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Adjuvant effect of TLR7 agonist adsorbed on aluminum hydroxide (AS37): A phase I randomized, dose escalation study of an AS37-adjuvanted meningococcal C conjugated vaccine

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ABSTRACT

An adjuvant system (AS37) has been developed containing a synthetic toll-like receptor agonist (TLR7a). We conducted a phase I randomized, observer-blind, dose-escalation study to assess the safety and immunogenicity of an investigational AS37-adjuvanted meningococcus C (MenC) conjugate vaccine in healthy adults (NCT02639351). A control group received a licensed MenC conjugate alum-adjuvanted vaccine. Eighty participants were randomized to receive one dose of control or investigational vaccine containing AS37 (TLR7a dose 12.5, 25, 50, 100 μg). All vaccines were well tolerated, apart from in the TLR7a 100 μg dose group, which had three reports (18.8%) of severe systemic adverse events. Four weeks after vaccination, human complement serum bactericidal assay seroresponse rates against MenC were 56–81% in all groups, and ELISA seroresponses were ≥81% for all AS37-adjuvanted vaccine groups (100% in 50 and 100 μg dose groups) and 88% in the control group. Antibody responses were maintained at six months after vaccination.

1. Introduction

Adjuvants are required to improve the immunogenicity of vaccines containing purified antigens and to facilitate the development of new vaccines [1]. An objective of many adjuvant discovery and development programs is to elicit more effective engagement of T-helper cells to optimize the quality, breadth and durability of antibody responses or to induce effector $CD4^+$ or $CD8^+$ T cells to kill intracellular pathogens [2,3]. One approach is to use agonists for toll-like receptors (TLRs) that activate innate immune cascades, mainly on antigen-presenting cells, and facilitate the generation of T-helper cell responses [4].

A new class of adjuvants, small molecule immune potentiators (SMIPs), has been identified that target TLRs [5]. TLR7/8 SMIPs include imidazoquinolines and benzazepines; most research has been on imidazoquinolines, such as imiquimod and resiquimod, which are potent cytokine inducers and have beneficial effects in the topical treatment of various skin disorders [6–8]. Imiquimod also significantly improved immunogenicity against an intradermal influenza vaccine when applied as a topical skin adjuvant [9]. There have however been reports of systemic adverse events (AEs) associated with topical imidazoquinoline use [10–14]. New formulations were needed to retain TLR7/8 adjuvants at the injection site and augment immune activation without inducing systemic cytokine release [6].

There have been promising developments in TLR7/8 delivery systems, including encapsulating nanoparticles [15,16] and lipid conjugation [17,18]. Another approach has been adsorption to aluminum hydroxide (alum) adjuvants. This includes a SMIP adjuvant system (AS37) containing a synthetic TLR7 agonist, TLR7a, with a benzonaphthyridine chemical scaffold, adsorbed to alum. Adjuvants based on insoluble salts of aluminum have been shown to be well tolerated and effective components of human vaccines over many decades of use [19].

Pre-clinical studies of AS37 demonstrated a superior capacity in eliciting an effective immune response when compared to alum-adjuvanted controls in animal models using several target pathogens

Abbreviations: AE, adverse event; CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; GMC, geometric mean concentration; GMT, geometric mean titer; hSBA, human complement serum bactericidal assay; IDMC, independent data monitoring committee; MenC, *Neisseria meningitidis* serogroup C; NOCD, new onset chronic disease; PPS, per-protocol set; SAE, serious adverse event; SMIP, small molecule immune potentiator; TLR, toll-like receptor

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[5,20–22]. This included a significantly higher persistence of protective antibody titers against *Neisseria meningitidis* serogroup C (MenC) 8 months after the last immunization in mice [21]. The adsorption of TLR7a to alum also significantly improved expansion of the memory B cell compartment in mice [23].

We now present results from a first-time-in-human study of AS37 to assess the safety, reactogenicity, and immunogenicity profiles of an investigational AS37-adjuvanted MenC-CRM_{197} conjugate vaccine (AS37-MenC-CRM_{197}) compared to those of the licensed MenC-CRM_{197} conjugate vaccine (*Menjugate*, GSK) in healthy adults. *Menjugate* has well established safety and immunogenicity profiles, and contains alum as adjuvant [24]. The alum dose was kept constant in each study group, enabling an assessment of the impact of different AS37 doses in the investigational vaccine groups.

2. Methods

2.1. Study design and participants

This was a phase I, randomized, observer blind, adjuvant dose escalation study performed at a single center in Germany between March 2016 and August 2017 (ClinicalTrials.gov identifier: NCT02639351). Healthy adults aged 18–45 years were enrolled, as determined by medical history, physical examination, and the results of screening tests. Inclusion and exclusion criteria are listed in the Supplementary Methods. The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. The protocols and associated documents were reviewed and approved by an independent ethics committee. All participants provided written informed consent before study entry. An independent data monitoring committee (IDMC) was appointed to oversee safety of the study participants.

Participants were randomized to receive a single intramuscular vaccination of either the licensed alum-adjuvanted MenC-CRM_{197} conjugate vaccine (*Menjugate*; control) or the investigational vaccine, AS37-MenC-CRM_{197}. For the dose escalation of AS37 (specifically, TLR7a), the participants were enrolled into one of four cohorts in a step-wise manner, with 20 participants (four receiving control vaccine and 16 the investigational vaccine) per cohort. Within each cohort, participants were randomized using an electronic data capture system in a 1:4 ratio to receive either the control MenC-CRM_{197} vaccine (four groups of four participants) or the investigational vaccine, with each cohort receiving increasing doses of TLR7a (12.5, 25, 50, or 100 μg; four groups of 16 participants). In each cohort, the first five participants (i.e., the first 1:4 randomization block) were vaccinated at a rate of one participant per day. Enrolment was then paused for review of the safety results up to Day 14 by the IDMC. The remaining 15 participants were then enrolled. The IDMC also reviewed safety results obtained through Day 29 before enrolment of the next cohort. Predefined criteria were in place to halt the study if there were safety concerns, based on Food and Drug Administration guidelines [25].

The study was conducted in an observer-blind manner, i.e. vaccine recipients and those responsible for the evaluation of any study endpoint were blinded to the administered vaccines and vaccines were prepared and administered by authorized medical personnel who did not participate in any of the study clinical evaluations or assays.

2.2. Study vaccines

A 0.5 mL single dose of the control vaccine contained MenC oligosaccharides (10 μg) conjugated to the protein carrier CRM_{197} (12.5–25.0 μg). Before administration, the diluent, containing alum, sodium chloride, and water for injection, was added to the lyophilized vaccine, containing mannitol and sodium phosphate. A 0.5 mL single dose of investigational vaccine had the same contents as the control vaccine with the addition in the diluent of TLR7a (12.5, 25, 50, or 100 μg), histidine (0.1–0.8 mg), and tris(hydroxymethyl)aminomethane (30–243 μg). The preparation and physicochemical characterization of AS37 (containing TLR7a adsorbed to alum) was described previously [26]; for all formulations, TLR7a was completely adsorbed to alum. The vaccines were injected in the deltoid muscle of the non-dominant arm.

2.3. Reactogenicity and safety

The primary safety objective was to assess the safety of the investigational vaccine as compared to that of the control MenC-CRM_{197} vaccine. Solicited local (induration, pain, erythema, and swelling) and systemic (arthralgia, chills, diarrhea, fatigue, fever, headache, loss of appetite, myalgia, nausea, rash, urticaria, and vomiting) AEs were recorded directly into the clinical database on the day of vaccination (Day 1, after the first 30 min following vaccination) and on diary cards for the following 13 days (to Day 14). Fever was defined as temperature ≥38.0 °C. Unsolicited AEs, serious AEs (SAEs), AEs of special interest, new onset chronic disease (NOCD), medically-attended AEs, and AEs leading to withdrawal were recorded throughout the study. AEs of special interest were as defined by the Committee for Medicinal Products for Human Use of the European Medicines Agency [27] and included anaphylaxis, Bell's palsy, convulsion, demyelination, encephalitis, Guillain-Barré syndrome, neuritis, and vasculitis. NOCD included conditions such as auto-immune disorders, allergies, type 1 diabetes, and asthma.

The intensity of each solicited symptom or AE was graded as mild, moderate, or severe. Severe induration, erythema, or swelling was defined as a diameter > 100 mm, severe temperature as > 39.5 °C, and all other severe AEs as preventing normal activities.

Hematological and biochemical parameters, measured before and 24 h after vaccination, and at Days 8 and 29 were graded 0–4 [25]. Abnormal laboratory findings that were judged by the investigator to be clinically significant were recorded as an AE or SAE.

An exploratory safety analysis was also conducted to assess plasma concentrations of TLR7a up to 72 h after vaccination, measured by liquid chromatography/tandem mass spectrometry, from participants who signed a separate informed consent form.

2.4. Humoral immunogenicity

Blood samples were taken before vaccination, at Days 8 and 29, and six months after vaccination (Day 181) for evaluation of the primary and secondary immunogenicity endpoints. Additional blood specimens were collected for assessment of exploratory endpoints before vaccination and at Days 1, 4, 8, 29, and 181 from participants who signed a separate informed consent form.

Immune responses were evaluated by human complement serum bactericidal assay (hSBA) directed against MenC [28] and by enzyme-linked immunosorbent assay (ELISA) to MenC polysaccharide [29]. An hSBA seroresponse was defined as a post-vaccination hSBA titer ≥8 for participants with a pre-vaccination hSBA titer < 4 or an increase of at least four times baseline hSBA level for those with pre-vaccination hSBA titer ≥4. An ELISA seroresponse was defined as an increase in antibody concentration of at least four times baseline concentration for participants with pre-vaccination concentration ≥0.06 μg/mL or post-vaccination concentration ≥0.12 μg/mL for those with pre-vaccination concentration < 0.06 μg/mL.

2.5. Statistical analysis

As this was a first-time-in-human study and because of the primary safety objective for the study, sample size was based on the probability of observing at least one participant with a given event, assuming a given frequency of AEs following vaccination. With 16 participants per cohort receiving the active vaccine, AEs with frequency ≥15% could be detected with at least 90% probability and AEs with frequency ≥10% could be detected with 81% probability. With four participants per

cohort receiving the control vaccine, AEs with frequency ≥30% could be detected with at least 80% probability.

Safety analyses were conducted on all exposed participants (total vaccinated cohort). The incidence of AEs per study group was calculated. The immunogenicity analysis was performed on the per-protocol set (PPS) for immunogenicity, including participants who received the study vaccine correctly and complied with study procedures. Geometric mean titers (GMTs) measured by hSBA, antibody geometric mean concentrations (GMCs) measured by ELISA, and seroresponse rates were estimated using two-sided 95% Clopper-Pearson confidence intervals (CIs). For the immunogenicity endpoints, the logarithmically (base 10) transformed antibody titers were modelled using an analysis of covariance model with a qualitative factor for the AS37 dose (0, 12.5, 25, 50, or 100 μg) and log (base 10) pre-vaccination titer as a covariate. The adjusted GMT and the two-sided 95% CIs of the GMT were estimated based on this model, as was the ratio of GMTs and corresponding CIs.

All analyses were performed using SAS software version 9.3.

3. Results

3.1. Study population

Eighty healthy adults aged 18–45 years were enrolled and vaccinated in the study, and were included in the safety analyses and PPS for immunogenicity. The demographic characteristics of the participants were similar across the five study groups (Table 1). All participants were Caucasian (European heritage), apart from one participant in the AS37-50 μg dose group, whose ethnic background was reported as unknown.

3.2. Reactogenicity and safety

The study vaccine formulations containing AS37 with TLR7a dose 12.5–50 μg were well tolerated, with no clear trend in the incidence of AEs (solicited or unsolicited) in relation to AS37 dose escalation. All participants in each group reported at least one solicited local AE (all reported injection site pain and a maximum of two participants per group reported other solicited local AEs; Fig. 1) and 44–75% reported at least one solicited systemic AE (Table 2). The most commonly reported solicited local AE in each group was pain and the most common solicited systemic AEs were headache and fatigue (Fig. 1). There was one report of severe pain in the AS37–100 μg group; all other local solicited AEs were mild to moderate in severity (Supplementary Table 1). In the AS37-100 μg group, there were three reports of severe solicited systemic AEs (18.8%), including one report each of headache and nausea and one report of fever with temperature > 40.0 °C on Days 5 and 6 that resolved by Day 11. In the other groups, one severe solicited systemic AE was reported (severe chills in AS37-25 μg group).

The number of participants experiencing unsolicited AEs was comparable between the study vaccine groups and the control MenC-CRM$_{197}$ vaccine group (Table 2). Most unsolicited AEs were reported by one participant each; only nasopharyngitis was reported by more than two participants in at least one group. One case of intermittent, mild dizziness (in AS37-25 μg group), which was considered as possibly or probably related to the study vaccine, was reported from Day 1 to Day 14 of the study.

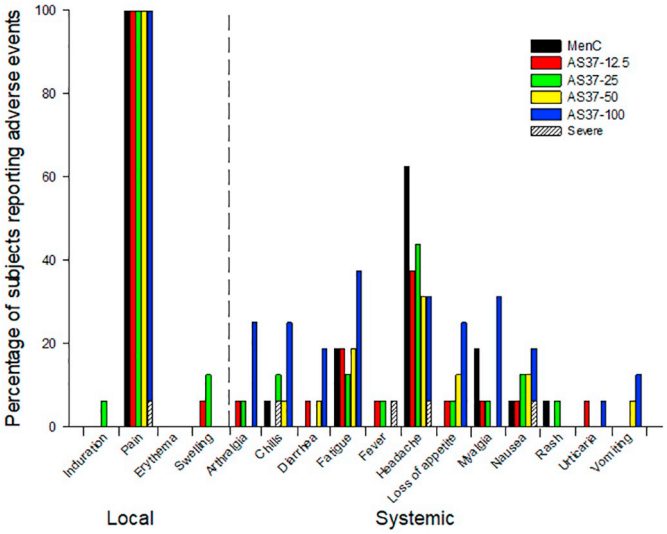

Fig. 1. Percentages of participants reporting solicited local (induration, pain, erythema, and swelling) and systemic (arthralgia, chills, diarrhea, fatigue, fever, headache, loss of appetite, myalgia, nausea, rash, urticaria, and vomiting) adverse events during the 14-day post-vaccination period (total vaccinated cohort: 16 participants in each group).
MenC, control alum-adjuvanted MenC-CRM$_{197}$ conjugate vaccine (*Menjugate*); AS37-12.5/25/50/100, investigational AS37-MenC-CRM$_{197}$ vaccine containing alum and TLR7a dose 12.5/25/50/100 μg.
Severe defined as diameter > 100 mm for induration, erythema, and swelling; fever with temperature > 39.5 °C; decreased oral intake with weight loss for loss of appetite; nausea leading to minimal or no oral intake; vomiting requiring outpatient hydration; at least 6 watery stools in 24 h or outpatient intravenous hydration required for diarrhea; rash/urticaria on most of the skin; preventing normal activities for all other adverse events. Severe event incidences include one report of fever with temperature > 40.0 °C in AS37-100 μg group.

There were no reports of an AE leading to withdrawal from the study, NOCD, or AEs of special interest. One SAE (joint injury in the control MenC-CRM$_{197}$ vaccine group) was reported, which was not considered related to vaccine administration. No clinically relevant laboratory abnormalities were observed during the study and none were reported as AEs.

The plasma concentration-time profile showed that levels of TLR7a slightly increased with dose, peaking at levels below 100 ng/L at the 8 h time point (Table 3). Thereafter, TLR7a concentrations decreased slowly up to the 72 h time point.

3.3. Immunogenicity

The immune response to vaccination was measured by hSBA directed against MenC and by ELISA to MenC polysaccharide. All vaccines

Table 1
Demographic characteristics of the study participants (total vaccinated cohort).

Characteristic	Control MenC group (*N* = 16)	AS37-MenC-CRM$_{197}$ group			
		AS37-12.5 (*N* = 16)	AS37-25 (*N* = 16)	AS37-50 (*N* = 16)	AS37–100 (*N* = 16)
Mean age, years (SD)	34.4 (7.85)	36.8 (7.51)	33.8 (6.89)	34.9 (5.18)	34.4 (6.72)
Age range, years	20–45	22–45	22–45	27–45	23–44
Male gender, n (%)	10 (62.5)	13 (81.3)	12 (75.0)	11 (68.8)	10 (62.5)

N, number of participants; n, number of participants in a specific category; SD, standard deviation.
Control MenC group received licensed alum-adjuvanted MenC-CRM$_{197}$ conjugate vaccine (*Menjugate*). AS37-MenC-CRM$_{197}$ groups received investigational AS37-adjuvanted MenC-CRM$_{197}$ conjugate vaccine. AS37 contained alum and TLR7a dose 12.5, 25, 50, or 100 μg.

Table 2
Participants reporting solicited adverse events (AEs) during the 2-week period after vaccination (Day 1 to Day 14) and unsolicited AEs during the whole study (total vaccinated cohort).

	Number (percentage) of participants				
	Control MenC group (N = 16)	AS37-MenC-CRM$_{197}$ group			
		AS37-12.5 (N = 16)	AS37-25 (N = 16)	AS37-50 (N = 16)	AS37-100 (N = 16)
At least one solicited AE[a]					
Local any grade	16 (100)	16 (100)	16 (100)	16 (100)	16 (100)
Local Severe	0	0	0	0	1 (6.3)
Systemic any grade	12 (75.0)	7 (43.8)	8 (50.0)	9 (56.3)	10 (62.5)
Systemic Severe	0	0	1 (6.3)	0	3 (18.8)[†]
At least one unsolicited AE					
Any unsolicited AE	9 (56.3)	7 (43.8)	10 (62.5)	6 (37.5)	9 (56.3)
Unsolicited AE related to vaccination	0	0	1 (6.3)	0	0
Severe unsolicited AE	0	0	0	0	0
Unsolicited AEs reported by ≥2 participants in at least one group					
Nasopharyngitis	3 (18.8)	3 (18.8)	2 (12.5)	1 (6.3)	3 (18.8)

N, number of participants. Control MenC group received licensed alum-adjuvanted MenC-CRM$_{197}$ conjugate vaccine (*Menjugate*). AS37-MenC-CRM$_{197}$ groups received investigational AS37-adjuvanted MenC-CRM$_{197}$ conjugate vaccine. AS37 contained alum and TLR7a dose 12.5, 25, 50, or 100 μg.

[a] Solicited local (induration, pain, erythema, and swelling) and systemic (arthralgia, chills, diarrhea, fatigue, fever, headache, loss of appetite, myalgia, nausea, rash, urticaria, and vomiting) AEs. Moderate defined as diameter 51–100 mm for induration, erythema, and swelling; fever with temperature 38.5–39.4 °C; decreased oral intake without weight loss for loss of appetite; nausea leading to decreased oral intake; > 2 vomiting episodes in 24 h; 4 or 5 loose stools in 24 h for diarrhea; rash/urticaria in at least 2 body regions without whole body involvement; interfering with normal activities for all other AEs. Severe defined as diameter > 100 mm for induration, erythema, and swelling; fever with temperature > 39.5 °C; decreased oral intake with weight loss for loss of appetite; nausea leading to minimal or no oral intake; vomiting requiring outpatient hydration; at least 6 watery stools in 24 h or outpatient intravenous hydration required for diarrhea; rash/urticaria on most of the skin; preventing normal activities for all other AEs. [†]Two severe AEs and one report of fever > 40.0 °C.

induced an immune response measured by hSBA (Table 4). The seroresponse rate measured by hSBA was 69–81% for AS37-adjuvanted vaccine groups at Day 29, which was comparable to that in the control MenC-CRM$_{197}$ group (75%). There was no apparent dose-response relationship with increasing TLR7a dose, with broad and overlapping 95% CIs for GMTs and GMT ratios (baseline: Day 29) among the study groups. The seroresponse rates were maintained at comparable titers in all groups at the six months post-vaccination time point (Table 4).

Antibody GMCs measured by ELISA showed a trend for higher MenC specific antibody titers in the groups receiving AS37 than in the control MenC-CRM$_{197}$ group, although 95% CIs overlapped (Table 5). Seroresponses at Day 29 measured by ELISA were 100% in the AS37-50 μg and 100 μg dose groups, 94% and 81% in the AS37-12.5 μg and AS37-25 μg dose groups, respectively, and 88% in the control MenC-CRM$_{197}$ group (Table 5). Comparable responses were observed at Day 181.

4. Discussion

This is the first report of the safety, reactogenicity, and immunogenicity of a vaccine including a novel SMIP adjuvant system (AS37) containing TLR7a adsorbed to alum, administered to healthy adults. The objective driving the development of AS37 was to achieve a vaccine adjuvant system with strong immunopotentiating properties and minimal side effects.

The safety and superior immunogenicity of AS37 over alum was demonstrated in pre-clinical studies [5,20–23]. Additionally, a recent comparative study evaluating the same TLR7 agonist molecule in nonhuman primates showed consistent adjuvanticity properties regardless of it being adsorbed to alum or coformulated with a lipidic nanoemulsion [30]. In the assessment of the safety and immunopotentiating properties of this novel adjuvant in humans, *Menjugate* was selected as control because of the extensive safety data available for this vaccine [24] and because it is also adsorbed to alum. Since the alum dose was constant in all groups, this enabled evaluation of the effects of TLR7a dose escalation in groups receiving the investigational AS37-MenC-CRM$_{197}$ vaccine.

The investigational vaccine formulations containing AS37 with TLR7a dose 12.5–50 μg had acceptable reactogenicity and safety profiles, which were comparable to those of the control MenC-CRM$_{197}$ vaccine. In the AS37-100 μg group, there were three reports of severe solicited systemic AEs. No clinically-significant abnormal laboratory values were reported and no participants withdrew from the study

Table 3
TLR7a geometric mean plasma concentrations measured by liquid chromatography/tandem mass spectrometry up to 72 h after vaccination.

	Control MenC group (N = 12)	AS37-MenC-CRM$_{197}$ group			
		AS37-12.5 (N = 14)	AS37-25 (N = 13)	AS37-50 (N = 15)	AS37-100 (N = 12)
GMC before vaccination, ng/L (95% CI)	2.50 (2.50–2.50)	2.50 (2.50–2.50)	2.50 (2.50–2.50)	2.50 (2.50–2.50)	2.50 (2.50–2.50)
GMC post-vaccination, ng/L (95% CI)					
1 h	2.50 (2.50–2.50)	17.24 (13 – 23)	27.06 (19–39)	30.41 (25–37)	37.12 (29–48)
2 h	2.50 (2.50–2.50)	16.65 (10–26)	22.87 (16–33)	32.10 (25–42)	38.27 (30–49)
4 h	2.50 (2.50–2.50)	11.96 (6.85–21)	20.48 (14–31)	30.39 (22–41)	38.88 (31–49)
8 h	2.50 (2.50–2.50)	18.01 (12–26)	32.12 (25–42)	50.34 (40–63)	82.58 (62–110)
24 h	2.50 (2.50–2.50)	15.69 (12 – 21)	28.80 (21–39)	48.29 (42–56)	63.47 (49–83)
72 h	2.50 (2.50–2.50)	2.72 (2.27–3.25)	6.97 (4.63–10)	16.38 (12–21)	35.63 (26–48)

GMC, geometric mean concentration; 95% CI, 95% confidence interval; N, number of participants who provided consent for exploratory assessments. Control MenC group received licensed alum-adjuvanted MenC-CRM$_{197}$ conjugate vaccine (*Menjugate*). AS37-MenC-CRM$_{197}$ groups received investigational AS37-adjuvanted MenC-CRM$_{197}$ conjugate vaccine. AS37 contained alum and TLR7a dose 12.5, 25, 50, or 100 μg.

Table 4
Anti-MenC antibody geometric mean titer (GMT) measured by human complement serum bactericidal assay (hSBA) before vaccination and one week (Day 8), four weeks (Day 29), and six months (Day 181) after vaccination (per-protocol set for immunogenicity).

	Control MenC group ($N = 16$[a])	AS37-MenC-CRM_{197} group			
		AS37-12.5 ($N = 16$)	AS37-25 ($N = 16$)	AS37-50 ($N = 16$)	AS37-100 ($N = 16$)
GMT (95% CI)					
Before vaccination	6.17 (3.37–11.28)	11.40 (6.23–20.86)	8.52 (4.66–15.59)	6.09 (3.33–11.13)	5.23 (2.86–9.57)
1 week post-vaccination	21.30 (8.80–51.53)	30.86 (12.61–75.50)	12.18 (5.04–29.47)	13.58 (5.61–32.87)	15.47 (6.20–38.65)
4 weeks post-vaccination	70.38 (25.83–191.79)	96.61 (34.97–266.89)	55.05 (20.19–150.06)	101.77 (37.34–277.39)	56.31 (20.55–154.29)
Ratio, 4 weeks:baseline post-vaccination	11.32 (3.60–35.57)	8.70 (2.77–27.36)	6.52 (2.08–20.51)	16.56 (5.27–52.07)	10.57 (3.36–33.23)
6 months post-vaccination	59.67 (29.61–120.27)	98.79 (49.66–196.52)	68.89 (34.94–135.83)	83.59 (42.40–164.78)	63.13 (31.91–124.91)
Seroresponse,[b] % (95% CI)					
1 week post-vaccination	37.5 (15.2–64.6)	31.3 (11.0–58.7)	37.5 (15.2–64.6)	31.3 (11.0–58.7)	33.3 (11.8–61.6)
4 weeks post-vaccination	75.0 (47.6–92.7)	75.0 (47.6–92.7)	56.3 (29.9–80.2)	81.3 (54.4–96.0)	68.8 (41.3–89.0)
6 months post-vaccination	80.0 (51.9–95.7)	75.0 (47.6–92.7)	56.3 (29.9–80.2)	87.5 (61.7–98.4)	75.0 (47.6–92.7)

95% CI, 95% confidence interval; N, number of participants. Control MenC group received licensed alum-adjuvanted MenC-CRM_{197} conjugate vaccine (*Menjugate*). AS37-MenC-CRM_{197} groups received investigational AS37-adjuvanted MenC-CRM_{197} conjugate vaccine. AS37 contained alum and TLR7a dose 12.5, 25, 50, or 100 μg.

[a] N = 15 for 6 months post-vaccination time point.

[b] Defined as post-vaccination titer ≥8 for participants with pre-vaccination hSBA titer < 4 or increase of at least four times baseline hSBA level for participants with pre-vaccination hSBA titer ≥4.

because of an AE. One SAE (joint injury) was reported that was not considered related to vaccine administration. TLR7a plasma levels slightly increased with adjuvant dose to a maximum level below 100 ng/L, suggesting limited systemic exposure to the SMIP, as expected from preclinical observations from rat and dog toxicokinetic models of TLR7 (unpublished results). For the 12.5–50 μg formulations, there was no clear trend in the occurrence of AEs (solicited or unsolicited) relative to adjuvant dose escalation. However, we cannot exclude the possibility that, with the highest dose formulation, the TLR7a plasma level may have contributed to the 18.8% rate of severe solicited systemic AEs in this group.

All vaccines induced an antibody immune response four weeks post-vaccination measured by hSBA, with no apparent dose-response relationship with increasing TLR7a dose. GMT and GMT ratio 95% CIs were large and overlapped among the control MenC-CRM_{197} and AS37-adjuvanted vaccine groups. There was a trend for higher MenC specific antibody concentrations by ELISA in the groups receiving AS37, with highest concentrations in the AS37-50 μg and AS37-100 μg dose groups, but again 95% CIs overlapped. Although the study was not powered to compare immunoresponse between groups, the ELISA assessment of antibody quantity may have revealed slight differences among groups that were not measurable with a less sensitive functional assay, such as hSBA. The seroresponse rate measured by hSBA was up to 81% four weeks after vaccination for the AS37-adjuvanted vaccine groups, which was comparable to the rate with the control MenC-CRM_{197} vaccine. For seroresponse measured by ELISA, rates were high in all groups four weeks post-vaccination, reaching 100% in the AS37-50 μg and AS37-100 μg dose groups and 88% in the control group. Seroresponse rates measured by both assays persisted in all groups at six months post-vaccination. There was no evidence of a faster seroresponse in the AS37-adjuvanted vaccine groups than in the control group one week after vaccination. Up to now, it has been difficult to demonstrate an added value of adjuvants in potentiating the immune response to glycoconjugates [21]. Interpretation of our immunoresponse observations should take into account that the control MenC-CRM_{197} vaccine has strong immunogenicity [24], as confirmed by results in our study, and both the control and investigational vaccine formulations contain alum in the adjuvant. Moreover, this study was conducted in young, healthy individuals, who typically have a robust immune response to the MenC vaccine [31] and therefore do not require a vaccine with additional adjuvantation. These factors are likely to have made it difficult to detect an improved immunoresponse with the inclusion of TLR7a in the investigational vaccine.

In conclusion, this phase I study of healthy adults demonstrated that investigational MenC-CRM_{197} vaccine formulations containing AS37

Table 5
Anti-MenC antibody geometric mean concentration (GMC) measured by enzyme-linked immunosorbent assay (ELISA) before vaccination and one week (Day 8), four weeks (Day 29), and six months (Day 181) after vaccination (per-protocol set for immunogenicity).

	Control MenC group (N = 16[a])	AS37-MenC-CRM_{197} group			
		AS37-12.5 (N = 16)	AS37-25 (N = 16)	AS37-50 (N = 16)	AS37-100 (N = 16)
GMC (95% CI)					
Before vaccination	0.199 (0.086–0.462)	0.287 (0.124–0.664)	0.407 (0.176–0.942)	0.207 (0.089–0.479)	0.107 (0.046–0.249)
1 week post-vaccination	1.445 (0.708–2.951)	2.005 (0.980–4.101)	2.169 (1.052–4.472)	1.668 (0.817–3.406)	2.897 (1.400–5.997)
4 weeks post-vaccination	9.207 (5.356–15.827)	15.946 (9.265–27.444)	12.753 (7.364–22.086)	19.322 (11.242–33.210)	21.944 (12.636–38.107)
6 months post-vaccination	3.432 (2.093–5.628)	5.201 (3.218–8.406)	5.556 (3.419–9.027)	7.131 (4.416–11.513)	7.570 (4.644–12.339)
Seroresponse[b], % (95% CI)					
1 week post-vaccination	62.5 (35.4–84.8)	68.8 (41.3–89.0)	50.0 (24.7–75.3)	68.8 (41.3–89.0)	68.8 (41.3–89.0)
4 weeks post-vaccination	87.5 (61.7–98.4)	93.8 (69.8–99.8)	81.3 (54.4–96.0)	100.0 (79.4–100.0)	100.0 (79.4–100.0)
6 months post-vaccination	86.7 (59.5–98.3)	87.5 (61.7–98.4)	75.0 (47.6–92.7)	100.0 (79.4–100.0)	100.0 (79.4–100.0)

95% CI, 95% confidence interval; N, number of participants. Control MenC group received licensed alum-adjuvanted MenC-CRM_{197} conjugate vaccine (*Menjugate*). AS37-MenC-CRM_{197} groups received investigational AS37-adjuvanted MenC-CRM_{197} conjugate vaccine. AS37 contained alum and TLR7a dose 12.5, 25, 50, or 100 μg.

[a] N = 15 for 6 months post-vaccination time point.

[b] Defined as increase in antibody GMC of at least four times baseline concentration for participants with pre-vaccination concentration ≥0.06 μg/mL or post-vaccination concentration ≥0.12 μg/mL for participants with pre-vaccination concentration < 0.06 μg/mL.

with TLR7a dose 12.5–50 μg have acceptable safety and reactogenicity profiles. All vaccine formulations induced immune responses that were maintained for six months. Despite the difficulty in showing additional enhancement in immunoresponse from AS37, the trend observed by ELISA is promising. Larger studies are required to confirm the immunopotentiating properties of this adjuvant candidate in different antigen models, and to investigate any association between systemic markers and the safety and immunogenicity of the vaccine. Future studies should also assess the advantages of this approach in relation to other TLR7/8 delivery systems, which are at earlier stages of development, such as encapsulating nanoparticles [15,16] and lipid conjugation [17,18].

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Data sharing

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

Disclosures

AGL, UDO, DTOH, GDG, ES, OF, and DM are employees of the GSK group of companies. UDO and OF hold restricted shares in the GSK group of companies. JO was an employee of the GSK group of companies and SB was a consultant employed by the GSK group of companies at the time of study conduct. TK and TF received fees through their institution for the conduct of the study.

Trademarks

Menjugate is a trademark owned by the GSK group of companies.

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Appendix A. Supplementary data

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