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Long-term immunogenicity of measles, mumps and rubella-containing vaccines in healthy young children: A 10-year follow-up

Stephane Carryn [a,*], Muriel Feyssaguet [b], Michael Povey [c], Emmanuel Di Paolo [b]

[a] R&D Center Belgium, GSK, Wavre, Belgium
[b] Clinical Laboratory Sciences, GSK, Rixensart, Belgium
[c] Biostatistics, GSK, Wavre, Belgium

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ABSTRACT

Measles and mumps outbreaks still occur in countries that have successfully implemented universal routine immunization programs. Measles outbreaks are mostly associated to absent or incomplete vaccination, whereas for mumps outbreaks the combined effects of waning of immunity and circulating new strains are incriminated. It is therefore increasingly useful to characterize the long-lasting immunity induced by measles-, mumps, and rubella (MMR)-containing vaccines.

In this 10-year study, 1887 healthy children aged 12–22 months, randomized to receive 1 or 2 doses of MMR-containing vaccines (*Priorix* or *Priorix-Tetra*; GSK), were included in an antibody persistence analysis. A total of 364 children in the 1-dose group received a second dose out of study according to their local vaccination schedule between Years 4 and 10 post-dose 1, and were included in a separate post-hoc analysis to evaluate the effect of the second dose when given later. Anti-measles, -mumps and -rubella antibody titers were measured by commercial ELISA kits (Enzygnost, Siemens) after each vaccine dose and at Years 1, 2, 4, 6, 8 and 10 post-vaccination.

Antibodies against measles and rubella declined moderately after vaccination but remained well above the seropositivity threshold after 10 years. The anti-measles antibody titers elicited by *Priorix-Tetra* remained about 2-fold higher throughout the study as compared with *Priorix*. A second dose of MMR vaccine later in life had a minor and transient effect on anti-measles and anti-rubella waning titers. In contrast, anti-mumps antibody levels remained relatively stable over the 10-year follow-up and a second dose of MMR vaccine, given anytime over the 10-year period, had a boosting effect on anti-mumps antibody titers and seropositivity rates.

In conclusion, 1 or 2 doses of MMR-containing vaccines given to children in their second year of life induced antibody responses against measles, mumps and rubella viruses that persisted at least up to 10 years post-vaccination.

Clinical trial registration number: NCT00226499.

1. Introduction

Implementation of universal routine vaccination programs with measles-, mumps- and/or rubella-containing vaccines has dramatically reduced the incidence of these diseases and their associated mortality and morbidity [1–4]. However, measles and mumps outbreaks still occur—each of them for different reasons—in regions where safe and effective vaccines are available [5,6]. For instance, only in the first half of 2018, over 41,000 children and adults were infected with measles in the World Health Organization (WHO) European region [7]. In the United States of America (USA), the number of outbreak cases was 349 for measles and 2,251 for mumps in 2018 (up to 29 December) [8,9].

Measles outbreaks occur mostly in unvaccinated individuals, thus a high vaccination coverage is the most important goal to prevent the disease. Mumps outbreaks, instead, can also occur in highly vaccinated communities. The main hypothesized cause for mumps outbreaks is a combination of the following: a high density of population (such as college campuses) that concentrate more susceptible subjects, the waning of antibody titers, a lower effectiveness of mumps-containing vaccines compared to measles and rubella vaccines, and currently circulating strains for which

* Corresponding author at: R&D Live Virus Vaccine, GSK, Avenue Fleming 20, 1300 Wavre, Belgium.
E-mail addresses: stephane.x.carryn@gsk.com (S. Carryn), muriel.m.feyssaguet@gsk.com (M. Feyssaguet), michael.x.povey@gsk.com (M. Povey), emmanuel.di-paolo@gsk.com (E. Di Paolo).

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cross-neutralization is less effective [6,10–12]. To prevent mumps outbreaks, not only is the completion of the recommended measles-mumps-rubella (MMR) vaccination schedule crucial, but also a third dose of MMR vaccine has been recently recommended in the USA to help reduce the number of cases during a mumps outbreak [13].

Another crucial point for the success of MMR vaccination programs is the degree of long-lasting immunity and effectiveness of the vaccines. It has been shown that levels of anti-measles, -mumps, or -rubella antibodies decline over time, and this happens faster after vaccination than when immunity is naturally acquired [14]. It is important to better understand vaccine-induced antibody persistence and how persistence patterns may influence the risk of vaccine failure.

In this study, we administered 1 or 2 doses of MMR-containing vaccines to healthy children using different vaccines and schedules with or without concomitant varicella vaccination. While study design details and results for efficacy, immunogenicity and safety of the varicella-containing vaccines administered have been previously published [15–17], this manuscript focuses on the assessment of measles, mumps and rubella immunogenicity up to 10 years post-vaccination.

A summary contextualizing the outcomes of this study is displayed in the Focus on the Patient Section (Fig. 1) for the convenience of health care professionals.

2. Materials and methods

2.1. Study design and participants

This was a phase III, observer-blind, randomized study where healthy children aged 12–22 months received one or two doses of MMR-containing vaccines with or without concomitant varicella immunization. Details of the study design have been previously published [15–17]. Of note, we used an accelerated schedule for the two doses of MMR-containing vaccines administered in the study (42-day interval between them) since the assessment of concomitant varicella immunization included in the study—and published in [15–17]—required a 42-day interval.

The study was conducted in Czech Republic, Greece, Italy, Lithuania, Norway, Poland, Romania, Russian Federation, Slovakia and Sweden according to the Declaration of Helsinki and the Good Clinical Practice guidelines (NCT00226499). Independent ethics review committees or institutional review boards at each site approved the study protocol, a summary of which is available at www.gsk-clinicalstudyregister.com (study IDs 100388, 103494, 104105, 104106). Inclusion criteria have been previously published [17]; they are listed in the Supplementary Materials and Methods.

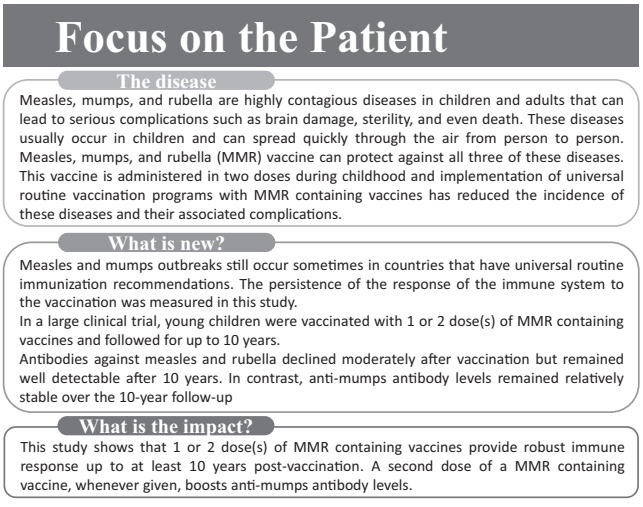

Fig. 1. Focus on the patient.

2.2. Study vaccines and procedures

The vaccines administered in this study were: combined measles, mumps, rubella and varicella vaccine MMRV (*Priorix-Tetra*, GSK); combined measles, mumps, and rubella vaccine MMR (*Priorix*, GSK); and monovalent varicella vaccine (V; *Varilrix*, GSK). The composition of these vaccines has been previously described [18–20]. Children were randomized 3:3:1 to receive by subcutaneous injection in the deltoid region, respectively, one of the following vaccination regimens: (a) 2 doses of MMRV at Day 0 and Day 42 (MMRV group), (b) 1 dose of MMR at Day 0 and 1 dose of V at Day 42 (MMR+V group) or (c) 2 doses of MMR at Day 0 and Day 42 (MMR group). Children were followed up for 10 years after receipt of their second vaccine at Day 42, with blood samplings done at Days 0, 42 and 84, and Years 1, 2, 4, 6, 8 and 10 of the study (Supplementary Fig. 1). Sera were stored and transported at −20 °C until assayed using standardized and validated procedures at a central laboratory (GSK Biologicals Global Vaccine Clinical Laboratory, Rixensart, Belgium for samples until Year 6; assays transferred to Néomed-Labs, Laval, Quebec, Canada for Year 8 and Year 10 samples).

Participants, parents/legally acceptable representatives (LARs), investigators and the personnel who assessed the study outcomes remained blinded to the intervention in all groups except for children (and their parents/LARs) of the MMR+V group in countries where the recommended national immunization program included a second dose of MMR vaccination at 4–8 years of age: Czech Republic, Italy, Lithuania, Romania, Russian Federation, Sweden.

Serum immunoglobulin G antibodies against measles, mumps and rubella viruses were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits specific to each serology (*Enzygnost*, Siemens [previously Dade Behring]).

2.3. Statistical analyses

The immunogenicity of the measles, mumps and rubella components of the study vaccines was assessed in the subset for MMR persistence, which included 1887 participants over the 10 countries. The subset for MMR persistence was identified through the Internet randomization system and consisted of up to 200 subjects from each country (however, children in Greece and the Russian Federation did not contribute to data beyond Year 2). Evaluation of antibodies against measles, mumps and rubella at each time point was based on the adapted according-to-protocol (ATP) cohort for MMR persistence, i.e. considering only the serology results of children in the subset for MMR persistence who respected all the visit intervals up to and including the time point considered and who were seronegative prior to vaccination.

Serological data from any subject who received an additional dose of MMR- or varicella-containing vaccines after Day 84 were censored from the day of additional dose receipt onwards for the main persistence analyses. However, serological data from participants in the MMR+V group who received a second dose of MMR-containing vaccine between Year 4 and the end of Year 10 (often in accordance with their country's specific vaccination schedule) were used in a post-hoc analysis to describe the persistence of antibodies when the second dose of MMR-containing vaccine was given later in life. These participants (a total of 364 children) were divided in three sub-groups according to the time window when they received their second dose of MMR-containing vaccine: in Year 4–6 (sub-group MMR+V 4–6; N = 208), Year 6–8

(sub-group MMR+V 6–8; N = 73) or Year 8–10 (sub-group MMR+V 8–10; N = 83). Too few subjects in the MMR+V group received a second dose of MMR-containing vaccine before Year 4 to justify the creation of a separate subgroup.

Antibody geometric mean concentrations (GMCs) were calculated for each group (and sub-group) and time point by taking the anti-log of the mean of the log antibody concentrations for each assay. Antibody concentrations below the assay cut-off (i.e. 150 mIU/mL for anti-measles, 231 U/mL for anti-mumps and 4 IU/mL for anti-rubella antibodies) were given an arbitrary value of half the cut-off value.

Seropositivity rates and associated 95% confidence intervals were calculated for each group (and sub-group) and time point for anti-measles, anti-mumps and anti-rubella. Seropositivity rate was defined as the percentage of participants with antibody concentration greater than or equal to the seropositivity threshold for each assay (i.e. 150 mIU/mL for anti-measles, 231 U/mL for anti-mumps and 4 IU/mL for anti-rubella antibodies).

All statistical analyses were descriptive only; no formal statistical hypotheses were formulated. Statistical analyses were performed using Statistical Analysis Software (SAS).

3. Results

3.1. Study participants

A total of 5803 children aged 12–22 months were enrolled and vaccinated between 1 September 2005 and 10 May 2006 [17]. Of these, 1887 were included in the subset for MMR persistence, from which 1647 were included in the adapted ATP cohort for MMR persistence. Main reasons for exclusion were having essential serological data missing and being initially seropositive or of unknown serostatus (Fig. 2).

Demographic characteristics of the participants were balanced among the 3 groups (Table 1). The mean age at enrollment was 14.6 months and 97.5% of participants were white/Caucasian.

3.2. Anti-measles persistence

Seropositivity rates for anti-measles antibody remained high (≥93.4%) throughout the ten years after vaccination across study groups (Fig. 3). Some children in the MMR+V group received a second dose of MMR-containing vaccine after Year 4 (subgroups MMR +V 4–6, MMR+V 6–8 and MMR+V 8–10). This second dose later in life did not have any impact on anti-measles seropositivity rate (Supplementary Fig. 2). At Year 10 after vaccination, ≥94.0% of children were still seropositive for anti-measles antibody.

In all groups, anti-measles antibody GMCs declined from Day 84 onwards (Fig. 4A). At Year 10, anti-measles antibody GMCs were 1857 mIU/mL in the MMRV group, 997 mIU/mL in the MMR+V group and 914 mIU/mL in the MMR group. The highest anti-measles antibody concentrations were observed, at all time points tested, in the MMRV group. At Day 84, children who received only 1 dose of MMR vaccine (MMR+V group) reached anti-measles GMCs similar to those seen in children who received 2 doses

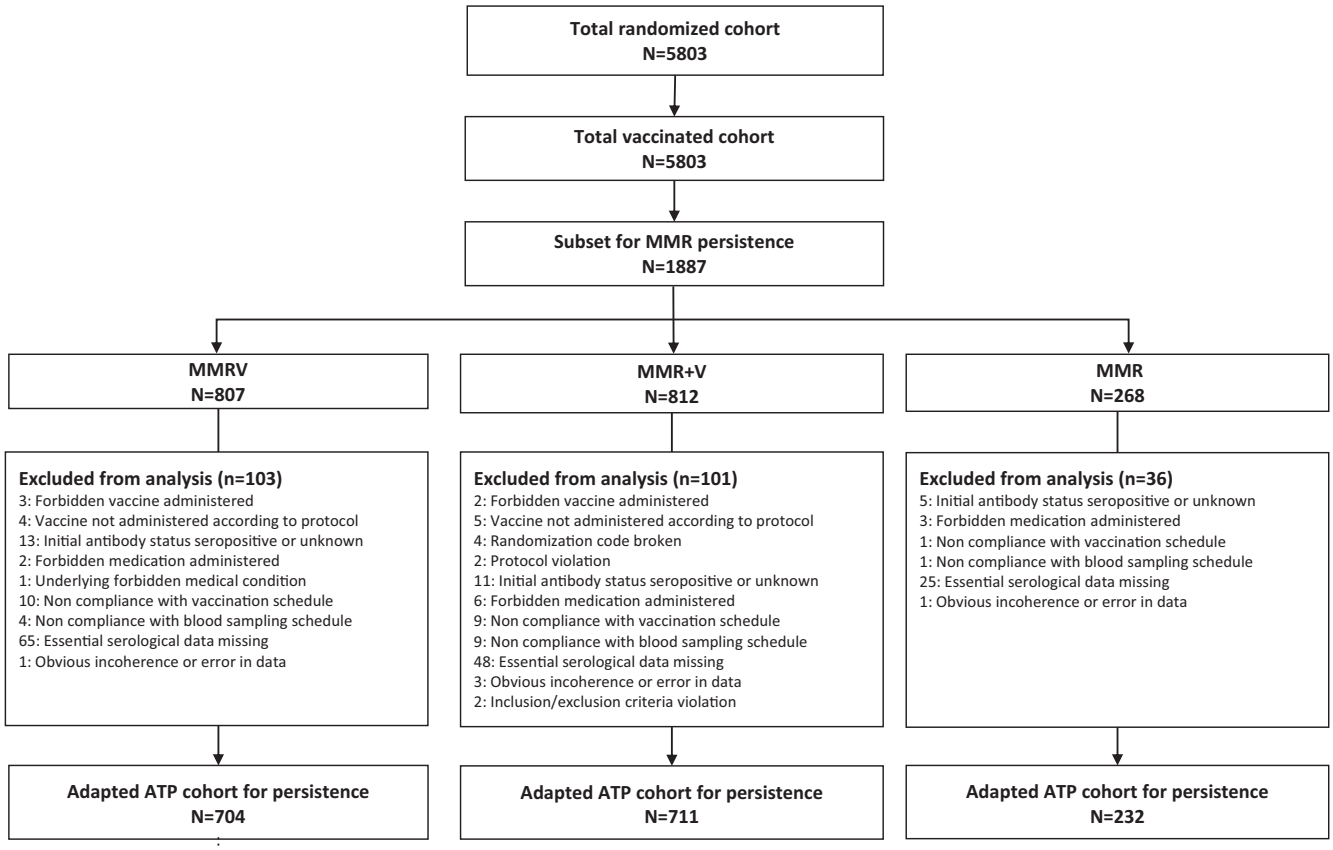

Fig. 2. Flow diagram of the study participants. ATP, according-to-protocol; MMRV, children who received two doses of the combined measles-mumps-rubella-varicella vaccine (at Day 0 and Day 42); MMR+V, children who received one dose of the combined measles-mumps-rubella vaccine (at Day 0) and one dose of monovalent varicella vaccine (at Day 42); MMR, children who received two doses of the measles-mumps-rubella vaccine (at Day 0 and Day 42); N, total number of children; n, number of children excluded.

Table 1
Demographic characteristics of the study participants (adapted according-to-protocol cohort for persistence, subset for MMR persistence).

Characteristic	MMRV N = 704	MMR+V N = 711	MMR N = 232
Age in months, mean (SD)	14.6 (2.4)	14.6 (2.5)	14.7 (2.5)
Female gender, n (%)	347 (49.3)	340 (47.8)	111 (47.8)
Race, n (%)			
White/Caucasian	682 (96.9)	695 (97.7)	229 (98.7)
Arabic/North African	10 (1.4)	3 (0.4)	1 (0.4)
Other[a]	12 (1.7)	13 (1.9)	2 (0.9)
Country, n (%)			
Czech Republic	88 (12.5)	87 (12.2)	29 (12.5)
Greece	72 (10.2)	69 (9.7)	18 (7.8)
Italy	74 (10.5)	74 (10.4)	20 (8.6)
Lithuania	79 (11.2)	83 (11.7)	26 (11.2)
Norway	19 (2.7)	19 (2.7)	4 (1.7)
Poland	79 (11.2)	81 (11.4)	27 (11.6)
Romania	65 (9.2)	66 (9.3)	25 (10.8)
Russian Federation	74 (10.5)	75 (10.5)	27 (11.6)
Slovakia	81 (11.5)	82 (11.5)	28 (12.1)
Sweden	73 (10.4)	75 (10.5)	28 (12.1)

MMRV, children who received two doses of the combined measles-mumps-rubella-varicella vaccine (at Day 0 and Day 42); MMR+V, children who received one dose of the combined measles-mumps-rubella vaccine (at Day 0) and one dose of monovalent varicella vaccine (at Day 42); MMR, children who received two doses of the measles-mumps-rubella vaccine (at Day 0 and Day 42); N, number of children in the group; n (%), number (percentage) of children in a given category; SD, standard deviation

[a] "Other": Black, East/South East Asian, American Hispanic and other heritage.

(MMR group). A second dose later in life appeared to have little or no effect on anti-measles antibody GMCs (Fig. 4B).

3.3. Anti-mumps persistence

Seropositivity rates for anti-mumps antibody ranged from 86.6% to 100% across study groups during the entire study period; at Year 10 after vaccination, ≥90.0% of children were still seropositive for anti-mumps antibody (Fig. 3C). The seropositivity rates peaked at Day 84 in the groups who received a second dose of MMR-containing vaccine at Day 42: 97.8% seropositivity rate in the MMRV group and 100% in MMR group. Similarly to the GMCS, in the MMR+V 4–6, MMR+V 6–8 and MMR+V 8–10 subgroups the seropositivity rates increased to ≥ 98.6% after receipt of their (later) second dose (Fig. 3D).

From Day 84 to Year 10, the levels of anti-mumps antibody remained relatively stable over time in the MMRV, MMR+V and MMR groups. At the end of the follow-up, GMCs were 889 U/mL, 1054 U/mL and 913 U/mL in the MMRV, MMR+V and MMR groups respectively (Fig. 4C).

Children who received the 2 doses of MMR-containing vaccine 42 days apart (groups MMRV and MMR) showed higher GMCs at Day 84 than children who received only one dose (group MMR +V). Children in the MMR+V group who received their second dose of MMR-containing vaccine later in life (sub-groups MMR+V 4–6, MMR+V 6–8 and MMR+V 8–10) reached similar post-dose 2 anti-mumps antibody GMCs as those observed in MMRV and MMR groups (Fig. 4C and D). This was observed regardless of when the second dose was given between Year 4 and Year 10. As observed for MMRV and MMR groups, a decrease in GMCs was observed after receipt of this later dose 2 in the sub-groups (Fig. 4C and D).

Among the children in the sub-groups MMR+V 4–6, MMR+V 6–8 and MMR+V 8–10 who did not show an anti-mumps seroresponse after the first dose, anti-mumps antibody GMCs after the second dose of the vaccine (whenever this was administered between Year 4 and 10) were comparable to those typically seen after the first dose at 12–15 months of age in other groups (Supplementary Table 1).

3.4. Anti-rubella persistence

Seropositivity rates for anti-rubella antibody remained high across study groups and sub-groups over time, with ≥96.6% of children being seropositive for anti-rubella antibodies at Year 10 (Fig. 3B).

Anti-rubella antibody GMCs at Day 84 were similar across groups irrespective of the number of vaccine doses received. After that point, antibody concentrations decreased gradually over time in MMRV, MMR+V and MMR groups, except for a sustainment of the levels at Year 1 in the MMR group and a small increase at Year 10 in the MMR+V group (Fig. 4E). At Year 10, anti-rubella antibody GMCs were 19 IU/mL, 29 IU/mL and 21 IU/mL in the MMRV, MMR+V and MMR groups respectively.

Children in sub-groups MMR+V 4–6, MMR+V 6–8 and MMR+V 8–10, who received their second dose of MMR-containing vaccine after Year 4, did not reach the same post-dose 2 anti-rubella antibody GMCs as children who received the second dose at Day 42 (Fig. 4C). However, receipt of the second dose later in life transiently reduced the speed of the decline in anti-rubella antibody levels over time (Fig. 4F). Sub-group MMR+V 8–10 also showed a transient sustainment in antibody levels at Year 1.

4. Discussion

This analysis showed that two doses of MMRV or 1 or 2 doses of MMR given to children in their second year of life induced antibody responses against measles, mumps and rubella viruses that persisted for up to 10 years post-vaccination. All groups showed high seropositivity rates 10 years post-vaccination, regardless of the MMR-containing vaccine regimen administered. Levels of anti-mumps antibodies remained relatively stable over the 10-year follow-up, whereas GMCs for anti-measles and anti-rubella antibodies moderately declined from Day 84 onward.

The evolution of anti-measles antibody levels over time was similar among groups, regardless of whether 1 or 2 doses of MMR had been administered. However, children in the MMRV group (who received 2 doses of MMRV 42 days apart) showed the highest GMCs at all time points. This is consistent with a previously published study where children who received 2 doses of MMRV showed anti-measles GMTs up to Day 84 post-vaccination that were 1.8-fold higher compared with children who received 2 doses of MMR 42 days apart with concomitant varicella vaccination at the first MMR dose [21]. In this regard, a recent study showed that post-vaccination fever—which is more frequently reported after MMRV vaccination than after MMR—is associated with higher antibody GMCs [22]. In agreement with this observation, in the previously published safety results of this study [17] we also found a higher rate of post-vaccination fever in children of the MMRV group compared to the rates observed in the MMR groups. In the present study, not only do we observe these expected higher GMCs in the MMRV group during the most immediate post-vaccination period, but we also show that these persist well until Year 10 post-vaccination. The evolution of GMC antibody levels in the MMR group was similar to that of the other groups over time, but absolute values as of Year 4 declined below the seropositivity threshold for some individuals in the MMR group (not reflected in the group calculation for GMCs), making the seropositivity rates as of that timepoint slightly lower than in the MMRV or MMR+V groups (Fig. 3A). However, this decline in seropositivity rates as of Year 4 was accompanied by a high variability (large 95% CIs), pointing to a potential confounding effect

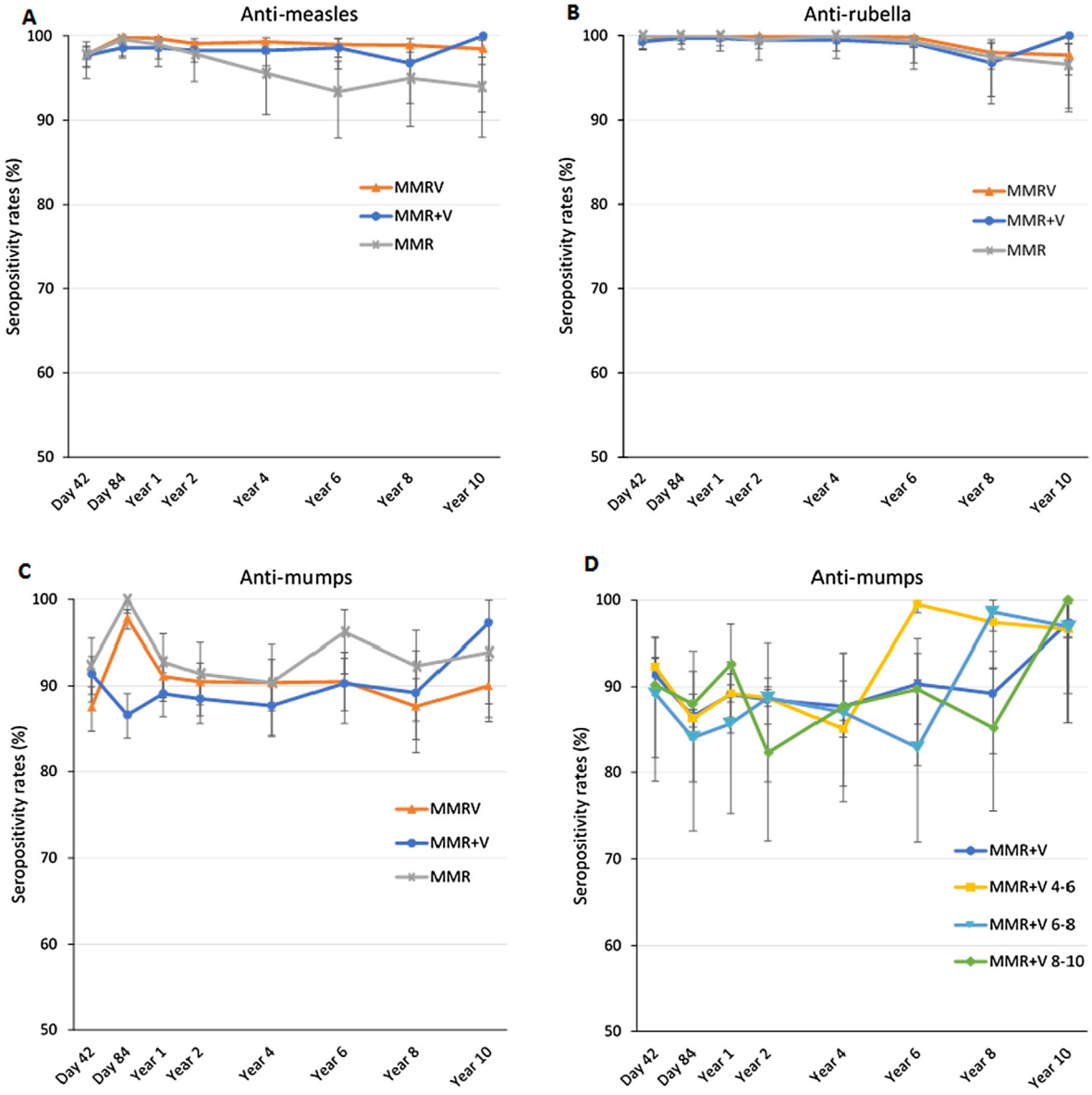

Fig. 3. Seropositivity rates from Day 42 to Year 10 for anti-measles, anti-mumps and anti-rubella antibodies for the MMRV, MMR+V and MMR groups (3A-C) and MMR+V sub-groups (3D) (adapted according-to-protocol cohort for persistence*, subset for MMR persistence). MMRV, children who received two doses of the combined measles-mumps-rubella-varicella vaccine (at Day 0 and Day 42); MMR+V, children who received one dose of the combined measles-mumps-rubella vaccine (at Day 0) and one dose of monovalent varicella vaccine (at Day 42) and who did not receive any second dose of MMR-containing vaccine until Year 10; MMR, children who received two doses of the measles-mumps-rubella vaccine (at Day 0 and Day 42); MMR+V 4–6, 6–8 and 8–10, children in the MMR+V group who received, in Year 4–6, 6–8 or 8–10, respectively, a second dose of MMR-containing vaccine in accordance with their country's specific vaccination schedule. Seropositivity rate, percentage of participants with antibody concentration equal to or above the seropositivity threshold (150 mIU/mL for anti-measles, 231 U/mL for anti-mumps and 4 IU/mL for anti-rubella antibodies). Error bars represent the upper and lower limits of the 95% confidence intervals. For each antigen and group/sub-group, the number of children at each time point are the same as in Fig. 4. * Children in the MMR+V group who received a second dose of MMR-containing vaccine in accordance with their country's specific vaccination schedule did not contribute to data in MMR+V group but were classified in the 3 sub-groups used in the post-hoc analysis: MMR+V 4–6, MMR+V 6–8 and MMR+V 8–10.

of a decrease in sample size at later timepoints. In any case, the percentage of subjects with anti-measles antibody concentrations above the seropositivity threshold remained higher than 93.3% at all timepoints and across study groups.

Anti-mumps antibody titers remained fairly stable over the 10-year period after both the 1-dose schedule (MMR+V group) and the 2-dose schedule (MMRV and MMR groups). This observation is not consistent with recent reports demonstrating waning of immunity of mumps vaccines [23,24]. However, none of these recent reports actually followed antibody titers in the same subjects over time, as done in the present study. One important observation is the rapid decline of antibody GMCs after the second dose down to levels similar to post-dose 1 (Fig. 4C). This might partially explain why the serological surveys are detecting waning over a long period of time and why only 11% of the waning could be explained by time in the model of Seagle et al. [25], which assumed a linear decline of antibody titers. Moreover, a very recent model based on a meta-analysis suggested that the average waning of immunity against mumps occurs 27 years (95% confidence interval: 16–51 years) post-vaccination [12]. Our study might therefore be not long enough to show actual waning of mumps serology.

The percentage of children seropositive for anti-mumps antibodies was in general lower than the seropositivity rate observed for anti-measles or anti-rubella antibodies, especially with only

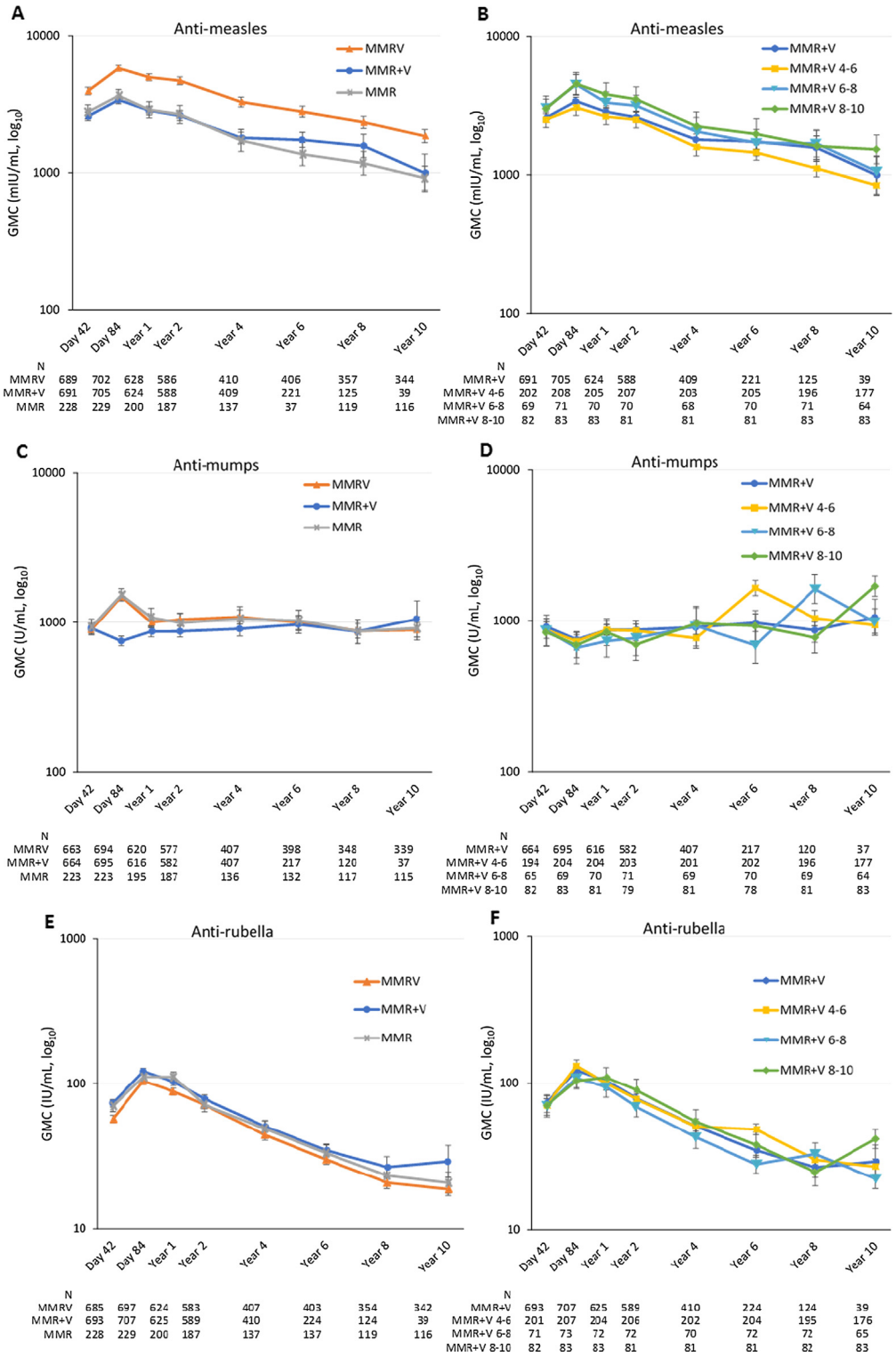

Fig. 4. Anti-measles, anti-mumps and anti-rubella antibody geometric mean concentrations from Day 42 to Year 10 for the MMRV, MMR+V and MMR groups (4A, 4C and 4E) and the MMR+V sub-groups (4B, 4D and 4F) (according-to-protocol cohort for persistence*, subset for MMR persistence). GMC, antibody geometric mean concentrations; IU, international unit; mIU, milli international unit; U, unit; MMRV, children who received two doses of the combined measles-mumps-rubella-varicella vaccine (at Day 0 and Day 42); MMR+V, children who received one dose of the combined measles-mumps-rubella vaccine (at Day 0) and one dose of monovalent varicella vaccine (at Day 42) and who did not receive any second dose of MMR-containing vaccine until Year 10; MMR, children who received two doses of the measles-mumps-rubella vaccine (at Day 0 and Day 42); MMR+V 4–6, 6–8 and 8–10, children in the MMR+V group who received, in Year 4–6, 6–8 or 8–10, respectively, a second dose of MMR-containing vaccine in accordance with their country's specific vaccination schedule; N, number of children with available results. * Children in the MMR+V group who received a second dose of MMR-containing vaccine in accordance with their country's specific vaccination schedule did not contribute to data in MMR+V group but were classified in the 3 sub-groups used in the post-hoc analysis: MMR+V 4–6, MMR+V 6–8 and MMR+V 8–10. Error bars represent the upper and lower limits of the 95% confidence intervals.

one dose of MMR-containing vaccine given. This observation agrees with what has been reported previously [21,26,27], and represents one of the reasons for recommending a 2-dose vaccination schedule of MMR-containing vaccines [28].

Anti-rubella antibody levels declined gradually over the 10-year follow-up period. However, seropositivity rates remained high. The small increase in GMCs observed in the MMR+V group at Year 10 may be attributable to a bias due to the thinning out of the cohorts rather than a real change in anti-rubella antibodies, therefore data in the MMR+V group from Year 6 to Year 10 have to be considered cautiously due to the low number of children after censoring data of subjects who received a second dose. The observation that anti-rubella antibody GMCs between Day 42 and Day 84 in the MMR+V group (1 dose) increased similarly to those in the MMRV and MMR groups (2 doses) could put into question the timing of immunogenicity assessment of the rubella component of the vaccines. However, the waning of antibody titer and the fact that rubella has a correlate of protection is making this questioning irrelevant [29].

Some children in this study received their 2 doses of MMR-containing vaccine at an interval longer than 42 days. These children were part of the MMR+V group and thus, per protocol, were given only 1 dose of MMR. However, and according to their country's vaccination schedule, they received a second dose of MMR-containing vaccine (not planned in the study) after Year 4. We performed a post-hoc analysis to evaluate the immune response of these children to the later second dose, and found different responses for each of the serologies tested. For anti-measles antibodies, a second dose after Year 4 had an almost negligible effect on antibody concentrations. For anti-rubella antibodies, a second dose slowed down the antibody waning. Although this effect in anti-rubella antibodies was visible in all sub-groups—that is, regardless of when the late second dose was administered—it was also of small magnitude and transient. For anti-mumps, the second dose did transiently boost the antibody levels, independently of the timing of that second dose (given between 42 days and 9–10 years after the first dose). Seropositivity rates increased in parallel to the antibody level increases. In agreement with this observation, *Priorix* has recently been shown to elicit high immune responses—which were not inferior to a comparator vaccine—when given to individuals $\geq$ 7 years old previously primed with a MMR-containing vaccine [30]. In our study, the increase in seropositivity rates after a (later) second dose reflects both children newly seroconverted (i.e., non-responders after dose 1) and children who responded to dose 1 but whose antibody levels waned below the seropositivity threshold before dose 2 (data not shown).

The anti-mumps antibody boost observed upon a second dose of MMR-containing vaccine after Year 4 is also in line with the recent use of this vaccine later in life in outbreak settings. Some epidemiologic studies have proved that a third dose of MMR-containing vaccine given during a mumps outbreak can help protect subjects at risk, and this piece of evidence has grounded a recent recommendation from the Advisory Committee on Immunization Practices of the USA to administer a third dose of a mumps-containing vaccine, in outbreak settings, for persons at risk who were previously vaccinated with 2 doses [13,31–33]. Interestingly, in these epidemiologic studies not only did a third dose of the vaccine help protect subjects at risk, but it also elicited a boost in anti-mumps antibody titers similar to what we observed in our study upon a second dose after Year 4. These multiple observations suggest that mumps vaccination is able to elicit a memory antibody response that can be recalled many years later. This is in accordance with the long-term persistence of cellular mediated immunity shown for the mumps component of the MMR vaccine, which may have triggered the efficient and fast generation of anti-mumps antibodies after the second dose administration [34,35]. On the contrary, although a lymphoproliferative response after MMR vaccination has also been reported for the measles component, and to a lesser extent for the rubella component [34], our data did not show any antibody boost for these vaccine antigens upon a second dose of MMR-containing vaccine. In any case, the lack of an established correlate of protection for mumps vaccines based on antibody titers makes it difficult to interpret the clinical impact of such boostability.

Even though some children in the MMR+V group in our study did not show an anti-mumps seroresponse after the first dose of MMR-containing vaccine administered at 12–22 months of age, all of them responded when a second dose was given after Year 4. This second dose allowed them to reach anti-mumps antibody GMCs above the seropositivity threshold, in the same range as the GMCs typically seen after a first dose at 12–22 months of age. This observation suggests that a catch-up dose of mumps-containing vaccine is important and elicits an efficient immune response in individuals with an incomplete vaccination schedule.

The boosting of antibody levels observed in the present study and the catch-up effect in children who did not respond to a first dose support the use of additional doses of mumps-containing vaccines at later ages to increase anti-mumps antibody levels, at least transiently (see also [36,37]), and to promote seroresponse in case of response failure at previous doses, which in turn could lead to a higher individual and community protection. Again, this is in line with the recent recommendation of a third dose of mumps-containing vaccine for individuals at risk in outbreak settings [13].

There are some limitations to this study. Firstly, 97.5% of the study population were white/Caucasian children. It has been reported that vaccine-induced measles and rubella immune responses are affected by ethnic differences, so the conclusions derived from this study on measles and rubella long-term immunogenicity might not necessarily be generalizable to children of all ethnicities [38–42]. Secondly, in this antibody persistence analysis we selected a subset of children (subset for MMR persistence) from the parent study, but two of the countries did not contribute to serological data after Year 2. However, we achieved a sample size as large as pre-specified in the study protocol, and participants showed balanced baseline characteristics among study groups. Thirdly, the number of children for whom results were available decreased over time. As expected in long follow-up studies, such as the present study, sample sizes may be low at the end of the follow-up and results at late time points should be considered with caution. In our study, this is particularly remarkable for the MMR+V group, as all children in this group who received a second dose of a MMR-containing vaccine out of protocol were censored from the analysis after their second dose. This is especially noticeable after Year 6, and in consequence the seropositivity rates and GMC results for the MMR+V group after that time point should be interpreted with caution. To partially account for that, and to understand the role of a second dose given later than in the other groups, a post-hoc analysis was conducted to investigate the persistence of antibodies in MMR+V children who received a (later) second dose of MMR-containing vaccine. Even though valuable results were obtained, the post-hoc analysis was restricted to a small number of subjects and did not account for the effect of potential natural infections, so the conclusions drawn from it should be taken with caution. In any case, all the results from the present study are descriptive only and no statistical hypotheses were set *a priori*.

The main strengths of this study are the large cohort considered, the long follow-up and the use of different MMR-containing vaccines and schedules. This provides a unique long-term descrip-

tive comparison of measles, mumps and rubella immunogenicity across different vaccines and vaccination schedules over a 10-year period.

As a conclusion, this study describes different profiles of long-term antibody persistence and boostability for each of the components of MMR-containing vaccines, depending also on the vaccine used and timing of doses. Importantly, we have shown that the responses obtained after receipt of 1 or 2 doses of MMR-containing vaccines remain well above the seropositivity thresholds up to 10 years post-vaccination, regardless of the vaccine given and the schedule used.

5. Trademark statement

Priorix, *Priorix-Tetra* and *Varilrix* are trademarks owned by or licensed to the GSK group of companies. *Enzygnost* is a trademark of Siemens (previously Dade Behring Marburg GmbH).

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Contributors

Michael Povey contributed as statistician to the method and selection development, the statistical data analyses, the reporting of data and the assessment of robustness of this manuscript. All authors contributed to the acquisition, review, interpretation of data and the drafting of the manuscript. They revised it critically for important intellectual content and approved the version to be published.

Declaration of Competing Interest

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: Stephane Carryn, Muriel Feyssaguet, Michael Povey and Emmanuel Di Paolo are employed by the GSK group of companies. Stephane Carryn and Emmanuel Di Paolo hold shares in the GSK group of companies as part of their employee remuneration.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.07.049.

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