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Safety and Immunogenicity of Human Serum Albumin-Free MMR Vaccine in US Children Aged 12–15 Months

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Background. M-M-RTMII (MMRII; Merck & Co) is currently the only measles-mumps-rubella (MMR) vaccine licensed in the United States. Another licensed vaccine would reinforce MMR supply. This study assessed the immunogenicity of a candidate vaccine (*Priorix*TM, GlaxoSmithKline Vaccines [MMR-RIT]) when used as a first dose among eligible children in the United States.

Methods. In this exploratory Phase-2, multicenter, observer-blind study, 1220 healthy subjects aged 12–15 months were randomized (3:3:3:3) and received 1 dose of 1 of 3 MMR-RIT lots with differing mumps virus titers (MMR-RIT-1 [4.8 log₁₀]; MMR-RIT-2 [4.1 log₁₀]; MMR-RIT-3 [3.7 log₁₀] CCID₅₀) or MMRII co-administered with hepatitis A vaccine (HAV), varicella vaccine (VAR) and 7-valent pneumococcal conjugate vaccine (PCV7). Immune response to measles, mumps, and rubella viruses was evaluated at Day 42 post-vaccination. Incidence of solicited injection site, general, and serious adverse events was assessed.

Results. Seroresponse rates for MMR vaccine viral components in MMR-RIT lots were 98.3–99.2% (measles), 89.7–90.7% (mumps), and 97.5–98.8% (rubella), and for MMRII were 99.6%, 91.1%, and 100%, respectively. Immune responses to HAV, VAR, and PCV7 were similar when co-administered with any of the 3 MMR-RIT lots or MMRII. There were no apparent differences in solicited or serious adverse events among the 4 groups.

Conclusions. Immune responses were above threshold levels for projected protection against the 3 viruses from MMR-RIT lots with differing mumps virus titers. MMR-RIT had an acceptable safety profile when co-administered with HAV, VAR, and PCV7.

Clinical Trials Registration. NCT00861744; *etrack*; 111870

Key words. co-administration; immunogenicity; measles; mumps; rubella.

INTRODUCTION

Despite the successful introduction of routine immunization with combined live attenuated measles-mumps-rubella (MMR) vaccines in the 1970s [1], outbreaks and generally increased prevalence of mumps and measles are still noted among the vaccinated and unvaccinated populations, respectively, across the United States [2–4].

Maintenance of high vaccine coverage rates remains an essential component of efforts to control these diseases. A 2-dose MMR vaccination schedule is recommended in the United States. Children receive dose-1 at 12–15 months concomitantly with other recommended vaccines, including hepatitis A vaccine (HAV), varicella vaccine (VAR), and pneumococcal conjugate vaccine (PCV) [5–8]. MMR dose-2 is administered at age 4–6 years to induce immune

responses in those who fail to respond to the initial dose. Two-dose catch-up schedules, with a minimum 4-week interval between MMR doses, are recommended for children and adolescents who miss the first dose [9]. As Merck's *M-M-R*TMII (MMRII), a human serum albumin-free vaccine, is currently the only MMR vaccine licensed in the United States, any interruption in its availability would pose a critical public health risk. Therefore, GlaxoSmithKline Vaccines is currently evaluating its trivalent MMR vaccine *Priorix*TM (MMR-RIT) for use in the United States. MMR-RIT is routinely given in over 100 countries from the second year of life onwards [10]. The formulation of MMR-RIT used in this study does not contain human serum albumin, thereby minimizing any theoretical risk of microbial contamination as compared to previous formulations [11]. This formulation is also consistent with the recommendation from the European Medicines Agency to eliminate the use of blood-derived products of human origin [12, 13].

This Phase-2 exploratory study assessed immunologic responses to 3 lots of MMR-RIT (containing a range of mumps virus titers) and to MMRII used as a first dose in 12–15-month-old children in the United States. The study was used as a preliminary evaluation of the minimum effective mumps virus titer for the candidate vaccine to allow planning of a Phase-3 study, and was used also to generate preliminary data on the safety and immunogenicity of co-administration of MMR-RIT with routine childhood vaccines: VAR, HAV, and 7-valent PCV (PCV7).

METHODS

This randomized, observer-blind, Phase-2 study was conducted at 51 centers in the United States in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. The study was approved by a national, regional, or investigational center institutional review board or independent ethics committee. Written informed consent was obtained from parents/guardians before enrollment. The study consisted of an active phase (immediate post-vaccination interval; Days 0–42), an extended safety follow-up phase (Days 43–180), and an antibody persistence phase ending approximately 2 years post-vaccination. Only the results of a planned analysis conducted for immunogenicity and safety data from the active phase are reported here.

Eligible healthy 12- to 15-month-old male and female subjects had not been previously immunized against (and had no previous history of) measles, mumps, rubella, varicella, and hepatitis A, and had received 3 doses of PCV7 within the first year of life (third dose administered ≥ 30

days before enrollment). Other key exclusion criteria included: exposure to measles, mumps, rubella, or varicella ≤ 30 days before study start; previous (≤ 30 days before study start) or planned administration of investigational products during the study; administration of other vaccines (except influenza and *Haemophilus influenzae* type b) ≤ 30 days before study vaccination until Day 42; chronic immunosuppressants/immune-modifying drugs, polyclonal immunoglobulins, or blood products received ≤ 6 months before study vaccination; immunosuppressive or immunodeficient conditions; contraindication to vaccination; a history of neurologic disorders or seizures; acute disease at enrollment; and severe chronic illness or major congenital defects.

Subjects visited the study site at Days 0, 42, 180, 365, and 730. At Day 0 (Visit 1), subjects were randomized using a blocking scheme (3:3:3:[1:1:1] ratio) to 1 of 4 parallel treatment groups: 3 groups received a single dose of 1 of 3 MMR-RIT lots (containing either high [MMR-RIT-1], medium [MMR-RIT-2], or low [MMR-RIT-3] RIT 4385 mumps strain titers); the fourth group received a single dose of 1 of 3 commercial lots of MMRII (Merck & Co Inc. [14]) (Table 1). The randomization list was generated at GlaxoSmithKline Biologicals using SAS[®] software. Treatment allocation was performed at the investigator site via a central internet-based randomization system. Subjects concomitantly received a single dose each of HAV (*Havrix*TM, GlaxoSmithKline Vaccines [15]), VAR (*Varivax*TM, Merck & Co. Inc. [16]), and PCV7 (*Prevnar*TM, Wyeth [17]) at Day 0. Immune response against measles, mumps, and rubella viruses was assessed at Day 42 (Visit 2). MMR-RIT or MMRII were administered subcutaneously into the upper right arm, VAR subcutaneously into the upper left arm, and HAV and PCV7

Table 1. Formulation of 3 Lots of Candidate MMR-RIT (Measles, Mumps, Rubella) Vaccine and Commercially Available Comparator Vaccine (MMRII, Merck & Co., Inc.)

Vaccine	Lot Number(s)	Log ₁₀ CCID ₅₀		
		Measles ^a	Mumps ^b	Rubella ^c
MMRII	1291X	4.0	4.8	4.2
	1255X	3.9	4.8	4.0
	1362X	3.8	4.8	4.1
MMR-RIT-1	AMJRB721A	3.8	4.8 (high)	3.9
MMR-RIT-2	DMJRA002A	4.1	4.1 (medium)	3.9
MMR-RIT-3	DMJRA003A	4.0	3.7 (low)	4.1

Abbreviation: CCID₅₀, Median cell culture infective dose.

Note: All CCID₅₀ values for vaccine components of MMRII and MMR-RIT were determined by GlaxoSmithKline.

^aMeasles was Schwarz strain for GlaxoSmithKline vaccines and *Moraten Edmonston-Enders* strain for MMRII.

^bMumps was RIT 4385 strain for GlaxoSmithKline vaccines and *Jeryl Lynn* for MMRII.

^cRubella strain was the same for MMRII and each MMR-RIT (ie, Wistar RA 27/3).

intramuscularly into the left and right thighs, respectively. A second HAV dose was administered at Day 180 (Visit 3). Vaccine recipients, parents/guardians, and those responsible for evaluation of study endpoints were blinded to study treatment. Vaccine reception, storage, preparation, reconstitution, and administration were performed by study personnel who did not participate in outcome evaluation.

Immunogenicity

Blood for antibody determination was obtained from subjects on Days 0 (pre-immunization), 42, 365, and 730. Analysis of blood obtained at Days 365 and 730 for evaluation of antibody persistence is ongoing and will be reported separately. Sera were stored at -20°C until assayed in a blinded manner at a central laboratory (GlaxoSmithKline Biologicals, Rixensart, Belgium). Mumps virus antibody response was determined using an in-house plaque-reduction assay (GlaxoSmithKline Biologicals [18, 19]) via neutralization of wild-type virus (Mu90LO1) in the presence of complement and anti-human globulin. Immunoglobulin (Ig) G antibodies to measles, rubella, and varicella-zoster virus (VZV) were measured with commercial enzyme-linked immunosorbent assay (ELISA) (Enzygnost, Dade Behring, Marburg GmbH, Germany); antibodies to hepatitis A virus were determined in a randomized subset of 50% of subjects. Antibodies to PCV7 pneumococcal serotypes were measured in the remaining 50% with an in-house ELISA (GlaxoSmithKline Biologicals [20]). Assay seronegativity cut-off values for antibodies to vaccine viral antigens were: measles <150 mIU/mL, mumps <24 ED₅₀, rubella <4 IU/mL, VZV <25 mIU/mL, hepatitis A <15 mIU/mL, and *Streptococcus pneumoniae* <0.05 $\mu\text{g/mL}$. The seronegativity cut-offs evaluated in this study were determined empirically as part of the assay validation protocol and were accepted by the US Food and Drug Administration (FDA). Post-vaccination seroresponses for MMR vaccine viral antigens in initially seronegative subjects were defined as antibody concentrations/titers of: measles ≥ 200 mIU/mL [21]; mumps ≥ 51 ED₅₀ (no known correlate of protection threshold) and rubella ≥ 10 IU/mL [22]. The seroresponse thresholds were accepted by the FDA as those defining active immunization offering clinical benefit.

VAR response was defined as a post-vaccination antibody concentration ≥ 75 mIU/mL in initially seronegative subjects. HAV response was defined as a post-vaccination antibody concentration ≥ 15 mIU/mL in initially seronegative subjects, or a ≥ 2 -fold increase in the pre-vaccination antibody concentration in initially seropositive subjects.

Reactogenicity and Safety

Reactogenicity and safety were assessed at each visit and via subject diary cards completed by parents/guardians.

Solicited injection site symptoms (pain, redness, swelling for study vaccines only) were recorded from Days 0–3. Solicited general symptoms (fever, rash, parotid/salivary gland swelling, febrile convulsions, irritability/fussiness, drowsiness, and loss of appetite), and unsolicited symptoms were recorded from Days 0–42. Serious adverse events (SAEs) were recorded throughout the study. Fever was assessed daily with a tympanic thermometer or rectally if the tympanic reading indicated fever ($\geq 38.0^{\circ}\text{C}$). For each reported symptom, parents/guardians were asked what medical attention (if any) the subject had received.

Statistics

This was a “hypothesis-generating” exploratory study conducted to provide estimations of response rates, which will be used to develop statistical criteria for a formal Phase-3 trial to support licensure of the candidate vaccine on the basis of non-inferior immunogenicity compared to the licensed comparator. All analyses in this study were descriptive, and no formal statistical comparison was prespecified.

Enrollment of 1200 subjects (300/group) was planned to ensure ≥ 240 evaluable subjects/group. Subjects in the MMRII group were randomized across 3 commercial MMRII lots; no lot-by-lot analysis was done and results were pooled. The primary analysis of immunogenicity was conducted on the according-to-protocol (ATP) cohort for immunogenicity, which included eligible subjects who had received the study vaccine via the correct administration route and complied with study procedures, and who were below the assay cut-off for at least 1 MMR vaccine antigen at baseline, with pre-vaccination and post-vaccination serology results available. Safety analysis was performed on the total vaccinated cohort (TVC), which included all vaccinated subjects.

The primary endpoint was seroresponse rates for antibodies to measles, mumps, and rubella viruses at Day 42; the proportions of subjects with antibody concentration/titer at or above specified assay cut-offs were calculated with exact 95% confidence intervals (CIs) both pre- and post-vaccination. Secondary endpoints included pre- and post-vaccination (Day 42) antibody concentration/titers, summarized by geometric mean concentrations/titers (GMC/Ts) with 95% CI. Exploratory analyses included standardized asymptotic 2-sided 95% CIs calculated for group differences (MMR-RIT group minus MMRII) in Day-42 seroresponse rates for antibodies to MMR viruses. In addition, 95% CIs for GMC ratios (MMR-RIT: MMRII) for antibodies to hepatitis A virus and PCV7 pneumococcal serotypes were obtained using an analysis of covariance model on the \log_{10} -transformed Day-42 concentrations. For the safety analysis, the number

and percentage of subjects reporting a symptom were calculated with exact 95% CIs. Symptoms were categorized according to intensity and relationship to study vaccine. All data processing and analyses were performed using SAS® version 9.2 (SAS Institute Inc., Cary, NC). Proc StatXact 8.1 derived exact 95% CIs for a proportion within a group as well as standardized asymptotic 95% CI for the group difference in proportions.

RESULTS

Subject Disposition and Baseline Demography

The first subject was enrolled on June 3, 2009, and the last visit of the active (43-day) phase was completed on July 21, 2010. Of 1259 enrolled subjects, 1224 were randomized and 4 did not receive study vaccine. The TVC consisted of 1220 subjects: MMR-RIT-1 (n = 304), MMR-RIT-2 (n = 304), MMR-RIT-3 (n = 304), and MMRII (n = 308). Of these, 1117 completed Day 42 and 103 were withdrawn (Figure 1).

Overall, of 1220 subjects in the TVC, 1026 subjects were included in the ATP-immunogenicity cohort (194 subjects were excluded): MMR-RIT-1 (n = 261); MMR-RIT-2 (n = 254); MMR-RIT-3 (n = 251), and MMRII (n = 260). Demographic characteristics of the 4 treatment groups were comparable between the TVC and ATP-immunogenicity cohorts. Mean (standard deviation) age in the TVC was 12.3 (0.71) months, 75.8% of subjects were white, and 51.1% were male. In the ATP-immunogenicity cohort, 100%, 86.2%, and 99.8%, of subjects were seronegative for measles, mumps and rubella antibody, respectively, before study vaccination; overall baseline seronegativity rates were comparable across the 4 groups (Table 2).

Immunogenicity

Seroresponse to MMR Vaccines. Measles virus seroresponse rates were 98.3–99.2% for MMR-RIT groups and 99.6% for the MMRII group; GMCs of measles virus antibodies were >2500 mIU/mL in all 4 groups. Day-42 seroresponse rates for mumps virus antibodies were 89.7% for

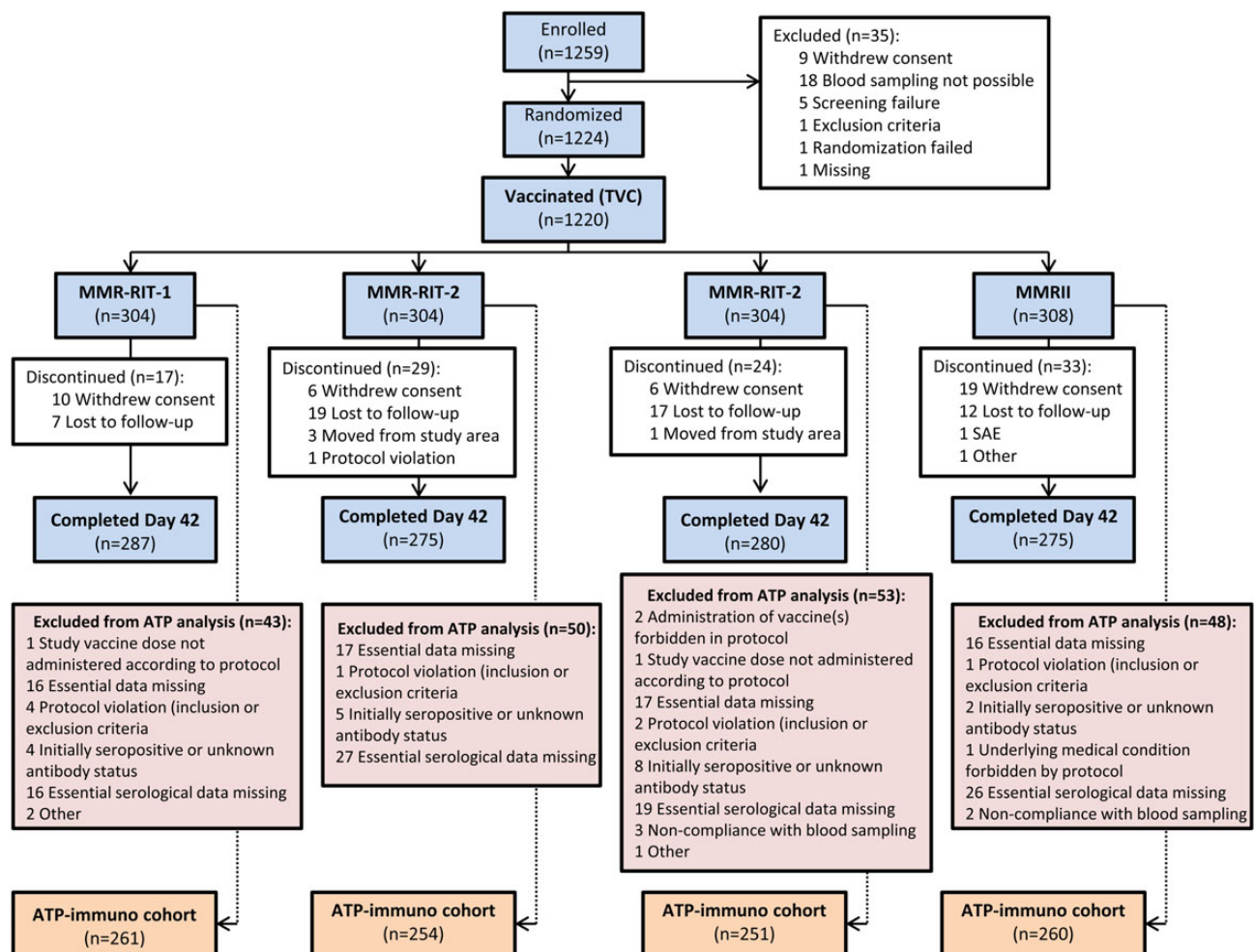


Figure 1. Disposition of subjects in the total vaccinated cohort (TVC) (enrolled = 1259 subjects, randomized = 1224 subjects, vaccinated = 1220 subjects). Abbreviations: MMR, measles-mumps-rubella; SAE, serious adverse event; ATP, according-to-protocol.

Table 2. Demographic and Baseline Data for the Total Vaccinated Cohort (TVC) and According-to-Protocol (ATP) Cohort for Immunogenicity

	MMR-RIT-1		MMR-RIT-2		MMR-RIT-3		MMR-II		Total	
	TVC N = 304	ATP N = 261	TVC N = 304	ATP N = 254	TVC N = 304	ATP N = 251	TVC N = 308	ATP N = 260	TVC N = 1220	ATP N = 1026
Age (mo), mean (SD)	12.4 (0.75)	12.4 (0.69)	12.4 (0.73)	12.4 (0.73)	12.2 (0.56)	12.2 (0.57)	12.4 (0.75)	12.4 (0.73)	12.3 (0.71)	12.3 (0.69)
Gender										
Female, n (%)	156 (51.3)	134 (51.3)	144 (47.4)	120 (47.2)	157 (51.6)	128 (51.0)	139 (45.1)	118 (45.4)	596 (48.9)	500 (48.7)
Male, n (%)	148 (48.7)	127 (48.7)	160 (52.6)	134 (52.8)	147 (48.4)	123 (49.0)	169 (54.9)	142 (54.6)	624 (51.1)	526 (51.3)
Antibody (cut-off point)	Pre-vaccination status of ATP cohort for immunogenicity, no. of seronegative subjects (%) ^a									
Measles (<150 mIU/mL)	MMR-RIT-1 N = 261 ^b	MMR-RIT-2 N = 254 ^b	MMR-RIT-3 N = 251 ^b	MMR-II N = 260 ^b	Total N = 1026 ^b					
Mumps (ED ₅₀ <24)	259/259 (100)	253/253 (100)	249/249 (100)	258/258 (100)	1019/1019 (100)					
Rubella (<4 IU/mL)	216/252 (85.7)	219/248 (88.3)	213/246 (86.6)	213/243 (86.6)	861/999 (86.2)					
	259/259 (100)	251/252 (99.6)	248/249 (99.6)	258/258 (100)	1016/1018 (99.8)					

Abbreviations: MMR, measles-mumps-rubella; N, total number of subjects; SD, standard deviation.

^aCalculated for subjects in TVC for which pre-vaccination status was known (ie, % = n/[n + seropositive] × 100, where n = no. of seronegative subjects).

^bNumber of subjects regardless of the status of baseline detection of antibody (ie, sum of seronegative, seropositive, and unknown).

MMR-RIT-3 (low mumps titer), 90.6% for MMR-RIT-2 (medium mumps titer), 90.7% for MMR-RIT-1 (high mumps titer), and 91.1% for MMR-II. Mumps virus antibody GMTs were at least 10-fold greater than the assay cut-off for seronegativity in all 4 groups. Day-42 rubella virus seroresponse rates were 97.5–98.8% for MMR-RIT groups and 100% for the MMR-II group. Observed rubella virus antibody GMCs for MMR-RIT groups (68.2–77.7 IU/mL) and MMR-II (89.4 IU/mL) were above the assay seronegativity cut-off of 10 IU/mL (Table 3).

Co-administration of VAR, HAV, and PCV7. Day-42 seroresponse rates for antibodies to VZV among initially seronegative subjects when VAR was co-administered with MMR-RIT or MMR-II were 95.8–98.0%. GMCs for VZV antibodies were >200 mIU/mL in all 4 groups. Day-42 response rates for antibodies to hepatitis A virus were 83.0–89.3% among initially seronegative vaccinees across the 4 groups. Day-42 GMCs adjusted for baseline serostatus for antibodies to hepatitis A virus or pneumococcal serotypes appeared to be similar for all 4 groups, as shown by calculated GMC ratios in Table 4.

Safety and Reactogenicity

Overall incidence of solicited and unsolicited symptoms in the TVC (Days-0–42) was 80.9% (95% CI: 76.0; 85.2) for MMR-RIT-1, 75.7% (70.4; 80.4) for MMR-RIT-2, 74.0% (68.7; 78.9) for MMR-RIT-3, and 75.3% (70.1; 80.0) for MMR-II. The most frequently observed solicited symptom (Days 0–3) at MMR-RIT and MMR-II injection sites was pain, reported in ≥24.5% of subjects in each group (Table 5), although Grade-3 pain (subject cried when the limb was moved/spontaneously painful) was reported in ≤1.5%. Two subjects (1 each in the MMR-RIT-2 and MMR-II groups) had Grade-3 injection site swelling (diameter >20 mm).

During Days 0–14, irritability or fussiness (overall incidence, 51.3–63.6%) and drowsiness (38.5–47.0%) were the most frequently reported solicited general symptoms (Table 5). Incidence of fever (rectal temperature ≥38.0°C) during Days 0–14 was 20.2–28.7% across the 4 groups; fever >39.5°C occurred in ≤3.5% subjects in any group. Incidence of fever requiring medical attention reported during Days 0–14 in each group was as follows: 6.0% (95% CI: 3.5; 9.4) for MMR-RIT-1, 8.0% (5.1; 11.9) for MMR-RIT-2, 8.5% (5.5; 12.4) for MMR-RIT-3, and 6.9% (4.2; 10.5) for MMR-II. Overall incidence of fever during Days 0–42 was 36.4–37.8% for MMR-RIT groups and 30.7% for MMR-II recipients (Table 5). For all groups, prevalence of fever peaked 5–12 days after study vaccination (Figure 2); in an exploratory *post hoc* analysis, the observed

Table 3. Seroresponse Rates and Geometric Mean Concentrations/Titers (GMC/Ts) for Antibodies to Measles, Mumps, and Rubella Viruses at Day 42 in Initially Seronegative Subjects (According-to-Protocol Cohort for Immunogenicity)

Measles (≥ 200 mIU/mL)				Mumps (≥ 51 ED ₅₀)				Rubella (≥ 10 IU/mL)			
Seroreponse		% Diff. vs. MMRII (95% CI)	GMC (95% CI)	Seroreponse		% Diff. vs. MMRII (95% CI)	GMC (95% CI)	Seroreponse		% Diff. vs. MMRII (95% CI)	GMC (95% CI)
N	n (%) (95% CI)			N	n (%) (95% CI)			N	n (%) (95% CI)		
247	245 (99.2) (97.1; 99.9)	-0.41 (-2.55; 1.50)	2799 (2545; 3078)	193	175 (90.7) (85.7; 94.4)	MMR-RIT-1 -0.47 (-6.42; 5.46)	242 (205; 287)	247	244 (98.8) (96.5; 99.7)	-1.21 (-3.51; 0.32)	72.2 (65.6; 79.6)
240	236 (98.3) (95.8; 99.5)	-1.27 (-3.85; 0.74)	2878 (2607; 3178)	202	183 (90.6) (85.7; 94.2)	MMR-RIT-2 -0.55 (-6.41; 5.35)	265 (222; 317)	238	235 (98.7) (96.4; 99.7)	-1.26 (-3.64; 0.27)	77.7 (70.4; 85.7)
240	236 (98.3) (95.8; 99.5)	-1.27 (-3.85; 0.74)	2593 (2350; 2861)	195	175 (89.7) (84.6; 93.6)	MMR-RIT-3 -1.40 (-7.47; 4.62)	253 (213; 301)	239	233 (97.5) (94.6; 99.1)	-2.51 (-5.37; -0.97)	68.2 (61.8; 75.3)
249	248 (99.6) (97.8; 100)	Reference	2950 (2698; 3224)	192	175 (91.1) (86.2; 94.8)	MMRII Reference	268 (224; 320)	249	249 (100) (98.5; 100)	Reference	89.4 (81.4; 98.2)

Abbreviations: MMR, measles-mumps-rubella; N, number of subjects with available results; n (%), number/percentage of subjects who seroconverted; 95% CI, 95% confidence interval; GMC/T, geometric mean concentrations/titers for measles, mumps, and rubella virus antibodies.

Table 4. Geometric Mean Concentrations (GMCs) for Varicella-Zoster Virus (VZV) Antibodies, and Baseline-Adjusted GMCs and GMC Ratios for Antibodies to Hepatitis A Virus and PCV7 Pneumococcal Serotypes at Day 42 (According-to-Protocol Cohort for Immunogenicity)

Antibody	MMRII		MMR-RIT-1		MMR-RIT-2		MMR-RIT-3	
	N	GMC (95% CI)	N	GMC (95% CI)	N	GMC (95% CI)	N	GMC (95% CI)
VZV	246	256 (240; 272)	245	246 (229; 263)	238	235 (217; 254)	240	256 (240; 272)
Antibody	MMRII		MMR-RIT-1		MMR-RIT-2		MMR-RIT-3	
	N	Adjusted GMC	N	Adjusted GMC	Adjusted GMC ratio ^a (95% CI)	N	Adjusted GMC	Adjusted GMC ratio ^a (95% CI)
Hepatitis A virus	124	42.0	117	33.9	0.81 (0.64; 1.02)	112	39.2	0.93 (0.74; 1.18)
S.PNEU-4	116	3.68	122	3.69	1.00 (0.82; 1.24)	125	3.78	1.03 (0.84; 1.26)
S.PNEU-6B	111	6.50	117	5.86	0.90 (0.75; 1.09)	122	5.87	0.90 (0.75; 1.09)
S.PNEU-9V	120	7.32	121	6.65	0.91 (0.76; 1.08)	125	7.23	0.99 (0.83; 1.17)
S.PNEU-14	118	7.87	127	8.91	1.13 (0.95; 1.35)	127	8.29	1.05 (0.88; 1.26)
S.PNEU-18C	119	6.58	123	6.29	0.96 (0.79; 1.15)	126	6.62	1.01 (0.84; 1.21)
S.PNEU-19F	115	2.39	122	2.41	1.01 (0.83; 1.22)	126	2.52	1.05 (0.87; 1.27)
S.PNEU-23F	113	10.21	121	9.56	0.94 (0.76; 1.16)	127	9.69	0.95 (0.77; 1.17)

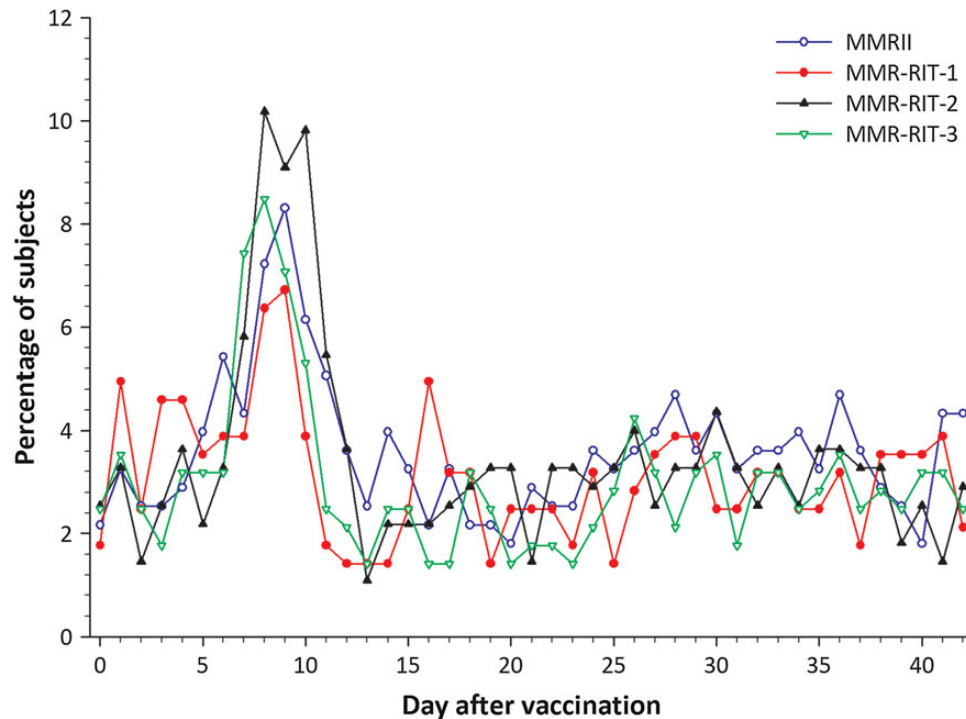
Abbreviations: Adjusted GMC, geometric mean antibody concentration adjusted for baseline antibody concentration; MMR, measles-mumps-rubella; N, Number of subjects with both pre- and post-vaccination results available; 95% CI, 95% confidence interval for the adjusted GMC ratio (ANCOVA model: adjustment for baseline concentration - pooled variance with more than 2 groups); S.PNEU, *Streptococcus pneumoniae*.

^aRatio of MMR-RIT lot: MMRII.

Table 5. Incidence of Solicited Injection Site (Days 0; 3) and General Symptoms During the 43-Day Post-vaccination Period (Total Vaccinated Cohort)

Symptom	MMR-RIT-1 (N = 283)		MMR-RIT-2 (N = 275)		MMR-RIT-3 (N = 283)		MMRII (N = 277)	
	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)
	Days 0; 3							
Pain	70	24.8 (19.9; 30.3)	70	25.5 (20.5; 31.1)	79	28.0 (22.9; 33.6)	67	24.5 (19.5; 30.0)
Redness	45	16.0 (11.9; 20.8)	47	17.2 (12.9; 22.1)	41	14.5 (10.6; 19.2)	47	17.2 (12.9; 22.1)
Swelling	20	7.1 (4.4; 10.7)	26	9.5 (6.3; 13.6)	19	6.7 (4.1; 10.3)	15	5.5 (3.1; 8.9)
	Days 0; 14							
Irritability/fussiness	180	63.6 (57.7; 69.2)	141	51.3 (45.2; 57.3)	150	53.0 (47.0; 58.9)	153	55.2 (49.2; 61.2)
Drowsiness	133	47.0 (41.1; 53.0)	106	38.5 (32.8; 44.6)	113	39.9 (34.2; 45.9)	109	39.4 (33.6; 45.4)
Loss of appetite	111	39.2 (33.5; 45.2)	77	28.0 (22.8; 33.7)	110	38.9 (33.2; 44.8)	94	33.9 (28.4; 39.8)
Fever (rectal temp. $\geq 38.0^{\circ}\text{C}$)	65	23.0 (18.2; 28.3)	79	28.7 (23.5; 34.5)	64	22.6 (17.9; 27.9)	56	20.2 (15.6; 25.4)
Fever (rectal temp. $>39.5^{\circ}\text{C}$)	10	3.5 (1.7; 6.4)	7	2.5 (1.0; 5.2)	9	3.2 (1.5; 6.0)	8	2.9 (1.3; 5.6)
	Days 0; 42							
Fever (rectal temp. $\geq 38.0^{\circ}\text{C}$)	103	36.4 (30.8; 42.3)	104	37.8 (32.1; 43.8)	104	36.7 (31.1; 42.7)	85	30.7 (25.3; 36.5)
Fever (rectal temp. $>39.5^{\circ}\text{C}$)	20	7.1 (4.4; 10.7)	14	5.1 (2.8; 8.4)	18	6.4 (3.8; 9.9)	13	4.7 (2.5; 7.9)
Localized/generalized rash	72	25.4 (20.5; 30.9)	74	26.9 (21.8; 32.6)	60	21.2 (16.6; 26.4)	69	24.9 (19.9; 30.4)
Parotid gland swelling	3	1.1 (0.2; 3.1)	3	1.1 (0.2; 3.2)	5	1.8 (0.6; 4.1)	2	0.7 (0.1; 2.6)

Abbreviations: MMR, measles-mumps-rubella; N, number of subjects having received the documented dose; n/%, number/percentage of subjects reporting a specified symptom; 95% CI, exact 95% confidence interval.

**Figure 2.** Prevalence of any fever from Day 0 to Day 42 after vaccination (total vaccinated cohort). Abbreviation: MMR, measles-mumps-rubella.

incidence of fever between Days 5 and 12 was 14.8% (95% CI: 10.9; 19.5) for MMR-RIT-1, 23.3% (18.4; 28.7) for MMR-RIT-2, 17.3% (13.1; 22.2) for MMR-RIT-3, and 14.8% (10.8; 19.5) for MMRII.

For Days 0–42, incidence of rash of any type varied between 21.2% and 26.9% across the 4 groups. Measles or rubella-like rashes were reported in MMR-RIT-1 (n = 6), MMR-RIT-2 (n = 7), MMR-RIT-3 (n = 5), and MMRII (n = 9) recipients; varicella-like rash was reported for

MMR-RIT-2 (n = 4) and MMRII (n = 2) subjects. Overall incidence of parotid-gland swelling was low (Table 5). There were 2 cases of febrile convulsion during Days 0–42. One MMR-RIT-2 recipient experienced a simple febrile convulsion at Day 29. This was not considered vaccine-related by the investigator since the peak prevalence of vaccine-related fever, and hence febrile convulsions, occurs in the second week following vaccination with measles-containing vaccines [23].

One MMRII recipient had a complex febrile seizure on Day 0, which led to hospitalization and the mother's withdrawal of the subject from the study; this event, classified as an SAE, was considered to be related to the study vaccine.

Of 15 SAEs reported in 11 subjects (Days 0–42), 2 SAEs were considered to be related to study treatment: febrile convulsion in a 12-month-old female MMRII recipient (described above), and idiopathic thrombocytopenic purpura (onset at Day 20) in a 13-month-old female MMR-RIT-2 recipient who was hospitalized for treatment and discharged after 3 days. Both subjects with vaccine-related SAEs were withdrawn from the study. All SAEs resolved without sequelae.

Concomitant Medication

Rates of concomitant medication use (Days 0–42) were the following: MMR-RIT-1 (76.6%), MMR-RIT-2 (72.0%), MMR-RIT-3 (74.0%), and MMRII (70.1%). Rates of antipyretic medication use were the following: MMR-RIT-1 (65.1%), MMR-RIT-2 (59.2%), MMR-RIT-3 (64.1%), and MMRII (57.5%).

DISCUSSION

This Phase-2 multicenter exploratory study assessed immune responses to the first dose of MMR-RIT with 3 differing mumps virus titers and to commercially available MMRII when concomitantly administered with HAV, VAR, and PCV7 in healthy children in the United States, aged 12–15 months. The results indicate that a single dose of any 1 of the 3 MMR-RIT lots elicited an acceptable immune response with respect to seroresponse rates to MMR viruses, 42 days post-vaccination. Previous randomized comparative studies have shown that MMR-RIT administered as a primary vaccination to children in the second year of life produced similar seroconversion rates for antibodies to MMR vaccine viruses compared to those seen with MMRII [24–28].

The current formulation of MMR-RIT used in this study, when co-administered to young children with other recommended vaccines (HAV, VAR, and PCV7), elicited measles and rubella virus antibody concentrations meeting the predefined threshold for seroresponse in $\geq 97.5\%$ MMR-RIT recipients, and in $\geq 99.6\%$ MMRII recipients when co-administered with the same vaccines.

Day-42 mumps virus antibody titers after vaccination with all 3 MMR-RIT lots met the seroresponse threshold in 90.7%, 90.6%, and 89.7% recipients, respectively, and no obvious dose–response relationship was observed. Of note, 13.8% of subjects were seropositive for mumps at baseline. This high baseline seropositivity rate is likely

attributed to complement enhancement of the mumps PRN assay rather than prior exposure to mumps or persistence of maternal antibodies. Complement-enhanced mumps PRN assays have been shown to have higher seroresponse rates than unenhanced assays [29], and this may also be reflected in the baseline seropositivity. Rubella seroresponse rates across all 4 groups were high (97.5–100%) and GMCs for antibodies to rubella virus following all 3 MMR-RIT lots were substantially higher than the cut-off level of ≥ 10 IU/mL and would provide effective immunization. Long-term follow-up in this study will allow the evaluation of antibody persistence until approximately 2 years post-vaccination.

The immune response to vaccines routinely co-administered with MMR dose-1 in the United States (HAV, VAR, and PCV7) was also assessed. Observed seroresponse rates for antibodies to VZV were consistently high ($\geq 95.8\%$) across all 4 treatment groups, and hepatitis A virus antibody response rates to HAV dose-1 were $\geq 83.0\%$ in each group. Furthermore, Day-42 baseline-adjusted GMCs for antibodies to hepatitis A virus and *Streptococcus pneumoniae* serotypes appeared comparable when these vaccines were administered with MMR-RIT or MMRII in this exploratory analysis of between-group GMC ratios.

MMR-RIT had an acceptable reactogenicity profile when co-administered with HAV, VAR, and PCV7. Injection site symptoms at the MMR injection site occurred in all 4 treatment groups within 4 days of vaccination, although the incidence of severe symptoms was low. Consistent with previous reports [25–28], vaccination with MMR-RIT or MMRII was associated with fever (rectal temperature $\geq 38.0^\circ\text{C}$) during the first 2 weeks, which peaked during days 5–12 after vaccine administration. MMR-RIT, which contains the Jeryl Lynn–derived RIT 4385 strain, has demonstrated a good reactogenicity profile in previous clinical trials [25–28, 30]. Accordingly, in this study, a low incidence of other solicited general symptoms, including measles/rubella- or varicella-like rash, parotid gland swelling, and febrile convulsions, was reported among both MMR-RIT and MMRII recipients during the follow-up period.

Strengths of this study include use of a computer-generated randomization list and blinding of study vaccines, thereby addressing potential biases in study conduct, and comparing the immune responses to the study vaccine with the US-licensed standard of care (MMRII). Furthermore, the MMR-RIT formulation used in this study was free of human serum albumin, thus eliminating the theoretical risk of microbial contamination associated with human serum albumin-containing vaccines [14].

Additionally, omitting a human blood-derived component from the MMR-RIT formulation might make it more socially acceptable. Lastly, concomitant administration of routine vaccines (HAV, VAR, and PCV7) did not affect the immunogenicity of MMR-RIT and vice versa.

Limitations of the study include the relatively small study population that is consistent with a Phase-2 planning study. Larger Phase-3 clinical trials are required in future to substantiate the immunogenicity and safety profile of MMR-RIT. Furthermore, extended follow-up studies are important to evaluate the long-term protection offered by MMR-RIT.

CONCLUSIONS

This Phase-2 study demonstrated an acceptable immune response to 3 candidate MMR-RIT lots containing differing mumps virus titers with respect to seroresponse rates to all 3 MMR virus components. There was no obvious dose-response relationship for the 3 mumps virus titers evaluated; based on the current results, all 3 lots would provide effective immunization, with an acceptable reactogenicity profile. MMR-RIT can be given concomitantly with HAV, VAR, and PCV7 without interfering with the immune response to these co-administered vaccines, as was shown for MMR-II. Confirmatory Phase-3 studies to support licensure of MMR-RIT on the basis of immunogenic non-inferiority to the licensed comparator are warranted.

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Potential conflicts of interest. C.D.C., B.L.I., and O.N., former and current employees of the sponsor, may own stock or stock options in the company. M.P. is an employee of GlaxoSmithKline group of companies. M.A.M. received travel support for a meeting for the study and payment for lectures, including service on speakers bureau (Merck). C.J.H. declares that his institution receives grants to conduct the trials for which he is the investigator; he has also received honoraria for lectures, including service on speakers bureau (Merck and Sanofi). S.G. received support for travel, consulting fees, payment for lectures, and fees for participation in review. M.L. has in the past, and is currently being paid by the sponsor as a principal investigator for vaccine research. C.D., A.C., S.C.T., R.J.F., A.Q.D.R., and G.B. have indicated they have no financial relationship or conflict of interest relevant to this article to disclose.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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MMR-II is a registered trademark of Merck & Co. Inc., Whitehouse Station, NJ, United States.

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Author Contributions

C.D.C. and B.I. conceived the study, designed the trial, and obtained research funding. All authors substantially contributed to the conception, the design, the acquisition of data, and the analysis and interpretation of data. M.M., C.D., M.L., C.H., S.G., A.C., S.C.T., R.J., and A.Q. recruited patients. M.P. provided statistical advice on study design and analyzed the data. All authors contributed substantially to the drafting of the article. They revised it critically for important intellectual content and approved the version to be published.

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