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The immunogenicity and safety of an investigational meningococcal serogroups A, C, W-135 and Y tetanus toxoid conjugate vaccine (ACWY-TT) compared with a licensed meningococcal tetravalent polysaccharide vaccine

A randomized, controlled non-inferiority study

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Abbreviations: ACWY-TT, investigational tetravalent serogroups A, C, W-135 and Y conjugate vaccine with all serogroups conjugated to the tetanus toxoid carrier protein; (S)AE, (serious) adverse event; ATP cohort, according-to-protocol cohort; CI, confidence interval; GMC, geometric mean concentration; GMT, geometric mean antibody titer; GSK, GlaxoSmithKline Biologicals; IMD, invasive meningococcal disease; MenPS, tetravalent meningococcal polysaccharide vaccine; rSBA, meningococcal bactericidal titers using rabbit complement as exogenous complement source; VR, vaccine response

Immunogenicity and safety of ACWY-TT compared with licensed ACWY polysaccharide vaccine (MenPS) in healthy adults, and lot-to-lot consistency of three ACWY-TT lots were evaluated in a phase III, open, controlled study. Adults aged 18–55 years were randomized to receive ACWY-TT (one of three lots) or MenPS. Serum bactericidal antibodies (rSBA) were measured pre- and 1 month post-vaccination. Adverse events (AEs) were assessed 4 days (solicited symptoms) and 31 d (unsolicited symptoms) post-vaccination. Serious AEs were reported up to 6 months after vaccination. The number of vaccinated subjects was 1,247 (ACWY-TT, n = 935; MenPS, n = 312). ACWY-TT lot-to-lot consistency and non-inferiority of ACWY-TT as compared with MenPS groups were demonstrated according to pre-specified criteria. The percentages of subjects with a vaccine response (VR = rSBA titer ≥ 1:32 in initially seronegative; ≥ 4-fold increase in initially seropositive) to ACWY-TT vs. MenPS were 80.1%/69.8% (serogroup A), 91.5%/ 92.0% (C), 90.2%/85.5% (W-135), 87.0%/78.8% (Y). Exploratory analyses showed that for serogroups A, W-135 and Y, VR rates and GMTs were significantly higher for ACWY-TT compared with MenPS. For each serogroup, ≥ 98.0% of subjects had rSBA titers ≥ 1:128. Grade 3 solicited AEs were reported in ≤ 1.6% of subjects in any group. The immunogenicity of ACWY-TT vaccine was non-inferior to MenPS for all four serogroups in adults, with significantly higher VR rates to serogroups A, W-135 and Y and an acceptable safety profile. Consistency of 3 ACWY-TT production lots was demonstrated. These data suggest that, if licensed, ACWY-TT conjugate vaccine may be used for protection against invasive meningococcal disease in healthy adults.

This study is registered at clinicaltrials.gov NCT00453986

Introduction

Invasive meningococcal disease (IMD) remains a global public health concern, with 0.5 million cases estimated to occur annually, of which at least 10% result in death.[1] *Neisseria meningitidis* serogroups A, B, C, W-135, and Y cause the majority of IMD globally, although the distribution of each serogroup varies. Epidemic IMD is most commonly due to serogroup A, whereas serogroups B, C Y and W-135 are more frequently implicated in endemic disease and sporadic outbreaks.[2]

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The epidemiology of IMD in much of South East Asia and the Middle East is incompletely described. In the Philippines, the distribution of serogroups causing IMD is not known, but a serogroup A outbreak was reported in Baguio City, Mt. Province and Ifugao between 2004 and 2005, with 33% mortality.[3] Serogroups A and W-135 currently predominate in the Middle East, and serogroup W-135 outbreaks have been reported in Hajj pilgrims and their contacts.[4-6] Serogroups B and C predominate in most of Europe, whereas in the US serogroup Y is also an important cause of disease.[2,7] In Africa, serogroup A is responsible for most major epidemics.[1,2] However, serogroups W-135 and serogroup X are important emerging causes of outbreaks within the African meningitis belt.[8-11]

IMD affects all age groups, and while the incidence of IMD is highest in infants, the burden of disease due to IMD in adults is substantial. Between 1998 and 2007 almost 43% of all IMD cases in the US were reported in adults 25 y of age and older.[7] Groups particularly at risk for IMD are travelers, notably Hajj pilgrims.[12]

Meningococcal polysaccharide vaccines have been available for use in adults for many years and are most frequently used for travelers to regions of high IMD incidence. Meningococcal polysaccharide vaccines are efficacious in preventing IMD in adults but do not elicit long-lasting immunological memory.[13] Antibody persistence only lasts for 3–5 y, but immune hyporesponsiveness may occur when polysaccharide vaccines are given more than once—this is particularly the case for serogroup C.[14] The induction of hyporesponsiveness is a key limitation for the use of polysaccharide vaccines for individuals who need to retain longer term immunity. Conjugation of the polysaccharides to carrier proteins overcomes many of the limitations associated with polysaccharide vaccines by inducing a T-cell dependent response, with resulting immune memory and boostability. Importantly, immune tolerance has not been demonstrated after repeated MenC conjugate vaccination, which has been in place in the UK for the past decade.[15,16]

The investigational tetravalent polysaccharide conjugate vaccine against *N. meningitidis* serogroups A, C, W-135 and Y, using tetanus toxoid as the carrier protein [ACWY-TT, GlaxoSmithKline Biologicals (GSK) Belgium] is immunogenic in toddlers, children and adolescents.[17-23] This partially double-blinded, controlled, non-inferiority study assessed the immunogenicity and safety of ACWY-TT in healthy adults between 18 and 55 y of age. Manufacturing consistency using three different manufacturing lots was established and pooled serological results were compared against the tetravalent polysaccharide vaccine control (MenPS: *Mencevax*™ ACWY, GSK).

Results

Study subjects. In total, 1,247 subjects were vaccinated with ACWY-TT (one of three lots) or MenPS, of which 1228 subjects completed the active phase. No subject withdrew from the study due to an adverse event (AE). There were 1,179 subjects included in the according to protocol (ATP) immunogenicity cohort (**Fig. 1**). More males were enrolled than females (**Table 1**). The demographic profiles of subjects in each group were comparable with respect to mean age, sex and race (**Table 1**).

Primary study objectives. The presence of serogroup-specific serum bactericidal activity (SBA; which is measured with a functional assay which measures the capability of test serum to kill a meningococcal strain when exogenous complement is added) above threshold levels derived from effectiveness studies is widely accepted as a surrogate for protection against IMD.[24,25] Lot-to-lot consistency of three ACWY-TT lots with respect to SBA geometric mean titers (using rabbit complement as the exogenous complement source: rSBA GMTs) was demonstrated. For all 12 pairwise comparisons, the 2-sided 95% confidence interval (CI) on the GMT ratio between lots was within the pre-specified interval for non-inferiority of [0.5; 2.0] for each pair of lots and for each serogroup (**Table 2**), justifying pooling of data in the ACWY-TT groups for evaluation of the other objectives.

Non-inferiority of ACWY-TT compared with MenPS in terms of the percentage of subjects with a vaccine response [VR, defined as an rSBA titer $\geq$ 1:32 in initially seronegative subjects (pre-vaccination titer $<$ 1:8), or a $\geq$ 4-fold increase over the pre-vaccination titer for initially seropositive subjects (pre-vaccination titer $\geq$ 1:8)] after vaccination was demonstrated: the lower limit of the 95% CI for the difference between groups was above the pre-specified non-inferiority limit of -10% for all four serogroups (**Table 3**).

Immunogenicity of ACWY-TT (pooled groups). Prior to vaccination, the percentage of subjects in the ACWY-TT group with rSBA titers $\geq$ 1:128 was 73.7% for serogroup A, 48.8% for C, 59.8% for W-135 and 79.0% for Y. The percentage of subjects in the MenPS group with rSBA titers $\geq$ 1:128 was 78.7% for serogroup A, 52.6% for C, 54.8% for W-135 and 77.8% for Y. Pre-vaccination rSBA titers for each serogroup were similar in the ACWY-TT and MenPS group (data not shown).

One month after vaccination, the percentage of subjects in both groups with rSBA titers $\geq$ 1:8 and rSBA titers $\geq$ 1:128 was $\geq$ 99.3% and $\geq$ 98.0%, respectively (data not shown). For each serogroup, $\geq$ 80.1% of ACWY-TT vaccinees had a VR (**Table 3**). An exploratory analysis showed that the VR was statistically significantly higher in the ACWY-TT group as compared with the MenPS group for serogroups A, W-135 and Y.

After vaccination with ACWY-TT rSBA GMTs increased by at least 20-fold for serogroups A, W-135 and Y and 109-fold for serogroup C (**Fig. 2**). The fold increase in GMTs observed after MenPS was at least 10-fold for serogroups A, W-135 and Y and 81-fold for serogroup C.

Exploratory analyses showed statistically significantly higher rSBA GMTs in the ACWY-TT group compared with the MenPS group for serogroups A, W-135 and Y.

The percentage of subjects with anti-tetanus antibody concentrations $\geq$ 0.1 IU/mL increased from 51.5% to 79.4% in the ACWY-TT group (ACWY contains 44 μg TT), but remained unchanged in the MenPS group (52.2% to 53.2%). Similarly, the anti-tetanus antibody geometric mean concentration (GMC) increased by 14-fold in the ACWY-TT group but did not increase in the MenPS group after vaccination (data not shown).

Immunogenicity in the 18–25 and 26–55 y age strata. An exploratory analysis of the primary objective by age stratum

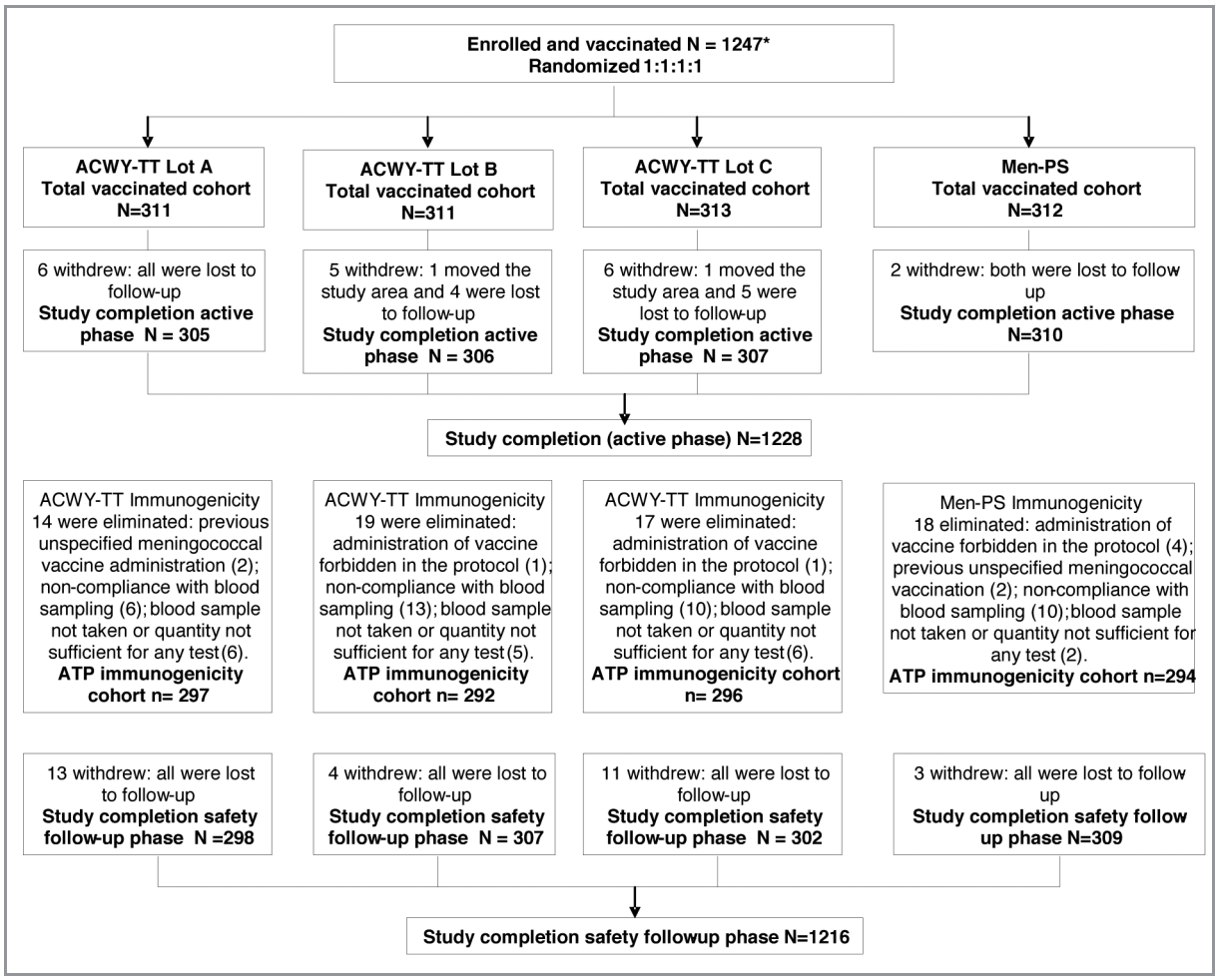

Figure 1. Subject flow through the study. *An additional 105 subjects were enrolled in the cohort evaluated for the co-administration of influenza vaccine (the analysis of co-administration with seasonal influenza vaccine will be presented in a separate publication).

(18–25 y and 26–55 y) showed that the lower limit of the 95% CI for the difference between ACWY-TT and MenPS groups in percentages of subjects with a VR in each age strata was above -10% for all four serogroups in both age strata (**Table 4**) .

Safety. Pain and headache were the most frequently reported local and general solicited symptoms within 4 d of vaccination, in both groups (**Table 5**). The percentage of subjects who reported pain, redness and swelling at the injection site was higher in

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

		ACWY lot A N = 311	ACWY lot B N = 311	ACWY lot C N = 313	ACWY-TT Pooled lots N = 935	MenPS N = 312
Characteristics	**Categories**	**Value/n (%)**	**Value/n (%)**	**Value/n (%)**	**Value/n (%)**	**Value/n (%)**
Age (years)	Mean	35.2	35.1	35.7	35.3	34.9
	SD	10.48	10.50	10.75	10.57	10.73
	Range	18–55	18–55	18–55	18–55	18–55
Gender	Female	135 (43.4)	139 (44.7)	133 (42.5)	407 (43.5)	151 (48.4)
	Male	176 (56.6)	172 (55.3)	180 (57.5)	528 (56.5)	161 (51.6)
Race	Southeast Asian	223 (71.7)	223 (71.7)	224 (71.6)	670 (71.7)	224 (71.8)
	Arabic/North African	88 (28.3)	88 (28.3)	87 (27.8)	263 (28.1)	88 (28.2)
	Other*	0 (0.0)	0 (0.0)	2 (0.6)	2 (0.2)	0 (0.0)

N, total number of subjects; Value, value of the considered parameter; n/%, number/percentage of subjects in a given category; SD, standard deviation; Other, Native Hawaiian/Pacific Islander or Caucasian/European heritage.

Table 2. Ratios of rSBA GMTs between ACWY-TT Lot groups one month after vaccination (ATP immunogenicity cohort)

							Adjusted GMT ratio*			
									95% CI	
Serogroup	ACWT-TT Lot	N	Adjusted GMT	ACWT-TT Lot	N	Adjusted GMT	Ratio order	Value	LL	UL
A	A	253	3851.3	B	244	3607.2	A / B	1.07	**0.88**	**1.29**
	A	253	3851.3	C	246	3582.6	A / C	1.07	**0.89**	**1.30**
	B	244	3607.2	C	246	3582.6	B / C	1.01	**0.83**	**1.22**
C	A	283	9945.1	B	275	8520.9	A / B	1.17	**0.91**	**1.50**
	A	283	9945.1	C	291	9393.5	A / C	1.06	**0.83**	**1.36**
	B	275	8520.9	C	291	9393.5	B / C	0.91	**0.71**	**1.16**
W-135	A	287	5380.8	B	286	5020.8	A / B	1.07	**0.87**	**1.33**
	A	287	5380.8	C	287	5534.7	A / C	0.97	**0.79**	**1.20**
	B	286	5020.8	C	287	5534.7	B / C	0.91	**0.73**	**1.12**
Y	A	294	7863.7	B	284	7204.0	A / B	1.09	**0.90**	**1.33**
	A	294	7863.7	C	284	7747.9	A / C	1.01	**0.83**	**1.24**
	B	284	7204.0	C	284	7747.9	B / C	0.93	**0.76**	**1.13**

Adjusted GMT, geometric mean antibody titer adjusted for age strata, baseline titer and whether or not influenza vaccine was co-administered; N, number of subjects with both pre- and post-vaccination results available. 95% CI, 95% confidence interval for the adjusted GMT ratio; ANCOVA model, adjustment for age strata, baseline titer and whether or not subjects were included in the analysis of co-administration with seasonal influenza vaccine—pooled variance with more than two groups; LL, lower limit; UL, upper limit. *Lot-to-lot consistency was demonstrated if for each pair of lots and for each serogroup, the two-sided 95% CI on the GMT ratio between lots was within the interval of [0.5; 2.0].

ACWY-TT recipients than in MenPS recipients (for swelling, the 95% CIs did not overlap), while the occurrence of general symptoms was similar in both groups. Notably, grade 3 local and general symptoms were infrequently reported in both groups.

The percentage of subjects reporting unsolicited symptoms during the 31-d follow-up period was 14.4% (95% CI 12.2%; 16.9%) in the ACWY-TT group and 15.1% (95% CI 11.3%; 19.5%) in the MenPS group. The percentage of subjects reporting a grade 3 unsolicited symptom was 1.4% (95% CI 0.7%; 2.4%) in the ACWY-TT group and 1.0% (95% CI 0.2%; 2.8%) in the MenPS group. Each individual grade 3 symptom was reported by only one subject, with the exception of toothache, which was reported by two subjects in the ACWY-TT group (data not shown).

Eight subjects (ACWY-TT group n = 7, 0.7%; MenPS group n = 1, 0.3%) reported 11 serious AEs (SAEs) after vaccination (10 in the ACWY-TT group and 1 in the MenPS group). Two of these events (reported by one subject) were considered to be related to vaccination: a subject in the ACWY-TT group reported abdominal pain and gastritis beginning 5 d after vaccination and required hospitalization. All SAEs resolved without sequelae. No deaths occurred during the study.

Table 3. Comparison between groups in rSBA vaccine response rate one month after vaccination (ATP immunogenicity cohort)

				% Vaccine response	Difference in vaccine response rate*
Serogroup	Group	N	n	% (95% CI)	% (95%CI)
A	ACWY-TT	743	595	80.1 (77.0; 82.9)	10.24 (**4.11**; 16.78)
	MenPS	252	176	69.8 (63.8; 75.4)	
C	ACWY-TT	849	777	91.5 (89.4; 93.3)	-0.49 (**-3.85**; 3.57)
	MenPS	288	265	92.0 (88.3; 94.9)	
W-135	ACWY-TT	860	776	90.2 (88.1; 92.1)	4.72 (**0.49**; 9.65)
	MenPS	283	242	85.5 (80.9; 89.4)	
Y	ACWY-TT	862	750	87.0 (84.6; 89.2)	8.19 (**3.24**; 13.69)
	MenPS	288	227	78.8 (73.6; 83.4)	

N, number of subjects with pre and post vaccination results; n/%, number/percentage of subjects with a vaccine response (defined as an rSBA titer ≥ 1:32 in subjects with pre-vaccination titer < 1:8, or a ≥ 4-fold increase in titer for subjects with pre-vaccination titer ≥ 1:8). 95% CI = 95% confidence interval. *ACWY-TT minus MenPS. Bold: the lower limit of the standardized asymptotic 95% CI is above the pre-specified non-inferiority limit of -10% for all four serogroups.

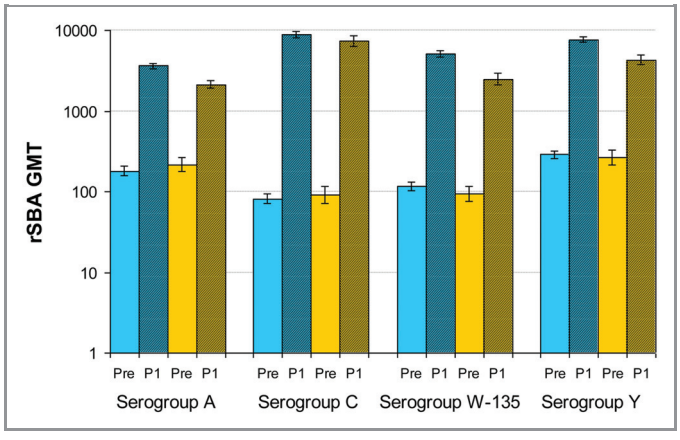

Figure 2. rSBA GMTs (with 95% CIs) before and one month after vaccination (ATP immunogenicity cohort). Pre, pre-vaccination (plain columns); P1, 1 mo post vaccination (hatched columns). Blue bars, ACWY-TT group; orange bars, MenPS group.

Table 4. Comparison between groups in rSBA vaccine response rate one month after vaccination stratified by age (exploratory analysis, ATP immunogenicity cohort)

		18–25 y subgroup				26–55 y subgroup			
			% Vaccine response		Difference in vaccine response rate*		% Vaccine response		Difference in vaccine response rate*
Serogroup	Group	N	n	% (95% CI)	% (95% CI)	N	n	% (95% CI)	% (95% CI)
A	ACWY-TT	177	147	83.1 (76.7; 88.3)	10.47 (-0.96; 23.70)	566	448	79.2 (75.6; 82.4)	10.20 (3.10; 17.82)
	MenPS	62	45	72.6 (59.8; 83.1)		190	131	68.9 (61.8; 75.4)	
C	ACWY-TT	204	193	94.6 (90.6; 97.3)	-5.39 (-9.40; -0.30)	645	584	90.5 (88.0; 92.7)	1.24 (-3.06; 6.49)
	MenPS	73	73	100 (95.1; 100)		215	192	89.3 (84.4; 93.1)	
W-135	ACWY-TT	209	193	92.3 (87.9; 95.6)	0.80 (-5.63; 10.07)	651	583	89.6 (86.9; 91.8)	6.06 (0.96; 12.05)
	MenPS	71	65	91.5 (82.5; 96.8)		212	177	83.5 (77.8; 88.2)	
Y	ACWY-TT	213	194	91.1 (86.4; 94.5)	5.94 (-1.92; 16.33)	649	556	85.7 (82.7; 88.3)	9.03 (3.10; 15.65)
	MenPS	74	63	85.1 (75.0; 92.3)		214	164	76.6 (70.4; 82.1)	

N, number of subjects with pre and post vaccination results; n/%, number/percentage of subjects with a vaccine response (defined as an rSBA titer $\geq$ 1:32 in subjects with pre-vaccination titer $<$ 1:8, or a $\geq$ 4-fold increase in titer for subjects with pre-vaccination titer $\geq$ 1:8). 95% CI, 95% confidence interval. *ACWY-TT minus MenPS.

At the conclusion of the 6-mo safety follow-up, the percentage of subjects who reported rash was 1.1% (95% CI 0.5%; 2.0%) in the ACWY-TT group and 1.0% (95% CI 0.2%; 2.8%) in the MenPS group. The percentage reporting an Emergency Room visit was 1.4% (95% CI 0.7%; 2.4%) in the ACWY-TT group and 0.3% (95% CI 0.0%; 1.8%) in the MenPS group. No subject reported new onset of chronic illness.

Discussion

Major disadvantages of meningococcal polysaccharide vaccines in adults include their short-lived protection and the induction of hyporesponsiveness on repeated exposure. These two factors combined make it difficult to maintain long-term protective antibody titers in adults in highly endemic regions using polysaccharide vaccines. Although the clinical implications of hyporesponsiveness are not well understood, there is a theoretical risk of increased disease susceptibility.[15] This may be particularly important in settings where meningococcal epidemics regularly occur: for example, during the Hajj pilgrimage and in countries within the African meningitis belt; and in older age groups when the immune response to vaccination is attenuated. Conjugate vaccines induce boostable responses and do not induce hyporesponsiveness on repeated exposure,[15] thereby overcoming the major limitations of polysaccharide vaccines.

Table 5. Percentage of subjects with solicited local and general symptoms reported during the 4 d (Days 0–3) post-vaccination period (total vaccinated cohort)

		ACWY–TT			MenPS		
Symptom	Intensity	N	n	% (95% CI)	N	n	% (95% CI)
Pain	All	927	180	19.4 (16.9–22.1)	310	42	13.5 (9.9–17.9)
	Grade 3	927	4	0.4 (0.1–1.1)	310	1	0.3 (0–1.8)
Redness (mm)	All	927	82	8.8 (7.1–10.9)	310	14	4.5 (2.5–7.5)
	$>$ 50 mm	927	12	1.3 (0.7–2.3)	310	0	0 (0–1.2)
Swelling (mm)	All	927	73	7.9 (6.2–9.8)	310	6	1.9 (0.7–4.2)
	$>$ 50 mm	927	10	1.1 (0.5–2)	310	0	0 (0–1.2)
Fatigue	All	927	114	12.3 (10.3–14.6)	310	30	9.7 (6.6–13.5)
	Grade 3	927	8	0.9 (0.4–1.7)	310	0	0 (0–1.2)
Fever(Axillary)	$\geq$ 37.5°C	927	37	4 (2.8–5.5)	310	14	4.5 (2.5–7.5)
	$>$ 39.5°C	927	2	0.2 (0.0–0.8)	310	2	0.6 (0.1–2.3)
GI symptoms	All	927	43	4.6 (3.4–6.2)	310	10	3.2 (1.6–5.9)
	Grade 3	927	2	0.2 (0–0.8)	310	1	0.3 (0–1.8)
Headache	All	927	151	16.3 (14.0–18.8)	310	44	14.2 (10.5–18.6)
	Grade 3	927	14	1.5 (0.8–2.5)	310	5	1.6 (0.5–3.7)

N, number of subjects with at least one documented dose; n/%, number/percentage of subjects reporting the symptom at least once, 95% CI, exact 95% confidence interval; Grade 3, Adverse events preventing normal activities; GI symptoms, gastrointestinal symptoms.

This study demonstrated lot-to-lot consistency of the ACWY-TT vaccine and showed that ACWY-TT was non-inferior to commercially available MenPS in terms of VR rates. Statistically significantly higher VR rates and rSBA GMTs were observed in ACWY-TT recipients for 3 out of 4 serogroups (exploratory analysis) suggesting a more robust immune response than that following MenPS.

Prior to vaccination the majority of adults were seropositive for rSBA against all four vaccine serogroups, despite only four individuals reporting a prior history of meningococcal polysaccharide vaccination (> 5 y previously). High pre-existing rSBA levels ≥ 1:8 have also been reported in other studies in adults conducted in the US (between 30–100% initially seropositive for each serogroup[26]) and in India (between 88–92% initially seropositive)[27]. Circulating meningococcal or cross-reacting strains causing asymptomatic nasopharyngeal carriage and/or cross-reactivity of antibodies with other bacteria may have contributed to the high seropositivity rate observed. The observation that IMD incidence decreases with age[7] suggests that the observed rSBA provides protection against meningococcal invasion. Notably, the percentage of subjects in the ACWY-TT group with rSBA titers ≥ 1:128 increased from between 48.8% and 79% for each serogroup pre-vaccination, to between 98.9% and 99.5% post vaccination, suggesting a benefit of vaccination in conferring immunity across all four serogroups. The high pre-vaccination seropositivity supports use of defined VRs as a key endpoint of the study, since it measures the percentage of participants that had a response to vaccination, rather than simply seropositivity.

Comparisons between studies that differ in design, population studied and in serological methods should be made cautiously. However, the results of this study showing robust immunogenicity of ACWY-TT in adults are broadly consistent with those of other ACWY-conjugate vaccines in the US and South America and a monovalent MenA-TT vaccine that has been developed for use in the African Meningitis Belt for which immunogenicity in adults was also demonstrated.[26-30]

The safety profile of ACWY-TT was acceptable, with a low reported incidence of grade 3 symptoms. Increased reactogenicity following vaccination with meningococcal conjugate vaccines conjugated to diphtheria toxoid and diphtheria toxoid variant (CRM197) as compared with a polysaccharide vaccine has been observed in adolescents and children,[31,32] and the monovalent MenA-TT conjugate was shown to have higher local reactogenicity than a quadrivalent MenPS vaccine.[30] Thus the higher incidence of local symptoms in ACWY-TT recipients as compared with MenPS recipients is not unexpected, and may be due to the TT component, for which local reactogenicity has been well described.[33]

Potential limitations of the study include the lack of a licensed conjugate ACWY vaccine as a control. This is because at the time of the study, none was licensed in the countries where the study took place. However, a head-to-head study of ACWY-TT and another ACWY-conjugate vaccine showed that the immune responses induced by the two vaccines and their respective safety profiles were comparable.[19] Additionally, the current study was conducted as open-blind with respect to receipt of ACWY-TT vs. MenPS control, primarily because the routes of vaccine administration were different (intramuscular route for the ACWY-TT vaccine and subcutaneous route for the MenPS control). However, the risk of bias in the analysis of immunogenicity was reduced since laboratory personnel were blinded as to age and group. Attribution of the relationship of AEs to vaccination could have been influenced by the open design, but reporting bias would more likely be against the investigational product. A final limitation was that numerous statistical comparisons were made without adjustment for multiplicity, increasing the risk that a significant difference may have arisen by chance alone.

ACWY-TT was immunogenic against all four meningococcal serogroups (A, C, W-135 and Y) in healthy adults 18–55 y of age, with VRs that were non-inferior to MenPS, and rSBA GMTs that were significantly higher than MenPS for serogroups A, W-135 and Y (exploratory analysis). These data suggest potential benefits of ACWY-TT conjugate vaccination over MenPS in this age-group, although this would need to be confirmed with antibody persistence studies. ACWY-TT had an acceptable safety profile in healthy adults, and lot-to-lot consistency of three ACWY-TT lots was demonstrated. These data suggest that, if licensed, ACWY-TT could provide enhanced protection against IMD in healthy adults.

Materials and Methods

Study design. This was a phase III, randomized, partially double-blinded, controlled, non-inferiority study conducted at one study center in Lebanon and in three centers in the Philippines (109067/NCT00453986) between April 2007 and May 2008. The study was conducted according to Good Clinical Practice and in accordance with the Declaration of Helsinki (1996). The protocol and associated documents were reviewed and approved by ethics committees at each study center. Written informed consent was obtained from subjects before study entry.

Enrolled adults received a single dose of one of three manufacturing lots of ACWY-TT (ACWY-TT group, lots A, B and C), or *Mencevax*™ ACWY (MenPS group), or ACWY-TT (lot A) co-administered with the seasonal influenza vaccine, (*Fluarix*™: GSK Biologicals, Coad group), respectively. Subjects in the main study cohort were randomized 1:1:1:1 to the ACWY-TT (three lots) and MenPS groups for the analysis of lot-to-lot consistency and immunogenicity and safety of ACWY-TT vs. MenPS. Safety and immunogenicity when ACWY-TT and seasonal influenza vaccine were co-administered (as assessed in the "influenza" cohort) is reported elsewhere.

Vaccines were numbered using a randomization list generated at GSK Biologicals and a blocking scheme ensured that balance between treatments was maintained. Randomization was performed using a central, web-based system. The randomization algorithm included a minimization procedure that ensured a balanced allocation between groups at individual centers and between age strata (18–25 y, 26–35 y, 36–45 y, 46–55 y).

The study was double-blind with respect to ACWY-TT lot, and open with respect to whether ACWY-TT or MenPS was administered. This is because ACWY-TT is administered intramuscularly, whereas MenPS is administered subcutaneously.

Study objectives. The co-primary objectives for the main study cohort were to demonstrate lot-to-lot consistency of the three ACWY-TT lots with respect to rSBA GMTs for meningococcal serogroups A, C, W-135 and Y, and to demonstrate non-inferiority of the rSBA VR induced by ACWY-TT compared with MenPS 1 mo post-vaccination.

The assessment of the reactogenicity and safety of the study vaccines was a secondary objective.

Study subjects. Subjects were not eligible if they were immunosuppressed from any cause, had previously been vaccinated with a meningococcal polysaccharide vaccine within the past 5 y or meningococcal conjugate vaccine at any time previously, had received tetanus toxoid within the last month, or had a history of meningococcal disease. Pregnant or lactating females were also excluded.

Vaccines. One 0.5 mL dose of ACWY-TT contained 5 μg of each meningococcal serogroups A, C, W-135 and Y polysaccharide conjugated to a total of approximately 44 μg TT. One 0.5 mL dose of *Mencevax*™ACWY contained 50 μg of each meningococcal serogroups A, C, W-135 and Y polysaccharide.

Immunogenicity assessment. Blood samples were collected from all subjects prior to and one month (21–48 d) after vaccination. Pre and post-vaccination sera were tested for rSBA for each meningococcal serogroup as previously described,[34] and for antibodies against tetanus toxoid with an enzyme-linked immunosorbent assay (ELISA).[35] The cut-off of the rSBA assay was a 1:8 dilution and was considered indicative of seroprotection.[24,25]

Rabbit complement source (rather than human complement) was used because of its wider availability, and because a cut-off for a population based protective titer (≥ 1:8) has been estimated post-surveillance data in the UK after licensure of meningococcal serogroup C conjugate.[25]

Safety and reactogenicity assessment. Diary cards were used to record the occurrence of local and general solicited AEs for 4 d after vaccination, and other (unsolicited) AEs for 31 d after vaccination. Symptom intensity of redness, swelling and fever was graded by millimeter of reaction and degrees Celsius of fever, respectively, and all other symptoms were graded by the subject using a pre-defined scale. SAEs were recorded throughout the study. A scripted phone call at 6 mo recorded the occurrence of any SAEs and other significant AEs (rash, new onset of chronic disease and adverse events resulting in an emergency room visit) that had occurred since the last study visit.

Statistical analyses. The analysis of immunogenicity was conducted on the ATP immunogenicity cohort that included all vaccinated subjects who complied with protocol-defined procedures.

In addition to the primary objectives, exploratory analyses were conducted. The 95% CIs of the rSBA GMT ratios between vaccine groups were calculated using an ANCOVA model on the $\log_{10}$ transformation of the titers, using the pre-vaccination $\log_{10}$ transformation of the titers, age strata, and whether or not subjects participated in the analysis of co-administration with seasonal influenza vaccine as covariates. Antibody titers below the cut-off of the assay were given an arbitrary value of half the cut-off for the purpose of GMT calculation. Vaccine groups were considered significantly different if the 95% CI for the GMT ratio between groups did not contain the value 1, or, if the asymptotic standardized 95% CI for the difference in threshold rates or VR rates between groups did not contain the value 0. Due to the multiplicity of endpoints, statistically significant findings from the exploratory analyses should be interpreted with caution.

The analysis of safety was performed on the total vaccinated cohort that included all vaccinated subjects. The incidence and intensity of symptoms were calculated with exact 95% CI for each group. A key safety objective was to demonstrate non-inferiority of ACWY-TT compared with MenPS in terms of grade 3 systemic symptoms based on a pooled analysis of safety with another similarly designed study in adolescents. The results of the pooled safety analysis have been reported elsewhere.[23]

With 285 subjects who received each vaccine lot, the overall power to meet the primary immunogenicity consistency objective was at least 98.8% if all lots elicited similar immune responses. With a sample size of 1,140 evaluable subjects overall, the study had 86.5% power to achieve the non-inferiority primary objective, assuming both vaccines induced identical VRs.

Analyses were performed using SAS® software version 9.1 (SAS Institute Inc.) and Proc StatXact 7.0.

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Disclosure of Potential Conflicts of Interest

M.R.A.L.R., G.D. and E.D. have received consulting fees and honoraria from GSK within the past three years. N.M. declares no conflict of interest. V.B., Y.B. and J.M. are employees of GSK Biologicals. Y.B. and J.M. report ownership of GSK Biologicals stocks and stock options.

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