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Ten-year follow-up on efficacy, immunogenicity and safety of two doses of a combined measles-mumps-rubella-varicella vaccine or one dose of monovalent varicella vaccine: Results from five East European countries

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ABSTRACT

Background: We assessed the 10-year efficacy, immunogenicity and safety of two doses of a combined measles-mumps-rubella-varicella vaccine (MMRV) or one dose of a monovalent varicella vaccine (V) in children from Czech Republic, Lithuania, Poland, Romania and Slovakia.
Methods: This was a phase IIIB follow-up of an observer-blind, randomized, controlled trial (NCT00226499). In phase A, healthy children aged 12–22 months from 10 European countries were randomized in a 3:3:1 ratio to receive two doses of MMRV (MMRV group), one dose of MMR followed by one dose of V (MMR + V group), or two doses of MMR (MMR; control group), 42 days apart. Vaccine efficacy (VE) against varicella (confirmed by viral DNA detection or epidemiological link and clinical assessment) was calculated with 95% confidence intervals using Cox proportional hazards regression model. Immunogenicity was assessed as seropositivity rates and geometric mean concentrations (GMCs). Solicited and unsolicited adverse events (AEs) and serious AEs (SAEs) were recorded.
Results: A total of 3705 children were vaccinated (1590, MMRV group; 1586, MMR + V group; 529, MMR group). There were 663 confirmed varicella cases (47, MMRV group; 349, MMR + V group; 267, MMR group). VE ranged between 95.4% (Lithuania) and 97.4% (Slovakia) in the MMRV group and between 59.3% (Lithuania) and 74% (Slovakia) in the MMR + V group. At year 10, seropositivity rates were 99.5%–100% in the MMRV group, 98%–100% in the MMR + V group and 50%–100% in the MMR control group, and the anti-VZV antibody GMCs were comparable between MMRV and MMR + V groups. The

Abbreviations: AEs, adverse events; ATP, according to protocol; CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; GMC, geometric mean concentrations; HR, hazard ratio; HZ, herpes zoster; IDMC, independent data monitoring committee; IU, international units; MMRV, measles-mumps-rubella-varicella; PCR, polymerase chain reaction; SAEs, serious adverse events; TVC, total vaccinated cohort; V, monovalent varicella vaccine; VE, vaccine efficacy; VZV, varicella zoster virus.

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occurrence of solicited and unsolicited AEs was similar across groups and no SAE was considered as vaccination-related. No new safety concerns were identified.
Conclusions: Our results indicated that two doses of varicella zoster virus-containing vaccine provided better protection than one dose against varicella and induced antibody responses that persisted 10 years post-vaccination.

1. Introduction

Varicella is a highly communicable vaccine-preventable disease caused by the varicella zoster virus (VZV), which typically affects children. After primary infection, VZV remains dormant in the nerve ganglia and may cause herpes zoster (HZ) in older adults upon endogenous reactivation [1].

Currently, only 12 European countries implemented universal varicella vaccination (UVV) programs [2,3]. In the absence of mass vaccination, the burden of varicella across European countries was estimated to 5.5 million cases annually, of which 3 million in children less than 5 years of age [4]. For the same age group, the estimated annual incidence of varicella cases per 100,000 population was 7707, 9468, 8974, 7108 and 9362 in the Czech Republic, Lithuania, Poland, Romania and Slovakia, respectively. The estimated annual hospitalization incidence rates ranged between 22 and 27 per 100,000 population in Romania and 29–36 in Lithuania, whereas the annual mortality incidence was less than 0.2 deaths per 100,000 in all age groups across all European countries [4].

Available varicella vaccines can be administered according to a one-dose or a two-dose schedule, based on national recommendations. The first dose can be administered between 11 and 18 months of age, followed, if adopted, by the second dose at 6 weeks to 3 months after the first dose or at 4–6 years of age [5]. While a single dose is effective in reducing the incidence of severe varicella, this schedule was associated with disease breakthrough [6–8] caused by primary vaccine failure and the declining exogenous exposure from children shedding wild-strain VZV [9]. Consequently, a two-dose schedule is recommended for optimal protection against varicella of any severity and to prevent the risk of breakthrough varicella and outbreaks [5,10].

In addition to monovalent vaccines against varicella, combination measles-mumps-rubella-varicella (MMRV) vaccines are available and their use may have several advantages from the separate MMR and V administration, such as increased vaccination compliance, more condensed immunization schedule and reduced associated costs. Administration of monovalent and combined varicella vaccines results in similar immunogenicity and safety outcomes, as previously shown in a metanalysis of 24 clinical trials [11].

A monovalent live-attenuated varicella vaccine (V; *Varilrix*, GSK) [12] and a combined MMRV vaccine (*Priorix-Tetra*, GSK) [13], containing the same Oka vaccine strain (minimum $10^{3.3}$ plaque forming units), are among the vaccines used for varicella immunization in European countries. In a phase IIIA, randomized, observer-blind, multicenter study, we assessed vaccine efficacy (VE) against varicella of two doses of MMRV and one dose of V given after MMR in children from 10 European countries [14]. Global results of follow-ups at years 3, 6 and 10 were previously published [14–16]. Here, we present results of vaccine efficacy, immune response persistence and safety after 10 years of follow-up for a subset of countries with similar ethnicity and different epidemiology (Czech Republic, Lithuania, Poland, Romania and Slovakia) to evaluate consistency with the overall study results.

A summary contextualizing the relevance, the results and the impact of our study is described in the Plain Language Summary (Fig. 1).

2. Methods

2.1. Study design and participants

This phase IIIB, observer-blind, controlled trial was conducted between 01 September 2005 and 15 December 2016 in 10 European countries. The overall results were previously published [14–16]. Here, we present results for the Czech Republic, Lithuania, Poland, Romania and Slovakia.

A detailed description of the study design was already published [14]. Briefly, children aged 12–22 months at the time of first vaccination were randomized in a 3:3:1 ratio to receive two doses of MMRV (MMRV group), one dose of MMR followed by one dose of V (MMR + V group), or two doses of MMR (MMR; control group), 42 days apart. The study consisted of two consecutive periods: phase A, extending between first day of vaccination and at least two years after the last vaccine dose, and phase B, extending between year 2 and year 10 following the last vaccine dose (study end). A combined phase A + B, covering the overall 10 years of the study was carried out between 6 weeks post-dose 2 and year 10.

Healthy children aged 12–22 months were enrolled in the study if they had no history of previous measles, mumps, rubella, varicella or herpes zoster diseases and vaccinations. Detailed inclusions and exclusion criteria were presented elsewhere [14]. The full protocol of this study is available at http://gsk-studyregister.com (ID 100388).

Randomization of vaccines was generated using the SAS software. Treatment allocation was performed at the investigator's site using a central randomization call-in system on internet. The phase A period of the study was carried out in an observer-blind manner. In phase B, children and their parents or guardians from the MMR + V group in Czech Republic, Lithuania and Romania were unblinded to receive a second dose of MMR vaccination at 4–8 years of age according to the national vaccination schedule. Further details regarding randomization and masking were published before [14].

Study vaccines were MMRV, MMR (*Priorix*, GSK), and V. Each 0.5 mL dose of MMRV and V contained after reconstitution $10^{3.7}$–$10^{4.2}$ plaque forming units of the live attenuated Oka strain. Vaccines were administered subcutaneously in the deltoid region of the left upper arm.

The study was registered on ClinicalTrials.gov (NCT00226499) and was conducted in accordance with the principles of Good Clinical Practice, the Declaration of Helsinki and applicable local regulations. The parents or guardians of all children gave written informed consent and, if capable, children signed an assent form for participation in phase B before study procedures. National, regional, or investigational review boards or ethics committees reviewed and approved the study protocol, protocol amendments and the informed consent form.

2.2. Objectives

The efficacy objectives were to assess the efficacy of two doses of MMRV (MMRV group) or one dose of V (MMR + V group) in preventing confirmed varicella cases during phase A + B and to deter-

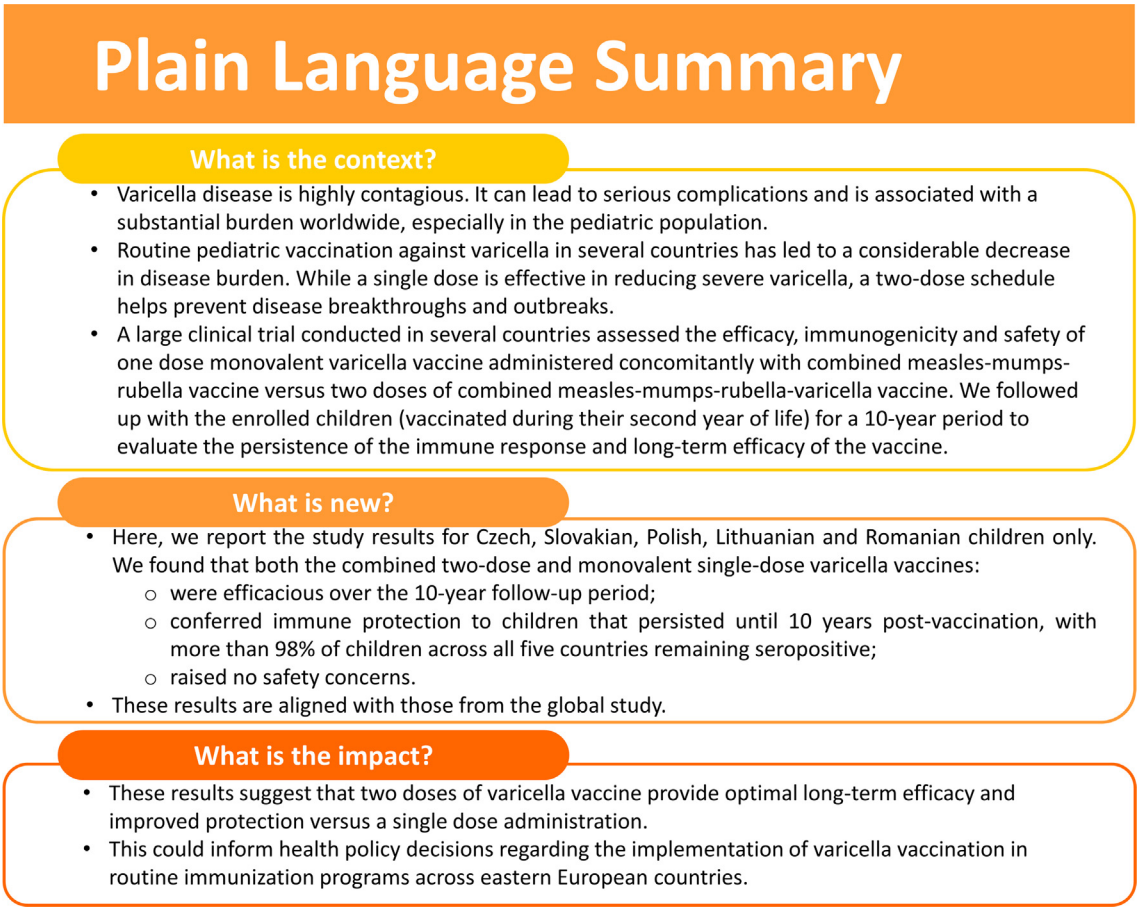

Fig. 1. Plain language summary.

mine the occurrence of complicated varicella cases, reported as serious adverse events, in all groups. Immunogenicity objectives were to assess at year 10 the VZV immune response in terms of seropositivity rates and anti-VZV antibody geometric mean concentrations (GMCs), in all groups. Safety objectives were to assess solicited local and general adverse events (AEs) and unsolicited AEs in a subset for reactogenicity analyses, and serious AEs (SAEs) in all children.

2.3. Efficacy assessment

VE was determined in the according-to-protocol (ATP) cohort for efficacy in phase A + B, which included all children with confirmed or probable varicella disease from 6 weeks post-dose 2 until year 10, who completed vaccination and respected all protocol requirements.

Parents or guardians were requested to immediately report any rash episode resembling varicella or HZ with onset any time after the first dose and to record the duration of the rash episode, the daily number of lesions/vesicles, the daily (highest) body temperature measured during the rash episode, and the date when the illness ended (i.e. the date at which the child resumed his/her normal activities). During an ascertainment visit, an investigator further documented the type and distribution of lesions and collected biological samples from the dermal lesions for VZV identification by polymerase chain reaction (PCR) amplification coupled with restriction fragment length polymorphism analysis.

All cases identified by the investigator as varicella-like rash were reviewed in a blinded manner by the independent data monitoring committee (IDMC). A varicella case was classified as confirmed when it met the clinical case definition [17] as determined by the investigator and the PCR result was positive, or when it met the clinical case definition as determined by the IDMC and was epidemiologically linked to a valid varicella or HZ case. A varicella case was classified as clinical when it met the clinical case definition as determined by the IDMC, but the PCR result was negative and was not epidemiologically linked to a valid varicella or HZ case.

2.4. Immunogenicity and safety assessment

Approximately 4 mL of blood were collected from all children before administration of dose 1 (pre-dose 1), at 84 days post-dose 1, and at study years 1, 2, 4, 6, 8, and 10. Serum anti-VZV antibodies were measured using an enzyme-linked immunosorbent assay (ELISA) as previously described [14,16]. Anti-VZV immune responses were assessed in the adapted ATP cohort for persistence, which included all children who completed vaccination, respected all protocol requirements and complied with the vaccination and blood sampling visit intervals. Serum anti-measles, -mumps, and -rubella antibodies were measured in the MMR subset of the adapted ATP cohort for persistence as previously presented [18].

The safety assessments for solicited local and general AEs, and unsolicited AEs were reported before [14]. Briefly, solicited local AEs were recorded within from day 0 to day 3, and solicited general and unsolicited AEs from day 0 to day 42 post-each vaccine dose. SAEs were reported throughout the entire study period. A SAE was defined as any medical event that resulted in death, was life-threatening, resulted in persistent of significant disability, required hospitalization or prolonged existing hospitalization. In

addition, all varicella complications such as secondary bacterial infection of the skin, cerebellar ataxia, encephalitis, pneumonia, hepatitis, appendicitis, arthritis, glomerulonephritis, orchitis and pericarditis were also considered SAEs. The SAE report included the suspected varicella case diagnosis together with the complication. An investigator assessed and reported the intensity and causality of each SAE. SAEs were assessed in the total vaccinated cohort (TVC), which included all children receiving at least one dose of study vaccine during phase A. AEs were assessed in a subset of the TVC [14].

2.5. Statistical analyses

Sample size considerations and VE calculations were previously described in detail [14,16]. All statistical analysis reported here were descriptive and performed using the SAS software (version 9.3, including Proc-StatXact, version 8.1 module).

VE for each comparison between a VZV-vaccinated group (MMRV and MMR + V) and the control group (MMR) was computed using the hazard ratio (HR) estimated with the Cox proportional hazards regression model [19], considering the individual follow-up time of each child and censored data. VE was calculated as 100×(1-HR) and reported with a two-sided 95% confidence interval (CI). To support the robustness of the VE assessment for confirmed varicella cases, the same computations were performed for clinical varicella cases in a *post-hoc* sensitivity analysis.

Anti-VZV antibody GMCs were calculated by taking the anti-log of the mean of the log concentration transformations of all values above the limit of quantification (40 mIU/mL). Prior to log-transformation, values between 25 mIU/mL and 40 mIU/mL were given a value of 25 mIU/mL, whereas values below the assay threshold (25 mIU/mL) were given an arbitrary value of 12.5 mIU/mL. VZV seropositivity rates at each timepoint were evaluated as the percentage of children with anti-VZV antibody concentrations above 25 mIU/mL. Both anti-VZV antibody GMCs and seropositivity rates were reported with two-sided 95% CIs, computed using the Clopper method [20]. Anti-measles, -mumps, and -rubella antibody GMCs and seropositivity rates were calculated as previously described [18].

Safety outcomes were presented as number and proportion (with two-sided 95% CI) of children for whom the event was reported.

3. Results

3.1. Participants

A total of 3705 Eastern European children aged 12–22 months were enrolled in the study and vaccinated (1590, 1586 and 529 in the MMRV, MMR + V and MMR groups, respectively). Of these, 3429 children were included in the ATP cohort for efficacy and 2560 in the adapted cohort for persistence completed the 10-year follow-up (Fig. 2). Groups were well balanced in terms of baseline characteristics (Table 1).

3.2. Vaccine efficacy

A total of 663 varicella cases (368 in Czech Republic, 75 in Lithuania, 94 in Poland, 8 in Romania and 118 in Slovakia) were confirmed for all groups during the 10-year follow-up period (Table 2).

In Romania, there were no confirmed varicella cases in the MMR control group, thus VE could not be estimated. For the remaining countries, VE against confirmed varicella was similar (97.2% in Czech Republic, 95.4% in Lithuania, 96.6% in Poland, 97.4% in Slovakia) for the group receiving two doses of VZV-containing vaccine (MMRV group). In the group receiving one dose of VZV-containing vaccine (MMR + V group), VE ranged between 59.3% in Lithuania and 74.0% in Slovakia (Table 2).

In the 10-year follow-up, VE against clinical varicella cases was similar to that against confirmed varicella cases. Children across all countries presented 704 cases of clinical varicella (389 in Czech Republic, 83 in Lithuania, 99 in Poland, 9 in Romania and 124 in Slovakia). VE against clinical varicella cases in the VZV-containing vaccine groups varied from 94.5% in Lithuania to 96.6% in Poland (MMRV group) and 63.6% in Lithuania to 73.3% in Slovakia (MMR + V group).

3.3. Immunogenicity

At year 10, anti-VZV antibody seropositivity rates for children receiving two doses of VZV-containing vaccine (MMRV group) ranged between 99.5% in Poland and 100% in Lithuania, Romania and Slovakia. After one dose of VZV-containing vaccine (MMR + V group), the percentage of seropositive children ranged between 98.0% in Lithuania and 100% in Romania. In the MMR control group, seropositivity rates varied from 50% in Slovakia to 100% in Romania (Fig. 3).

Anti-VZV antibody GMCs in the MMRV group tended to be higher compared to the ones in the MMR + V group from day 42 post-dose 2 (i.e., day 84 of the study) until year 6, after which GMCs were comparable between groups. In the MMR control group, anti-VZV antibody GMCs were lower than those in the MMR + V and V groups at all time points, but similar values were observed at year 10 in Lithuania, Poland and Romania (Fig. 3).

At year 10, seropositivity rates varied between 98.2% and 100%, 66.7% and 100%, and 95.2% and 100% for anti-measles, -mumps and -rubella antibodies, respectively (Supplementary Figs. 1-3). Across all countries, anti-measles antibody GMCs declined from day 84 onwards in all groups and tended to be higher in the MMRV group than those in the MMR + V and MMR groups (Supplementary Fig. 1). Anti-mumps antibody GMCs remained relatively stable from day 84 to year 10 across all groups and countries (Supplementary Fig. 2). Anti-rubella antibody GMCs were similar across groups and decreased gradually over time among all countries (Supplementary Fig. 3).

3.4. Safety

Redness was the most frequently reported solicited local AE across all groups, followed by pain and swelling. The incidence of redness was the highest in the MMRV group and ranged from 4.8% in Lithuania to 26.7% in Czech Republic post-dose 1, and from 4.5% in Romania to 43.3% in Czech Republic post-dose 2. Increases in the rates of all solicited local AEs post-dose 2 were observed for most countries in the MMRV group, but not in the MMR + V and MMR groups (Fig. 4). Among solicited general AEs, fever was reported most frequently post-dose 1 across groups and varied from 42.7% in Romania to 74.4% in Czech Republic in the MMRV group, from 41.3% in Romania to 55.8% in Slovakia in the MMR + V group, and from 37.9% in Lithuania to 53.8% in Romania in the MMR control group. Rash ranged between 1.3% (Romania) and 17.9% (Slovakia) post-dose 1 and decreased post-dose 2 in most countries across groups (Fig. 4). No cases of salivary gland swelling or meningism were reported following each dose. Grade 3 fever was reported for 3.4%–21.4% of children following each

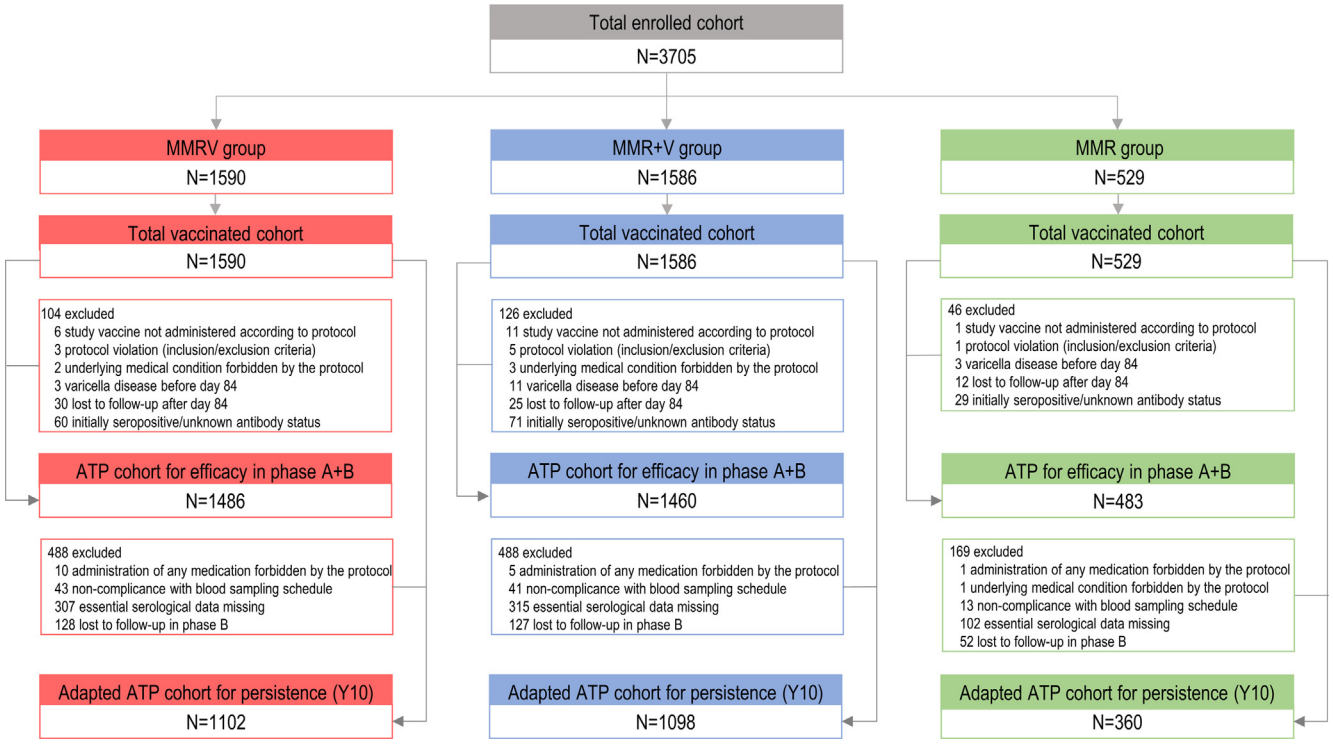

Fig. 2. Participant flow chart N, number of children; ATP, according-to-protocol; MMRV, group receiving two doses of the combined measles, mumps, rubella, and varicella vaccine; MMR + V, group receiving one dose of combined measles, mumps, and rubella vaccine followed by one dose of monovalent varicella vaccine; MMR, control group receiving two doses of combined measles, mumps, and rubella vaccine; Y, year.

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

	MMRV group N = 2489	MMR + V group N = 2487	MMR group N = 827
Age in months, mean (±SD)	14.3 (2.5)	14.2 (2.5)	14.3 (2.5)
Male sex, n (%)	1335 (53.6)	1264 (50.8)	426 (51.5)
Race or ethnicity, n (%)			
White/Caucasian	2430 (97.6)	2446 (98.4)	818 (98.9)
Arabic/North African	24 (1.0)	7 (0.3)	3 (0.4)
Other	35 (1.4)	34 (1.4)	6 (0.7)
Country, n (%)			
Czech Republic	552 (22.2)	549 (22.1)	185 (22.4)
Lithuania	278 (11.2)	276 (11.1)	93 (11.2)
Poland	407 (16.4)	404 (16.2)	135 (16.3)
Romania	143 (5.7)	145 (5.8)	47 (5.7)
Slovakia	210 (8.4)	212 (8.5)	69 (8.3)
Contact with other children, n (%)			
At least one sibling at home	717 (28.8)	652 (26.2)	209 (25.3)
Attending a day care centre	596 (23.9)	605 (24.3)	209 (25.3)
Attending a childminder	158 (6.3)	170 (6.8)	62 (7.5)
At least once a week contact*	2242 (90.1)	2251 (90.5)	752 (90.9)

N, total number of children; n (%), number (percentage) of children in a given category; SD, standard deviation; MMRV, group receiving two doses of the combined measles, mumps, rubella, and varicella vaccine; MMR + V, group receiving one dose of combined measles, mumps, and rubella vaccine followed by one dose of monovalent varicella vaccine; MMR, control group receiving two doses of combined measles, mumps, and rubella vaccine.*With other children without a known positive history of varicella disease or vaccination.

dose across all groups in all countries, whereas the incidence of other grade 3 AEs was limited.

Unsolicited AEs were reported for 13.3%–51.7% of children post-dose 1 and by 9.3%–48.1% of children post-dose 2 across groups in all five countries. Grade 3 unsolicited AEs were reported by less than 10.2% of children following each dose and no more than 6.9% of the unsolicited AEs were ascertained as vaccination-related.

Across the five Eastern European countries, 2361 SAEs were reported for 1287 children throughout the study. Among these, infections and infestations were the most frequently recorded, accounting for 45.8% of SAEs. During the entire study period, 38 febrile seizures (16 in the MMRV group, 19 in the MMR + V group and 3 in the MMR group) were reported for 33 Eastern European children and accounted for 1.0%–1.8% of SAEs across all groups. Two of the SAEs reported in these countries were considered causally related to vaccination by the investigator and occurred in phase A of the study [16], while no SAE was assessed as causally related in phase B. Additionally, two deaths were reported in phase A of the study [16], but neither of these occurred in the countries presented here. At year 10, there were six confirmed cases of HZ, and no cases of complicated varicella were reported across groups, over all countries.

4. Discussion

In this study including more than 3000 children from five Eastern European countries, we assessed the long-term efficacy, immunogenicity and safety of two doses or one dose of a tetravalent combination vaccine and a monovalent vaccine containing the same live attenuated Oka strain.

Over the 10-year follow-up period, more than seven times less cases of breakthrough varicella occurred in children receiving two doses of VZV-containing vaccine (MMRV group) compared with the ones receiving one dose of V (MMR + V group). VE against confirmed varicella cases was > 95.4% for children receiving two doses

Table 2
Estimates of vaccine efficacy against confirmed varicella cases (according-to-protocol cohort for efficacy).

	Czech Republic			Lithuania			Poland			Romania			Slovakia		
	MMRV group	MMR + V group	MMR group	MMRV group	MMR + V group	MMR group	MMRV group	MMR + V group	MMR group	MMRV group	MMR + V group	MMR group	MMRV group	MMR + V group	MMR group
n/N	28/525	196/516	144/171	5/256	40/255	30/86	6/385	46/368	42/116	1/121	7/126	0/42	7/199	60/195	51/68
T (years)[a]	4954	3848	724	2311	2056	609	3241	2928	737	633	612	199	1877	1537	350
Attack rate (95% CI) per 100 PY[b]	0.6 (0.4–0.8)	5.1 (4.4–5.9)	19.9 (16.9–23.4)	0.2 (0.1–0.5)	1.9 (1.4–2.7)	4.9 (3.4–7.0)	0.2 (0.1–0.4)	1.6 (1.2–2.1)	5.7 (4.2–7.7)	0.2 (0.0–1.1)	1.1 (0.5–2.4)	0.0 (NE)	0.4 (0.2–0.8)	3.9 (3.0–5.0)	14.6 (11.1–19.2)
Vaccine efficacy (95% CI)	97.2% (95.7–98.1)	73.5% (67.0–78.7)	–	95.4% (88.0–98.2)	59.3% (34.7–74.7)	–	96.6% (92.0–98.6)	71.4% (56.5–81.2)	–	NE	NE	–	97.4% (94.2–98.8)	74.0% (62.1–82.2)	–

n, number of children for whom ≥ 1 varicella case was reported; N, total number of children; T, total time to event; CI, confidence interval; PY, person-year; MMRV, group receiving two doses of the combined measles, mumps, rubella, and varicella vaccine; MMR + V, group receiving one dose of combined measles, mumps, and rubella vaccine followed by one dose of monovalent varicella vaccine; MMR, control group receiving two doses of combined measles, mumps, and rubella vaccine; NE, not estimated because there were no confirmed varicella cases in the MMR control group.

[a] Total time to event was calculated as the sum of the follow-up period expressed in years and censored at first occurrence of an event in each group.

[b] The person-year rate was calculated as the number of children for whom ≥ 1 varicella case was reported in each group divided by the total time to event.

and > 59.3% for one dose as compared to the MMR control group. These estimates were consistent with the global results reported for the present study (95.4% after two doses and 67.2% after one dose) [16]. Our results suggest that two doses of VZV-containing vaccine provided optimal long-term efficacy and superior protection than one dose, as previously indicated in meta-analyses of observational studies (VE of 91% after two doses of MMRV and 77% after one dose of V) [6] and of randomized controlled trials (VE of 79% after two doses and 63% after one dose) [8].

Anti-VZV antibodies persisted up to 10 years after vaccination in children receiving MMRV and V, with > 98% of children in both groups across all five countries remaining seropositive. These results are comparable with those reported in the global study [16]. However, the increase of anti-VZV antibody GMCs over the 10-year follow-up, observed in children (including those from the MMR control group even in countries where no varicella cases were reported) from both current and global study, might suggest an under-ascertainment of varicella cases or subclinical infections [16]. Anti-measles, -mumps, and -rubella antibodies also persisted up to 10 years in the MMRV, MMR + V and MMR groups, with most children across all five countries remaining seropositive. The evolution of GMCs for anti-measles, -mumps, and -rubella antibodies over time was similar among groups and countries and with that reported in the global study [18].

The safety profile was comparable between groups and similar among countries and with that from the global study [16]. Redness at the injection site (in 4.5%–43.3% of children post-dose 2) and fever (in 42.7%–74.4% of children post-dose 1) were the most frequently reported solicited AEs, in accordance with previous findings [11,12,21]. Unsolicited AEs occurred in less than 52% of the children across all five countries. Febrile seizures represent the most important safety concern following MMRV vaccination. Several studies have shown that a first dose of MMRV is associated with an increased risk of febrile seizures up to 12 days post-vaccination compared with separate MMR and V administrations [22–25]. A more recent meta-analysis reported an approximately 2-fold increase in risk of seizure or febrile seizure 7–10 days or 5–12 days after MMRV in children 12–23 months of age [11]. However, no evidence of an increased risk was found neither during the six weeks after vaccination in children 4–6 years of age nor after the MMRV vaccine was given as the second dose of measles-containing vaccine [11,26]. In the present study, the incidence of fever was similar between children receiving MMRV and those receiving MMR as first doses, and febrile seizures were reported for 0.9% of children across all five countries. No cases of salivary gland swelling or meningism, were reported.

Across all 10 countries in which the study was conducted, eight SAEs were assessed by the investigator as vaccine-related [14,16]. Of these, two SAEs were reported in the Eastern European countries and occurred in phase A of the study [16]. Two accidental deaths were reported throughout the study [16], but they were not treatment-related and did not occur in the countries presented here. During the 10-year follow up, there were six cases of HZ and no cases of complicated varicella reported in neither the current and global study [16].

Despite the proved efficacy of UVV programs in reducing the incidence of varicella cases and hospitalization rates [6–8,27,28], European vaccination policies are heterogenous and vary between UVV (Andorra, Austria, Cyprus, Finland, Germany, Greece, Hungary, Iceland, Italy, Latvia, Luxembourg and Spain), targeted vaccination of high-risks or susceptible groups (Belgium, Czech Republic, Lichtenstein and Poland), and no vaccination [2,3]. This study provides evidence in support of varicella vaccination at national level and specific for each country population and shows that vaccine efficacy against varicella is largely unbiased by country specific characteristics. Moreover, country-level

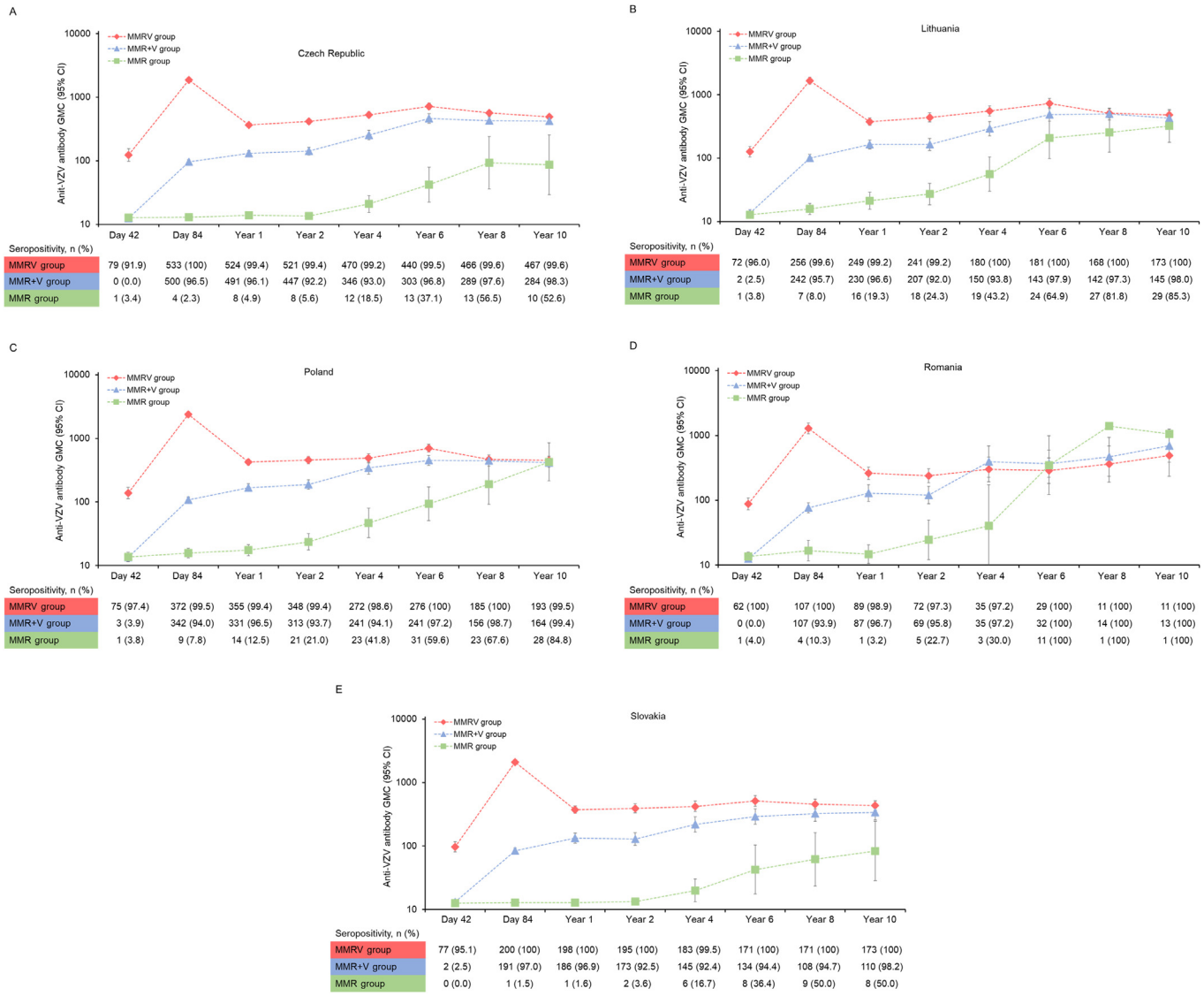

Fig. 3. Anti-varicella zoster virus antibody persistence during 10 years of follow-up in the Czech Republic (A), Lithuania (B), Poland (C), Romania (D) and Slovakia (E) (adapted according-to-protocol cohort for persistence) GMC, geometric mean concentration; VZV, varicella zoster virus; CI, confidence interval; n (%), number (percentage) of children with anti-VZV antibody GMC equal to or above the seropositivity threshold (25 mIU/mL); MMRV group, group receiving two doses of the combined measles, mumps, rubella, and varicella vaccine; MMR + V group, group receiving one dose of combined measles, mumps, and rubella vaccine followed by one dose of monovalent varicella vaccine; MMR group, control group receiving two doses of combined measles, mumps, and rubella vaccine.

VE data may help clarify the impact of varicella vaccination in these settings with similar ethnicity and different epidemiology and inform health policy makers regarding the implementation of varicella vaccination in routine immunization programs across Eastern European countries. While the effectiveness of a one-dose schedule has been demonstrated, the two-dose schedule ensures optimal protection against all severity varicella, limiting VZV transmission and reducing the risk of breakthrough cases and outbreaks, as previously observed [6–8,28,29]. A shorter interval between two doses seems to be more effective at preventing primary vaccine failure and breakthrough varicella cases than longer dosing intervals [9]. However, the optimal timing of the second dose may depend on vaccine coverage and national immunization programs [9].

A strength of the study is the long-term follow-up conducted in an extensive number of children, with active control group, from varicella endemic countries, where UVV has not been implemented. A potential limitation of the study is the relatively high proportion of children excluded from the efficacy and immunogenicity analyses at the end of the study, although this was expected due to the long-term follow up. Additionally, results presented per country were only descriptive, and any comparison should therefore be interpreted with caution.

5. Conclusions

Similar to the observations from the overall study, two doses of live attenuated VZV-containing vaccine administered during the second year of life provided long-term efficacy against varicella of any severity in children from East European countries and induced anti-VZV antibodies that persisted until 10 years post-vaccination. Our results indicated that two doses provided better long-term protection versus a single dose administration.

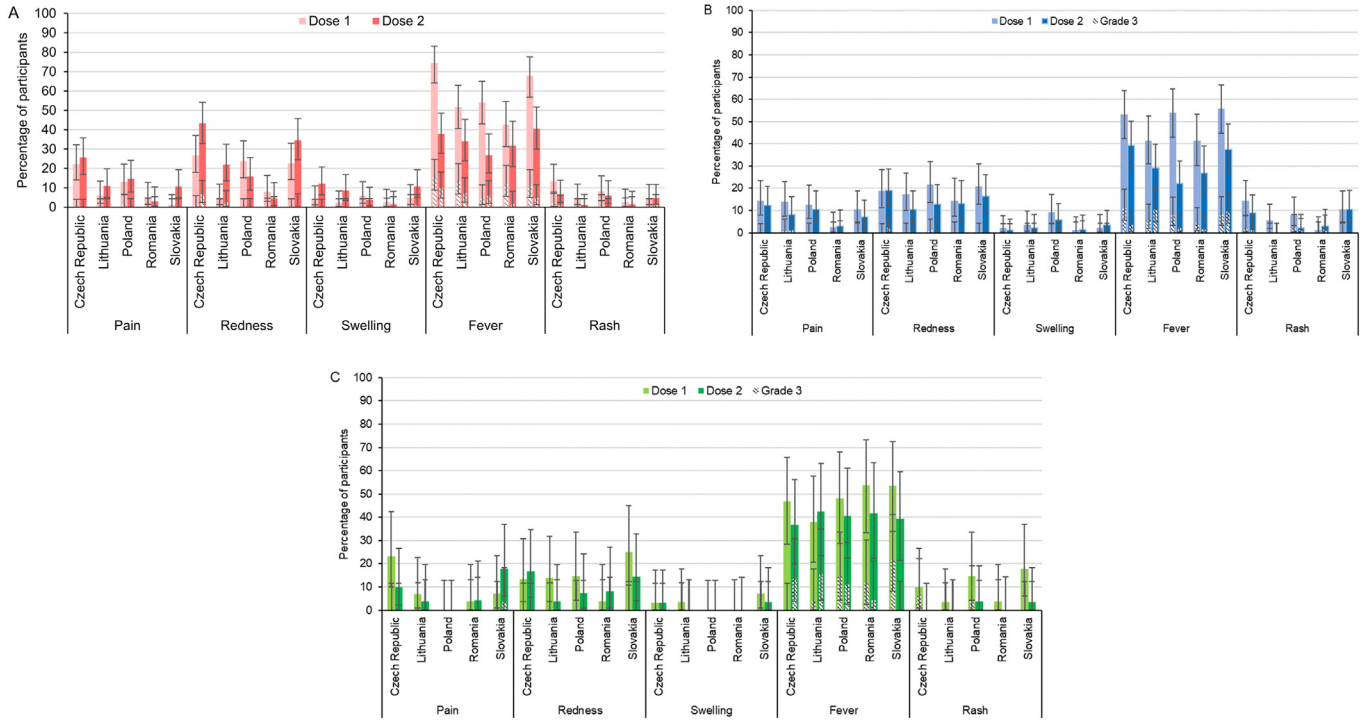

Fig. 4. Incidence of solicited local adverse events from day 0 to day 3, and of solicited general adverse events from day 0 to day 42, after each dose in the MMRV (A), MMR + V (B) and MMR (C) groups (total vaccinated cohort, subset). MMRV group, group receiving two doses of the combined measles, mumps, rubella, and varicella vaccine; MMR + V group, group receiving one dose of combined measles, mumps, and rubella vaccine followed by one dose of monovalent varicella vaccine; MMR group, control group receiving two doses of combined measles, mumps, and rubella vaccine. A grade 3 adverse event was defined as a crying child when the limb was moved, or the limb was spontaneously painful for pain; >20 mm in diameter for redness and swelling; >39.5 °C measured rectally or > 39.0 °C measured axillary for fever. For intensity of rash, grade 3 or 4 were defined as 101–500 and > 500 lesions.

6. Trademark statement

Varilrix, *Priorix* and *Priorix-Tetra* are trademarks owned by or licensed to the GSK group of companies.

7. Funding statement

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8. Data sharing statement

The protocol of this study is available at gsk-studyregister.com (ID 100388). Anonymized individual participant data and study documents can be requested for further research from www.clinicalstudydatarequest.com.

9. Author contributions

All authors either participated in the design (PP, RP), implementation (DP, DV, GL, HCa, HCz JB, JW, MN, MS, PP, RC, RP, RR, SM, VU) or analysis and interpretation (GC, JW, MAH, MP, PP, RP, RC) of the study, as well as the development of this manuscript. All authors had full access to the data and granted their final approval of the paper before submission.

Declaration of Competing Interest

GC, MAH, MP are employees of the GSK group of companies, and GC and MAH hold shares in the GSK group of companies as part of the employee remuneration. DV, JW, MS, RP, VU have received grants from the GSK group of companies during the conduct of the study. JW, MS have received consulting and lecture fees from the GSK group of companies. MS has received non-financial support from the GSK group of companies to attend scientific meetings, outside the submitted work. DV reports to have received personal fees by Biovomed during the conduct of the study. DV moreover reports to have received personal fees from the GSK group of companies, MSD, Sanofi and Pfizer, outside the submitted work. RR reports that her institution received fees during the conduct of the study. DP, GL, HCa, HCz, JB, NM, PP, RC, SM have not received any consulting fees and declare that they have no conflicts of interest and have no non-financial interest to declare.

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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.2021.03.085.

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