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A new fully liquid presentation of MenACWY-CRM conjugate vaccine: Results from a multicentre, randomised, controlled, observer-blind study

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ABSTRACT

Background: The currently licensed quadrivalent MenACWY-CRM conjugate vaccine presentation consists of two vials (lyophilised MenA and liquid MenCWY) to be reconstituted before injection. A new fully liquid formulation in a single vial has been developed to further improve the vaccine presentation. Since the MenA structure is subject to hydrolytic degradation, this study was conducted to compare the immunogenicity and safety of the investigational MenACWY-CRM liquid vaccine with the licensed vaccine.
Methods: In this multicentre, randomised, controlled, observer-blind, phase 2b study, 979 healthy adults were administered a single dose of MenACWY-CRM liquid presentation or the currently licensed MenACWY-CRM vaccine. MenA free saccharide generation was accelerated to approximately 30% in the liquid presentation and MenA polysaccharide O-acetylation was reduced to approximately 40%, according to a controlled procedure. Immunological non-inferiority of the MenACWY-CRM liquid to the licensed vaccine, as measured by human serum bactericidal assay (hSBA) geometric mean titres (GMTs) against MenA 1 month post-vaccination, was the primary study objective. Safety assessment was among the secondary objectives.

Abbreviations: AE, adverse event; CI, confidence interval; CRM_{197}, cross-reacting material 197; FS, free saccharide; GMT, geometric mean titre; hSBA, human serum bactericidal assay; IMD, invasive meningococcal disease; LLOQ, lower limit of quantitation; LOD, limit of detection; MenACWY-CRM, quadrivalent meningococcal glycoconjugate vaccine; MenA, meningococcal serogroup A; MenC, meningococcal serogroup C; MenW, meningococcal serogroup W; MenY, meningococcal serogroup Y; SAE, serious adverse event.

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Results: Immune responses against each serogroup were similar between the two vaccine groups and was non-inferior for MenA. Adjusted hSBA GMTs for MenA were 185.16 and 211.33 for the MenACWY-CRM liquid presentation and currently licensed vaccine presentation, respectively. The between-group ratio of hSBA GMTs for MenA was 0.88, with a two-sided 95% confidence interval lower limit of 0.64, greater than the prespecified non-inferiority margin of 0.5, thus meeting the primary study objective. Both vaccines were well tolerated. No serious adverse events were considered related to vaccination.
Conclusions: The levels of MenA free saccharide and polysaccharide O-acetylation did not affect the immunogenicity of the fully liquid presentation, which was demonstrated to be non-inferior to the immunogenicity of the currently licensed MenACWY-CRM vaccine against MenA. The immunogenicity, reactogenicity and safety profiles of the two vaccine presentations were similar.

1. Introduction

Invasive meningococcal disease (IMD), caused by *Neisseria meningitidis* (meningococcus), is characterised by a rapid onset and case fatality rates of 4–20% for treated cases [1]. Moreover, a high proportion of IMD survivors are affected by a broad range of physical, neurological and psychological sequelae and reduced quality of life that can persist years after infection [2]. Of the 12 meningococcal serogroups identified, half (serogroups A, B, C, W, X and Y) are responsible for almost all IMD cases globally [3]. The quadrivalent meningococcal glycoconjugate vaccine MenACWY-CRM (*Menveo*, GSK), that uses non-toxic diphtheria cross-reacting material 197 (CRM_{197}) as carrier protein, induces active immunisation against IMD caused by meningococcal serogroups A, C, Y and W [4]. MenACWY-CRM was first licensed in 2010 and has well established safety and immunogenicity profiles [4].

The licensed MenACWY-CRM vaccine requires reconstitution of the lyophilised meningococcal serogroup A (MenA-CRM_{197}) component with the liquid serogroups C, W and Y (MenCWY-CRM_{197}) component just before injection [5]. A fully liquid presentation of MenACWY-CRM vaccine has been developed to ease the administration process and avoid potential reconstitution errors. During the manufacturing process, conjugates are produced by covalent attachment of the serogroup oligosaccharides to CRM_{197}. Development stability studies have indicated that, in aqueous solution, the percentage of unconjugated or free saccharide (FS) increases and the O-acetylation level of the MenA oligosaccharide decreases over time at 2–8 °C [6]. This is because the structure of MenA conjugated polysaccharide is particularly labile in aqueous solution in terms of the glycosidic linkage and susceptibility to hydrolytic degradation [7,8]. FS and O-acetylation contents may therefore have an impact on the immunogenicity of conjugate vaccines. Studies conducted using human sera and mouse immunisation found immunogenicity was reduced with the removal of O-acetyl groups from MenA [9]. However, a controlled clinical study involving 1170 healthy adults, comparing two lots of MenACWY-tetanus toxoid conjugate vaccine with different MenA O-acetylation levels (68% versus 92%) and polysaccharide unconjugated vaccine with 82% MenA O-acetylation, showed no impact on vaccine immunogenicity [10].

In the present study, we tested the non-inferiority of immune responses against MenA induced by an investigational fully liquid MenACWY-CRM presentation as compared to the currently licensed MenACWY-CRM vaccine presentation. The investigational liquid vaccine underwent an artificial accelerated controlled ageing process to achieve approximately 30% MenA FS, a concentration higher than the one set for the licensed vaccine at end of shelf-life (<25%), and de-O-acetylation of MenA polysaccharide to approximately 40%. Immune responses against serogroups C, W and Y, and the reactogenicity and safety of the study vaccines were also evaluated.

2. Materials and methods

2.1. Study design

This was a randomised, controlled, observer-blind phase 2b clinical study conducted at 30 centres in five countries (Australia, Belgium, Canada, Germany and Italy) between September 2018 and June 2019 (ClinicalTrials.gov Identifier: NCT03652610). A study summary is available at www.gsk-studyregister.com (study identifier, 205343). The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice and approved by the appropriate ethics committees. All participants provided written informed consent before enrolment.

Participants were allocated to one of two study groups at each site via a central randomisation system using a minimisation procedure accounting for centre. Randomisation of supplies within blocks was performed at GSK using MATerial Excellence (MATEX), a program developed by GSK for use with SAS software (Cary, NC, USA). Due to differences in the presentation of the study vaccines, the study was conducted in an observer-blind manner, i.e., vaccine recipients and those responsible for the evaluation of any study outcome were unaware of which vaccine was administered, while each vaccine was prepared and administered by authorised medical personnel who did not participate in any of the study's clinical evaluations or assays.

There were two study visits (at vaccination and approximately 1 month [28 days] afterwards) and three telephone calls were made to each participant at 2 weeks (Day 15), 3 months (Day 91) and 6 months (Day 181) after vaccination to follow-up on vaccine safety during study participation.

2.2. Outcome measures

The primary study objective was to demonstrate non-inferiority of the fully liquid MenACWY-CRM vaccine presentation to the currently licensed lyophilised-liquid presentation (*Menveo*), as measured by human serum bactericidal assay (hSBA) geometric mean titres (GMTs) against MenA at approximately 1 month following vaccination. The primary outcome measure was the between-group ratio of hSBA GMTs at Day 29. The main secondary immunogenicity outcomes reported in this paper are the serogroup-specific hSBA GMTs at 1 month post-vaccination, the percentage of participants with hSBA titre $\geq$8 (the titre considered as threshold for seroprotection [4]) pre- and post-vaccination and the percentage with a four-fold increase in hSBA titre post-vaccination. Other immunogenicity results, such as pre-vaccination hSBA GMTs and percentage of participants with hSBA titres $\geq$ lower limit of

quantitation (LLOQ) pre- and post-vaccination, can be found at ClinicalTrials.gov (https://www.clinicaltrials.gov/ct2/show/results/NCT03652610). Clinical tolerability and safety of the two vaccine presentations were analysed as secondary study outcomes.

2.3. Study participants and vaccines

Healthy adults aged 18–40 years were to be enrolled and randomised to one of two study groups, receiving a single 0.5 mL intramuscular dose of either the investigational MenACWY-CRM liquid vaccine (MenACWY liquid group) or the licensed MenACWY-CRM vaccine (MenACWY licensed group) in the deltoid region of the non-dominant arm. The licensed MenACWY-CRM vaccine contains 10 μg of MenA-CRM_{197} and 5 μg each of serogroup C (MenC-CRM_{197}), serogroup W (MenW-CRM_{197}) and serogroup Y (MenY-CRM_{197}) and is prepared by mixing the lyophilised MenA-CRM_{197} component (including as excipients, sucrose and potassium dihydrogen phosphate) with the liquid MenCWY-CRM_{197} component (including as excipients, sodium dihydrogen phosphate monohydrate, disodium phosphate dihydrate, sodium chloride and water for injection) just before injection. The investigational liquid presentation of the MenACWY-CRM vaccine contained the same CRM-conjugated meningococcal serogroup components as the licensed vaccine. As the MenA component is not lyophilised in the liquid vaccine, sucrose and potassium dihydrogen phosphate were not included as excipients.

An approximate 30% level of MenA FS and 40% O-acetylation was obtained in the investigational MenACWY-CRM liquid vaccine by storing it at approximately 22.5 °C (±2°C) for around 2 months (59 days) during the usual storage period at 2–8 °C [6]. The FS and O-acetylation levels of the fully liquid vaccine were constantly monitored before and during study conduct via HPAEC-PAD (high-performance anion-exchange chromatography with pulsed amperometric detection) for FS and 1H:NMR (proton nuclear magnetic resonance) for O-acetylation.

Participants were to be excluded from study enrolment if they had received any meningococcal vaccine, had a history of meningococcal disease or had contact, within 60 days of enrolment, with an individual with meningococcal disease. Other exclusion criteria included immune system disorders resulting from any cause or immune-mediated disease, known adverse reactions to vaccine components, receipt of immunoglobulins or blood products within 180 days before enrolment, receipt of a long-acting immune-modifying drug at any time during the study, receipt of an investigational product within 30 days before enrolment, receipt of any other vaccine within 7 days (inactivated vaccines) or 14 days (live vaccines) before enrolment or planned receipt of any vaccine within 28 days of study vaccine administration, and any acute or chronic condition that could interfere with the results of the study. Pregnant or breastfeeding women were excluded from enrolment, and women of child-bearing potential had to commit to using birth control measures from 30 days before vaccination until 1 month after vaccination.

2.4. Serological analyses

Two blood samples of 20 mL each were collected before and approximately 1 month after vaccination (Days 1 and 29). The induction of bactericidal antibodies directed against *N. meningitidis* serogroups A, C, W and Y was determined by a validated hSBA (MenACWY agar-overlay hSBA). Titres were expressed as the reciprocal of the dilution resulting in 50% inhibition. The hSBA clinical testing was performed by GSK, Clinical Laboratory Sciences, Wavre, Belgium.

2.5. Safety analyses

Participants were observed at the study centres for 30 min after vaccination for immediate reactions. Solicited local (erythema, induration and pain at the injection site) and systemic (arthralgia, chills, fatigue, headache, loss of appetite, myalgia and nausea) adverse events (AEs) and body temperature were to be reported by participants on electronic diaries for 7 days following vaccination. The severity of solicited AEs was classified as mild, moderate (interfering with normal activity or, for loss of appetite, eating less than usual, or for erythema and induration, >50 mm to ≤100 mm diameter) or severe (preventing normal activity or, for loss of appetite, not eating at all, or for erythema and induration, >100 mm diameter). Fever was defined as body temperature ≥38 °C.

Unsolicited AEs reported within 30 min after vaccination and during the 29-day post-vaccination period were recorded. Serious AEs (SAEs), medically-attended AEs and AEs leading to withdrawal were reported over the entire study period of 6 months and the causal relationship of AEs to vaccination was assessed by study investigators.

2.6. Statistical analysis

With 437 evaluable participants in each study group, there was 90% power to reject the null hypothesis that the between-group ratio of hSBA GMTs against MenA for the MenACWY liquid group to the MenACWY licensed group was ≤0.5 (given an underlying ratio of 0.8 and assuming a common standard deviation on log scale of 0.929). Anticipating a dropout rate of 10%, enrolment of approximately 972 participants (486 per group) was planned.

The primary immunogenicity analyses were conducted on the per-protocol population for immunogenicity (per-protocol set), defined as all participants who received study vaccination correctly, had no major protocol deviations and who had assay results available for at least one serogroup at the appropriate time points. Non-inferiority of the investigational vaccine to the licensed vaccine was to be concluded if the lower limit of the two-sided 95% confidence interval (CI) for the between-groups ratio of adjusted hSBA GMTs against MenA was greater than 0.5 (non-inferiority margin) 1 month post-vaccination.

GMTs were adjusted using an analysis of covariance model with pre-vaccination titre as covariate and group and country as factors.

Analysis of all secondary immunogenicity objectives were not associated to formal statistical objectives, and aimed to provide a comprehensive picture of the immune responses. For each serogroup, percentages of participants with a four-fold increase in post-vaccination hSBA titre and percentage of participants with hSBA titre ≥8 and associated two-sided 95% Clopper-Pearson CIs [11] were analysed. Differences in percentages between groups were calculated using the method of Miettinen and Nurminen [12]. The four-fold increase in hSBA titre post-vaccination was defined as: i) four times the limit of detection (LOD) or LLOQ (whichever was greater) for individuals with pre-vaccination titres below the LOD, ii) four times the LLOQ for individuals with pre-vaccination titres between LOD and LLOQ (inclusive), or iii) four times the pre-vaccination titre for individuals with pre-vaccination titres greater than LLOQ. The LOD was 4 for each serogroup and the LLOQ was 5 for serogroup A, 6 for serogroup C, 7 for serogroup W and 6 for serogroup Y.

All participants who received study vaccination and provided safety data were included in the safety analyses, which were descriptive.

Statistical analyses were performed using SAS Life Science Analytics Framework version 9.4 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Study population

Of the 983 healthy adults enrolled and randomised, 979 were vaccinated: 490 received the investigational liquid vaccine and 489 received the licensed vaccine. The study was completed by 487 and 484 participants, respectively; of those randomised, four withdrew, one was lost to follow-up and one was withdrawn for other reason in the MenACWY liquid group and six withdrew in the MenACWY licensed group. A total of 938 participants were included in the per-protocol set for immunogenicity analyses (Fig. 1). Overall, demographic characteristics were similar between the groups (Fig. 2).

3.2. Immunogenicity

At 1 month post-vaccination, MenA hSBA adjusted GMTs were 185.16 in the MenACWY liquid group and 211.33 in the MenACWY licensed group (Table 1). The ratio of GMTs against MenA between the liquid presentation and licensed vaccine groups was 0.88, with 0.64 as lower limit of the two-sided 95% CI, greater than the non-inferiority margin of 0.5, thus fulfilling the primary immunogenicity objective.

Adjusted hSBA GMTs against serogroups C, W and Y post-vaccination were similar between the two vaccine groups and ranged between 62.83 (serogroup Y) and 161.54 (serogroup C) in MenACWY liquid group, and between 51.57 (serogroup W) and 135.78 (serogroup C) in MenACWY licensed group. Point estimates for the between-group ratios of GMTs were above 1 for all three serogroups: 1.19 for serogroups C and Y, and 1.23 for serogroup W (Table 1).

Non-inferiority criteria for serogroups C, W and Y were not defined in the study protocol. However, by applying the same non-inferiority limit as used for MenA, non-inferiority would be demonstrated for these serogroups also, as the lower limit of the two-sided 95% CI for the between-group ratios of GMTs were 0.84, 0.96 and 0.90 for MenC, MenW and MenY, respectively, all greater than 0.5 (Table 1).

Percentages of participants with hSBA titre ≥8 at baseline varied according to serogroup, being lowest for MenA (8.7% for MenACWY liquid and 11.2% for MenACWY licensed) and highest for MenC (53.8% and 54.4%) and MenW (39.8% and 45.7%). For MenY, 22.7% (MenACWY liquid) and 25.5% (MenACWY licensed) of participants had hSBA titre ≥8 at baseline. One month post-vaccination, percentages of participants with hSBA titre ≥8 ranged between 73.3% (MenW) and 82.8% (MenA) in the MenACWY liquid group and between 73.1% (MenW) and 86.4% (MenA) in the MenACWY licensed group (Fig. 3).

A four-fold increase in GMTs compared to baseline was observed in 44.7% (MenW) to 79.8% (MenA) of participants in the MenACWY liquid group and 40.2% (MenW) to 83.7% (MenA) of the MenACWY licensed group (Fig. 3).

3.3. Reactogenicity and safety

The percentage of participants reporting at least one solicited local AE was 41.7% in the MenACWY liquid group and 38.2% in the MenACWY licensed group, with pain at injection site being the most frequently reported (Fig. 4), and severe pain reported only by two (0.4%) participants in both groups. The percentage reporting at least one systemic AE was 48.5% and 49.9%, in the two groups, respectively; the most commonly reported solicited systemic AEs were fatigue and headache in 159 (32.5%) and 158 (32.3%) participants in the MenACWY liquid group and 159 (32.6%) and 165 (33.9%) participants in the MenACWY licensed group (Fig. 4). Solicited local and systemic AEs were generally mild to moderate in intensity; few participants reported severe solicited

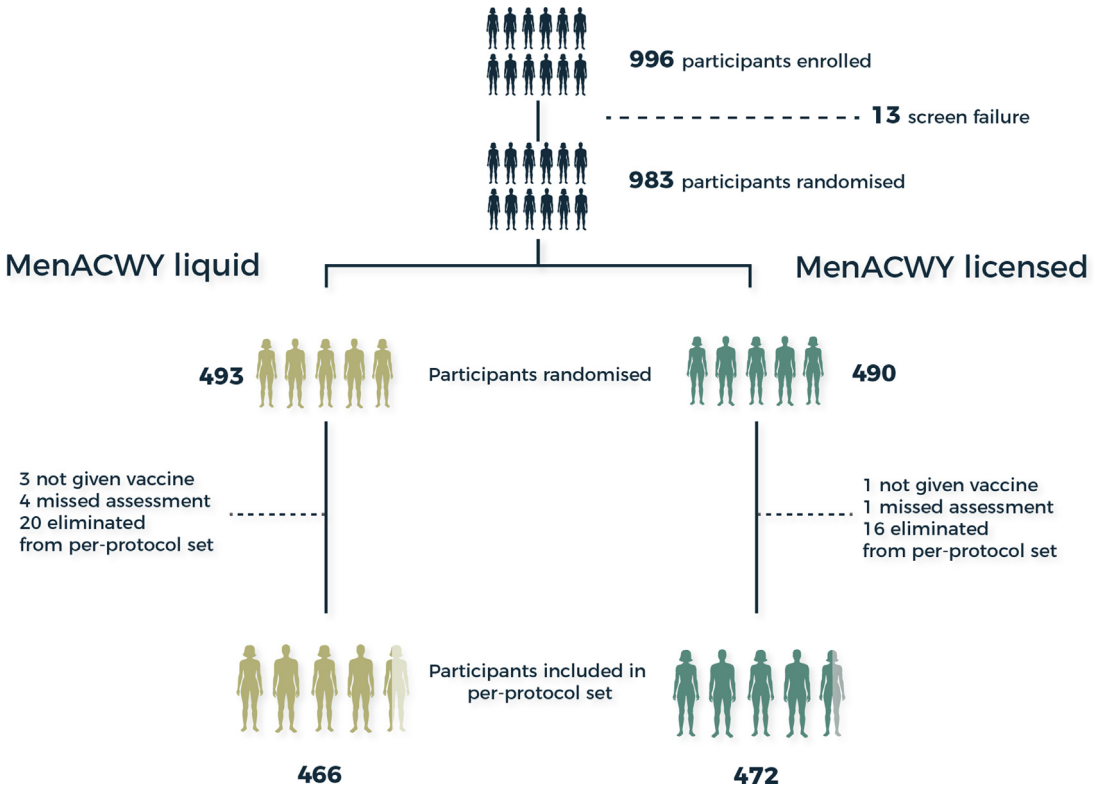

Fig. 1. Participant disposition flowchart.

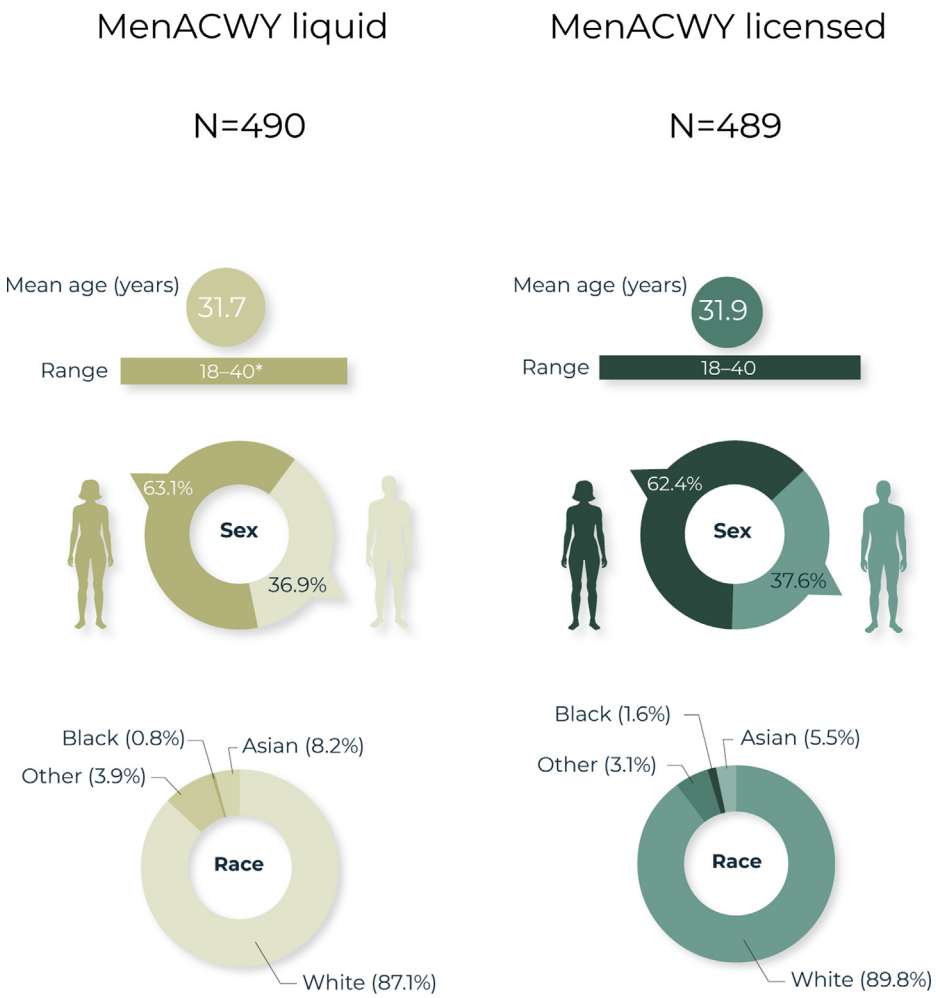

Fig. 2. Demographic characteristics of participants who received the investigational MenACWY-CRM fully liquid vaccine or licensed MenACWY-CRM vaccine. * One participant in MenACWY liquid group was aged 44 years at enrolment and was excluded from the per-protocol immunogenicity analysis. MenACWY, quadrivalent meningococcal conjugate vaccine; N, number of participants.

Table 1
Adjusted hSBA geometric mean titres (GMTs) 1 month post-vaccination and their between-group ratios.

Serogroup	MenACWY liquid group		MenACWY licensed group		GMT ratio, MenACWY liquid: MenACWY licensed (95% CI)
	N	GMT (95% CI)	N	GMT (95% CI)	
A	386	185.16 (147.90–231.81)	404	211.33 (169.61–263.32)	0.88 (**0.64**–1.20)
C	437	161.54 (126.34–206.55)	441	135.78 (106.39–173.29)	1.19 (0.84–1.68)
W	445	63.40 (52.97–75.88)	443	51.57 (43.07–61.74)	1.23 (0.96–1.58)
Y	452	62.83 (51.39–76.81)	455	52.59 (43.05–64.25)	1.19 (0.90–1.58)

CI, confidence interval; GMT, geometric mean titre; MenACWY, quadrivalent meningococcal glycoconjugate vaccine; N, number of participants who received vaccination and had assay results available.
MenACWY liquid group received the investigational MenACWY-CRM fully liquid vaccine and MenACWY licensed group received the licensed MenACWY-CRM vaccine (per-protocol population for immunogenicity).
In bold: Lower limit of two-sided 95% CI > non-inferiority margin of 0.5, thus fulfilling primary immunogenicity study objective.

AEs (Fig. 4). Fever (body temperature ≥38 °C) was reported by seven (1.4%) participants in the MenACWY liquid group and 10 (2.1%) in the MenACWY licensed group. There were no reports of fever ≥40 °C.

Percentages of participants reporting unsolicited AEs within 1 month or 6 months after vaccination were similar between the two study groups (Fig. 5). The most frequently reported unsolicited AEs in the 29-day post-vaccination period were nasopharyngitis (2.2% in MenACWY liquid group and 2.0% in MenACWY licensed group), upper respiratory tract infection (2.0% and 1.2%, respectively) and headache (1.8% and 2.9%, respectively). In the 29-day post-vaccination period, the most frequent unsolicited AEs considered related to vaccination in the MenACWY liquid group were injection site pain (reported by four participants, 0.8%), injection site erythema, dizziness and headache (three participants, 0.6%, each). The most frequently reported unsolicited AEs considered related to vaccination in the MenACWY licensed group were injection site erythema and headache (four participants, 0.8%, each).

Over the entire study period of 6 months, a similar percentage of participants in both groups reported a medically-attended unsolicited AE during the study (Fig. 5). Seven SAEs were reported by six participants in the MenACWY liquid group: incomplete sponta-

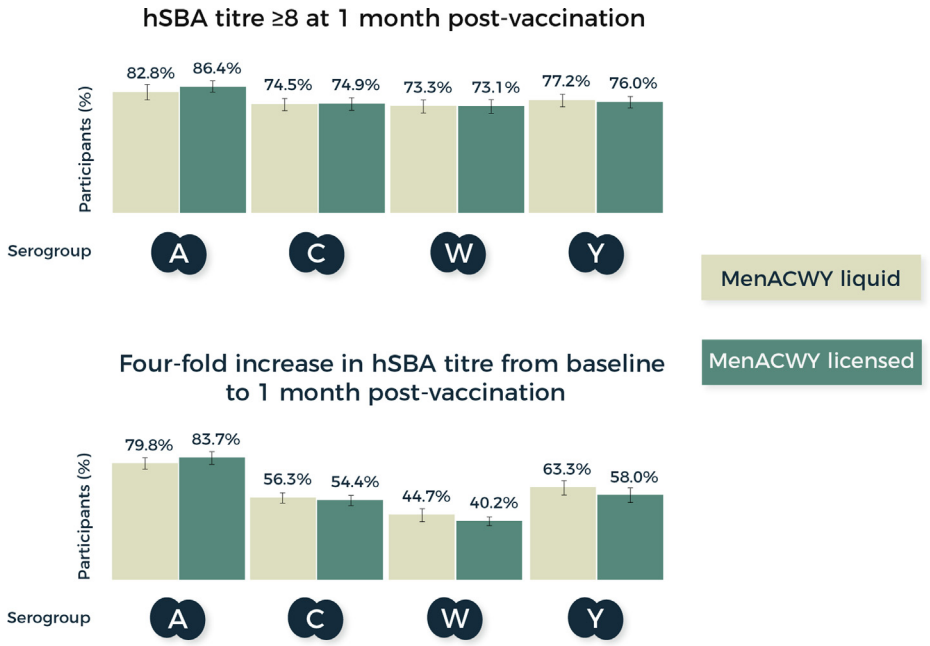

Fig. 3. Percentages of participants (with 95% confidence intervals) with human serum bactericidal assay (hSBA) titre ≥8 at 1 month post-vaccination and four-fold increase in hSBA titre from baseline to 1 month post-vaccination against serogroups A, C, W and Y (per-protocol population for immunogenicity). MenACWY, quadrivalent meningococcal glycoconjugate vaccine. Number of participants with data available on hSBA titre ≥8: 406, 443, 454 and 460 for serogroups A, C, W and Y, respectively, for MenACWY liquid group, and 428, 446, 457 and 463, respectively, for MenACWY licensed group. Number of participants with data available on four-fold increase in hSBA titre: 386, 437, 445 and 452 for serogroups A, C, W and Y, respectively, for MenACWY liquid group, and 404, 441, 443 and 455, respectively, for MenACWY licensed group.

neous abortion, spontaneous abortion, dengue fever, migraine, attempted suicide, and (in a single participant) ruptured ovarian cyst and pleural effusion. There were nine SAEs reported by nine participants in the MenACWY licensed group: atrial flutter, hyperthyroidism, hernia, biliary colic, dengue fever, jaw fracture, wrist fracture, uterine cancer and ectopic pregnancy. None of the SAEs were assessed as related to vaccination. No participant was withdrawn from the study due to an AE and no deaths were reported.

4. Discussion

A fully liquid presentation of MenACWY-CRM vaccine, which does not require reconstitution before injection, has been developed to simplify vaccine administration. In this study of the liquid vaccine, we examined if changes in FS and O-acetylation content had an impact on the immunogenicity of the MenA component, the vaccine constituent modified from lyophilised to liquid in the investigational presentation. The fully liquid MenACWY-CRM vaccine was aged artificially under controlled conditions (storage at 22.5 °C for around 2 months) to achieve approximately 30% MenA FS and reduced O-acetylation (approximately 40% O-acetylation level of the MenA moiety). The aim was to demonstrate that, even when tested under stress conditions of MenA degradation, the fully liquid vaccine was as immunogenic as the licensed vaccine presentation that had not been aged.

The study was successful in demonstrating non-inferiority of immune responses of the liquid versus licensed vaccine against MenA, as measured by between-group ratio and adjusted 95% CI. Also, the immunogenicity of the improved vaccine presentation against serogroups C, W and Y was similar to that of the licensed vaccine. Point estimates for the between-group GMT ratios were above 1 for all three serogroups and, although not formally planned, when the same non-inferiority limit as used for MenA was applied, non-inferiority would also be met for serogroups C, W and Y.

These results in healthy adults confirm prior clinical results generated with a different MenACWY-tetanus toxoid conjugate vaccine with different degrees of MenA degradation (de-O-acetylation) [10]. This provides evidence that, despite findings from pre-clinical models showing O-acetylation is essential to the immunogenic epitopes of the MenA polysaccharide [9], O-acetylation of MenA up to the levels achieved in our study (~40%) and MenA FS percentages of approximately 30% do not have a significant impact on the vaccine's ability to induce bactericidal immune responses in adults.

The immunogenicity of MenACWY-CRM in both groups was consistent with reports from previous *Menveo* studies, which showed the induction of robust immune responses when administered in healthy adults [5]. Notably, for both MenACWY liquid and licensed, hSBA GMTs against MenA 1 month post-vaccination were much higher than those reported in previous clinical trials [13,14]. In a study conducted in the US, the GMTs against MenA were 31 [14], while in another study conducted in Argentina and Colombia, the GMTs were 77 [13] 1 month after vaccination. This is likely to be due to differences in the validated serological assay adopted in previous *Menveo* studies. In the present clinical trial, the hSBA was an agar-overlay assay with a higher throughput than the previously-used manual tilt hSBA used to support *Menveo* licensure. Moreover, the hSBA in the present trial included a different MenA indicator strain (3125) [15]. This strain was selected instead of the former F8238 MenA antigen to optimise the hSBA and allow the detection of bactericidal antibodies against most invasive MenA strains expressing the L(3,7)9 or L10 immunotype, such as 3125, rather than against a strain usually observed to be involved in carriage, such as F8238 [15].

Between vaccine groups, the percentage of participants with hSBA titres ≥8 and the percentage of those with a four-fold increase in hSBA titres post-vaccination were similar for each serogroup. A four-fold increase was observed in 80%, 56%, 45% and 63% of participants in the MenACWY liquid group for serogroups A, C, W and Y, respectively, with similar percentages in the

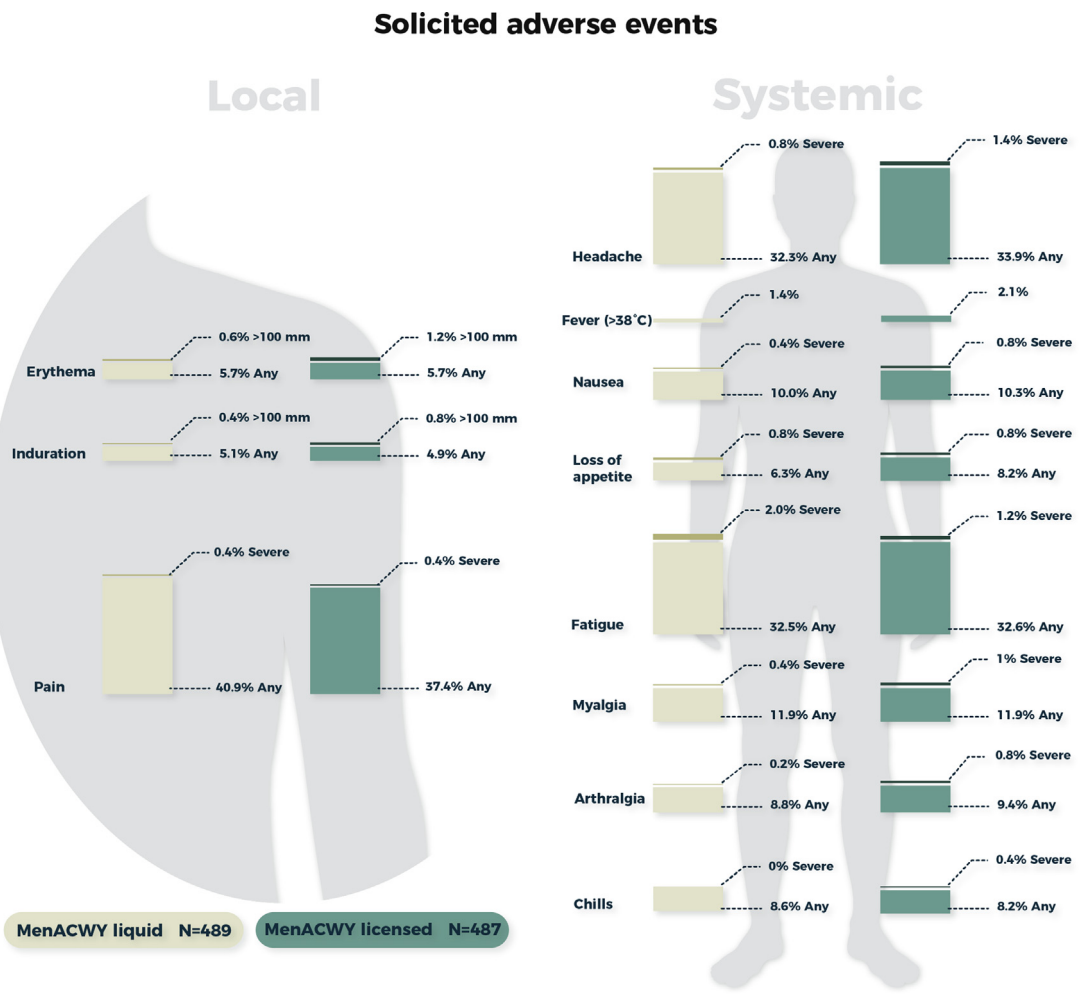

Fig. 4. Percentages of participants reporting solicited local and systemic adverse events within 7 days of vaccination (solicited safety population). MenACWY, quadrivalent meningococcal glycoconjugate vaccine; N, number of participants. Severe defined as preventing normal activity or, for loss of appetite, not eating at all.

MenACWY licensed group. Interpretation of these values needs to take into account the relatively high percentages of adults with hSBA titre ≥8 before vaccination for serogroups C, W and Y: 54%, 40% and 23% in the MenACWY liquid group and comparable percentages (54%, 46% and 26%) in the licensed vaccine group. Considering the exclusion criterion related to prior meningococcal vaccination, this is likely to be due, at least in part, to natural exposure; it is known that serogroups C, W and Y have caused a large proportion of IMD outbreaks in the regions involved in this study (Europe, North America and Australasia) in recent years [3].

The reactogenicity and safety profile of the liquid presentation was similar to that of the licensed vaccine and both presentations were well tolerated, consistent with the safety data reported in other trials of the licensed MenACWY-CRM vaccine in adults [5,13,14]. No SAEs related to vaccination were reported during the 6-month follow-up period and there were no withdrawals due to an AE.

Interpretation of the overall results of this study is partly limited by the fact that non-inferiority was not planned to be formally assessed for serogroups C, W and Y. However, these serogroup components of the investigational presentation were identical to those of the licensed vaccine and the descriptive analyses indicate similar immunogenicity results for all serogroups in both groups, irrespective of the vaccine presentation.

5. Conclusion

When comparing an improved fully liquid MenACWY-CRM vaccine presentation with the currently licensed presentation (lyophilised MenA reconstituted with liquid MenCWY), the immunogenicity of the new presentation was non-inferior for the modified component (MenA) and similar for the unmodified components (serogroups C, W, Y). These results were obtained with a liquid vaccine that was aged artificially under controlled conditions to include approximately 30% MenA FS and 40% MenA O-acetylation, in order to establish vaccine release (and shelf-life) specifications. The clinical tolerability and safety profile of the two presentations was similar and no safety concerns arose with administration of the improved vaccine presentation. The single vial fully liquid MenACWY-CRM vaccine, once licensed, will offer a more convenient presentation of the quadrivalent CRM-conjugated meningococcal vaccine, to further support the public health attempt in preventing IMD due to *N. meningitidis* serogroups A, C, W and Y.

Declaration of Competing Interest

TA, SVH, MA, BK, BdW, GFDD, MC, PVS, EF, ML and MP are employed by the GSK group of companies. TA, MA, BdW, PVS, EF,

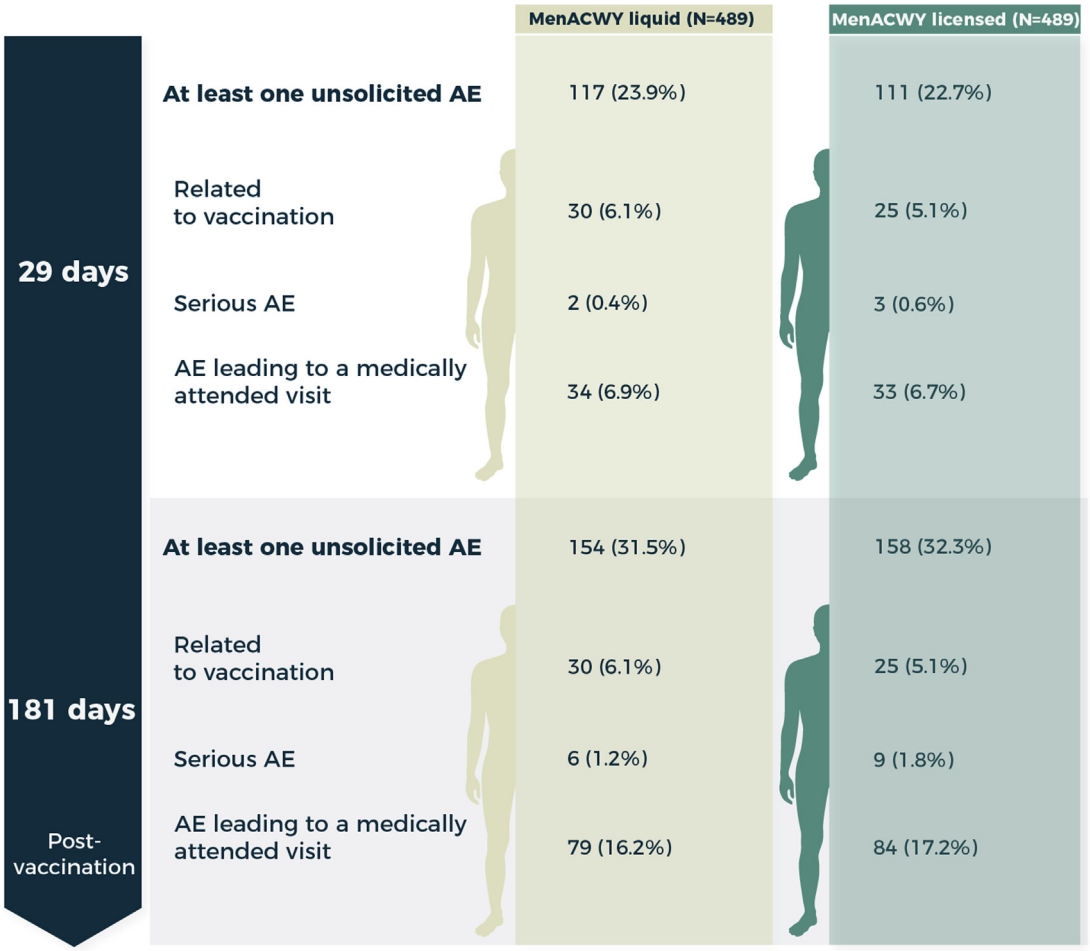

Fig. 5. Number (percentage) of participants reporting unsolicited adverse events (AEs) during 1-month and 6-month post-vaccination periods (unsolicited safety population). MenACWY, quadrivalent meningococcal glycoconjugate vaccine; N, number of participants.

ML and MP hold shares in the GSK group of companies. CV reports her institution received payments from the GSK group of companies, MSD and Pfizer, outside the submitted work. ILR reports her institution received payments from the GSK group of companies for the conduct of the study. TN reports payments from the GSK group of companies, Merck, Sanofi Pasteur and Seqirus, outside the submitted work. TA, SVH, MA, BK, BdW, GFDD, MC, PVS, EF, ML, MP, CV, ILR and TN declare no other financial and non-financial relationships and activities. JV, TS, GG, MF, GI, TFS, AMN and SC declare no financial and non-financial relationships and activities and no conflicts of interest.

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Authors' contributions

TA, MA, EF, ML and MP were involved in the study conception and design. CV, ILR, JV, TS, GG, MF, GI, TFS, AMN, TN, SC, TA, SVH, BK, BdW and MP were involved in acquisition and generation of data and/or performed the study. CV, TA, GFDD, MC, PVS, ML and MP were involved in data analysis and data interpretation. All authors contributed substantially to the development of the manuscript and approved the final version. All authors attest they meet the ICMJE criteria for authorship.

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Trademark

Menveo is a trademark owned by or licensed to the GSK group of companies.

Data sharing statement

Anonymised individual participant data and study documents can be requested for further research from www.clinicalstudydatarequest.com.

References

[1] Wang B, Santoreneos R, Giles L, Haji Ali Afzali H, Marshall H. Case fatality rates of invasive meningococcal disease by serogroup and age: a systematic review and meta-analysis. Vaccine 2019;37(21):2768–82. https://doi.org/10.1016/j.vaccine.2019.04.020.

[2] Olbrich KJ, Müller D, Schumacher S, Beck E, Meszaros K, Koerber F. Systematic review of invasive meningococcal disease: sequelae and quality of life impact on patients and their caregivers. Infect Dis Ther 2018;7(4):421–38. https://doi.org/10.1007/s40121-018-0213-2.

[3] Parikh SR, Campbell H, Bettinger JA, Harrison LH, Marshall HS, Martinon-Torres F, et al. The everchanging epidemiology of meningococcal disease worldwide and the potential for prevention through vaccination. J Infect 2020;81(4):483–98. https://doi.org/10.1016/j.jinf.2020.05.079.

[4] Keshavan P, Pellegrini M, Vadivelu-Pechai K, Nissen M. An update of clinical experience with the quadrivalent meningococcal ACWY-CRM conjugate vaccine. Expert Rev Vaccines 2018;17(10):865–80. https://doi.org/10.1080/14760584.2018.1521280.

[5] Bröker M, Cooper B, Detora LM, Stoddard JJ. Critical appraisal of a quadrivalent CRM_{197} conjugate vaccine against meningococcal serogroups A, C W-135 and Y (Menveo) in the context of treatment and prevention of invasive disease. Infect Drug Resist 2011;4:137–47. https://doi.org/10.2147/idr.s12716.

[6] Moriconi A, Onnis V, Aggravi M, Parlati C, Bufali S, Cianetti S, et al. A new strategy for preparing a tailored meningococcal ACWY conjugate vaccine for clinical testing. Vaccine 2020;38(23):3930–3. https://doi.org/10.1016/j.vaccine.2020.04.005.

[7] Berti F, Romano MR, Micoli F, Pinto V, Cappelletti E, Gavini M, et al. Relative stability of meningococcal serogroup A and X polysaccharides. Vaccine 2012;30(45):6409–15. https://doi.org/10.1016/j.vaccine.2012.08.021.

[8] Beresford NJ, Martino A, Feavers IM, Corbel MJ, Bai X, Borrow R, et al. Quality, immunogenicity and stability of meningococcal serogroup ACWY-CRM_{197}, DT and TT glycoconjugate vaccines. Vaccine 2017;35(28):3598–606. https://doi.org/10.1016/j.vaccine.2017.03.066.

[9] Berry DS, Lynn F, Lee C-H, Frasch CE, Bash MC. Effect of O acetylation of *Neisseria meningitidis* serogroup A capsular polysaccharide on development of functional immune responses. Infect Immun 2002;70(7):3707–13. https://doi.org/10.1128/IAI.70.7.3707-3713.2002.

[10] Lupisan S, Limkittikul K, Sosa N, Chanthavanich P, Bianco V, Baine Y, et al. Meningococcal polysaccharide A O-acetylation levels do not impact the immunogenicity of the quadrivalent meningococcal tetanus toxoid conjugate vaccine: results from a randomized, controlled phase III study of healthy adults aged 18 to 25 years. Clin Vaccine Immunol 2013;20(10):1499–507. https://doi.org/10.1128/CVI.00162-13.

[11] Clopper CJ, Pearson ES. The use of confidence or fiducial limits illustrated in the case of the binomial. Biometrika 1934;26(4):404–13.

[12] Miettinen O, Nurminen M. Comparative analysis of two rates. Stat Med 1985;4(2):213–26. https://doi.org/10.1002/(ISSN)1097-025810.1002/sim.v4:210.1002/sim.4780040211.

[13] Stamboulian D, Lopardo G, Lopez P, Cortes-Barbosa C, Valencia A, Bedell L, et al. Safety and immunogenicity of an investigational quadrivalent meningococcal CRM_{197} conjugate vaccine, MenACWY-CRM, compared with licensed vaccines in adults in Latin America. Int J Infect Dis 2010;14(10):e868–75. https://doi.org/10.1016/j.ijid.2010.03.017.

[14] Reisinger KS, Baxter R, Block SL, Shah J, Bedell L, Dull PM. Quadrivalent meningococcal vaccination of adults: phase III comparison of an investigational conjugate vaccine, MenACWY-CRM, with the licensed vaccine, Menactra. Clin Vaccine Immunol 2009;16(12):1810–5. https://doi.org/10.1128/CVI.00207-09.

[15] Poolman JT, De Vleeschauwer I, Durant N, Devos N, Feron C, Lestrate P, et al. Measurement of functional anti-meningococcal serogroup a activity using strain 3125 as the target strain for serum bactericidal assay. Clin Vaccine Immunol 2011;18(7):1108–17. https://doi.org/10.1128/CVI.00549-10.