-2 group). Adverse events leading to an ER visit were reported by 10.6% and

TABLE 1. Demographic Characteristics of Subjects in the According to Protocol Immunogenicity and Persistence at Year 1 Cohorts

Characteristic		Vaccination Phase*		Year 1 Follow-up Phase	
		ACWY-1	ACWY-2	ACWY-1	ACWY-2
	N	136	146	111	123
Age (mo)	Mean (standard deviation)	9.0 (0.0)	9.0 (0.0)	24.5 (0.99)	24.6 (1.01)
	Range	9	9	23–27	23–28
Sex, n (%)	Male	68 (50.0)	72 (49.3)	58 (52.3)	62 (50.4)
	Female	68 (50.0)	74 (50.7)	53 (47.7)	61 (49.6)
Race, n (%)	Caucasian/European heritage	81 (59.6)	79 (54.1)	70 (63.1)	63 (51.2)
	African/African American	9 (6.6)	6 (4.1)	7 (6.3)	6 (4.9)
	American Indian/Alaskan Native	12 (8.8)	16 (11.0)	8 (7.2)	16 (13.0)
	Central/South Asian	5 (3.7)	4 (2.7)	3 (2.7)	4 (3.3)
	East Asian	3 (2.2)	1 (0.7)	2 (1.8)	0 (0.0)
	South East Asian	8 (5.9)	8 (5.5)	6 (5.4)	7 (5.7)
	Native Hawaiian/Pacific Islander	3 (2.2)	2 (1.4)	1 (0.9)	2 (1.6)
	Arabic/North African	1 (0.7)	0 (0.0)	1 (0.9)	0 (0.0)
	Other	14 (10.3)	30 (20.5)	13 (11.7)	25 (20.3)

Other includes 1 subject of Hispanic descent and 43 subjects of mixed race/multiracial descent.
*Data refer to status at enrolment at visit 1 for all subjects.

TABLE 2. Percentage of Subjects With hSBA Titers ≥1:4 and ≥1:8 (According to Protocol Immunogenicity Cohorts for Primary Vaccination and Persistence at Year 1)

Serogroup	Group	Timing	N	n	% [95% CI]	n	% [95% CI]
hSBA					≥1:4		≥1:8
A	ACWY-1	P1	132	115	87.1 [80.2; 92.3]	105	79.5 [71.7; 86.1]
		Year 1	102	23	22.5 [14.9; 31.9]	21	20.6 [13.2; 29.7]
	ACWY-2	P1	128	90	70.3 [61.6; 78.1]	81	63.3 [54.3; 71.6]
		P2	138	122	88.4 [81.9; 93.2]	122	88.4 [**81.9**; 93.2]
		Year 1	108	29	26.9 [18.8; 36.2]	28	25.9 [18.0; 35.2]
C	ACWY-1	P1	130	123	94.6 [89.2; 97.8]	123	94.6 [89.2; 97.8]
		Year 1	104	91	87.5 [79.6; 93.2]	91	87.5 [79.6; 93.2]
	ACWY-2	P1	127	115	90.6 [84.1; 95.0]	115	90.6 [84.1; 95.0]
		P2	137	137	100 [97.3; 100]	137	100 [**97.3**; 100]
		Year 1	113	103	91.2 [84.3; 95.7]	103	91.2 [84.3; 95.7]
W-135	ACWY-1	P1	118	63	53.4 [44.0; 62.6]	60	50.8 [41.5; 60.2]
		Year 1	104	93	89.4 [81.9; 94.6]	93	89.4 [81.9; 94.6]
	ACWY-2	P1	117	24	20.5 [13.6; 29.0]	22	18.8 [12.2; 27.1]
		P2	143	142	99.3 [96.2; 100]	142	99.3 [**96.2**; 100]
		Year 1	112	111	99.1 [95.1; 100]	111	99.1 [95.1; 100]
Y	ACWY-1	P1	132	79	59.8 [51.0; 68.3]	74	56.1 [47.2; 64.7]
		Year 1	110	89	80.9 [72.3; 87.8]	88	80.0 [71.3; 87.0]
	ACWY-2	P1	131	52	39.7 [31.3; 48.6]	49	37.4 [29.1; 46.3]
		P2	146	145	99.3 [96.2; 100]	145	99.3 [**96.2**; 100]
		Year 1	120	111	92.5 [86.2; 96.5]	111	92.5 [86.2; 96.5]

Bold values indicate that the lower limit of the exact 95% CI is above the predefined limit of 90% for serogroup C and 80% for serogroups A, W-135 and Y.
N indicates number of subjects with available results; n, number of subjects with titer above the specified cutoff; P1, 1 month postvaccination (dose 1 in the ACWY-2 group); P2, 1 month postdose 2 in the ACWY-2 group; Year 1, 1 year after the last vaccine dose.

16.9% of subjects in the ACWY-1 and ACWY-2 group, respectively. Seventeen subjects (10.6%) in the ACWY-1 group had at least 1 ER visit during the study. The most common diagnoses associated with the ER visits were infections (upper respiratory tract infection, viral infection, croup) as well as dehydration, pyrexia, febrile convulsion and injuries. An ER visit was reported by 32 subjects (16.9%) in the ACWY-2 group. The most common diagnoses were diarrhea, vomiting and pyrexia, as well as infections (gastroenteritis, pneumonia, upper respiratory tract infection and viral infection) and injuries. No deaths occurred during the study.

In order to identify whether the longer follow-up period contributed to the greater number of adverse event reports after vaccination in the ACWY-2 group, we conducted a post hoc analysis considering only adverse events reported after the second dose of ACWY-TT to study end (ie, 6 months extended safety follow-up) on subjects who received 2 vaccine doses (N = 172). The following adverse events were reported during the 6-month period from 12 to 18 months of age: any SAEs (0.6%), new onset of chronic disease (18.0%), rash (23.3%) and ER visits (11.0%) (Table 3).

DISCUSSION

MenACWY-TT (Nimenrix) is licensed in Europe as a single dose schedule for individuals as of 12 months of age. This study evaluated a 2-dose schedule at 9 and 12 months of age compared with a single dose at 12 months of age to see if vaccination could be

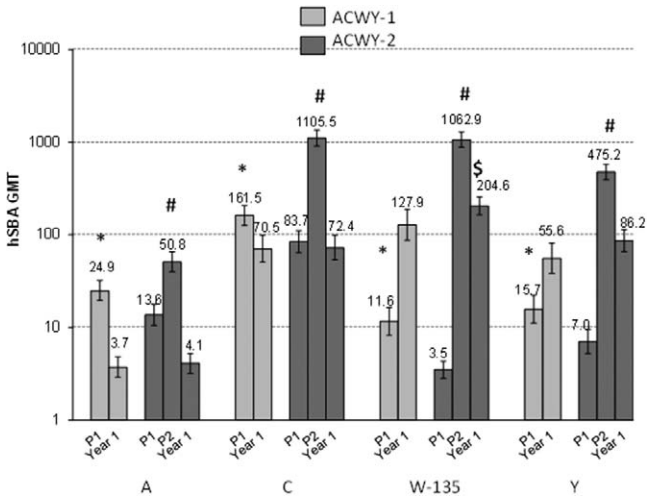

FIGURE 2. hSBA GMTs after vaccination with 1 or 2 doses of MenACWY-TT (according to protocol immunogenicity cohorts for primary vaccination and persistence at Year 1). Y axis indicates log-10 scale hSBA GMT; vertical lines, 95% CIs; P1, 1 month postvaccination (dose 1 in the ACWY-2 group); P2, 1 month postdose 2 in the ACWY-2 group; Year 1, 1 year after vaccination; *, statistically significantly difference between 1 dose administered at 9 or 12 months of age (ACWY-1 group versus the ACWY-2 group after the first dose, exploratory analysis); #, statistically significant difference between 2 doses (ACWY-2 group) and 1 dose at 12 months of age (ACWY-1 group, exploratory analysis); $, statistically significant difference between the ACWY-2 group and the ACWY-1 at Year 1 (exploratory analysis).

initiated within the first year of life. A single dose of MenACWY-TT at 9 months of age was immunogenic in the majority of subjects for serogroups A and C, with lower responses observed for serogroups W-135 and Y. By contrast, hSBA titers ≥1:8 were observed in the majority of subjects for all serogroups when the single dose of MenACWY-TT was administered at 12 months of age. The highest hSBA GMTs for all serogroups were observed when MenACWY-TT was administered according to a 2-dose schedule, which provided the additional benefit of initiating vaccination at less than 1 year of age.

Comparisons between studies in different populations that use different assays should be made cautiously. Bearing this caveat in mind, our findings are consistent with studies of MenACWY-DT and MenACWY-CRM$_{197}$ where responses to serogroups W-135 and Y were low after a single dose administered before 12 months of age.[10,24,28] When a second dose was administered several months after the first one, the percentages of subjects with hSBA ≥1:8 increased to at least 88.4% for all serogroups, and GMTs tended to exceed those observed in subjects who received their first vaccine dose in the second year of life. For MenACWY-DT, which is licensed for use in a 2-dose schedule from 9 months of age, the percentage with hSBA ≥1:8 after 1 dose at 9 months of age was between 66.7% and 85.4% for serogroups A and C and between 20.6% and 26.7% for serogroups W-135 and Y.[10] The percentages of subjects increased to at least 88.9% for all serogroups after a second dose administered at 12 months of age.[10] A lower percentage of subjects with hSBA ≥1:8 for serogroup Y (57.1%) versus serogroup C (94.3%) was also observed in toddlers 12 to 15 months of age administered a single dose of HibMenCY-TT vaccine.[9,29]

Persistence of functional antibodies appears critical for maintenance of protection against IMD, with reductions in the percentage of children with rSBA titers above the accepted correlates of protection associated with waning effectiveness of monovalent meningococcal serogroup C conjugate vaccines.[7,30] After 1 year, persistence of hSBA titers ≥1:8 was observed for serogroups C, W-135 and Y in ≥80% of subjects after 1 or 2 MenACWY-TT doses. The reduction in hSBA titers for serogroup A over time was much greater than observed for the other serogroups, consistent with 21-month persistence in adolescents after vaccination with MenACWY-DT or MenACWY-CRM$_{197}$.[30] The absolute percentage of children who maintained hSBA titers ≥1:8 for serogroups C, W-135 and Y 1 year after vaccination was higher after 2 doses than after 1 dose, although the trends were only significant in the exploratory analysis for serogroups W-135 and Y. This trend will

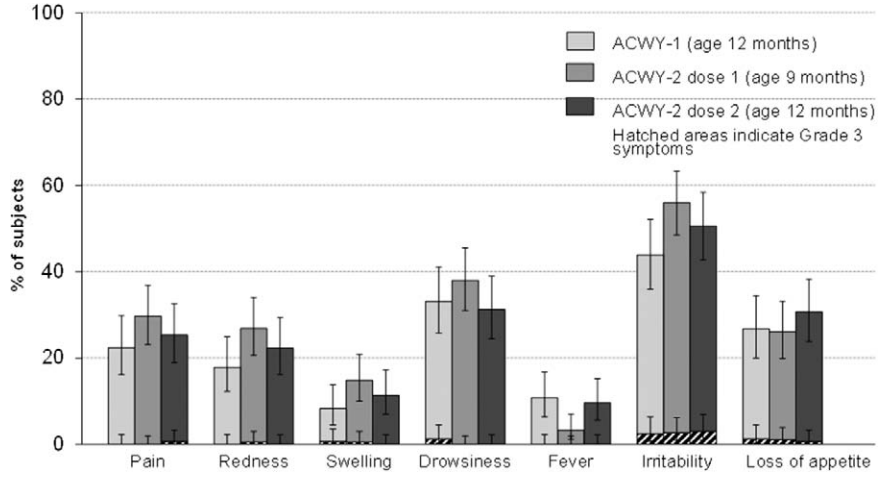

FIGURE 3. Percentage of subjects with solicited local and general symptoms reported during 8-day follow-up after each dose (total vaccinated cohort). Grade 3 Pain: Cried when limb was moved/spontaneously painful; Grade 3 Redness and swelling: >30 mm; Grade 3 Irritability: Crying that could not be comforted/prevented normal activity; Grade 3 Drowsiness: Drowsiness that prevented normal activity; Grade 3 Fever: temperature >40.0°C by any route; Grade 3 Loss of appetite: Did not eat at all. Vertical lines indicate exact 95% CIs.

TABLE 3. Percentage of Subjects Reporting New Onset of Chronic Disease Classified by Medical Dictionary for Regulatory Activities Preferred Term From Dose 1 up to Study End (Total Vaccinated Cohort)

	Total Cohort Analysis				Post Hoc Analysis: 6-Month Follow-Up From 12 Months Of Age	
	ACWY-1 N = 160		ACWY-2 N = 189		ACWY-2 N = 172	
Preferred Term	n	% [95% CI]	n	% [95% CI]	n	% [95% CI]
At least 1 symptom	19	11.9 [7.3; 17.9]	38	20.1 [14.6; 26.5]	31	18.0 [12.6; 24.6]
Allergy to arthropod bite	0	0.0 [0.0; 2.3]	1	0.5 [0.0; 2.9]	1	0.6 [0.0; 3.2]
Drug hypersensitivity	4	2.5 [0.7; 6.3]	0	0.0 [0.0; 1.9]	0	0.0 [0.0; 2.1]
Food allergy	1	0.6 [0.0; 3.4]	3	1.6 [0.3; 4.6]	2	1.2 [0.1; 4.1]
Milk allergy	1	0.6 [0.0; 3.4]	1	0.5 [0.0; 2.9]	1	0.6 [0.0; 3.2]
Multiple allergies	0	0.0 [0.0; 2.3]	1	0.5 [0.0; 2.9]	1	0.6 [0.0; 3.2]
Otitis media chronic	0	0.0 [0.0; 2.3]	1	0.5 [0.0; 2.9]	1	0.6 [0.0; 3.2]
Lactose intolerance	0	0.0 [0.0; 2.3]	1	0.5 [0.0; 2.9]	0	0.0 [0.0; 2.1]
Muscular weakness	1	0.6 [0.0; 3.4]	0	0.0 [0.0; 1.9]	0	0.0 [0.0; 2.1]
Asthma	4	2.5 [0.7; 6.3]	10	5.3 [2.6; 9.5]	9	5.2 [2.4; 9.7]
Bronchial hyperreactivity	1	0.6 [0.0; 3.4]	4	2.1 [0.6; 5.3]	4	2.3 [0.6; 5.8]
Rhinitis allergic	3	1.9 [0.4; 5.4]	3	1.6 [0.3; 4.6]	2	1.2 [0.1; 4.1]
Dermatitis allergic	0	0.0 [0.0; 2.3]	3	1.6 [0.3; 4.6]	2	1.2 [0.1; 4.1]
Dermatitis atopic	1	0.6 [0.0; 3.4]	4	2.1 [0.6; 5.3]	3	1.7 [0.4; 5.0]
Drug eruption	2	1.3 [0.2; 4.4]	4	2.1 [0.6; 5.3]	3	1.7 [0.4; 5.0]
Eczema	3	1.9 [0.4; 5.4]	6	3.2 [1.2; 6.8]	4	2.3 [0.6; 5.8]
Rash	0	0.0 [0.0; 2.3]	1	0.5 [0.0; 2.9]	1	0.6 [0.0; 3.2]

At least 1 symptom indicates at least 1 symptom experienced (regardless of the Medical Dictionary for Regulatory Activities Preferred Term); N, number of subjects with at least 1 administered dose; n/%, number/percentage of subjects reporting the symptom at least once.

be explored further in the long-term antibody persistence study planned to continue for another 4 years.

An unexpected finding of our study was the increase in antibodies at the 1-year persistence time point for serogroups W-135 and Y in the ACWY-1 group. The reasons for this increase are not clear, nor are the reasons why this phenomenon was observed only in the 1-dose and not the 2-dose group. One possibility is that the peak immune response to serogroups W-135 and Y may occur later than 1 month after vaccination. Serum sampling time points between 1-month and 1-year postvaccination were not obtained, so this hypothesis could not be tested in the present study but is a potential investigation for future studies.

We measured antibody responses for each serogroup using hSBA and rSBA. Postmarketing surveillance data and paired comparisons of sera using rSBA and hSBA suggest that rSBA titers of 1:8 correlate well with efficacy and with hSBA titers of 1:4 for serogroup C.[25–27] A recent study in adolescents showed poor correlation between rSBA and hSBA for serogroups A, W-135 and Y,[31] although correlations with protective efficacy were not made. Using hSBA, we observed robust immune responses for all 4 serogroups after a single ACWY-TT dose given either at 9 months or 12 months of age, with apparent benefits in terms of percentages reaching threshold rates and GMTs when a single dose was administered later in life. Although hSBA appeared to differentiate between 1 and 2 doses in terms of threshold levels and GMTs for all serogroups, only the response to serogroup C was higher after 2 doses using rSBA. Although we observed a degree of concordance between conclusions using rSBA and hSBA, further investigation into protective thresholds for serogroups A, W-135 and Y are needed.

Two doses of MenACWY-TT had a clinically acceptable safety profile. The percentage of subjects reporting fever and loss of appetite increased after the second dose, although the 95% CIs overlapped. The reactogenicity profile of MenACWY-TT in this study was similar to previous reports of MenACWY-TT administered to toddlers.[12,14] We observed a higher percentage of new onset of chronic disease, notably asthma, ER visits and rash in subjects in the ACWY-2 group. A post hoc analysis using the same length of follow-up time for both groups showed that some, but not all, of the differences between groups were accounted for by the different follow-up periods.

The annual incidence of asthma in 0–4 years is estimated at 23.4/1000.[32] Using this estimate and considering a 6-month follow-up period, we would have expected to observe 3 new cases in each group. In the post hoc analysis, we observed 4 cases in the ACWY-1 group and 9 in the ACWY-2 group. However, several important considerations should be kept in mind. This analysis did not take into account differences in vaccine exposure at different ages between the 2 groups. Furthermore, this study did not account for possible risk factors such as genetic background and allergen exposure that may have differed between the study groups. Finally, this study was not designed or powered to evaluate for differences in individual adverse events. Ongoing monitoring of asthma and atopy after ACWY-TT vaccination will be required.

The percentage of subjects reporting "diaper rash" and "rash" (unspecified) was also higher in the ACWY-2 group. For all other new onset of chronic disease and ER visits, no disease patterns or clustering were observed and the reports encompassed a wide range of organ systems and symptoms.

Limitations of this study include its open design, which may have led to bias in reactogenicity reporting. We were also unable to compare immune responses with a control group that received an active comparator because no meningococcal vaccine was licensed for use in US infants at the time of the study. The numerous exploratory statistical comparisons were performed without adjustment for multiplicity. Although these comparisons allow for the identification of trends, interpretation of individual statistical results should be made with caution. The safety follow-up period was different between treatment groups, limiting the interpretation of possible differences in adverse event reporting rates. Finally, because we chose to limit the number of blood draws due to the young age of the subjects, we did not obtain prevaccination blood samples and were not able to measure the increase in antibodies postvaccination relative to prevaccination titers. It is also possible that the improved responses at 12 months of age compared with 9

months of age were due to a further waning of maternal antibody, but this hypothesis could not be tested due to the absence of prevaccination samples.

In summary, this study suggests that MenACWY-TT could be administered to infants as early as 9 months of age to induce protection to all 4 meningococcal serogroups in the first year of life, although a second dose of MenACWY-TT should be administered to these infants to optimize protection against serogroups W-135 and Y. Further investigation of the kinetics of the immune responses in this age group is warranted.

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