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Tetravalent meningococcal serogroups A, C, W-135 and Y conjugate vaccine is well tolerated and immunogenic when co-administered with measles–mumps–rubella–varicella vaccine during the second year of life: An open, randomized controlled trial

Timo Vesikari[a,*], Aino Karvonen[a], Veronique Bianco[b], Marie Van der Wielen[b], Jacqueline Miller[c]

[a] Vaccine Research Center, University of Tampere Medical School, Tampere, Finland
[b] GlaxoSmithKline Biologicals, Belgium
[c] GlaxoSmithKline Biologicals, United States

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ABSTRACT

Co-administration of meningococcal ACWY-tetanus toxoid conjugate vaccine (ACWY-TT) with MMRV vaccine was investigated in 1000 12–23-month old children randomized (3:3:1:1) to receive co-administered ACWY-TT + MMRV, or a single dose of ACWY-TT, MMRV or MenC-CRM$_{197}$. Non-inferiority of ACWY-TT to MenC-CRM$_{197}$ and non-inferiority of ACWY-TT + MMRV to ACWY-TT and MMRV alone, and the immunogenicity of serogroups AWY were demonstrated according to pre-defined criteria. Fever reactions in ACWY + MMRV and MMRV groups were comparable. ACWY-TT can be co-administered with MMRV without affecting immunogenicity or safety profiles of either vaccine.

This study has been registered at www.clinicaltrials.gov NCT00474266.

1. Introduction

Between 1999 and 2004, around 50,000 cases of invasive meningococcal disease (IMD) were reported across Europe, with a case fatality rate of 8% [1]. The incidence of IMD peaks during infancy and early childhood, and again during adolescence. During 2004, the incidence of IMD in Europe among children was between 6–7/100,000 in 1–4 year olds, approximately 3/100,000 in 15–19 year olds and less than 1/100,000 in adult age groups [1]. There are five major disease-causing serogroups (A, B, C, W-135 and Y) [2,3]. Most endemic IMD in Europe is due to serogroup B [1]. However, no broadly protective vaccine targeting serogroup B is currently available [4]. The contribution of serogroup C as the cause of IMD varies across European countries, ranging from 0% (Greece, Latvia, Malta, Slovenia) to 55% (Italy) in 2004 [1]. IMD due to serogroup A has been reported in Estonia, Greece, Norway and Slovenia [5]. Serogroups W-135 and Y together cause between 0% and 23% of IMD cases, with W-135 predominating in Austria and Slovenia, and serogroup Y predominating in Malta and Scandinavia [5].

The distribution of meningococcal serogroups causing IMD across Europe is diverse and evolving [1,6]. Outbreaks due to strain importation have been reported, such as the spread of IMD in 2000 due to serogroup W-135 that began in Hajj pilgrims [7]. The evolving nature of IMD and the risk for strain importation highlights the need for effective vaccines with broad serogroup coverage.

Monovalent meningococcal serogroup C (MenC) conjugate vaccines have been shown to be immunogenic and effective in preventing IMD due to serogroup C in infants and young children in the UK, The Netherlands, Canada, Spain and Australia [8–12].

Tetravalent (ACWY) conjugate vaccines potentially offer broader protection against four of the five main disease-causing serogroups. To date, two tetravalent conjugate vaccines are licensed for use (*Menactra*® and *Menveo*®). However, neither is licensed for use in children less than 2 years of age [13,14]. Effective multivalent conjugate vaccines for infants and toddlers are therefore needed to provide protection to the age group most at risk of IMD.

GlaxoSmithKline Biologicals (GSK) has developed a tetravalent ACWY vaccine with all serogroups conjugated to tetanus toxoid (ACWY-TT). ACWY-TT has been shown to be immunogenic in clinical trials conducted in adolescents, children and toddlers with a safety profile comparable to that of a licensed meningococcal vaccine [15,16]. Notably, ACWY-TT was highly immunogenic in 12- to

* Corresponding author. Tel.: +358 50 524 6530; fax: +358 3 3551 8450.
E-mail address: timo.vesikari@uta.fi (T. Vesikari).

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23-month-old children [15,17], suggesting that prevention of IMD through vaccination with this vaccine may be feasible in toddlers.

Vaccines targeting measles, mumps, rubella (MMR) and varicella (V) are frequently administered during the second year of life in Europe and other industrialized countries. Recently developed combined MMRV vaccines may replace MMR in some settings [18] although the higher incidence of febrile convulsions after MMRV has delayed the progress of MMRV introduction [19].

It is generally accepted that increasing the complexity of vaccination calendars by increasing the number of injections or the number of visits required reduces compliance with vaccination programmes and therefore vaccine coverage [20,21]. The implementation of ACWY-TT into vaccination calendars would be facilitated if ACWY-TT could be co-administered with other childhood vaccines, including MMR or MMRV at the same vaccination visit. This study was designed to compare the immune response induced by ACWY-TT to that induced by a licensed MenC conjugate vaccine, to demonstrate the immunogenicity of ACWY-TT to serogroups A, W-135, and Y in toddlers, and to assess the immunogenicity and safety of ACWY-TT when co-administered with MMRV during the second year of life. Since fever following vaccination is reported more frequently after MMRV than after separately administered MMR and V [22,23], MMRV may be regarded as the most challenging vaccine for co-administration.

2. Methods

2.1. Study design

This phase 3 study (NCT00474266) was conducted in 14 study centres in Finland between June 2007 and March 2008. The study was conducted according to good clinical practice and in accordance with the Somerset West 1996 version of the Declaration of Helsinki. The protocol and associated documents were reviewed and approved by the ethics committee of Pirkanmaa Hospital District. Written informed consent was obtained from the parents/guardians of children before study procedures were performed.

The study was conducted in two phases: a vaccination phase and a 6 month extended safety follow-up phase. Children were randomized (3:3:1:1) to one of four treatment groups (Table 1). All children received one dose of a meningococcal vaccine and two doses of MMRV (MMRV doses were administered either 6 weeks or 12 weeks apart). Children in the first two investigational treatment groups receiving ACWY-TT at the first vaccination visit were administered the first dose of MMRV either co-administered with ACWY-TT at Visit 1 (ACWY + MMRV group) or 6 weeks after ACWY-TT at Visit 2 (ACWY-TT group).

Children in the two control groups received *Meningitec*® (MenC-CRM$_{197}$, Pfizer, formerly Wyeth) and two MMRV doses. The MenC group received MenC-CRM$_{197}$ at Visit 1 followed by two MMRV doses at Visits 2 and 3. The MMRV group received the first MMRV dose at Visit 1 and MenC-CRM$_{197}$ at Visit 2, followed by a final MMRV dose at Visit 3.

This study design allowed for the evaluation of four co-primary objectives: the non-inferiority of the immune response to MenC following ACWY-TT administered alone compared to *Meningitec*®; the evaluation of the immunogenicity of ACWY-TT administered alone against serogroups A, W-135, and Y; and the evaluation of the effect of co-administration of ACWY-TT and MMRV versus the separate administration of each vaccine. Licensed MenC-CRM$_{197}$ vaccine was used as the control meningococcal vaccine because there are no tetravalent meningococcal conjugate vaccines currently available for children under 2 years of age.

The study was open in design because the study vaccines differed both in appearance and route of administration, and because the ACWY + MMRV group received two vaccinations at the first visit, whereas the other groups received a single injection.

Randomization was performed using a central, web-based system. The randomization algorithm used a minimization procedure to ensure balanced allocation between groups at individual centres. Sub-randomizations were used to identify subsets for immunological testing.

2.2. Study objectives

The co-primary objectives of the study were to demonstrate the non-inferiority of ACWY-TT compared to MenC-CRM$_{197}$ in terms of post-vaccination serogroup C bactericidal antibody titres (rSBA-MenC) $\geq$1:8; to demonstrate the immunogenicity of ACWY-TT to serogroups A, W-135 and Y in terms of post-vaccination rSBA titres $\geq$1:8; and to demonstrate the non-inferiority of ACWY-TT co-administered with MMRV compared to ACWY-TT and MMRV given alone in terms of rSBA titres $\geq$1:8 for the ACWY-TT antigens and seroconversion rates for the MMRV antigens.

The criterion for non-inferiority of ACWY-TT compared to MenC-CRM$_{197}$ was a lower limit (LL) $\geq -10\%$ for the two-sided standardized asymptotic 95% confidence interval (CI) for the group difference (ACWY-TT group minus the MenC group) in the percentages of subjects with post-vaccination rSBA-MenC titre $\geq$1:8. For the other serogroups, the criterion used to define immunogenicity of ACWY-TT was a LL $\geq$90% for the two-sided exact 95% CIs for percentages of subjects in the ACWY-TT group with post-vaccination rSBA titre $\geq$1:8.

The criterion for non-inferiority of ACWY + MMRV compared to the ACWY-TT group or to the MMRV group respectively, was a LL $\geq -10\%$ for the two-sided standardized asymptotic 95% CI for the group difference in the percentage of subjects who reached rSBA titre $\geq$1:8 for each group, or who seroconverted for antibodies against measles, mumps, rubella and varicella after the first MMRV dose.

MMRV seroconversion rates were derived from the observed seropositivity rates at post-vaccination, using the calculation $(P1 - P0)/(1 - P0)$, where $P1$ was the percentage of seropositive subjects in the study group and $P0$ was the percentage of subjects seropositive in the pooled MenC and ACWY-TT groups at Day 42.

Secondary objectives included the immunogenicity of MMRV 42 days after the second dose of MMRV vaccine, and assessment of the safety profile of ACWY-TT relative to MenC-CRM$_{197}$, and the safety profile of ACWY-TT when co-administered with MMRV as compared to the separate administration of the two vaccines.

2.3. Study subjects

Children in good health and between 12 and 23 months of age were eligible. Children had to have completed routine childhood vaccinations at 12 months of age. Children who were immunosuppressed from any cause; had previously received vaccination against *Neisseria meningitidis*; had a history of meningococcal disease; or who had been vaccinated against or exposed to measles, mumps, rubella, varicella or zoster within 30 days prior to vaccination, were ineligible to participate.

2.4. Vaccines

One 0.5 mL dose of ACWY-TT contained 5 μg of each meningococcal serogroup A, C, W-135 and Y polysaccharide and approximately 44 μg of tetanus toxoid. The lyophilized vaccine was reconstituted with saline and administered intramuscularly into the left thigh. One 0.5 mL dose of *Meningitec*® contained

Table 1
Study design.

Group	Visit 1 (Day 0)	Visit 2 (Day 42)	Visit 3 (Day 84)	Visit 4 (Day 126)	Visit 5 (Month 6)
ACWY + MMRV	ACWY-TT and MMRV	–	MMRV	†	‡
ACWY-TT	ACWY-TT	MMRV	MMRV	†	‡
MMRV	MMRV	MenC-CRM_{197}	MMRV	†	‡
MenC	MenC-CRM_{197}	MMRV	MMRV	†	‡
Blood sampling	*	*	–	**	–

* All subjects.
** 30% of subjects in the ACWY + MMRV and MMRV groups only; Diary cards were returned at Visit 2.
† Telephone contact or Visit 4 for AE reporting at the end of the active phase.
‡ Telephone contact for extended safety follow-up.

10 μg of meningococcal serogroup C polysaccharide conjugated to CRM_{197} protein (mutant diphtheria toxoid) and was administered intramuscularly into the left thigh. The MMRV vaccine (*Priorix-Tetra*™, GSK Biologicals, Belgium) contained ≥$10^{3.0}$ $CCID_{50}$ Schwarz measles strain, ≥$10^{4.4}$ $CCID_{50}$ RIT 4385 mumps strain, ≥$10^{3.0}$ $CCID_{50}$ RA 27/3 rubella strain and ≥$10^{3.3}$ PFU OKA varicella strain. MMRV was administered subcutaneously into the right upper arm.

2.5. Immunogenicity assessment

Blood samples were collected from all subjects prior to vaccination at Visit 1 and on Day 42. A subset of subjects in the ACWY + MMRV and MMRV groups had blood samples collected 42 days after the second MMRV dose (Day 126, Table 1).

Sera collected prior to the first vaccination were tested for rSBA-MenC in a randomized subset of 50% of subjects, and for rSBA-MenA, rSBA-MenW-135, rSBA-MenY in the other 50% of subjects.

Sera collected at Day 42 from all subjects in the ACWY + MMRV and ACWY-TT groups were tested for serum bactericidal activity using rabbit complement (rSBA) for each meningococcal serogroup [24]. Sera collected at Day 42 from all subjects in the MMRV and MenC groups were tested for rSBA-MenC; 50% of subjects in each group were also tested for rSBA-MenA, rSBA-MenW-135, rSBA-MenY. Sera from all groups at Day 42, and from 30% of subjects in the ACWY + MMRV and MMRV groups at Day 126, were tested for measles, mumps, rubella and varicella antibodies.

Assay cut-offs were 1:8 and 1:128 dilutions. An antibody titre ≥1:8 is considered indicative of seroprotection for rSBA-MenC [24]. This has previously been applied to the other serogroups [25]. The rSBA-MenA assay was performed using the 3125 strain with an L10 immunotype, the rSBA-MenC assay was performed using the C11 strain, the rSBA-MenW-135 assay was performed using the MP01240070 strain and the rSBA-MenY assay was performed using the S-1975 strain.

Antibodies against measles, mumps and rubella were measured using commercial immunoassays (*Enzygnost*™, Dade-Behring, Marburg, Germany) according to the manufacturer's instructions. Varicella antibodies were measured by an indirect immunofluorescence assay (*Virgo*™, Hemagen Diagnostics, Columbia, Maryland, with modifications). Varicella titres were expressed as the reciprocal of the last dilution considered as positive. The cut-off for each assay (seropositivity) was: 150 mIU/mL for measles, 231 U/mL for mumps, 4 IU/mL for rubella and 1:4 dilution for varicella. All assays were performed at GSK Biologicals' laboratories. Laboratory personnel were blinded as to group allocation.

Subjects with an rSBA-MenC titre below 1:8 1 month after vaccination with ACWY-TT or MenC-CRM_{197} were offered an extra dose of a licensed meningococcal serogroup C conjugate vaccine.

After study completion remaining available sera from the ACWY + MMRV, ACWY-TT and MenC groups were re-tested for SBA using human complement source [26]. hSBA titres ≥1:4 are associated with protection against disease due to serogroup C [27], and this threshold is assumed to extend to other serogroups. In this study, hSBA titres ≥1:8 are reported as a conservative threshold of seroprotection [27].

2.6. Safety and reactogenicity assessment

The occurrence of local symptoms of pain, redness and swelling at the injection site, and general symptoms of fever ≥38.0 °C (rectal route), irritability/fussiness, drowsiness and loss of appetite were recorded using diary cards for 4 days after the first vaccination. Symptoms generally associated with MMRV administration, including fever, parotid/salivary gland swelling, rash, and symptoms of meningitis including febrile convulsions, were recorded for 43 days after the first vaccine dose. All other (unsolicited) adverse events within 43 days after the first vaccination (Days 0–42) were also recorded. Serious adverse events (SAEs) were reported for an extended safety follow-up period of 6 months after the first vaccination.

Parents/guardians of children were given a ruler to measure the diameter of local reactions for 4 days, and a digital thermometer to measure temperature for 15 days (Days 0–14) after the first dose. Parents/guardians were also given a screening temperature pad to further screen for fever from Day 15 to Day 42 after the first vaccination. If the sensitive pad indicated fever, then temperature was to be measured using the digital thermometer supplied.

Symptom intensity was graded by the parents on a scale of 0–3, where Grade 0 was absent, Grade 1 was 'mild', Grade 2 was 'moderate', and Grade 3 was 'severe'. Grade 3 symptoms were defined as follows; redness and swelling >30 mm in diameter; for pain, if the subject cried when the affected limb was moved or the limb was spontaneously painful; fever >40.0 °C (rectal route); for loss of appetite, if the subject was not eating at all; and, for all other symptoms, preventing normal activity. The relationship between vaccination and all solicited general symptoms or unsolicited adverse events following vaccination was assessed by the investigator. All solicited local symptoms reported were automatically considered to be related to vaccination.

2.7. Statistical analyses

With 840 subjects evaluable for the immunogenicity analysis (315 in the ACWY + MMRV and ACWY-TT groups, 105 in the MMRV and MenC groups), the global power to meet all four co-primary objectives was at least 86.0%.

The analysis of immunogenicity was performed on the according to protocol cohort, which included subjects who had complied with all protocol-defined procedures and had data available for at least one immunogenicity endpoint. The geometric mean antibody concentration/titres (GMC/GMTs) were calculated by taking

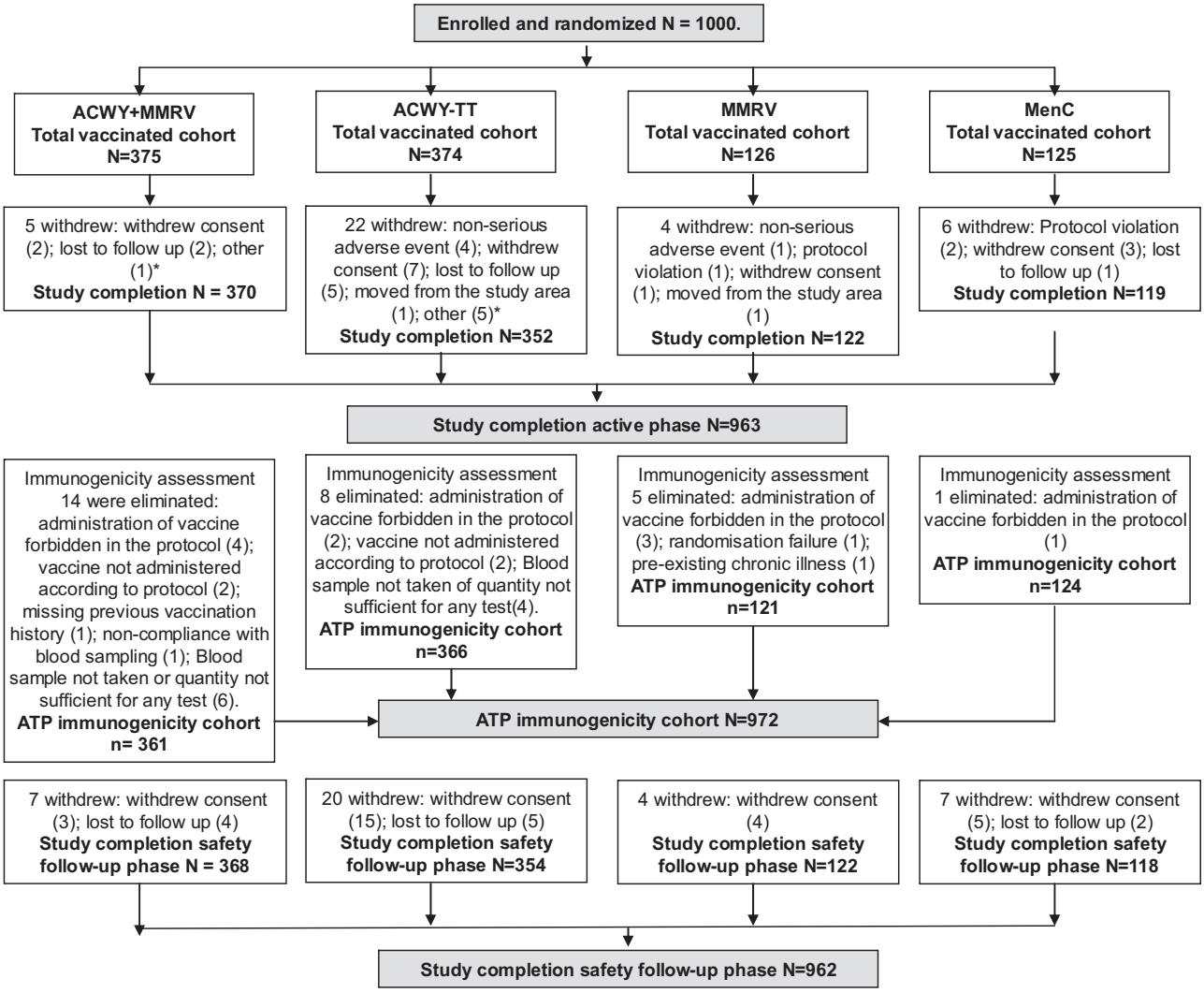

Fig. 1. Subject flow through the study. (*) 'Other' reasons included subjects who did not return for a visit, a subject with previous anaphylactic shock due to egg allergy, and a case of consent withdrawal due to varicella infection between Visit 1 and Visit 2.

the anti-log of the mean of the $\log_{10}$ concentration/titre transformations. Antibody concentrations or titres below the cut-off of the assay were given an arbitrary value of half the cut-off for the purpose of GMC/GMT calculation.

Exploratory analyses compared the immune response 42 days after the first dose between groups for the meningococcal antigens and MMRV components. Treatment groups were considered statistically significantly different if the standardized asymptotic 95% CI for the difference in rates between the two vaccine groups did not contain the value 0, or if the 95% CI for the GMT/GMC ratio between the two groups did not contain the value 1. The study was considered successful if all primary objectives were met simultaneously. Note that no adjustment for multiplicity of tests of secondary objectives was made, and significant results in the secondary analyses should be interpreted with caution.

The analysis of safety was conducted on the total vaccinated cohort, which included all vaccinated subjects according to the treatment received. The incidence and intensity of each solicited local and general symptom was calculated with exact 95% CI for each group. Analyses were performed using SAS® software version 9.1 (SAS Institute Inc., Cary, NC, United States) and Proc StatXact 7.0.

Table 2
Demographic characteristics of enrolled and vaccinated subjects (total vaccinated cohort).

Characteristic		ACWY + MMRV	ACWY-TT	MMRV	MenC
N					
Age (months)	Mean (SD)	14.7 (1.50)	14.6 (1.49)	14.6 (1.41)	14.4 (1.47)
	Range	12–18	12–18	12–18	12–19
Sex	Male *n* (%)	195 (52.0)	200 (53.5)	58 (46.0)	65 (52.0)
	Female *n* (%)	180 (48.0)	174 (46.5)	68 (54.0)	60 (48.0)
Race	White – Caucasian/European heritage	98.4%	99.5%	97.6%	98.4%
	White Arabic/North African	0.5%	0.0%	0.8%	0.0%
	Other	1.1%	0.5%	1.6%	1.6%

Other = White/West African, White/Egyptian, White/Black, Finnish/Egyptian, Finnish/American, European/African, European/North-African, Algerian/Finnish, African /Finnish, Arabic/Finnish.

3. Results

3.1. Study subjects

One thousand subjects were enrolled and vaccinated (Fig. 1). 37 subjects withdrew from the study during the vaccination phase and 38 subjects during the extended follow-up safety phase. Five subjects withdrew because of a non-serious adverse event (Fig. 1). One subject in the ACWY-TT group withdrew because of bronchitis that began 48 days after the first vaccination. Two subjects in the ACWY-TT group withdrew because of development of rash (a varicella rash that began 37 days after the first vaccination, and an atopic rash and varicella rash that began 6 and 38 days, respectively, after the first vaccination). None of these events were considered related to vaccination by the investigator. Two subjects withdrew due to adverse events considered to be related to vaccination by the investigator: one subject in the ACWY-TT group experienced Grade 3 headache that began 2 days after vaccination and lasted for 4 days, and another subject in the MMRV group developed Grade 3 fever that began 8 days after vaccination and lasted for 1 day. Safety was assessed in all 1000 subjects who were vaccinated (total vaccinated cohort). Of these, 28 subjects were eliminated from the immunogenicity analysis, leaving 972 subjects in the ATP immunogenicity cohort.

The four treatment groups were comparable in terms of demographic characteristics (Table 2).

3.2. Immunogenicity

3.2.1. Primary study objectives

3.2.1.1. Non-inferiority of ACWY-TT to MenC-CRM$_{197}$ and immunogenicity of ACWY-TT to serogroups A, W-135 and Y. The two co-primary objectives pertaining to the immunogenicity of ACWY-TT administered alone were reached. ACWY-TT was shown to be non-inferior to MenC-CRM$_{197}$: the LL of the two-sided standardized asymptotic 95% CI for the group difference in the percentages of subjects with rSBA-MenC titre ≥1:8 was above the pre-defined limit of ≥−10% (group difference 2.20% [95% CI 0.29; 6.78]).

ACWY-TT was also shown to be immunogenic in terms of serogroups A, W-135 and Y: the LLs of the two-sided exact 95% CI for percentages of subjects with rSBA titre ≥1:8 was ≥90% for each serogroup (Table 3).

3.3. Non-inferiority of co-administration of ACWY-TT and MMRV

The two co-primary objectives pertaining to the co-administration of ACWY-TT and MMRV were reached. ACWY-TT with MMRV was shown to be non-inferior to ACWY-TT alone. The LLs of the 95% CIs for the group differences in the percentages of subjects with rSBA titres ≥1:8 were above the pre-defined limit of ≥−10% for all four serogroups (Table 3). ACWY-TT co-administered with MMRV was shown to be non-inferior to MMRV alone. The LLs of the 95% CIs for the group difference in percentages of subjects who seroconverted for antibodies against measles, mumps, rubella and varicella were ≥−10% (Table 4).

3.3.1. Meningococcal serogroup A, C, W-135 and Y rSBA antibody responses

Prior to vaccination, the percentage of subjects with rSBA antibody titres ≥1:8 ranged from 34.0% to 45.3% for MenA, 21.7% to 27.0% for MenC, 42.9% to 49.2% for MenW-135 and 54.8% to 68.3% for MenY in the four study groups.

Forty-two days after vaccination with ACWY-TT, at least 99.7% of subjects had rSBA titres ≥1:8 against each vaccine serogroup, and at least 99.4% also had titres ≥1:128 against serogroups A, W-135 and Y (Table 3). Against serogroup C, 94.4% and 95.8% of the

Table 3
Percent of subjects with rSBA titres ≥1:8 and ≥1:128 42 days after the first vaccine dose in each treatment group (ATP immunogenicity cohort).

Group	ACWY + MMRV			ACWY-TT			MenC			Difference (ACWY + MMRV minus ACWY-TT groups)
Serogroup	*N*	% ≥1:8 [95% CI]	% ≥1:128 [95% CI]	*N*	% ≥1:8 [95% CI]	% ≥1:128 [95% CI]	*N*	% ≥1:8 [95% CI]	% ≥1:128 [95% CI]	% ≥1:8 [95% CI]†
A	360	100 [99.0; 100]	99.7 [98.5; 100]	354	99.7 [**98.4**; 100]	99.7 [98.4; 100]	51	45.1 [31.1; 59.7]	33.3 [20.8; 47.9]	0.28 [−0.78; 1.58]
C	357	100* [99.0; 100]	94.4* [91.5; 96.5]	354	99.7* [98.4; 100]	95.8* [93.1; 97.6]	121	97.5 [92.9; 99.5]	70.2 [61.3; 78.2]	0.28 [−0.79; 1.58]
W-135	360	100 [99.0; 100]	100 [99.0; 100]	354	100 [**99.0**; 100]	99.4 [98.0; 99.9]	58	50.0 [36.6; 63.4]	25.9 [15.3; 39.0]	0.00 [−1.06; 1.07]
Y	359	100 [99.0; 100]	99.7 [98.5; 100]	354	100 [**99.0**; 100]	99.7 [98.4; 100]	59	54.2 [40.8; 67.3]	35.6 [23.6; 49.1]	0.00 [−1.06; 1.07]

N = number of subjects with results available; % = percentage of subjects with concentration/titre within the specified range; 95% CI = 95% confidence interval; Bold = LL of 95% CI is above pre-defined limit of 90% for rSBA-MenA, rSBA-MenW-135 and rSBA-MenY.
[95% CI]† Standardized asymptotic 95% confidence interval – LL of 95% CI is above the non-inferiority limit of −10% for rSBA-MenA, rSBA-MenC, rSBA-MenW-135 and rSBA-MenY.
* Statistically significantly higher compared to MenC group (exploratory analysis).

Table 4
Immune responses to MMRV 42 days after each MMRV dose in the ACWY + MMRV and MMRV treatment groups (ATP immunogenicity cohort).

Antibody	Cut-off	Group	Timing	*N*	% ≥cut-off [95% CI]	GMC/T [95% CI]	Difference in seroconversion rate** (ACWY + MMRV minus MMRV group)
Measles	≥150 mIU/mL	ACWY + MMRV	PI(D42)	361	100 [99.0; 100]	4273.4 [4018.4; 4544.6]	0.00 [−**1.06**; 3.17]
			PII(D126)	37	100 [90.5; 100]	7113.8 [5335.6; 9484.7]	
		MMRV	PI(D42)	118	100 [96.9; 100]	4457.3 [3976.3; 4996.6]	
			PIII(D126)	7	100 [59.0; 100]	8699.8 [4865.3; 15556.2]	
Mumps	≥231 U/mL	ACWY + MMRV	PI(D42)	349	87.7 [83.8; 90.9]	662.9 [598.4; 734.4]	4.06 [−**2.82**; 12.46]
			PII(D126)	37	100 [90.5; 100]	3351.2 [2658.2; 4224.7]	
		MMRV	PI(D42)	116	83.6 [75.6; 89.8]	710.1 [583.8; 863.8]	
			PIII(D126)	7	100 [59.0; 100]	3334.1 [1933.1; 5750.5]	
Rubella	≥4 IU/mL	ACWY + MMRV	PI(D42)	361	100 [99.0; 100]	43.1* [40.0; 46.5]	0.00 [−**1.06**; 3.18]
			PII(D126)	37	100 [90.5; 100]	87.2 [74.8; 101.6]	
		MMRV	PI(D42)	118	100 [96.9; 100]	53.2 [46.6; 60.7]	
			PIII(D126)	7	100 [59.0; 100]	117.0 [73.8; 185.5]	
Varicella	≥1:4	ACWY + MMRV	PI(D42)	333	97.9 [95.7; 99.2]	152.8 [133.5; 174.8]	3.36 [−**0.28**; 9.50]
			PII(D126)	36	100 [90.3; 100]	4175.6 [3064.0; 5690.6]	
		MMRV	PI(D42)	111	94.6 [88.6; 98.0]	128.8 [99.1; 167.4]	
			PIII(D126)	7	100 [59.0; 100]	3360.1 [1646.5; 6857.0]	

N = number of subjects with results available; GMC/GMT = geometric mean antibody concentration or titre calculated on all subjects; % = percentage of subjects with concentration/titre within the specified range; 95% CI = 95% confidence interval; PI(D42) = 42 days post-dose 1; PIII(D126) = 42 days post-dose 2 at Day 126. Bold = LL of 95% CI is above pre-defined limit of ≥−10% for measles, mumps, rubella and varicella seroconversion rates after the first MMRV dose.

* Statistically significantly lower compared to the MMRV group (exploratory analysis).

** Seroconversion 42 days after vaccination with MMRV derived from $(P1 - P0)/(1 - P0)$ where $P1$ = percentage of seropositive subjects post-vaccination in the study group and $P0$ = percentage of subjects seropositive post-vaccination in the pooled MenC and ACWY-TT groups.

subjects in the ACWY + MMRV and ACWY groups had rSBA-MenC titres ≥1:128, respectively; both were significantly higher than in the MenC group. In the MenC group, 97.5% of subjects had rSBA-MenC titres ≥1:8 and 70.2% had titres ≥1:128.

rSBA GMTs in the ACWY + MMRV and ACWY-TT groups increased by at least 47.8-fold against each of the four vaccine serogroups after vaccination. The rSBA-MenC GMT in the MenC group increased 27.9-fold (Table 5). Exploratory analyses showed that the rSBA-MenC GMT adjusted for pre-vaccination measurements was statistically significantly higher both in the ACWY + MMRV and ACWY-TT groups than in the MenC group (Table 5). Exploratory analyses did not detect any statistically significant differences between the ACWY + MMRV and ACWY-TT groups in terms of percentage of subjects reaching either cut-off, or the rSBA GMT, for any of the four vaccine serogroups.

3.3.2. Meningococcal serogroup A, C, W-135 and Y hSBA antibody responses

Prior to vaccination, 2.0% of subjects at most had hSBA titres ≥1:8 in the tested study groups (Table 6). Post-vaccination, at least 77.2% of the subjects in the ACWY + MMRV and ACWY-TT groups had hSBA titres ≥1:8 against each vaccine serogroup. Exploratory comparisons showed that the percentage of subjects with titres ≥1:8 and GMTs was statistically significantly higher in the ACWY + MMRV and ACWY-TT groups compared to the MenC group for hSBA-MenC and was statistically significantly higher in the ACWY + MMRV group compared to the ACWY-TT group for hSBA-MenA.

3.3.3. Immune response to MMRV

Forty-two days after the first dose of MMRV, all subjects in the ACWY + MMRV and MMRV groups had seroconverted for antibodies against measles and rubella, 87.7% and 83.6% in each group, respectively, had seroconverted for antibodies against mumps and 97.9% and 94.6% of subjects in each group had seroconverted for antibodies against varicella.

Table 4 shows seropositivity rates after the first and second MMRV doses in the ACWY + MMRV and MMRV groups. All subjects in the subset of 30% of subjects from the ACWY + MMRV and MMRV groups tested after the second dose of MMRV, were seropositive for each vaccine component.

Exploratory analyses did not detect any statistically significant differences between the ACWY + MMRV and MMRV groups 42 days post-vaccination, with the exception of the anti-rubella GMC, which was statistically significantly lower in the ACWY + MMRV group compared to the MMRV group (95% CI for the group GMC ratio [0.697, 0.945]).

3.4. Safety

3.4.1. Clinically significant adverse events and serious adverse events within 6 months of vaccination

SAEs were reported by five subjects (three subjects [0.8%] in the ACWY + MMRV group and two subjects [0.5%] in the ACWY-

Table 5
rSBA geometric mean titres 42 days after the first vaccination in each treatment group (ATP immunogenicity cohort).

Group	ACWY + MMRV		ACWY-TT		MenC	
Serogroup	*N*	GMT [95% CI]	*N*	GMT [95% CI]	*N*	GMT [95% CI]
A	360	2085.9 [1905.3; 2283.6]	354	2205.0 [2007.8; 2421.6]	51	24.3 [13.4; 44.1]
C	357	519.0* [470.9; 571.9]	354	477.6* [437.3; 521.6]	121	212.3 [170.0; 265.2]
W-135	360	2055.8 [1871.0; 2258.9]	354	2681.7 [2453.1; 2931.6]	58	25.1 [14.6; 43.1]
Y	359	2282.4 [2051.3; 2539.5]	354	2729.4 [2472.7; 3012.8]	59	31.4 [18.4; 53.6]

N = number of subjects with results available. 95% CI = 95% confidence intervals. GMT = geometric mean antibody titre calculated on all subjects.

* Statistically significantly higher compared to MenC group (exploratory analysis based on adjusted GMT ratio).

Table 6
Percent of subjects with hSBA titres ≥1:8 and hSBA GMTs before and 42 days after the first vaccine dose in each treatment group (ATP immunogenicity cohort).

Group	ACWY + MMRV				ACWY-TT			MenC		
Serogroup	Time point	*N*	% ≥1:8 [95% CI]	GMT [95% CI]	*N*	% ≥1:8 [95% CI]	GMT [95% CI]	*N*	% ≥1:8 [95% CI]	GMT [95% CI]
A	Pre	357	2.0 [0.8; 4.0]	2.1 [2.1; 2.2]	356	2.0 [0.8; 4.0]	2.1 [2.0; 2.1]	122	0.8 [0.0; 4.5]	2.0 [2.0; 2.1]
	Post	348	83.9* [79.6; 87.6]	33.7* [28.9; 39.2]	338	77.2 [72.4; 81.6]	19.0 [16.4; 22.1]	117	0.9 [0.0; 4.7]	2.0 [2.0; 2.1]
C	Pre	359	0.3 [0.0; 1.5]	2.0 [2.0; 2.0]	363	0.8 [0.2; 2.4]	2.0 [2.0; 2.1]	122	0.8 [0.0; 4.5]	2.0 [2.0; 2.1]
	Post	346	98.0† [95.9; 99.2]	209.1† [183.8; 238.0]	341	98.5† [96.6; 99.5]	196.0† [175.4; 219.0]	116	81.9 [73.7; 88.4]	40.3 [29.5; 55.1]
W-135	Pre	353	1.1 [0.3; 2.9]	2.1 [2.0; 2.1]	357	0.6 [0.1; 2.0]	2.1 [2.0; 2.1]	123	0.0 [0.0; 3.0]	2.0 [2.0; 2.0]
	Post	337	82.8 [78.3; 86.7]	57.3 [47.0; 69.9]	336	87.5 [83.5; 90.8]	48.9 [41.2; 58.0]	114	0.9 [0.0; 4.8]	2.0 [2.0; 2.2]
Y	Pre	344	1.2 [0.3; 3.0]	2.1 [2.0; 2.1]	349	1.4 [0.5; 3.3]	2.1 [2.0; 2.1]	122	0.8 [0.0; 4.5]	2.1 [1.9; 2.2]
	Post	333	81.4 [76.8; 85.4]	38.7 [32.2; 46.7]	329	79.3 [74.5; 83.6]	30.9 [25.8; 37.1]	117	1.7 [0.2; 6.0]	2.1 [1.9; 2.3]

N = number of subjects with results available; % = percentage of subjects with titre ≥1:8; 95% CI = 95% confidence interval; GMT = geometric mean antibody titre.

* Statistically significantly higher compared to the ACWY-TT group (exploratory analysis).

† Statistically significantly higher compared to the MenC group (exploratory analysis). For GMTs, the exploratory analysis was based on the GMT ratio adjusted for baseline concentrations.

TT group) during the 43-day post-vaccination period after dose 1. None were considered to be causally related to vaccination. A further 23 subjects reported SAEs in the extended follow-up safety phase (between 1.6% and 2.7% of subjects in each group) from Day 42 post-dose 1 up to the end of the extended safety follow-up period. None of the events were considered causally related to vaccination by the investigator. No deaths occurred during the study.

Unsolicited symptoms (both serious and non-serious) reported within 43 days of the first vaccination were reported by 64.8% of subjects in the ACWY + MMRV group, 60.2% in the ACWY-TT group, 68.3% in the MMRV group and 54.4% in the MenC group. Unsolicited symptoms considered by the investigator to be related to vaccination were reported by 30.7%, 15.0%, 28.6% and 12.8% of the subjects in the ACWY + MMRV, ACWY-TT, MMRV and MenC groups, respectively. The most frequently reported unsolicited symptoms with a causal relationship to vaccination were irritability in the ACWY + MMRV group (8.8%) and the MMRV group (7.9%), and diarrhoea in the ACWY-TT group (4.5%) and MenC group (8.8%).

3.4.2. Local symptoms within 4 days of vaccination

Redness at the injection site was the most frequently reported local symptom after each dose administered at Visit 1 (Fig. 2). Redness and swelling of Grade 3 intensity were reported after vaccination with ACWY-TT by 4.4% and 4.1% of subjects, respectively, and by 0.8% each after MenC-CRM_{197}. No Grade 3 local reactions were reported after MMRV.

3.4.3. General symptoms within 4 days of vaccination

Irritability was the most frequently reported solicited general symptom in each treatment group after the first vaccine dose (Fig. 3). General solicited symptoms of Grade 3 intensity were infrequent, reported by 1.6% of subjects or fewer in each group. Fever with rectal temperature >40 °C was reported by one subject (on Day 3 after vaccination in the MMRV group).

3.4.4. MMRV-specific symptoms within 43 days of vaccination

MMRV-specific symptoms were recorded for 43 days after the first dose. During this period, fever was reported by 78.7% of subjects in the ACWY + MMRV group and 79.8% of subjects in the MMRV group. In these groups fever was most prevalent between Day 4 and Day 10 after vaccination, peaking on Day 8 (Fig. 4). In groups that did not receive MMRV at the first vaccination visit, fever was reported by 44.7% (ACWY-TT group) and 45.2% (MenC group) of subjects. Fever >40 °C was reported in 5.3% and 4.0% of the subjects in the ACWY + MMRV and MMRV groups, respectively.

Rash (any) after the first vaccine dose was reported by 31.7% of the subjects in the ACWY + MMRV and 29.0% in the MMRV group, 18.0% in the ACWY-TT group and 19.4% in the MenC group. Measles/rubella like rash was reported by 3.7% of subjects in the ACWY + MMRV group, by 3.2% of subjects in the MMRV group and by none of the subjects in the ACWY-TT and MenC groups. A varicella-like rash was reported by 2.4% of subjects in the ACWY + MMRV, MMRV and MenC groups, and by 1.4% of subjects in the ACWY-TT group. 'Symptoms of meningitis' were reported by one subject each in the ACWY + MMRV and MenC groups. One subject experienced a febrile convulsion 26 days after vaccination with ACWY and MMRV. The other subject experienced febrile convulsions 34 and 41 days after receiving MenC-CRM_{197}. These events were Grade 3 in intensity, were considered not related to vaccination by the investigator and both of the subjects sought medical advice. None of the subjects reported parotid/salivary gland swelling.

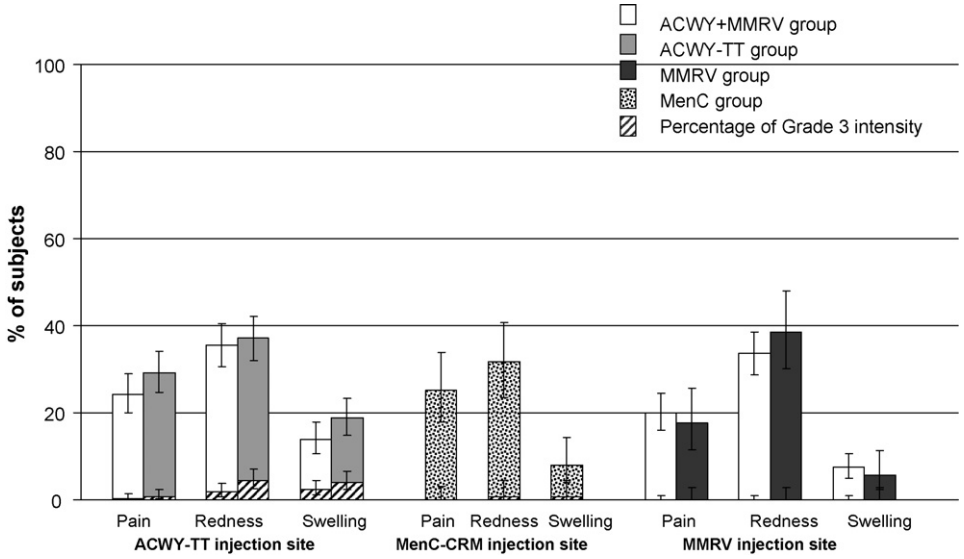

Fig. 2. Percentage of children with local solicited symptoms reported within 4 days after the first vaccine dose (Total vaccinated cohort) (Vertical bars = 95% CI).

4. Discussion

Monovalent MenC conjugate vaccines have made progress in IMD control in countries where use of these vaccines has been widespread [8–12]. However, broader serogroup coverage using tetravalent ACWY conjugate vaccines could provide protection against the other major disease-causing serogroups in children less than 2 years of age. GSK Biologicals' investigational ACWY-TT vaccine has been shown to be immunogenic for all four serogroups, with a clinically acceptable safety profile in toddlers after a single dose [15,17]. In the present study, a single dose of ACWY-TT was non-inferior to a licensed monovalent MenC-CRM$_{197}$ vaccine in terms of the percentage of subjects with post-vaccination rSBA-MenC titres ≥1:8, the accepted correlate of protection [24]. Despite containing less polysaccharide antigen than MenC-CRM$_{197}$, the response to the MenC component of ACWY-TT was higher in terms of the percentage of subjects reaching titres ≥1:128 and GMTs. In addition, immunogenicity to the other three serogroups was demonstrated, with >99% of ACWY-TT recipients achieving post-vaccination rSBA titres ≥1:8.

This is the first study to investigate the feasibility of co-administration of ACWY-TT with MMRV. Pre-specified non-inferiority criteria in terms of the rSBA and MMRV antibody responses were met. In addition, exploratory analyses did not detect any significant differences in immune responses between the ACWY + MMRV co-administration group and the ACWY-TT and MMRV groups, except in terms of the anti-rubella GMCs, which were significantly lower in the ACWY + MMRV group as compared to the MMRV group. The clinical relevance of this finding is questionable, since no difference was observed in the anti-rubella seropositivity or seroconversion rates. This finding is expected to have minimal clinical impact, since the anti-rubella GMC in the co-administered group (43.1 IU/mL) is well above the 10 IU/mL threshold used to define seroresponse. In addition, rSBA GMTs to serogroups W-135 and Y tended to be lower in the co-administration group. Again, the clinical impact is expected to be limited, since >99% of subjects in the ACWY + MMRV group attained rSBA titres ≥1:128 for both serogroups.

The relative infrequency of meningococcal disease and the unpredictable nature of the epidemiology make efficacy trials

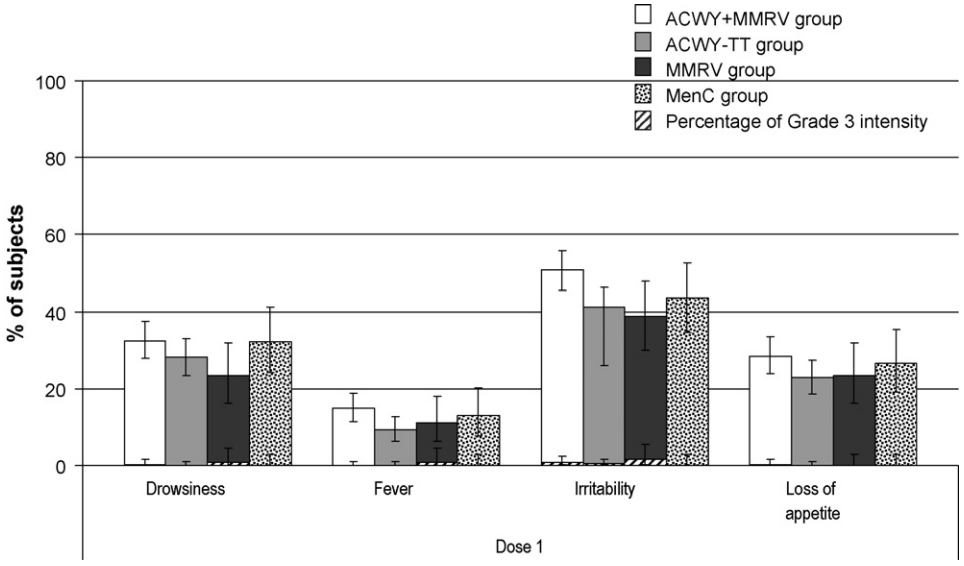

Fig. 3. Percentage of children with general solicited symptoms reported within 4 days after the first vaccine dose (Total vaccinated cohort) (Vertical bars = 95% CI).

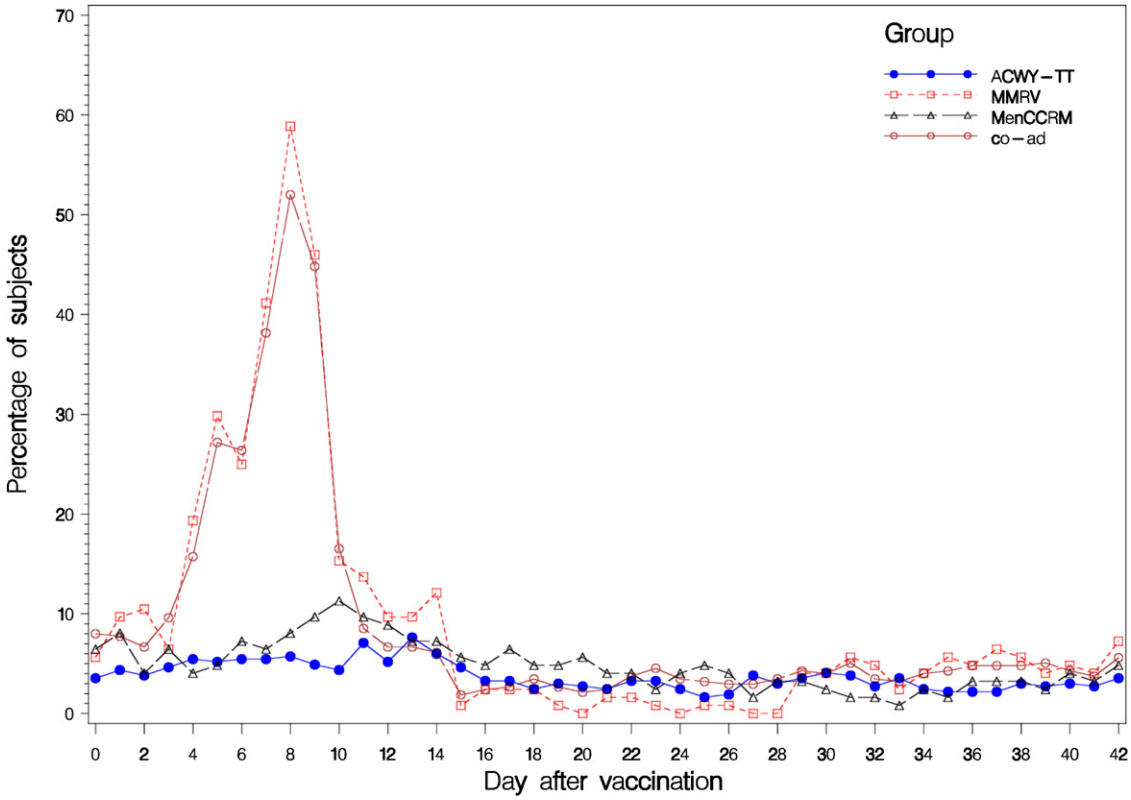

Fig. 4. Prevalence of any fever (rectal temperature ≥38.0 °C) by day during the 43-day (Days 0–42) post-vaccination period after the first vaccine dose (Total vaccinated cohort).

unfeasible. The efficacy of meningococcal conjugate vaccines can be inferred from demonstration of immunogenicity. There is general consensus that immunogenicity should be measured by a functional assay measuring SBA, but there is no consensus on the source of complement to be used [28–30]. Currently, SBA assays are performed using exogenous complement obtained from either rabbit or human sources. Although the original correlate of protection was defined in terms of hSBA titres ≥1:4 [27], the original assay is no longer available to serve as a benchmark of clinical protection or as a reference for the standardization of other assays, which greatly complicates comparison of results amongst different laboratories. In contrast, the rSBA-MenC assay developed at the Health Protection Agency in the United Kingdom was used to license all three available MenC conjugate vaccines. During post-licensure surveillance, rSBA titres ≥1:8 were confirmed as the rSBA-MenC antibody threshold that best correlated to protection against disease [31]. Due to a large percentage of initially seropositive subjects against some serogroups [32], a secondary analysis of immunogenicity using sera tested by hSBA was performed to more completely characterize the immunogenicity induced by ACWY-TT. High percentages of subjects reaching the conservative 1:8 threshold for each serogroup after vaccination confirm the immunogenicity of a single dose of ACWY-TT in toddlers. Statistically significantly higher hSBA-MenC titres as compared to the licensed MenC conjugate vaccine, confirm the results obtained with the rSBA assay.

Over the 43-day follow-up period, the occurrence of fever and rash in the ACWY + MMRV and MMRV groups were within the same range, and higher than that observed in the ACWY-TT and MenC groups. In line with the known timing of fever in association with MMRV vaccines [33,22], the prevalence of fever in the ACWY + MMRV and MMRV groups peaked between 4 and 10 days after vaccination with MMRV. A delayed febrile response and occurrence of transient rash have been linked to viral replication following measles vaccination [34]. In general, a higher proportion (60% on Day 8) of children experienced fever than were previously reported after MMR, indicating a possibility for the higher incidence of fever with MMRV as compared with MMR [35]. This is line with previous reports. Higher rates of fever following administration of MMRV compared with separately administered MMR and monovalent varicella vaccine have been described, thought to reflect increased measles virus replication following MMRV compared to the component vaccines [36]. Furthermore, a higher incidence of febrile seizures has been reported with another MMRV vaccine as compared to the separate administration of MMR and V [19].

The present study has the limitation of being open in design, but the potential impact of this on immunogenicity data was minimized by blinding laboratory personnel during the immunogenicity analysis. Bias in safety reporting cannot be excluded, but would be most likely in favour of licensed versus investigational vaccines. There was a higher drop-out rate in the ACWY-TT group than in the other groups, but this is mitigated by the lower drop-out rate in the ACWY + MMRV group, since it is likely that the co-administration group represents the highest risk scenario for the occurrence of an increased rate of adverse events. A further limitation is that MenC vaccine conjugated to TT was not used as control, despite the fact that MenC-TT results in the highest rSBA-MenC GMTs as compared to MenC-CRM_{197} [37–39]. However, access to MenC-TT is difficult due to limited supply. The MenC-CRM_{197} vaccine used (*Meningitec*®) is a valid control, since MenC-CRM vaccines have been demonstrated to be effective in reducing IMD disease due to serogroup C in toddlers [11]. A study to compare ACWY-TT to a licensed MenC-TT conjugate is ongoing.

The results of this study, along with previous studies in toddlers and young children [15,17], indicate that the ACWY-TT investigational vaccine is highly immunogenic when administered a single dose between 12 and 23 months of age. To our knowledge, this is

the first study demonstrating the acceptable co-administration of a tetravalent meningococcal conjugate vaccine with MMRV, which will be an important component in facilitating the inclusion of such vaccines into routine immunization schedules. There have been previous studies with a combined *Haemophilus influenzae* type b and *N. meningitidis* serogroup C conjugate vaccine (*Menitorix*®, GSK Biologicals), which demonstrated no interference the between MenC and MMR antigens [40].

A growing body of evidence attests to the immunogenicity of the ACWY-TT candidate vaccine both in older populations and during the second year of life [15,16,18]. This study also demonstrates that ACWY-TT can be co-administered with MMRV between 12 and 23 months of age without affecting the immunogenicity or safety profiles of either vaccine. Administration of both vaccines at the same visit would facilitate uptake and coverage of both vaccines, without the need for additional vaccination visits.

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