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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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
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 Hauag between 2004 and 2005, with 35% mortality.14 Serogroups A and W-135 currently predominate in the Middle East, and serogroup W-135 outbreaks have been reported in Haji pilgrims and their contacts.15 Serogroups B and C predominate in most of Europe, whereas in the US serogroup Y is also an important cause.16 In Africa, serogroup A is responsible for most major epidemics.17 However, serogroups W-135 and serogroup X are important emerging causes of outbreaks within the African meningitis belt.18

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 Haji pilgrims.19

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.20 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, resulting in 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.21,22

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 using tetanus toxoid as the carrier protein [ACWY-TT, GlaxoSmithKline Biologicals (GSK) Belgium] is immunogenic in toddlers, children and adolescents.23,24 This partially double-blind, 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 accepted as a surrogate for protection against IMD.25,26 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 ≥ 1:32 in initially seronegative subjects (pre-vaccination titer < 1:8)], or a ≥ 4-fold increase over the pre-vaccination titer for initially seropositive subjects (pre-vaccination titer ≥ 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 ≥ 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 ≥ 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 ≥ 1:8 and rSBA titers ≥ 1:128 was ≥ 99.3% and ≥ 98.0%, respectively (data not shown). For each serogroup, ≥ 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 ≥10-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 ≥ 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...
(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

| Characteristics Categories | ACWY lot A N = 311 | ACWY lot B N = 311 | ACWY lot C N = 313 | ACWY-TT Pooled lots N = 935 | MenPS N = 312 |
|---------------------------|---------------------|---------------------|---------------------|-----------------------------|---------------|
| Age (years)               | Mean                | 35.2                | 35.1                | 35.7                        | 35.3          |
|                           | SD                  | 10.48               | 10.50               | 10.75                       | 10.57         |
|                           | Range               | 18–55               | 18–55               | 18–55                       | 18–55         |
| Gender                    | Female              | 135 (43.4)          | 139 (44.7)          | 133 (42.5)                  | 407 (43.5)    |
|                           | Male                | 176 (56.6)          | 172 (55.3)          | 180 (57.5)                  | 528 (56.5)    |
| Race                      | Southeast Asian     | 223 (71.7)          | 223 (71.7)          | 224 (71.6)                  | 670 (71.7)    |
|                           | Arabic/North African| 88 (28.3)           | 88 (28.3)           | 87 (27.6)                   | 263 (28.1)    |
|                           | Other*              | 0 (0.0)             | 0 (0.0)             | 2 (0.6)                     | 2 (0.2)       |

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.

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

| Serogroup | ACWY-TT Lot | N       | Adjusted GMT | ACWY-TT Lot | N       | Adjusted GMT | Ratio order | Value   | LL  | UL  |
|-----------|-------------|---------|--------------|-------------|---------|--------------|-------------|---------|-----|-----|
| A         | A           | 253     | 385.1        | B           | 244     | 3607.2       | A / B       | 1.07    | 0.88| 1.29|
| A         | A           | 253     | 385.1        | 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|
| C         | 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 ratio between lots was within the interval of [0.5; 2.0].

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

| 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) |
| C         | MenPS       | 252     | 176     | 69.8 (63.8; 75.4)  |
| W-135     | ACWY-TT     | 860     | 776     | 90.2 (88.1; 92.3)  | 4.72 (0.49; 9.65) |
| Y         | MenPS       | 283     | 242     | 85.5 (80.9; 89.4)  |

Adjusted GMT ratio: 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. *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.
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. 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, thereby overcoming the major limitations of polysaccharide vaccines.

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

| 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 | 488| 79.2 (75.6; 82.4) | 10.20 (3.10; 17.82) |
|           | MenPS     | 62  | 45 | 72.6 (59.8; 85.1) | 1.90 (0.71; 5.46) | 190 | 131| 68.9 (61.8; 75.6) | 1.10 (0.10; 4.31) |
| 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) | 0.80 (0.53; 1.07) | 215 | 192| 89.3 (84.4; 93.1) | 1.24 (0.10; 4.31) |
| W-135     | ACWY-TT   | 209 | 193| 92.3 (87.9; 95.6) | 0.80 (0.53; 1.07) | 651 | 583| 89.6 (86.9; 91.8) | 6.06 (0.96; 12.05) |
|           | MenPS     | 71  | 71 | 100 (95.1; 100) | 0.80 (0.53; 1.07) | 212 | 177| 83.5 (77.8; 88.2) | 6.06 (0.96; 12.05) |
| Y         | ACWY-TT   | 213 | 194| 91.1 (86.4; 94.5) | 5.94 (-1.92; 16.33) | 649 | 556| 85.7 (82.7; 88.8) | 9.03 (3.10; 15.65) |
|           | MenPS     | 74  | 63 | 85.1 (75.0; 92.3) | 5.94 (-1.92; 16.33) | 214 | 164| 76.6 (70.4; 82.1) | 9.03 (3.10; 15.65) |

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 $\geq$1:8, or a $\geq$4-fold increase in titer for subjects with pre-vaccination titer $<1:8$). 95% CI, 95% confidence interval. *ACWY-TT minus MenPS.

**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)

| Symptom | Intensity | ACWY-TT | MenPS | ACWY-TT | MenPS |
|---------|-----------|---------|-------|---------|-------|
|         | 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 (6.2–9.6) | 310 | 6 | 1.9 (0.7–4.2) |
|         | > 50 mm 927 | 10 | 1.1 (0.5–2.2) | 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 (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 (0.0–0.8) | 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 indications 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) and in India (between 88–92% initially seropositive). 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 IM vaccination decreases with age suggests that the observed rSBA reactivity of antibodies with other bacteria may have contributed to the high seropositivity rate observed. The observation that IMD 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-blind, 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 (MenPS group), or ACWY-TT (lot A) co-administered with the seasonal influenza vaccine, (FluarixTM: 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, 56–45 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 immunocompromised or 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 serogroup A, C, W-135 and Y polysaccharide conjugated to a total of approximately 44 μg TT. One 0.5 mL dose of Menrixm<sup>TM</sup>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, and for antibodies against tetanus toxoid with an enzyme-linked immunosorbent assay (ELISA). The cut-off of the rSBA assay was a 1:8 dilution and was considered indicative of seroprotection.<sup>24,25</sup>

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 local and general solicited AEs for 4 d after vaccination, and other (unsolicited) AEs for 31 d after vaccination.

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Analyses were performed using SAS<sup>©</sup> software version 9.1 (SAS Institute Inc.) and Proc StatXact 7.0.

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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References

1. Pollock A. J. Global epidemiology of meningococcal disease and vaccine efficacy. Pediatr Infect Dis J 2006; 25(9):843-59. PMID:17035822; http://dx.doi.org/10.1097/01.inf.0000235621.20511.db

2. Harrison LH, Trotter CL, Ramsay ME. Global epidemiology of meningococcal disease. Vaccine 2004; 22(suppl 3): B5-63. PMID:15477762; http://dx.doi.org/10.1016/j.vaccine.2004.05.007

3. World Health Organization. Epidemic and pandemic alert and response (EPR). Meningococcal Disease in the Philippines – update 4 [Internet]. [cited 2009 Dec 30]; Available from: http://www.who.int/epi characters.html

4. Wilder-Smith A, Goh KT, Barkham T, Paton NI. Hajj and meningococcal disease and vaccine efficacy. Pediatr Infect Dis J 2004; 23(Suppl):S274-9; PMID:15597069

5. Miller E, Galbraith D, Ramsey M. Planning, registration, and implementation of an immunization campaign against meningococcal group C disease in the UK: a case study. Vaccine 2001; 20(4):358-67; PMID:1147916; http://dx.doi.org/10.1016/S0264-410X(01)00429-3

6. Oppong P, Lubsche B, Poon J. Intramuscular vs. subcutaneous administration of meningococcal A, C, W-135, and Y tetanus toxoid conjugate vaccine given in the second year of life and in young children. Vaccine 2010; 28:744-53; PMID:20387737; http://dx.doi.org/10.1016/j.vaccine.2010.04.064

7. Banerjee R, Banerjee R, Emory K, Banerjee V, Fratila LR, Miller JM. Immunogenicity and safety of an investigational quadrivalent meningococcal A,C,W-135,Y conjugate vaccine in healthy adolescents and young adults 16 to 25 years of age. Pediatr Infect Dis J 2011; 30:414-22; PMID:21349505; http://dx.doi.org/10.1097/INF.0b013e31820b0765

8. Verheijen T, Kersters M, Banik V, Van den Wildt M, Miller J. Tetravalent meningococcal (groups A, C, W-135 and Y) conjugate vaccine in children: safety and immunogenicity of a single dose of 20 microg serogroup A and W-135 conjugate, 5 microg serogroup C conjugate, 2 microg serogroup Y conjugate and 20 microg diphtheria toxoid. Pediatr Infect Dis J 2011; 30:412-4; PMID:21349506; http://dx.doi.org/10.1097/INF.0b013e31820b0767

9. Memish ZA, Dhaif G, Montalban J, Vayghan VP, Jafari H, Doherty AP et al. Immunogenicity of a single dose of 20 microg serogroup A and W-135 conjugate, 5 microg serogroup C conjugate, 2 microg serogroup Y conjugate and 20 microg diphtheria toxoid. Pediatr Infect Dis J 2011; 30:411-2; PMID:21349767; http://dx.doi.org/10.1097/INF.0b013e31820b0760

10. Krid M, Patrian-Chenomoto J, Aghahosseini U, Tchelitchew-Simonier J, Hamer M, Mower I, et al. An intranasal quadrivalent meningococcal vaccine containing Neisseria meningitidis A, C, W-135 and Y strains and a licensed tetravalent meningococcal A, C, W-135 and Y conjugate vaccine co-administered to infants. J Infect Dis 2010; 201:2209-16; PMID:20271880; http://dx.doi.org/10.1086/653568

11. Deforges DP, Gomp G, Babila RB, Anderson EJ. Induction of immunologic reactivity in adults by meningococcal C polysaccharide vaccine. J Infect Dis 1996; 174:870-4; PMID:9782562; http://dx.doi.org/10.1086/353344

12. Andrews N, Banerjee R, Miller JM. Validation of serological correlates of protection for meningococcal C conjugate vaccine by using efficacy estimates from postmarketing surveillance in England. Clin Diag Lab Immunol 2003; 10:786-9; PMID:12965994

13. Campbell JD, Eshrow S, King JC, J, Papp T, Ryall R, Bourd E, Miano, Safety, immunogenicity, and immune persistence of a meningococcal polysaccharide-diphtheria toxoid conjugate vaccine given in healthy adults. Pediatr Infect Dis J 2000; 19:1164-51; PMID:10864777; http://dx.doi.org/10.1097/00006252-199711000-00020

14. Kolho et al. Multi-year evaluation of the safety and immunogenicity of the meningococcal A,C,W-135,Y conjugate vaccine in healthy Italian adults. Vaccine 2007; 25(Suppl 1):A1-7; PMID:17532316; http://dx.doi.org/10.1016/j.vaccine.2007.04.050

15. Keningst et al. Induction of immune reactivity in adults by meningococcal C polysaccharide vaccine. J Infect Dis 1996; 174:870-4; PMID:9782562; http://dx.doi.org/10.1086/353344

16. Andrews N, Banerjee R, Miller JM. Validation of serological correlates of protection for meningococcal C conjugate vaccine by using efficacy estimates from postmarketing surveillance in England. Clin Diag Lab Immunol 2003; 10:786-9; PMID:12965994

17. Campbell JD, Eshrow S, King JC, J, Papp T, Ryall R, Bourd E, Miano, Safety, immunogenicity, and immune persistence of a meningococcal polysaccharide-diphtheria toxoid conjugate vaccine given in healthy adults. Pediatr Infect Dis J 2000; 19:1164-51; PMID:10864777; http://dx.doi.org/10.1097/00006252-199711000-00020

18. Kolho et al. Multi-year evaluation of the safety and immunogenicity of the meningococcal A,C,W-135,Y conjugate vaccine in healthy Italian adults. Vaccine 2007; 25(Suppl 1):A1-7; PMID:17532316; http://dx.doi.org/10.1016/j.vaccine.2007.04.050

19. Keningst et al. Induction of immune reactivity in adults by meningococcal C polysaccharide vaccine. J Infect Dis 1996; 174:870-4; PMID:9782562; http://dx.doi.org/10.1086/353344