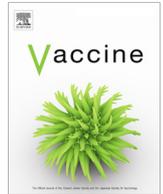




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Immunogenicity and safety of measles-mumps-rubella vaccine at two different potency levels administered to healthy children aged 12–15 months: A phase III, randomized, non-inferiority trial

The MMR-161 Study Group¹

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ABSTRACT

Background: The potency of live viral vaccines decreases over time. We compared the immunogenicity and safety of GSK measles-mumps-rubella vaccine (MMR-RIT) formulations at two different potencies with that of the commercially-available MMR II formulation.

Methods: In this phase III observer-blind clinical study (NCT01681992), 4516 healthy children aged 12–15 months were randomized (1:1:1 ratio) to receive one dose of MMR-RIT at the minimum potency used for this study (MMR-RIT-Min) or MMR-RIT at the second lowest potency used for this study (MMR-RIT-Med), or control MMR II vaccine. A second dose (MMR-RIT or MMR II) was administered 42 days after the first. The study had 10 co-primary objectives to evaluate MMR-RIT versus MMR II immunogenicity via a hierarchical procedure. Anti-measles and anti-rubella antibodies were measured by ELISA and anti-mumps antibodies by ELISA and unenhanced plaque reduction neutralization test (PRNT).

Results: Each formulation induced immune responses to all vaccine antigens after each MMR dose. While the primary objectives for MMR-RIT-Min were not met, MMR-RIT-Med induced immune responses as measured by ELISA against the three vaccine antigens that met pre-specified non-inferiority criteria. The immune response following MMR-RIT-Med against mumps measured by PRNT failed the non-inferiority criterion for seroresponse rate: the 97.5% confidence interval lower limit (–10.94%) was beyond the pre-defined limit of –10%. Immune responses were comparable among groups post-dose 2. No safety concerns were identified, and MMR-RIT and MMR II vaccines had similar reactogenicity and safety profiles.

Conclusions: One dose of MMR-RIT formulation with lower potency (MMR-RIT-Med) induced a non-inferior immune response compared to commercial MMR II vaccine, measured by ELISA in one-year-old children. Non-inferiority was not demonstrated in terms of immune response against mumps virus measured by unenhanced PRNT, although the difference was of uncertain clinical relevance. After the second dose, immune responses were comparable among the MMR-RIT and MMR II groups.

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Abbreviations: AE, adverse event; ATP, according-to-protocol; CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; GMC, geometric mean concentration; GMT, geometric mean titer; HAV, hepatitis A vaccine; IgG, immunoglobulin G; LAR, legally acceptable representative; LL, lower limit; MMR, measles-mumps-rubella; MMR-RIT-Min, MMR-RIT vaccine at the minimum potency used for this study; MMR-RIT-Med, MMR-RIT vaccine at the second lowest potency used in this study; NOCD, new onset chronic disease; PCV13, 13-valent pneumococcal conjugate vaccine; PRNT, plaque reduction neutralization test; SAE, serious adverse event; SAS, Statistical Analysis Systems; US, United States; VAR, varicella vaccine; WHO, World Health Organization.

¹ See Contributors section. Corresponding author: Federico Martinon-Torres, MD, PhD, Translational Pediatrics and Infectious Diseases, Pediatrics Department, Hospital Clínico Universitario de Santiago de Compostela, A Choupana s.n., 15706 Santiago de Compostela, Spain. E-mail address: federico.martinon.torres.

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1. Introduction

The World Health Organization (WHO) and United States (US) Centers for Disease Control and Prevention recommend universal vaccination of children with two doses of live attenuated combined measles-mumps-rubella (MMR) vaccine [1–4]. Although vaccine coverage rates are generally high in most countries [5,6], measles and mumps outbreaks still occur [1,7–12].

In the US, MMR II (*M-M-R II*, Merck & Co., Inc.) is the only MMR vaccine available. Another MMR vaccine, MMR-RIT (*Priorix*, GSK), is licensed for use in individuals aged 9 months and older [13] in over 100 countries outside the US. Both vaccines have a shelf life of two years under specified packaging and storage conditions [13,14].

As the potency of live viral vaccines tends to decay over time [15], it is important to demonstrate adequate immunogenicity at a potency typical of end of shelf-life [9], to provide reassurance on the vaccine's continued capability to confer protective immunity. We conducted a study in which the immunogenicity and safety of two MMR-RIT formulations with lower potency were compared with the commercially-available MMR II formulation when administered to children aged 12–15 months. The first MMR-RIT dose was at either the minimum potency or the second lowest potency used for this study, while commercial MMR-RIT was administered as a second dose. The immunogenicity and safety of the MMR vaccines were also compared after the second dose.

2. Methods

2.1. Study design and participants

This phase III randomized, observer-blind, controlled clinical study (NCT01681992) was conducted in 81 centers in six countries (Czech Republic, Finland, Malaysia, Spain, Thailand, and US) between October 2012 and August 2015.

The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines, and each local site was approved by a national, regional, or investigational center institutional review board or independent ethics committee. The study's purpose, procedures, and parental responsibilities were explained in detail to each parent or legally acceptable representative (LAR) who expressed interest in participating in the study. Written informed consent was obtained from parents/LARs before enrollment.

Healthy children aged 12–15 months who had not been immunized against (and had no history of) measles, mumps, rubella, varicella, or hepatitis A were enrolled. Exclusion criteria are listed in the Supplement. Children were randomized, using a blocking scheme (1:1:1 ratio), to receive either one dose of MMR-RIT at

the minimum potency used for this study (MMR-RIT-Min) or MMR-RIT at the second lowest potency used for this study (MMR-RIT-Med), or one dose of control MMR II vaccine (Fig. 1, Table S1). MMR-RIT-Min had the lowest potency tested in a MMR-RIT clinical study. To ensure a robust control group, the MMR II vaccine was procured in pairs of lots: >10 MMR II lots were used in the study, effectively establishing a standard response curve to MMR II. The randomization list was generated at GSK using MATerial EXcellence (MATEX), a program developed by GSK for use with Statistical Analysis Systems (SAS) software. Treatment allocation was performed at each site via a central internet-based randomization system. Due to differences in vaccine appearance and storage, the study was conducted in an observer-blind manner, i.e. neither the investigator nor the subject/parent/LAR was aware of which vaccine was received and staff handling study vaccines were not involved in the assessment of study endpoints.

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.vaccine.2018.07.076>.

Other vaccines were administered according to national immunization program schedules. All children received concomitant single doses of hepatitis A vaccine (HAV; *Havrix*, GSK) and varicella vaccine (VAR; *Varivax*, Merck & Co., Inc.) with the first MMR dose. Children enrolled in the US also received a dose of 13-valent pneumococcal conjugate vaccine (PCV13; *Prevnar 13*, Pfizer), having already received three PCV13 doses, with the last dose at least 60 days before study entry. MMR and VAR doses were administered subcutaneously and HAV and PCV13 were administered intramuscularly. As the first MMR dose could have a lower potency, which could induce a lower immune response, a second MMR dose (MMR-RIT or MMR II) was administered 42 days after the first to ensure protection of children (Fig. 1; Table S1).

The study had 10 co-primary objectives to evaluate immunogenicity after the first dose of MMR-RIT compared to MMR II vaccine, as described in the statistical analyses section. Secondary objectives included evaluation of immunogenicity by

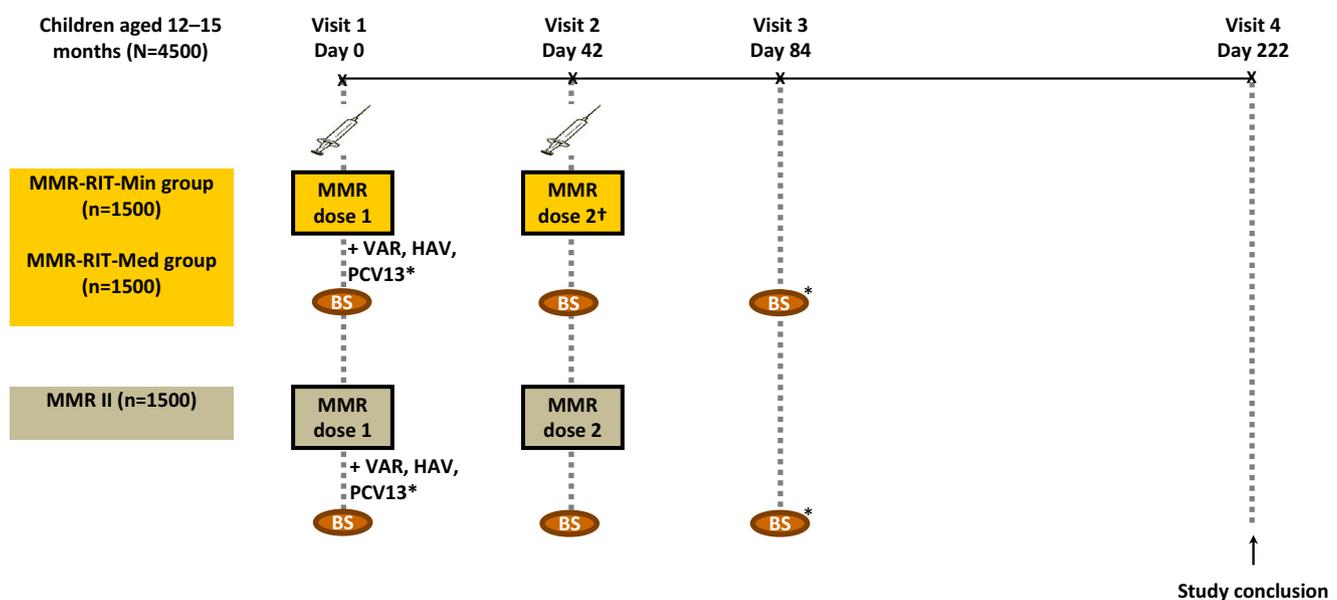


Fig. 1. Study design. Healthy children aged 12–15 months were randomized (1:1:1 ratio) to receive one dose of either MMR-RIT at the minimum potency used for this study (MMR-RIT-Min) or MMR-RIT at the second lowest potency used for this study (MMR-RIT-Med), or one dose of MMR II vaccine. All children received concomitant single doses of hepatitis A vaccine (HAV) and varicella vaccine (VAR). Children enrolled in the US also received a dose of 13-valent pneumococcal conjugate vaccine (PCV13). A second dose (MMR-RIT or MMR II) was administered 42 days after the first. BS, Blood sample; N, planned number of study participants; n, planned number of participants in each study group. †Only for children enrolled in US. ‡Commercial MMR-RIT.

enzyme-linked immunosorbent assay (ELISA) after the second MMR dose in children enrolled in the US, and the assessment of vaccine safety and reactogenicity in all children.

2.2. Immunogenicity assessment

Immunogenicity assessments were performed on blood samples taken before the first vaccination, 42 days after the first dose, and (for children enrolled in the US) 42 days after the second dose (Fig. 1). Sera were stored at -20°C until assayed. Immunoglobulin G (IgG) antibodies to measles and rubella were measured using a commercial ELISA, *Enzygnost* (Dade Behring Marburg GmbH, Germany) at NEOMED-LABS Inc., Quebec, Canada. IgG antibodies to mumps were measured using a quantitative purified protein derivative ELISA (Merck, USA) at PPD Inc., PA, USA. The tests were performed and interpreted according to the manufacturers' instructions.

Anti-mumps antibody concentrations were also determined by plaque reduction neutralization test without complement and without anti-IgG enhancement (unenanced PRNT; GSK) to assess the production of neutralizing antibodies, as described elsewhere [16].

Pre-vaccination samples were defined as seronegative to the different viral antigens if assay results were below 150 mIU/mL for measles, 5 EU/mL (ELISA) or 2.5 ED₅₀ (PRNT) for mumps, and 4 IU/mL for rubella. Post-vaccination seroresponses in initially seronegative children were defined as antibody concentrations/titers ≥ 200 mIU/mL for measles, ≥ 10 EU/mL (ELISA) or ≥ 4 ED₅₀ (PRNT) for mumps, and ≥ 10 IU/mL for rubella. These seroresponse thresholds were accepted by the US Food and Drug Administration as defining active immunization offering clinical benefit.

2.3. Reactogenicity and safety assessments

Reactogenicity and safety were assessed at each visit and via diary cards completed by parents/LARs. Solicited injection site symptoms (pain, redness, and swelling) were recorded for four days (Days 0–3) after the first vaccine dose. Some solicited general symptoms (irritability/fussiness, drowsiness, and loss of appetite) were recorded for 15 days while other solicited general symptoms (fever, rash, parotid/salivary gland swelling, and febrile convulsions), and unsolicited symptoms were recorded for 43 days after each vaccination. Fever was defined as temperature $\geq 38.0^{\circ}\text{C}$.

Serious adverse events (SAEs) and adverse events (AEs) of specific interest (new onset chronic disease [NOCD, see Supplement], AEs prompting emergency room or medically-attended visits) were recorded throughout the study. The intensity of each solicited symptom or AE was graded on a scale from 0 to 3 (see Supplement).

2.4. Statistical analyses

Considering that up to 20% of enrolled participants could be non-evaluable, it was planned to enroll 4500 children (Fig. 1) to obtain 3600 evaluable children (1200 in each MMR-RIT group and 1200 in MMR II group). This gave >99% power for meeting the co-primary objectives for each MMR-RIT vaccine under the hypothesis of no difference in immunogenicity between MMR-RIT and MMR II; the endpoint of anti-mumps antibody titers by PRNT drove the sample size calculation.

Primary analyses were conducted on the according-to-protocol (ATP) cohort for immunogenicity, including eligible children who received the study vaccine correctly and complied with study procedures, and were below the assay cut-off for at least one MMR vaccine antigen before vaccination. Percentages of children reaching the predefined immunological thresholds were deter-

mined with exact 95% confidence intervals (CIs). ELISA antibody geometric mean concentrations (GMCs) and PRNT antibody geometric mean titers (GMTs) were calculated with 95% CIs. Reactogenicity and safety analyses were performed on the total vaccinated cohort, including all vaccinated subjects. Incidences of AEs were calculated with exact 95% CIs.

Asymptotic standardized 97.5% CIs were computed for group differences in seroresponse rate [17] and percentage of children with antibody titer/concentration above each specific cut-off. The 97.5% CI for the group GMC ratio was computed using an ANOVA model on the logarithm-transformed concentrations, with vaccine group and country as fixed effects. To keep the global type I error of this study below 2.5%, a hierarchical procedure with adjustment of the nominal type I error was used for the study's 10 co-primary objectives. As described below, the first five related to MMR-RIT-Min and the second five to MMR-RIT-Med.

The first primary objective was to demonstrate non-inferiority of MMR-RIT-Min compared to MMR II in terms of seroresponse rates (by ELISA) for measles, mumps, and rubella 42 days after the first dose (Day 42). Criteria for non-inferiority were reached if the lower limit (LL) of the two-sided 97.5% CI on the group difference (MMR-RIT-Min minus MMR II) was -5% or higher. The second primary objective was to demonstrate non-inferiority of MMR-RIT-Min compared to MMR II in terms of antibody GMCs to the different viral antigens by ELISA at Day 42. Criteria for non-inferiority were reached if the LL of the two-sided 97.5% CI on the group ratio (MMR-RIT-Min over MMR II) was ≥ 0.67 . The third primary objective was to demonstrate an acceptable immune response of MMR-RIT-Min in terms of seroresponse rates for viral antigens at Day 42, which was reached if the LL of the two-sided 97.5% CI was $\geq 90\%$.

The fourth primary objective was to demonstrate non-inferiority of MMR-RIT-Min compared to MMR II in terms of seroresponse rates for mumps virus determined by PRNT at Day 42, which was shown if the LL of the two-sided 97.5% CI on the group difference was -10% or higher. The fifth primary objective was to demonstrate non-inferiority of MMR-RIT-Min compared to MMR II in terms of GMT for antibodies to mumps virus (by PRNT) at Day 42, which was shown if the LL of the two-sided 97.5% CI on the GMT ratio (MMR-RIT-Min over MMR II) was ≥ 0.67 .

Primary objectives 6–10 were the same as objectives 1–5, but comparing MMR-RIT-Med with MMR II. To conclude on objectives 6–10, if one or more of objectives 1–5 associated to MMR-RIT-Min were not met, a Bonferroni adjustment was to be used, hence the use of 97.5% CIs for all primary objectives. Primary objective 5 could only be reached if all associated criteria were met and objectives 1–4 had been reached. Likewise, primary objective 10 could only be reached if all the associated criteria were met and objectives 6–9 had been reached.

Statistical analyses were performed using SAS version 9.3 on SAS Drug Development 4.3.

3. Results

3.1. Study participants

We enrolled 4535 children, of whom 4516 were randomized and vaccinated with MMR-RIT-Min (1493 children), MMR-RIT-Med (1497), or MMR II (1526); 4297 children completed the study. The main reasons for discontinuation were consent withdrawal and lost to follow-up (Fig. 2). The ATP cohort for immunogenicity post-dose 1 included 4117 children (Fig. 2) and the ATP cohort for immunogenicity post-dose 2 included 764 children enrolled in the US (Figure S1). Demographic characteristics were similar among the study groups (Table 1).

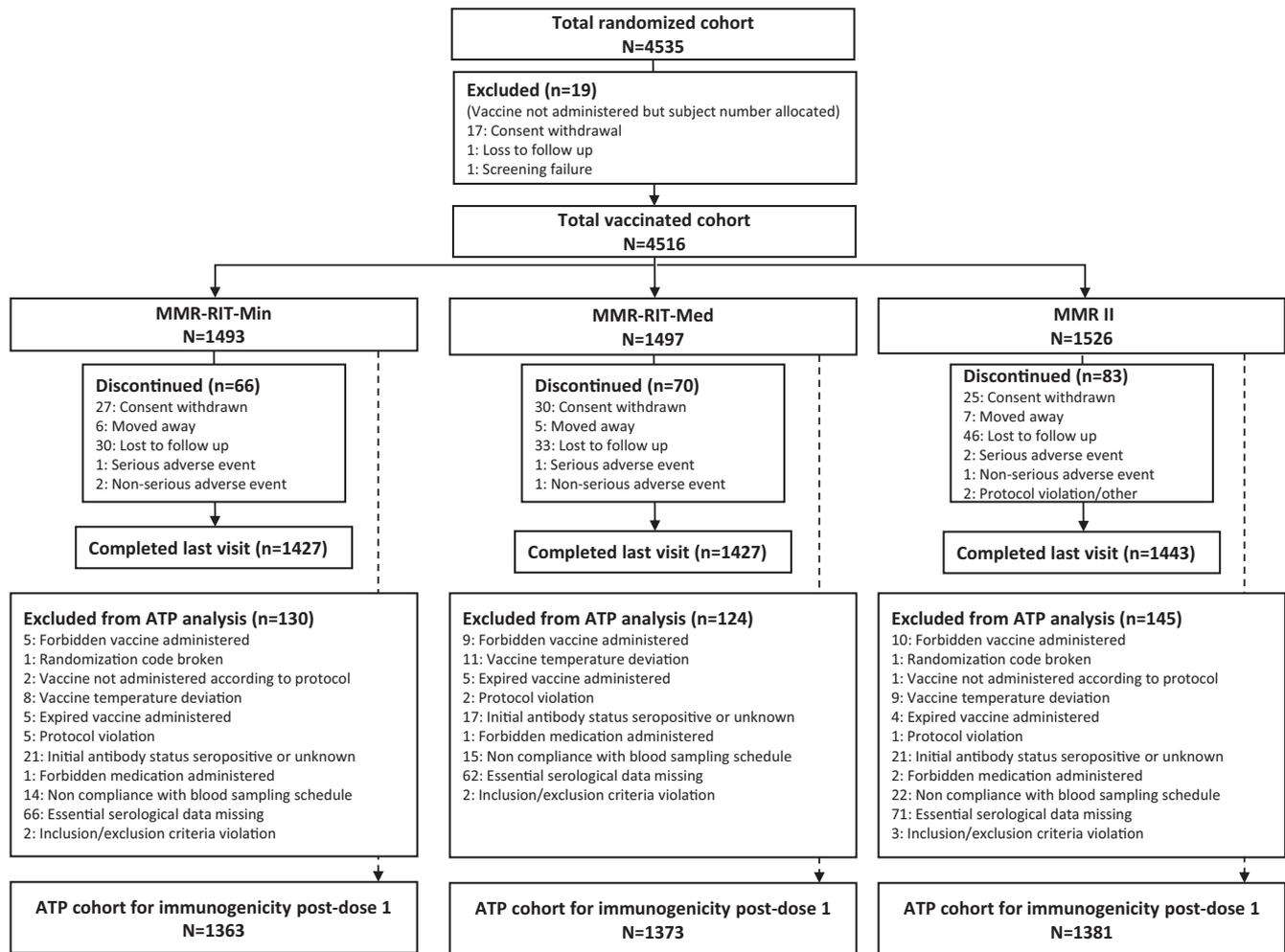


Fig. 2. Disposition of study participants in the post-dose 1 cohort. ATP, according-to-protocol; N, number of participants; n, number of participants in a given category.

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

Characteristic	MMR-RIT-Min (N = 1493)	MMR-RIT-Med (N = 1497)	MMR II (N = 1526)
Age (months) at dose 1, mean (SD)	12.6 (0.9)	12.6 (0.9)	12.6 (0.9)
Male gender, n (%)	789 (52.8)	779 (52.0)	768 (50.3)
Race, n (%)			
European heritage	1017 (68.1)	1022 (68.3)	1052 (68.9)
African heritage	45 (3.0)	53 (3.5)	46 (3.0)
Asian heritage	366 (24.5)	366 (24.4)	370 (24.2)
Other	65 (4.4)	56 (3.7)	58 (3.8)

N, number of children; n (%), number (percentage) of children with specified characteristic; SD, standard deviation.

3.2. Immunogenicity

3.2.1. Non-inferiority of the immune response to MMR-RIT-Min/Med versus MMR II

Seroresponse rates were comparable among the three groups for each vaccine antigen (Table 2). The LL of the two-sided 97.5% CI for difference in seroresponse between MMR-RIT-Min and MMR II was within the protocol definition of non-inferiority (-5% or higher) for anti-mumps and anti-rubella antibodies tested with ELISA (LL -1.91% and -3.11% , respectively), but outside this definition for anti-measles antibodies (LL -7.65%).

As the first objective was not met, subsequent primary objectives related to MMR-RIT-Min were not assessed. Continuation of the hierarchical analyses defined in the study protocol evaluated the primary objectives for MMR-RIT-Med. Objective 6 was met, showing non-inferiority in seroresponse for anti-measles, anti-mumps, and anti-rubella antibodies tested with ELISA (Table 2). Objectives 7 and 8 for MMR-RIT-Med were also met: non-inferiority was demonstrated in anti-measles, anti-mumps, and anti-rubella antibody GMCs and acceptable immune responses. Objective 9 was not met, since the seroresponse rate of anti-mumps neutralizing antibodies tested by PRNT in MMR-RIT-Med recipients was outside of the protocol definition of non-inferiority: the LL of the two-sided 97.5% CI for group difference was beyond -10% (-10.94% ; Table 2). Primary objective 10 was therefore not assessed for MMR-RIT-Med.

3.2.2. Immunogenicity after second MMR dose

In the cohort of children enrolled in the US, immune responses were comparable among the study groups in terms of seroresponse rates and GMCs for antibodies to the different viral antigens 42 days after the second MMR dose (Table 3). The seroresponse rate was at least 98.4% against each MMR viral antigen in each group.

3.3. Reactogenicity and safety

Frequencies of solicited local symptoms after the first and second MMR doses and solicited general symptoms after the first dose

Table 2

Non-inferiority of MMR-RIT-Min/Med versus MMR II in terms of seroresponse rates and adjusted GMC/GMT ratios, and acceptable response rates to measles, mumps, and rubella 42 days after the first MMR dose (ATP cohort for immunogenicity post-dose 1).

SRR and acceptable response ^a	MMR-RIT-Min (97.5% CI)	MMR-RIT-Med (97.5% CI)	MMR II (97.5% CI)	Difference MMR-RIT-Min vs MMR II ^b (97.5% CI)	Difference MMR-RIT-Med vs MMR II ^b (97.5% CI)
Measles ELISA (%)	90.8 (88.9, 92.5)	94.2 (92.6, 95.5)	96.3 (95.0, 97.3)	-5.48 (-7.65, -3.43)	-2.08 (-3.96, -0.27)
Mumps ELISA (%)	97.4 (96.2, 98.3)	97.3 (96.0, 98.2)	97.8 (96.7, 98.7)	-0.42 (-1.91, 1.04)	-0.58 (-2.11, 0.91)
Rubella ELISA (%)	96.8 (95.5, 97.7)	97.3 (96.1, 98.2)	98.5 (97.6, 99.1)	-1.71 (-3.11, -0.42)	-1.18 (-2.50, 0.05)
Mumps PRNT (%)	71.2	73.4	80.6	-9.41 (-13.20, -5.62)	-7.22 (-10.94, -3.49)
GMC/GMT	MMR-RIT-Min	MMR-RIT-Med	MMR II	MMR-RIT-Min/MMR II ratio ^c (97.5% CI) MMR-RIT-Med/MMR II ratio ^c (97.5% CI)	
Measles ELISA GMC (mIU/mL)	2221.5	2553.8	2798.9	0.79 (0.72, 0.88)	0.91 (0.83, 1.01)
Mumps ELISA GMC (EU/mL)	57.8	59.4	70.6	0.82 (0.76, 0.89)	0.84 (0.78, 0.91)
Rubella ELISA GMC (IU/mL)	55.9	55.6	63.0	0.89 (0.83, 0.95)	0.88 (0.83, 0.95)
Mumps PRNT GMT (ED ₅₀)	9.4	10.2	15.6	0.60 (0.53, 0.68)	0.65 (0.57, 0.74)

ATP, according-to-protocol; ELISA, enzyme-linked immunosorbent assay; GMC, geometric mean antibody concentration measured by ELISA; GMT, geometric mean antibody titer measured by PRNT; PRNT, plaque reduction neutralization test; SRR, seroresponse rate, defined as percentage of initially seronegative children with antibody concentration/titer ≥200 mIU/mL for measles, ≥10 EU/mL (ELISA) or ≥4 ED₅₀ (PRNT) for mumps, and ≥10 IU/mL for rubella; 97.5% CI, asymptotic standardized 97.5% confidence interval.

Number of children with available results for SRR, acceptable responses and GMC/GMT for anti-measles antibodies: 1361 for MMR-RIT-Min, 1366 for MMR-RIT-Med, 1378 for MMR II group; for anti-mumps (ELISA): 1161 for MMR-RIT-Min, 1131 for MMR-RIT-Med, 1155 for MMR II group; for anti-rubella: 1359 for MMR-RIT-Min, 1366 for MMR-RIT-Med, 1376 for MMR II group; and for anti-mumps (PRNT): 1252 for MMR-RIT-Min, 1265 for MMR-RIT-Med, 1287 for MMR II group.

^a Percentage of children with acceptable immune response in terms of SRR for measles (number of children with available results 1361 for MMR-RIT-Min, 1366 for MMR-RIT-Med, 1378 for MMR II group), mumps as measured by ELISA (1161, 1131, 1155, respectively), and rubella (1359, 1366, 1376, respectively), which was demonstrated if the lower limit of two-sided 97.5% CI of SRR ≥ 90%. The study design did not foresee calculation of 97.5% CIs for the SRRs for anti-mumps by PRNT.

^b Difference in SRR, calculated as SRR in MMR-RIT-Min or MMR-RIT-Med group minus SRR in MMR II group. Non-inferiority criterion: lower limit of two-sided 97.5% CI ≥ -5% for measles, mumps (ELISA), and rubella or ≥ -10% for mumps PRNT.

^c GMC/GMT ratio calculated as MMR-RIT-Min or MMR-RIT-Med GMC/GMT over MMR II GMC/GMT, adjusted for country. Non-inferiority criterion: lower limit of two-sided 97.5% CI ≥0.67.

Table 3

Percentage of children with anti-measles, anti-mumps, and anti-rubella antibody seroresponses 42 days after the second MMR-RIT or MMR II dose (ATP cohort for immunogenicity post-dose 2; children enrolled in US).

	MMR-RIT-Min ^a		MMR-RIT-Med ^b		MMR II ^c	
	SRR ^d , % (95% CI)	GMC (95% CI)	SRR ^d , % (95% CI)	GMC (95% CI)	SRR ^d , % (95% CI)	GMC (95% CI)
Measles	99.6 (97.7, 100)	4803.5 mIU/mL (4290.4, 5378.0)	98.4 (96.1, 99.6)	4557.7 mIU/mL (4061.5, 5114.4)	98.4 (96.1, 99.6)	4453.9 mIU/mL (3951.9, 5019.8)
Mumps	99.1 (96.7, 99.9)	88.9 EU/mL (80.4, 98.3)	100 (98.2, 100)	94.1 EU/mL (85.3, 103.8)	98.6 (95.9, 99.7)	86.4 EU/mL (77.4, 96.5)
Rubella	99.6 (97.7, 100)	112.7 IU/mL (104.1, 122.0)	99.6 (97.9, 100)	110.7 IU/mL (102.9, 119.1)	99.6 (97.8, 100)	110.9 IU/mL (101.8, 120.8)

ATP, according-to-protocol; GMC, geometric mean antibody concentration; SRR, seroresponse rate; 95% CI, 95% confidence interval.

Number of children with available results: (measles) 245 for MMR-RIT-Min, 258 for MMR-RIT-Med, 257 for MMR II group; (mumps) 216, 199, 212, respectively; (rubella) 245, 259, 255, respectively.

^a Children received one dose of MMR-RIT-Min or MMR-RIT-Med followed 42 days later by one dose of MMR-RIT.

^b Children received two doses of MMR II 42 days apart.

^c Percentage of initially seronegative children with antibody concentrations on ELISA of ≥200 mIU/mL for measles, ≥10 EU/mL for mumps, and ≥10 IU/mL for rubella.

were similar among the MMR-RIT and MMR II groups (Fig. 3). The frequency of fever reported within 43 days post-vaccination was similar among groups, reported in 40–42% of MMR recipients in each group after the first dose and 32–34% after the second dose (Fig. 3).

After each dose, incidences of MMR-specific solicited general symptoms, febrile convulsion and parotid/salivary gland swelling, were under 0.5% in each group (Table S2). Localized or generalized rash was reported in similar percentages of children among all groups after dose 1 (22–23%) and dose 2 (9–10%).

Unsolicited AEs were reported in around half of children in each group after each dose (51.0% [95% CI: 48.5, 53.6] in MMR-RIT-Min,

53.0% [50.5, 55.6] in MMR-RIT-Med, 50.9% [48.4, 53.5] in MMR II group after dose 1; 46.0% [43.4, 48.6], 48.0% [45.4, 50.6], and 46.5% [44.0, 49.1], respectively, after dose 2). SAEs were reported in 91 of 1493 children (6.1%) in MMR-RIT-Min, 102 of 1497 (6.8%) in MMR-RIT-Med, and 92 of 1526 (6.0%) in the MMR II group. Two SAEs were considered by the investigator as related to vaccination: one child in the MMR-RIT-Med group had severe pyrexia (axillary temperature >39.5 °C), which was reported six days after the first vaccine dose and lasted six days, and a child in the MMR II group had moderate toxic skin eruption 15 days after the first dose, which lasted five days. Both children were hospitalized and recovered without sequelae. Frequencies of NOCD and AEs

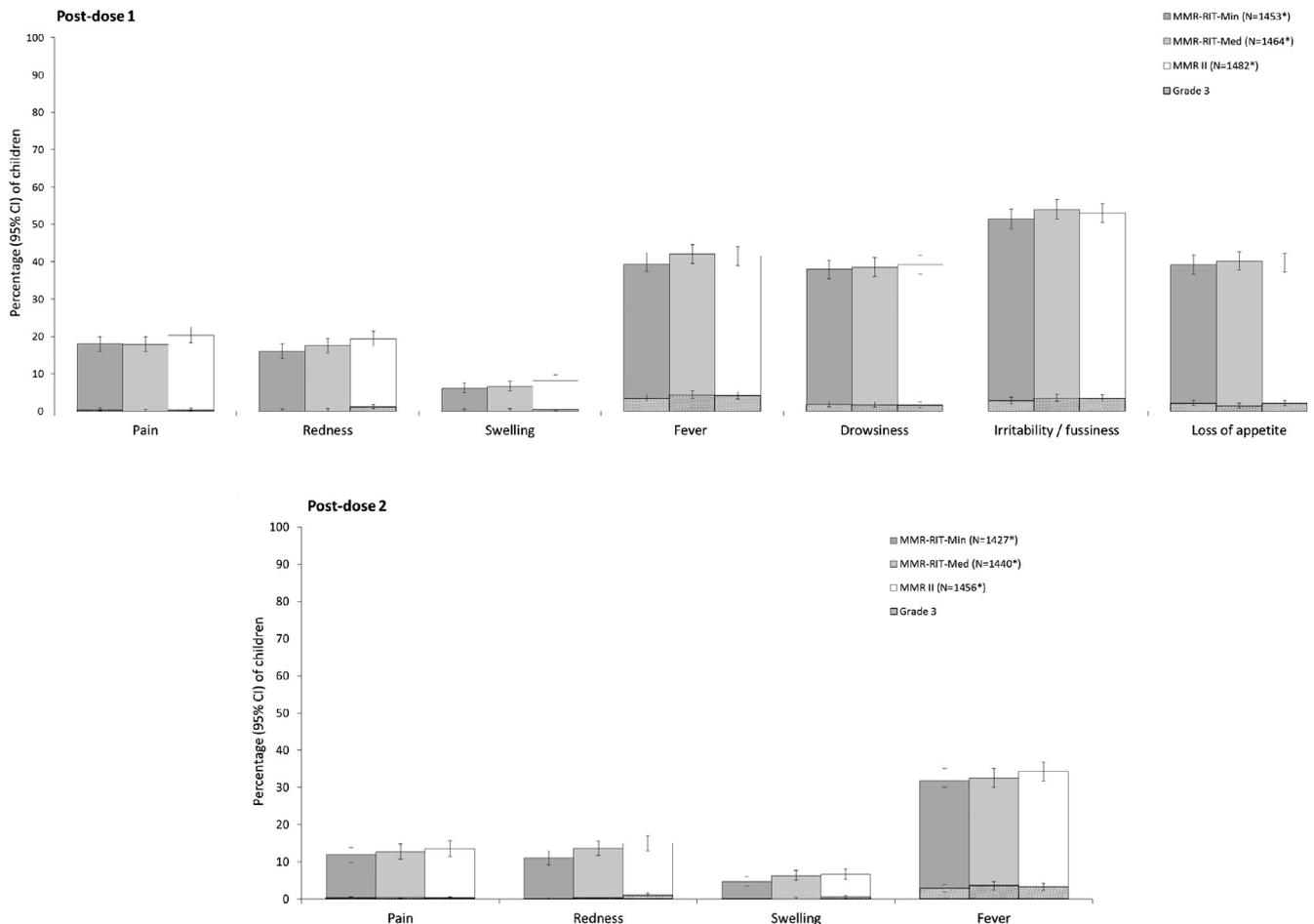


Fig. 3. Incidences of solicited local symptoms (pain, redness, and swelling) during 4-day period after each MMR dose, solicited general symptoms (drowsiness, irritability/fussiness, and loss of appetite) during 15-day period after the first dose, and fever during 43-day period after each dose (total vaccinated cohort). *Except for post-dose 1 fever, drowsiness, irritability/fussiness, and loss of appetite, for which MMR-RIT-Min (N = 1454), MMR-RIT-Med (N = 1466), and MMR II (N = 1486), and except for post-dose 2 fever, for which MMR-RIT-Min (N = 1426), MMR-RIT-Med (N = 1443), and MMR II (N = 1455). Grade 3 defined as crying when limb was moved or limb was spontaneously painful (pain), diameter >20 mm (redness and swelling), temperature >39.5 °C (fever), preventing normal activity (drowsiness and irritability), crying inconsolably (irritability), and not eating at all (loss of appetite). 95% CI, exact 95% confidence intervals; N, number of children.

that required an emergency room or medically-attended visit were similar between groups (see Supplement).

Three deaths occurred, none of which were considered as related to vaccination. Two children (one in MMR-RIT-Min group and one in MMR II group) died because of drowning 171 and 153 days, respectively, after dose 2. The third child had pyelonephritis reported as starting five days before MMR-RIT-Med vaccination and died 14 days after vaccination. The child's medical history showed suspected autosomal recessive polycystic kidney disease.

Further details on safety outcomes of this study are available at <https://www.gsk-clinicalstudyregister.com/files2/115649-Clinical-Study-Results-Summary.pdf>.

4. Discussion

In this study of healthy toddlers, a MMR-RIT formulation at the second lowest potency used in this study induced robust immune responses to all vaccine antigens. While the primary objectives for MMR-RIT at the minimum potency used for this study (MMR-RIT-Min) were not met, MMR-RIT at the second lowest potency used for this study (MMR-RIT-Med) induced immune responses as measured by ELISA against the three vaccine antigens that met all non-inferiority criteria versus MMR II. The immune

response following MMR-RIT-Med against mumps measured by PRNT did not meet the non-inferiority criterion for seroresponse rate by a small margin of uncertain clinical relevance. After the second MMR dose, immune responses were comparable among groups in terms of seroresponse rates and GMCs for antibodies to measles, mumps, and rubella viruses. Seroresponse rates were above 98.0% for each antigen in each group and consistent with immune responses reported in other studies of children administered a second MMR-RIT dose in the second year of life [18–20]. No safety concerns were identified and the reactogenicity profile of the MMR-RIT vaccine was acceptable when coadministered with HAV, VAR, and (in the US) PCV13. Reactogenicity and safety were in line with what has been reported globally for MMR-RIT and the MMR II vaccines [16,21,22]. The results of this study and choice of MMR-RIT-Med as the specification for MMR-RIT should have no impact on the manufacturability of MMR-RIT as its viral content is compatible with the acceptable range of potencies between end of shelf-life and maximum potency specification.

Non-inferiority was not demonstrated in terms of immune response against mumps virus measured by PRNT. The PRNT was an unenhanced in-house test developed by GSK that is likely to be a more rigorous test of immunogenicity to mumps vaccination than a previously used complement- and IgG-enhanced assay [23,24]. In addition, the new assay determines neutralizing

antibodies to the wild-type strain Mu-90/LO1, which is considered better than using a vaccine-specific mumps strain when approximating the potency of vaccine-induced antibody to the circulating wild-type strain [25,26]. However, no reliable correlate for protective neutralizing antibody titers has been established for mumps [27]. Because PRNT has different principles than ELISA, they cannot be directly compared, and successful bridging with the current ELISA threshold for protection is yet to be achieved. ELISA is the most widely accepted assay for assessing mumps immunogenicity but cannot distinguish neutralizing from non-neutralizing antibodies [28].

In terms of limitations, this study was not designed to evaluate the comparator MMR II vaccine at different potencies during its shelf-life. This is a limitation since the potency of all live virus vaccines decreases up to their expiry date [15,29] and the potencies of the MMR II lots used in this study were not tested. Precise differences in potency of the MMR-RIT-Min/Med formulations and MMR II are therefore unknown and it is unknown if non-inferiority would have been demonstrated if each vaccine had been tested at the same potency. Moreover, as far as we are aware, the anti-mumps antibody response after MMR II with reduced potency has not been tested with an assay as stringent as the unenhanced PRNT used in the present study. Also, since all children received HAV, VAR, and (for children enrolled in US) PCV13 vaccines, the safety and reactogenicity of MMR when administered alone could not be assessed.

In conclusion, one dose of a MMR-RIT formulation with lower potency induced a non-inferior immune response compared to standard MMR II vaccine, as measured by ELISA in one-year-old children. The PRNT assay seems to be a more sensitive assay, able to discriminate a slight dose-response effect across the potencies investigated. After the second dose, immune responses against measles, mumps, and rubella viruses were comparable among the MMR-RIT and MMR II groups, canceling pre-existing differences due to potency, if any. The safety profiles of all MMR-RIT vaccine formulations were similar to that of MMR II vaccine.

5. Trademark statement

Priorix and *Havrix* are trademarks of GSK group of companies. *M-M-R II* and *Varivax* are trademarks of Merck & Co., Inc. *Prevnar 13* is a trademark of Pfizer Inc. *Enzygnost* is a trademark of Dade Behring Marburg GmbH.

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Contributors

Within the MMR-161 study group, the following fulfilled the ICMJE criteria to be considered as authors (in alphabetical order): Study investigators: Ahonen, Anitta (Vaccine Research Center, University of Tampere, Tampere, Finland); Berry, Andrea (Center for Vaccine Development, Institute for Global Health, University of Maryland School of Medicine, Baltimore, MD, USA); Chatterjee, Archana (Department of Pediatrics, University of South Dakota, Sanford School of Medicine/Sanford Children's Specialty Clinic, Sioux Falls, SD, USA); Clifford, Robert (Coastal Pediatric Associates,

Charleston, SC, USA); Diaz Perez, Clemente (School of Medicine, Medical Sciences Campus, University of Puerto Rico, San Juan PR, Puerto Rico); Diez-Domingo, Javier (Centro Superior de Investigación en Salud Pública, Valencia, Spain); Haney, Byron (Family Health Care of Ellensburg, Ellensburg and Pacific Northwest University, Ellensburg, WA, USA); Harrison, Christopher J (Children's Mercy Hospital and Clinics, Kansas City, MO, USA); Kerdpanich, Angkool Phirangkul (Division of Infectious Diseases, Department of Pediatrics, Phramongkutklo Hospital, Bangkok, Thailand); Lee, Jimmy KF (Hospital Sultanah Nur Zahirah, Kuala Terengganu, Malaysia); Leonardi, Michael (Palmetto Pediatrics, North Charleston, SC, USA); Martínón-Torres, Federico (Hospital Clínico Universitario, Santiago de Compostela, Spain); Miranda, Mariano (Pediatrics Department, Hospital de Antequera, Antequera, Spain); Perez Porcuna, Xavier Maria (Manlleu Primary Care Center, Manlleu, Spain); Phongsamart, Wanatpreeya (Pediatric Infectious Diseases Unit, Department of Pediatrics, Faculty of Medicine Siriraj Hospital, Madiol University, Bangkok Noi, Bangkok, Thailand); Sharifah Huda, Engku Alwi (Hospital Sultanah Nur Zahirah, Kuala Terengganu, Malaysia); Toh, Teck-Hock (Department of Pediatrics and Clinical Research Centre, Sibu Hospital, Sibu, Sarawak, Malaysia); Twigg, Jerry (Dixie Pediatrics, Saint George, UT, USA); Ulied Arminana, Angels (Centre d'Atenció Primària, EBA Centelles, Barcelona, Spain); Varman, Meera (Pediatric Infectious Disease, Creighton University, Omaha, NE, USA); Zissman, Edward (Children's Research, Altamonte Springs, FL, USA). GSK Vaccines staff: Caplanusi, Adrian (GSK, Wavre, Belgium); Carryn, Stephane (GSK, Wavre, Belgium); Henry, Ouzama (GSK, Rockville, USA); Povey, Michael (GSK, Wavre, Belgium).

Conflict of interest

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: Stephane Carryn, Ouzama Henry, and Michael Povey are employed by the GSK group of companies. Adrian Caplanusi was an employee of the GSK group of companies at the time of the study conduct. Stephane Carryn and Ouzama Henry hold shares in the GSK group of companies as part of their employee remuneration. Andrea Berry's institution received payment from the GSK group of companies for her participation as a principal investigator in the trial. Her institution has received payment from the GSK group of companies, NIAID, Novartis, and Pfizer to conduct vaccine or epidemiological trials. Archana Chatterjee's institution received a grant from the GSK group of companies for her participation as a principal investigator in the trial. Clemente Diaz Perez's institution (UPR Medical Sciences Campus) received payment from the GSK group of companies to support partially the execution of the study. Javier Diez-Domingo received personal fees from the GSK group of companies and MSD for participation as a board member, grant from Sanofi Pasteur MSD, and non-financial support from Sanofi-Pasteur for ESWI meeting (2017). Byron Haney received fees and non-financial support from the GSK group of companies. Christopher J Harrison's institution received grant from the GSK group of companies for his participation as a principal investigator in the trial and for another vaccine trial and grant funding from Pfizer for his investigator role in a project. He has also received reimbursement and honorarium from Pfizer for the presentation of data. Michael Leonardi has received grant funding from the GSK group of companies for his participation as principal investigator in the study. He also received grant funding from Merck, Medimmune, and Novartis. Federico Martínón-Torres's institution received payment from the GSK group of companies for his participation as a principal investigator in the trial. The institution also received fees from Ablynx, Janssen, the GSK group of companies, Regeneron,

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Authors' contributions

Ouzama Henry contributed to the conception, design, and planning of the study. Michael Povey contributed as statistician to the method and selection development, the statistical data analysis, the reporting of data, and the assessment of robustness of this manuscript. All authors contributed to the acquisition and review of the data. All study investigators from the MMR-161 study group recruited patients. All authors contributed to the interpretation of data and the drafting of the report. They revised it critically for important intellectual content and approved the version to be published.

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