Protection against varicella with two doses of combined measles-mumps-rubella-varicella vaccine versus one dose of monovalent varicella vaccine: a multicentre, observer-blind, randomised, controlled trial

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Summary

Background Rates of varicella have decreased substantially in countries implementing routine varicella vaccination. Immunisation is possible with monovalent varicella vaccine or a combined measles-mumps-rubella-varicella vaccine (MMRV). We assessed protection against varicella in naive children administered one dose of varicella vaccine or two doses of MMRV.

Methods This study was done in ten European countries with endemic varicella. Healthy children aged 12–22 months were randomised (3:3:1 ratio, by computer-generated randomisation list, with block size seven) to receive 42 days apart (1) two doses of MMRV (MMRV group), or (2) MMR at dose one and monovalent varicella vaccine at dose two (MMR+V group), or (3) two doses of MMR (MMR group; control). Participants and their parents or guardians, individuals involved in assessment of any outcome, and sponsor staff involved in review or analysis of data were masked to treatment assignment. The primary efficacy endpoint was occurrence of confirmed varicella (by detection of varicella zoster virus DNA or epidemiological link) from 42 days after the second vaccine dose to the end of the first phase of the trial. Cases were graded for severity. Efficacy analyses were per protocol. Safety analyses included all participants who received at least one vaccine dose. This trial is registered with ClinicalTrials.gov, number NCT00226499.

Findings Between Sept 1, 2005, and May 10, 2006, 5803 children (mean age 14·2 months, SD 2·5) were vaccinated. In the efficacy cohort of 5285 children, the mean duration of follow-up in the MMRV group was 36 months (SD 8·8), in the MMR+V group was 36 months (8·5) and in the MMR group was 35 months (8·9). Varicella cases were confirmed for 37 participants in the MMRV group (two moderate to severe), 243 in the MMR+V group, and 201 in the MMR group. Second cases occurred for three participants (all in the MMR+V group). Varicella cases were moderate to severe for two participants in the MMRV group, 37 in the MMR+V group (one being a second case that followed a mild first case); and 117 in the MMR group. Efficacy of two-dose MMRV against all varicella was 94·9% (97·5% CI 92·4–96·6), and against moderate to severe varicella was 99·5% (97·5–99·9). Efficacy of one-dose varicella vaccine against all varicella was 65·4% (57·2–72·1), and against moderate to severe varicella (post hoc) was 90·7% (85·9–93·9). The most common adverse event in all groups was injection-site redness (up to 25% of participants). Within 15 days after dose one, 57·4% (95% CI 53·9–60·9) of participants in the MMRV group reported fever of 38°C or more, by contrast with 44·5% (41·0–48·1) with MMR+V, and 39·8% (33·8–46·1) with MMR. Eight serious adverse events were deemed related to vaccination (three MMRV, four MMR+V, one MMR). All resolved within the study period.

Interpretation These results support the implementation of two-dose varicella vaccination on a short course, to ensure optimum protection from all forms of varicella disease.

Funding GlaxoSmithKline Vaccines.

Introduction

Varicella is a highly contagious disease caused by the varicella zoster virus (VZV). Universal vaccination with live-attenuated varicella vaccine in the USA since 1995, and Uruguay since 1999, has substantially reduced the incidence of varicella and related admissions to hospital and deaths.[1–3] However, breakthrough varicella occurs in children vaccinated with one dose.[4] In 2007, a two-dose live varicella-vaccine schedule was recommended by the USA's Advisory Committee on Immunization Practices.[5] This schedule could include a live monovalent varicella vaccine, or a combination measles-mumps-rubella-varicella vaccine (MMRV).[5]

Countries considering implementation of universal varicella immunisation must decide whether to offer one or two doses, the timing of a second dose, and whether to use MMRV as a potential way to enhance compliance.[6,7] MMRV vaccines have been licensed with two-dose schedules in most European countries on the basis of comparative immunogenicity to monovalent varicella vaccines only.[6] No differences in anti-VZV immunogenicity have been identified between MMRV and

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monovalent varicella vaccine administered concomitantly with MMR.[8–11] Nevertheless, MMRV's efficacy for prevention of varicella has not been confirmed.

We did a randomised clinical trial to assess the protection against varicella afforded by administration of two doses of MMRV or one dose of monovalent varicella vaccine. To achieve more robust efficacy estimates than in previous multiyear live varicella-vaccine trials,[12–15] the first phase of this trial (reported here) was observer-blind and used a concurrent control group, active surveillance for varicella, and rigorous case confirmation and severity-grading procedures. This trial was initiated in countries with endemic varicella among children aged 12–22 months, permitting a stringent test of vaccine efficacy. We also assessed immunogenicity and safety of the vaccine regimens.

Methods

Study design and participants

This study is the first phase (Sept 1, 2005, to June 29, 2009) of an observer-blind, randomised, controlled trial. The study was done in 111 study centres in Europe: Czech Republic (22), Greece (11), Italy (nine), Lithuania (nine), Norway (five), Poland (ten), Romania (nine), Russia (14), Slovakia (17), and Sweden (five).

See Online for appendix

An eligible participant was a healthy child aged 12–22 months at the time of the first vaccination; had a negative history of varicella, mumps, measles, and rubella diseases and vaccinations; and was either (1) at home with at least one sibling (with negative history of varicella disease and vaccination), or (2) attending a childminder (where at least one child was without a known positive history of varicella disease and vaccination), or (3) playing for more than 5 min weekly with children without a known positive history of varicella disease and vaccination, or (4) registered to attend a daycare centre from 24 months of age. An eligible participant's parents or guardians had direct access to a telephone and were deemed by the investigator of being capable of complying with the requirements of the trial protocol.

The trial was approved by Research Ethics Committees of all participating countries. In the Czech Republic, Greece, Italy, Lithuania, Norway, Romania, Russia, Slovakia, and Sweden the trial was done in accordance with the Declaration of Helsinki and good clinical practice guidelines. Written informed consent was obtained from the parents or guardians of all children before trial entry. An Independent Data Monitoring Committee (IDMC) monitored the study and classified suspected varicella cases. Deviations from good clinical practice guidelines in Poland were investigated by GSK Vaccines. The findings of GSK Vaccines' investigation are shown at the end of the Methods.

Procedures

Eligible participants were randomly allocated (3:3:1 ratio) to one of three treatment groups: group MMRV, in which MMRV (Priorix-Tetra, GlaxoSmithKline Vaccines [GSK], Rixenstart, Belgium) was administered at doses one and two; group MMR+V, in which MMR was administered at dose one (Priorix, GSK) and monovalent varicella vaccine (Varilrix, GSK) at dose two; or group MMR, in which MMR (Priorix) was administered at doses one and two. Doses were administered 42 days apart (day 0 and day 42). After completion of this first phase of the clinical trial, group MMR+V participants were offered the second dose of MMR in accordance with the immunisation schedule of their respective country.

200 participants per country were included in a subset used for additional immunogenicity and reactogenicity analyses. According to the country, this subset consisted of either the first 200 participants enrolled irrespective of the study centre, or 200 participants enrolled at selected centres.

All study vaccines were lyophilised and refrigerator-stable, to be reconstituted before injection with supplied diluent. Three lots each of MMRV and of monovalent varicella vaccine and one lot of MMR were used (appendix p 1). All vaccines were administered subcutaneously in the deltoid region of the left arm.

The primary efficacy endpoint was the occurrence of confirmed varicella from 42 days after the second vaccine dose to the end of the first phase of the trial. The secondary efficacy endpoint was the occurrence of confirmed varicella graded by severity over the same time period. Other secondary endpoints were anti-VZV antibody concentrations at day 0, day 42, day 84, year 1, and year 2; occurrence of solicited injection-site symptoms within 4 days after each dose; occurrence of solicited fever within 15 days and 43 days after each dose and other solicited general symptoms within 43 days after each dose; and occurrence of herpes zoster and of any serious adverse event to the end of the first phase of the trial. Three immunogenicity secondary endpoints and three health-economics secondary endpoints were not considered in this Article and will be presented elsewhere; these endpoints were measles, mumps, and rubella antibody titres in a subset of patients at day 0, day 84, year 1, and year 2; and for the varicella cases during the study, the time lost from work by parents or guardians to provide care; the attendance time lost to day care, childminder, or school or any extra-curricular activities; and the time spent by a paid caregiver to provide care.

Parents or guardians of participants were requested to seek assessment of their child for any rash illness suggestive of varicella or herpes zoster and to report such rash illnesses of others in the household. These requests were repeated through monthly contact after the child completed vaccination. For participants with a rash illness, a detailed description of the case was recorded with type of rash (including photographs), duration of rash, intensity of rash, presence of fever during the rash event, disease complications, and (to identify an epidemiological link) information about contact with, and

characterisation of, potential index cases. Vesicular dermal lesions were sampled by unroofing the lesion and collecting the fluid by capillary action. Dry lesions (papules or crusts) were sampled by unroofing and swabbing, or by collecting scabs or crusts from lesions. VZV DNA was detected and determined by restriction fragment length polymorphism (RFLP) analysis of the PCR amplified product. PCR detection of β-globin DNA served as a positive control for adequate lesion sampling.[16] All cases of varicella-like rash[17,18] assessed by the investigator were referred to the IDMC for final blinded classification (figure 1). The IDMC's assessment of clinical criteria and of disease severity was done without knowledge of the VZV-PCR/RFLP result or of the history of an epidemiological link (ie, exposure to a valid varicella index case within 30 days of rash onset). A varicella case was confirmed when the VZV-PCR/RFLP result was positive or when the case met clinical criteria and had an epidemiological link. Varicella severity grading was based on the scoring system of Vázquez and colleagues[19] with modifications: mild disease, 7 points or fewer; moderately severe disease, 8–15 points; severe disease, 16 points or more. The modifications were that more than 500 lesions was attributed 8 points; both interstitial pneumonia and encephalitis were attributed 10 points, macular and papular lesions were scored separately, each at 2 points; and pain in the back or abdomen was not scored.

Anti-VZV antibody concentrations were established in blood samples by ELISA (Enzygnost; Siemens, Marburg, Germany; appendix p 3). A seronegative participant had an anti-VZV antibody concentration less than 25 mIU/mL. A seroresponse was defined as a post-vaccination antibody concentration 50 mIU/mL or more in a particpant who was seronegative before vaccination.

For participants in the reactogenicity subset of the total vaccinated cohort and after each dose, injection-site reactions (pain, redness, and swelling) were solicited daily for 4 days and general symptoms (fever [rectal temperature ≥38·0°C or axillary temperature ≥37·5°C], rash, parotid or salivary gland swelling, and any signs of meningism [including febrile seizures]) were solicited for 43 days. Unsolicited adverse events were also recorded for 43 days (appendix p 4). For all participants, any serious adverse event (which included an adverse event that resulted in persistent or substantial incapacity, admission to hospital, or death) or any withdrawal attributed to adverse events were recorded in detail during the whole study period. All solicited local symptoms were deemed causally related to vaccination. Causality of solicited general symptoms, unsolicited symptoms, and serious adverse events was assessed by the investigator.

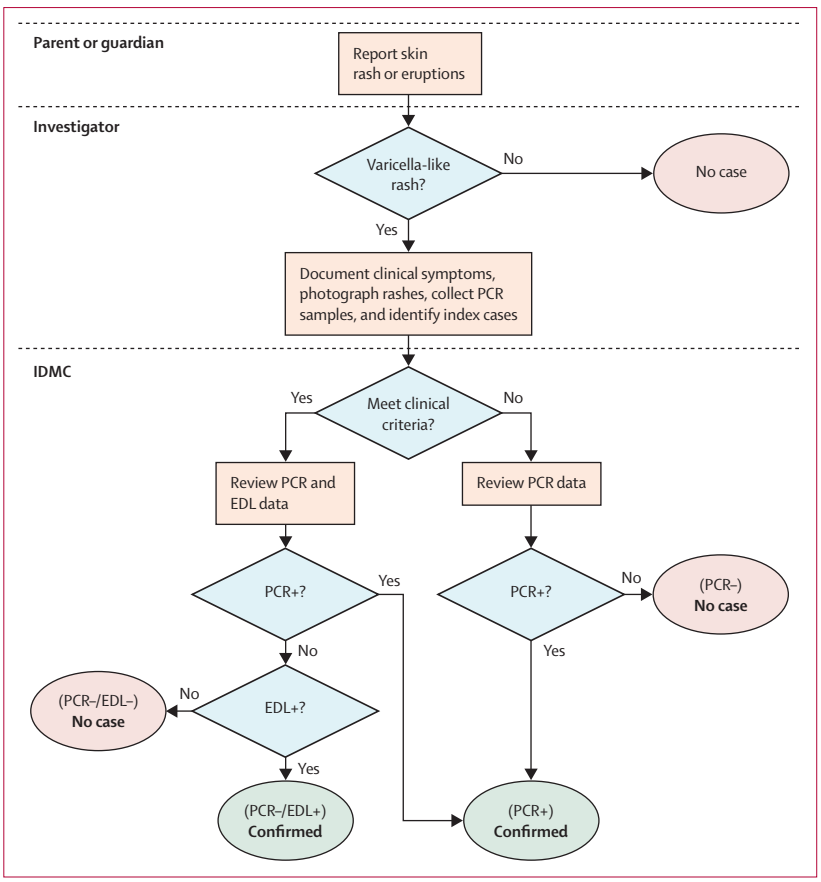

Figure 1: **Schematic description of the procedure followed to confirm varicella cases**
IDMC=Independent Data Monitoring Committee. EDL=epidemiological link. PCR+ indicates a case for which VZV-PCR/RFLP is positive and β-globin-PCR is positive. PCR– encompasses one of three case outcomes: VZV-PCR/RFLP negative and β-globin-PCR positive; VZV-PCR/RFLP negative and β-globin-PCR negative (ie, PCR result invalid); or missing sample.

Randomisation and masking

A randomisation list with a block size of seven was generated at GSK with SAS software (version 8.2) and was used to ascribe a unique treatment number for the two-vaccine dose regimen with a blocking scheme respecting the 3:3:1 treatment allocation ratio. The blocking scheme also took into account the three vaccine lots used for MMRV and monovalent varicella vaccine. The blocking scheme was respected in the distribution of vaccine treatments to each study centre. Each eligible participant was allocated (via the internet at the study centre) a unique treatment number, with a minimisation algorithm accounting for study centre and containing a random component. The vaccine was prepared out of view of the participants' parents or guardians and of the individuals who were responsible for assessing and recording the reactogenicity of the vaccines. All individuals involved in the assessment of any outcome after vaccination (whether efficacy or safety) were unaware of the treatment administered. Sponsor staff involved in review or analysis of data were also unaware of the treatment assignments.

Statistical analysis

Statistical power calculations (with PASS software [version 2000], one-sided proportion) were based on vaccine efficacy being determined as a proportion,

calculated as 1–(the proportion of cases in the test group [either MMRV or MMR+V] divided by the proportion of cases in the control group [MMR]). In view of the 3:3:1 randomisation and assuming live varicella vaccine efficacies of 0·8 (ie, 80%) in both MMRV and MMR+V groups, 144 confirmed varicella cases in the entire cohort (39[MMRV]+39[MMR+V]+66[MMR]) was calculated to provide 89% power to reject at least one of the null hypotheses with a one-sided test (ie, H_0: vaccine efficacy <60%) and a Bonferonni correction for multiple comparisons giving a type I error of 1·25%. In view of this outcome and assuming a 2 year follow-up, exclusion of 20% of participants from the per-protocol analysis, and a conservative annual attack rate of 5% for children in the MMR control group, the number of eligible participants to be enrolled was calculated to be 5754.

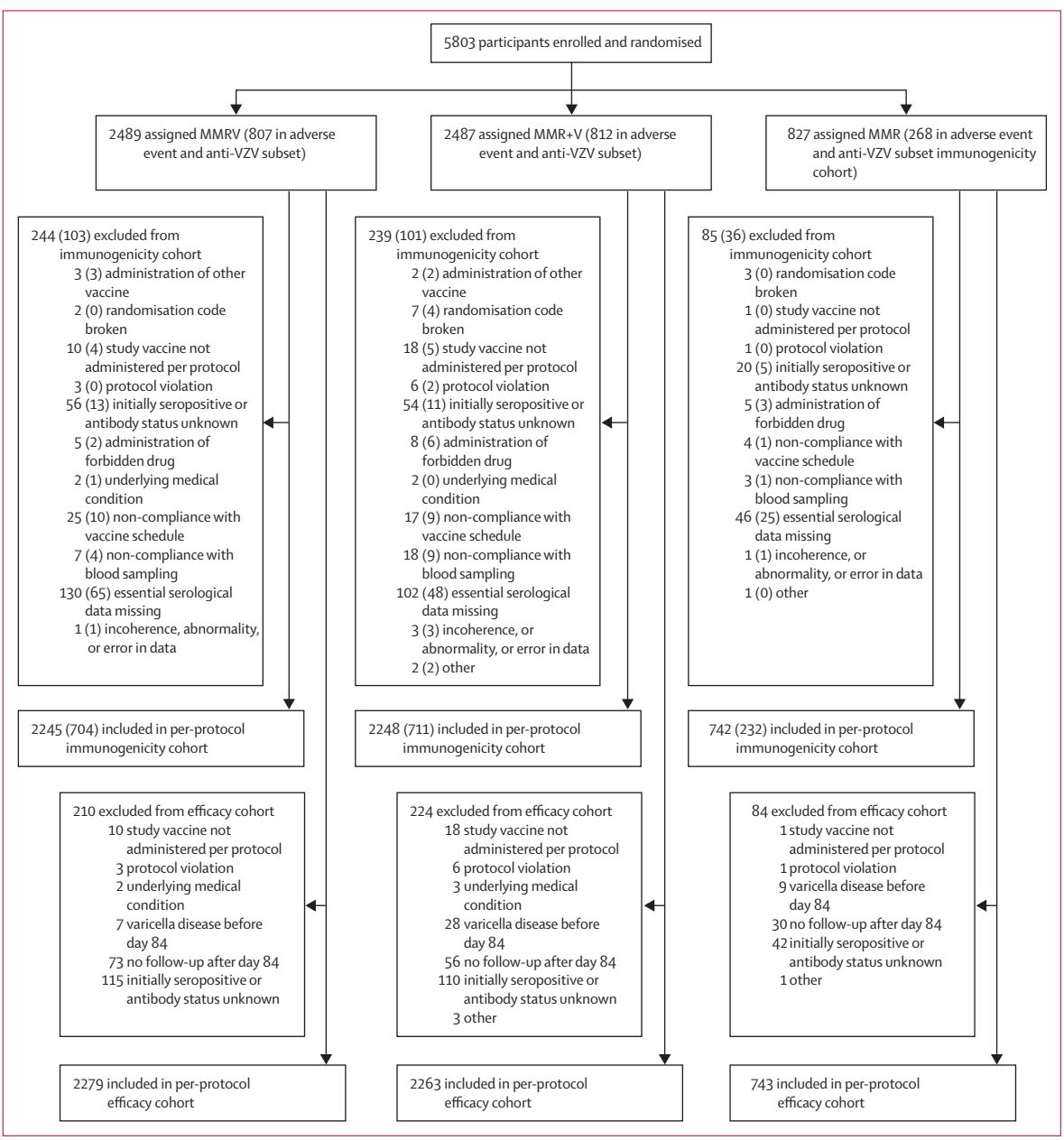

Figure 2: **Allocation and elimination of participants during course of study**
Seropositive participants were excluded from the per-protocol cohorts. Numbers in parentheses correspond to the subsets used to analyse unsolicited and solicited adverse events up to 43 days after each vaccine dose (as part of the total vaccinated cohort), and to analyse anti-varicella-zoster-virus responses at day 42 (as part of the per-protocol immunogenicity cohort). No enrolled participant was excluded from the vaccination cohort. Exclusion criteria are listed in appendix p 2. MMRV=measles-mumps-rubella-varicella combined vaccine. MMR+V=measles-mumps-rubella combined vaccine plus monovalent varicella vaccine. MMR=measles-mumps-rubella combined vaccine only.

Vaccine efficacies against confirmed varicella, from 42 days after dose two until the end of the study period, were estimated in the per-protocol efficacy cohort. The primary objective for one dose of varicella vaccine or two doses of MMRV was to exclude a lower limit of the two-sided 97·5% CI for vaccine efficacy against all grades of varicella that was less than 60%. The secondary objective for one dose of varicella vaccine or two doses of MMRV was to exclude a lower limit of the two-sided 97·5% CI for vaccine efficacy against moderate varicella that was less than 70% and against severe varicella that was less than 80%. Vaccine efficacy was calculated as 100×(1–hazard ratio [HR]). HRs and CIs were estimated with a Cox regression model in which the treatment group was the only regressor. 97·5% CIs were used because a Bonferroni correction was applied giving a nominal one-sided type I error of 1·25%. To control the effect of multiple objectives on the type I error rate, the secondary objective was assessed in a confirmatory manner when the primary objective was reached for the given vaccine. 95% CIs were used for the exploratory analysis of vaccine efficacy by country.

Anti-VZV antibody responses were analysed in the per-protocol immunogenicity cohort (figure 2). The analysis of safety was done on the total vaccinated cohort for serious adverse events and on the reactogenicity subset for solicited and unsolicited adverse events within 42 days after each dose. Computation of the exact 95% CI for proportions within a treatment group assumed independence between doses.[20] Computation of 95% CIs for the geometric mean concentrations (GMCs) used an ANOVA model on $\log_{10}$ transformed data assumed to be normally distributed with unknown variance. SAS (version 9.2, including Proc-StatXact, version 8.1 module) was used for all computations. This trial is registered with ClinicalTrials.gov, number NCT00226499.

Deviations from good clinical practice guidelines

On May 31, 2012, GSK Vaccines (the Sponsor) was made aware of deviations from good clinical practice guidelines affecting GSK Vaccines studies done in Poland between 2005 and 2011. The Sponsor submitted a report on their investigation into the present study to *The Lancet* on Oct 22, 2013. Deviations from good clinical practice guidelines, other notable findings, and corrective actions taken after the investigation are described below. The clinical trial is planned to follow-up participants for 10 years and is therefore ongoing.

An addendum to the informed consent form (ICF; for a subset of participants [dated Oct 3, 2005] and for all participants [dated Jan 6, 2006]) was implemented at sites before Ethics Committee and Regulatory Authority approval. The ICF addendum was implemented to correct the requirements for temperature recording. The original ICF incorrectly stated that temperature recording should begin on day 4. This was not consistent with the protocol which required temperature recording to begin on day 0. Since the addendum did not involve an emergency safety update, it should not have been implemented before the appropriate approvals. The Sponsor has notified the Ethics Committee and Regulatory Authority of this deviation. The Sponsor has also undertaken additional training to ensure that investigators are aware that changes to ICFs should not be implemented before the appropriate approval unless there is a need to inform and protect participants from probable harm on the basis of the availability of new safety information.

Ten sites in Poland lacked monitoring visit reports for at least one on-site monitoring visit undertaken by local representatives of the Sponsor. Because of the lack of monitoring visit reports for some visits, the Sponsor could not, upon study conclusion, verify the validity of all the data collected at these sites. As a result, all sites in Poland were remonitored by the Sponsor between November, 2012, and June, 2013. This re-monitoring included review of ICFs, eligibility criteria, documentation of serious

	MMRV N=2279	MMR+V N=2263	MMR N=743	Total N=5285
Age (months)	14·2 (2·5)	14·2 (2·4)	14·2 (2·5)	14·2 (2·5)
Sex				
Girls	1057 (46·4%)	1109 (49·0%)	359 (48·3%)	2525 (47·8%)
Boys	1222 (53·6%)	1154 (51·0%)	384 (51·7%)	2760 (52·2%)
Race				
White	2227 (97·7%)	2225 (98·3%)	736 (99·1%)	5188 (98·2%)
Black	8 (0·4)	7 (0·3%)	1 (0·1%)	16 (0·3%)
Arabic/north African	21 (0·9%)	7 (0·3%)	2 (0·3%)	30 (0·6%)
East/southeast Asian	7 (0·3%)	6 (0·3%)	1 (0·1%)	14 (0·3%)
South Asian	2 (0·1%)	2 (0·1%)	0	4 (0·1%)
American Hispanic	1 (<0·1%)	5 (0·2%)	2 (0·3%)	8 (0·2%)
Japanese	1 (<0·1%)	0	0	1 (<0·1%)
Other	12 (0·5%)	11 (0·5%)	1 (0·1%)	24 (0·5%)
Country				
Czech Republic	525 (23·0%)	516 (22·8%)	171 (23·0%)	1212 (22·9%)
Greece	115 (5·0%)	111 (4·9%)	31 (4·2%)	257 (4·9%)
Italy	106 (4·7%)	108 (4·8%)	35 (4·7%)	249 (4·7%)
Lithuania	256 (11·2%)	255 (11·3%)	86 (11·6%)	597 (11·3%)
Norway	74 (3·2%)	76 (3·4%)	25 (3·4%)	175 (3·3%)
Poland	385 (16·9%)	368 (16·3%)	116 (15·6%)	869 (16·4%)
Romania	121 (5·3%)	126 (5·6%)	42 (5·7%)	289 (5·5%)
Russia	378 (16·6)	392 (17·3%)	130 (17·5%)	900 (17·0%)
Slovakia	199 (8·7%)	195 (8·6%)	68 (9·2%)	462 (8·7%)
Sweden	120 (5·3%)	116 (5·1%)	39 (5·2%)	275 (5·2%)
Care type at enrolment				
At least one sibling at home	655 (28·7%)	591 (26·1%)	191 (25·7%)	1437 (27·2%)
Attending day care centre	525 (23·0%)	546 (24·1%)	187 (25·2%)	1258 (23·8%)
Attending a child minder	148 (6·5%)	154 (6·8%)	57 (7·7%)	359 (6·8%)
At least once a week contact*	2051 (90·0%)	2052 (90·7%)	679 (91·4%)	4782 (90·5%)

Data are n (%) or mean (SD). MMRV=measles-mumps-rubella-varicella combined vaccine. MMR+V=measles-mumps-rubella combined vaccine plus monovalent varicella vaccine. MMR=measles-mumps-rubella combined vaccine only. *With other children without a known positive history of varicella disease or vaccination.

Table 1: **Demographic characteristics of the per-protocol cohort**

adverse events, and review of data collected for documentation of clinical varicella cases for all participants. Additionally, at nine of the ten sites, full source data verification for 20% of participants was done; at one of the ten sites, full source data verification was not done because the site had already been closed. All ICFs for this site were checked and deemed valid. The re-monitoring effort at the nine sites that were still open showed that the ICFs for all participants were valid and that all participants met the required eligibility criteria. Full source data verification of the sample of 20% of participants was deemed acceptable. However, it was identified that some serious adverse events were not reported within 24 h after notification to the investigator, as stipulated in the protocol. The issue of late reporting of serious adverse events is covered below. Additionally, for one site, most suspected clinical varicella cases (15 of 21) had no photograph associated with the case as stipulated in the protocol. Although this restricted the information available to the study's adjudication committee tasked with reviewing clinical information, including photographs, to confirm suspected cases of varicella, most of those cases (ten of 15) had a skin lesion sample available for confirmation by polymerase chain reaction (PCR). Sites were re-trained on the need to report serious adverse events in a timely fashion and the need to photograph skin lesions when children presented with suspected cases of varicella. The Sponsor has informed the Regulatory Authority and Ethics Committee of these issues regarding monitoring oversight of the study at these ten sites and has also submitted the results of site re-monitoring to the Regulatory Authorities and Ethics Committee.

	n/N	Total time to event (years)	Attack rate (97·5% CI) per 100 person years	Vaccine efficacy (97·5% CI)	p value	H_0
MMRV						
All	37/2279	6690	0·6 (0·4–0·8)	94·9 (92·4–96·6)	<0·0001	Vacccine efficacy <60%
Moderate to severe	2/2279	6740	0·0 (0·0–0·1)	99·5 (97·5–99·9)	<0·0001	Vacccine efficacy <80%
MMR+V						
All	243/2263	6455	3·8 (3·3–4·3)	65·4 (57·2–72·1)	0·1265	Vacccine efficacy <60%
Moderate to severe	37/2263	6698	0·6 (0·4–0·8)	90·7 (85·9–93·9)	..	Vacccine efficacy <70%
MMR						
All	201/743	1934	10·4 (9·1–11·9)	..	..	..
Moderate to severe	117/743	2047	5·7 (4·8–6·9)	..	..	..

The time to event was the time from 42 days after dose two to the time of the event's first occurrence. In the two vaccine efficacy calculations, the event was either any (all) varicella grades or only moderate to severe varicella, respectively. When there was no varicella event, time to event was censored at the end of the first phase of the trial (June 29, 2009) or at the last visit (if the participant withdrew). The date of the varicella case was the date of (in order of preference for available data) rash onset (the appearance of the first lesion), telephone contact to the investigator, or investigator's ascertainment visit. Testing the null hypothesis (H_0) for vaccine efficacy against moderate to severe disease (secondary objective) was conditional on the rejection of the null hypothesis (H_0) for vaccine efficacy against all disease (primary objective). Therefore the p value is not shown for MMR+V efficacy against moderate to severe disease. MMRV=measles-mumps-rubella-varicella combined vaccine. MMR+V=measles-mumps-rubella combined vaccine plus monovalent varicella vaccine. MMR=measles-mumps-rubella combined vaccine only.

Table 2: **Vaccine efficacy against varicella in the per-protocol efficacy cohort**

The Sponsor requires that investigators report serious adverse events to the company within 24 h of their notification. During this investigation, three sites in Poland were identified as having failed to report serious adverse events within the required 24 h period. Five serious adverse events in three participants occurred before the end of enrolment and were reported more than 24 h after the investigator was notified: otitis media and gastroenteritis (reported 10 months after investigator notification), and gastroenterocolitis, rhinopharyngitis, and laryngitis (reported within 3–12 days of notification). The investigators did not deem any of these events to be related to the study vaccines. The Sponsor believes that more timely reporting of these common paediatric infections, which are not deemed to be new safety signals for the vaccines administered in the trial, would not have affected the informed consent information. Therefore the delayed reporting would not have affected a parent's willingness to enrol a child in the study. The Sponsor has re-trained investigators on the timely reporting of serious adverse events.

Study participants were given gifts at visits that occurred after the end of the vaccination phase of the study starting at visit 3 (day 84). These gifts included one of the following at each visit: a skipping rope, a ball, a children's encyclopaedia, bubble bath and sponge, a puzzle, a game, or a book. Each study participant received a total of five gifts. Study ICFs included information about these gifts, and these documents were approved by the Ethics Committee and Regulatory Authority. This practice was perceived as possibly in conflict with local laws in Poland which forbids financial inducements in clinical trials. Furthermore, while internal Sponsor policies at the start of the study did not preclude the distribution of such nominal gifts, policies that took effect during the course of the study prohibit gift giving to clinical trial participants. The practice of gift giving was halted in the context of the good clinical practice compliance issues that were detected during the investigation. By the end of 2013, the parents of study participants were notified that children would no longer receive gifts at subsequent study visits. In view of the low value of the items provided, the Sponsor does not believe that the items are likely to have resulted in inappropriate inducement. The practice and its suspension have been reported to the relevant Ethics Committee and Regulatory Authority.

In Poland, annual progress reports were not routinely generated by local representatives of the Sponsor and therefore were not submitted to the Ethics Committee or Regulatory Authority throughout the duration of the study as required by International Conference on Harmonisation good clinical practice 8.3.19.

The Sponsor informed the Ethics Committee and Regulatory Authority in Poland of this issue on Nov 12,

2012. Thereafter, the annual progress reports for the most recent years were submitted on Feb 4, 2013. It should be noted that while annual progress reports were not submitted, annual safety reports had been provided and submitted to the Ethics Committee and Regulatory Authority throughout the study. The Sponsor has implemented corrective actions to ensure timely submission of annual progress reports to the appropriate Regulatory Authority and Ethics Committee.

Role of the funding source

The trial was sponsored and designed by GSK but amended with input from an IDMC consisting of relevant experts. Investigators generated and reported the data for analysis by GSK statisticians according to a prespecified analysis plan. All authors had complete access to the analysed data (without compromising the trial blinding), participated in the drafting and reviewing of the report, and vouch for the accuracy and completeness of this report. RP, SE, VU, LG, JW, ON, and PW had final responsibility for the decision to submit for publication. GSK covered the costs associated with the development and publishing of the manuscript, including writing assistance.

Results

Between Sept 1, 2005, and May 10, 2006, 5803 participants were enrolled (figure 2). Most (56·4%) of the 5285 participants in the efficacy cohort were from the Czech Republic, Poland, and Russia (table 1). The overall mean age was 14·2 months (range 11–22 months) at the time of enrolment. 98·2% were white with a male to female ratio of 1·0 to 0·9. The three treatment groups were much the same in terms of age, race, and sex, and of the proportion of children with different levels of contact based on care type.

In the per-protocol efficacy cohort (5285 children), the mean duration of follow-up in the MMRV group was 36 months (SD 8·8), in the MMR+V group was 36 months (8·5) and in the MMR group was 35 months (8·9). The percentage of participants with a reported contact with varicella or zoster disease, or both, was

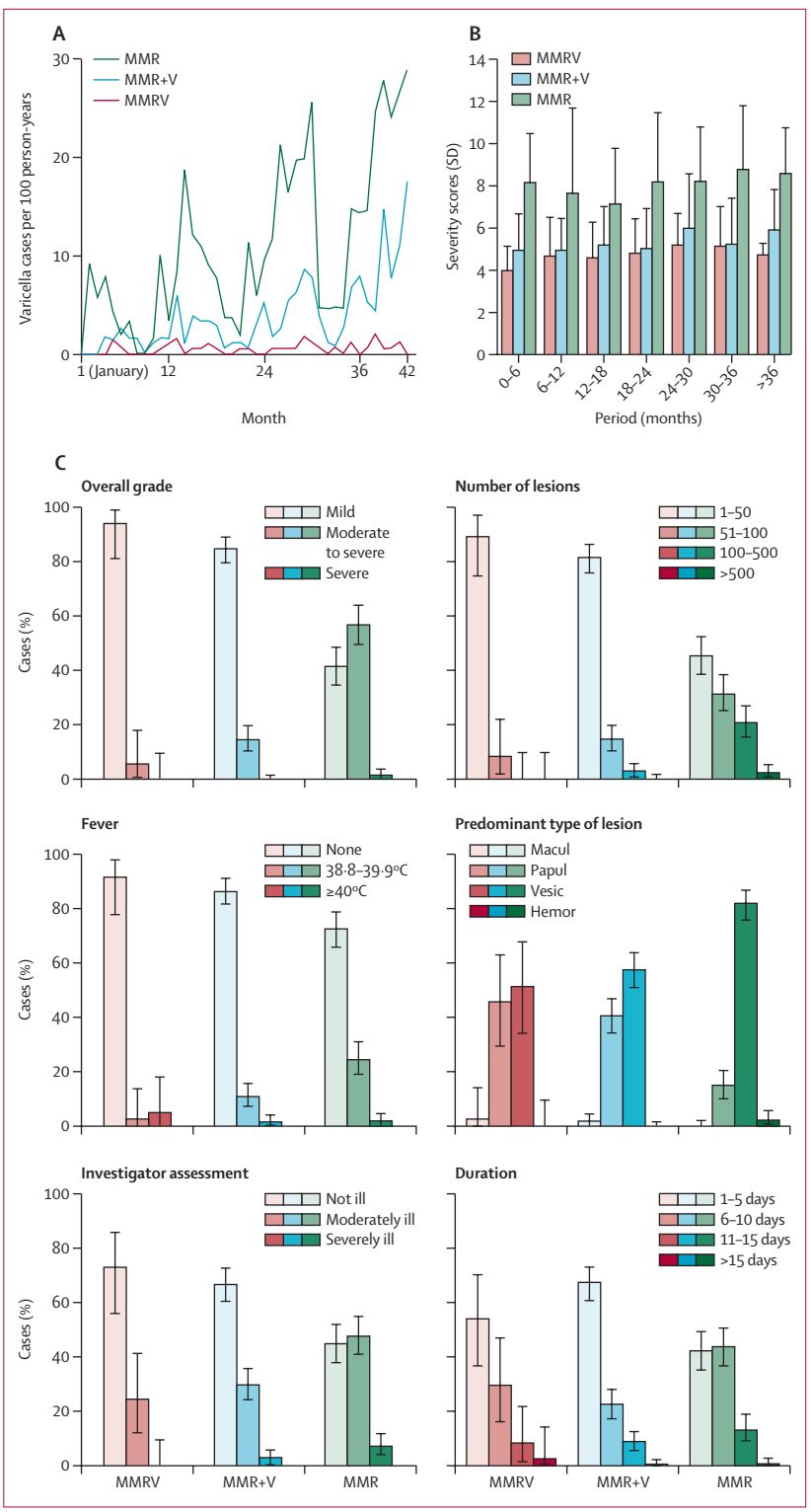

Figure 3: **Varicella case grading and seasonal rates of infection in the per-protocol cohort for efficacy**

(A) Varicella case rate (per 100 person years) for each treatment group for the months starting January, 2006, and ending June, 2009. (B) Mean severity scores for each treatment group during blocks of 6 months over course of study. Error bars are SD. (C) Overall grading for varicella with respect to the percentage of confirmed cases within a treatment group, and the grading within some of the categories. An overall score was determined from the following illness attributes (individual scores given in parentheses): rash, number of lesions, 1–5 (1), 51–100 (2), 101–500 (4), and >500 (8); the character of most lesions, macular (2), papular (2), vesicular (4), and haemorrhagic (10); fever, 38·8°C–39·9°C (1), and ≥40°C (3); interstitial pneumonia (10); encephalitis (10); and a subjective assessment by the investigator, participant does not seem ill (0), participant seems moderately ill (2), and participant seems severely ill (5). Disease gradings were: mild disease, ≤7 points; moderately severe disease, 8–15 points; and severe disease, ≥16 points. Graph bars within a treatment group from right to left and with progressively more shading follow increases in grading. Error bars are 95% CI. Note, all values and assessments refer to the first-varicella case only, because three participants (all in the MMR+V group) had second cases of varicella. MMRV=measles-mumps-rubella-varicella combined vaccine. MMR+V=measles-mumps-rubella combined vaccine plus monovalent varicella vaccine. MMR=measles-mumps-rubella combined vaccine only.

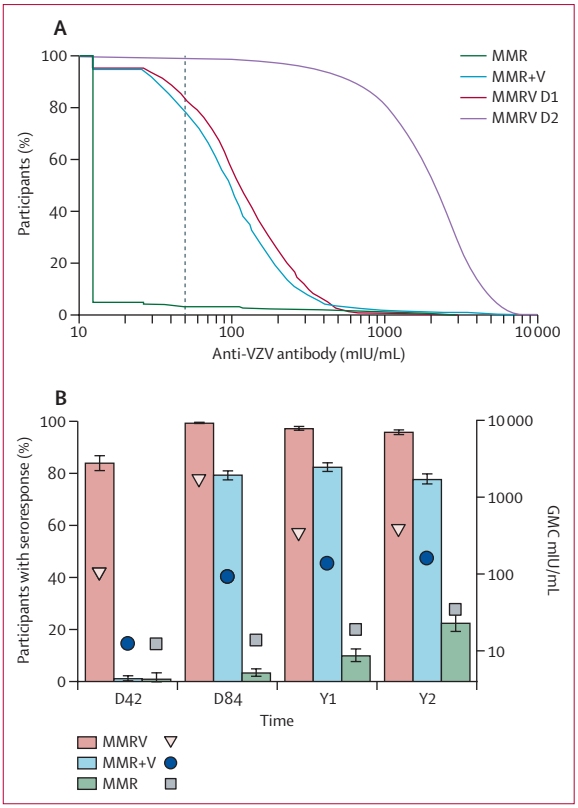

Figure 4: **Anti-VZV antibody responses to vaccination**
(A) Reverse cumulative curves describing the percentage of particpants with respect to anti-VZV antibody concentrations, 42 days after dose 2 (day 84) in the MMR and MMR+V groups and after dose 1 and dose 2 in the MMRV group. Vertical dashed line shows the seroresponse cutoff of 50 mIU/mL. (B) Percentage of particpants with seroresponses to vaccination (vertical bars, left axes) and geometric mean concentrations (GMCs; symbols, right axes) in the three treatment groups at day 42, day 84, year 1 and year 2. Error bars are 95% CI and for GMCs are masked by the size of the symbols. Data at day 42 come from the subset of the per-protocol cohort for immunogenicity. Data at day 84, year 1, and year 2 come from the entire per-protocol cohort for immunogenicity. VZV=varicella zoster virus. MMRV=measles-mumps-rubella-varicella combined vaccine. MMR+V=measles-mumps-rubella combined vaccine plus monovalent varicella vaccine. MMR=measles-mumps-rubella combined vaccine only. D=day. Y=year.

19·4% (144 of 743) in the MMR group, 20·9% (474 of 2263) in the MMR+V group, and 23·1% (527 of 2279) in the MMRV group. In the MMR (control) group, 201 participants had varicella cases, giving an incidence of 10·4 per 100 person years (table 2); of these, more than half were graded as moderately severe or severe. In the MMR+V group (one-dose live varicella vaccine), 243 participants had varicella cases, giving an incidence of 3·8 per 100 person years. In the MMRV group (two-dose live varicella vaccine), 37 participants had varicella cases, giving an incidence of 0·6 per 100 person years. Notably, most live varicella vaccine recipients had first cases that were mild (MMR+V group, 207 of 243; MMRV group, 35 of 37) compared with the MMR control group (84 of 201). Three participants had second cases of varicella (all in the MMR+V group). For one participant, the first case was mild and the second case was moderate. For the two other participants, all the cases were mild. The mean varicella severity grades of all cases were 5·5 (SD 2·1) in the MMR+V group and 4·8 (1·5) in the MMRV group, and were also indicative of mild disease, by contrast with 8·1 (2·9) in MMR control group, indicative of moderately severe disease. Efficacy of two-dose vaccination against all varicella was 94·9% and against moderate to severe varicella was 99·5%. For one-dose, efficacy was 65·4% against all varicella and 90·7% against moderate to severe disease (table 2). Hence, risk of breakthrough varicella was 6·9 times (95% CI 4·9–9·8) less likely with two doses of live varicella vaccine (MMRV group) than with one dose (MMR+V group). We identified no evidence that vaccine efficacy estimates were different in the individual participating countries from the overall estimates (appendix p 5). Two doses of live varicella vaccine also seemed to substantially reduce the seasonal fluctuations in varicella incidence (figure 3A). For one or two doses of live varicella vaccine, we noted no evidence that the respective degrees of protection against varicella changed during the follow-up period (appendix p 6). Moreover, we recorded no obvious increase in mean severity scores in either of the live varicella vaccine groups compared with the MMR group (figure 3B).

The difference in disease severity grading was apparent in many of the contributing grading categories, including fever, number and predominant type of lesions, and the investigator's assessment (figure 3C). Moreover, many varicella cases (16 of 37, 43·2%) in the MMRV group were confirmed by epidemiological link, by contrast with only 9·5% (23 of 243) of (first) cases in the MMR+V group and 6·5% (13 of 201) of cases in the MMR group. Confirmation by epidemiological link, in addition to direct VZV-DNA detection, was used to increase sensitivity of case finding, especially in mild breakthrough cases in which lesions would be more difficult to sample and in which viral DNA might be reduced in quantity or cleared more rapidly. Notably, for 15 of 16 cases in the MMRV group and 12 of 23 cases in MMR+V group confirmed by epidemiological link, no VZV-DNA was detected in lesion samples, whereas no VZV-DNA was detected for only two of 13 cases from the MMR group (although sample quality was adequate because β-globin DNA was detected). Besides the 29 VZV-DNA negative cases confirmed by epidemiological link, we recorded 20 cases without a sample (one MMRV; nine MMR+V; ten MMR) and three cases with inadequate sample quality (two MMR+V; one MMR).

Anti-VZV responses 42 days after one or two doses of live varicella vaccines were broadly distributed, yet seemed to be different, suggested by the rightward incremental shifts in the reverse cummulative curves in accordance with the number of live varicella-vaccine doses (figure 4A). Distribution of anti-VZV responses, and seroresponse rates and GMCs, seemed to be much the same after one dose of MMRV or monovalent varicella vaccines (figure 4). Moreover, 42 days after dose

	N (local)	N (fever)	Participants (% [exact 95% CI])							
			All grades				Grade 3			
			Redness (day 0–3)	Pain (day 0–3)	Swelling (day 0–3)	Fever (day 0–14)	Redness (day 0–3)	Pain (day 0–3)	Swelling (day 0–3)	Fever (day 0–14)
Dose 1										
MMRV	782	784	17·5% (14·9–20·4)	9·5% (7·5–11·7)	4·9% (3·5–6·6)	57·4% (53·9–60·9)	0·4% (0·1–1·1)	0·1% (0·0–0·7)	0·4% (0·1–1·1)	12·9% (10·6–15·4)
MMR+V*	796	795	19·3% (16·7–22·3)	10·4% (8·4–12·8)	4·6% (3·3–6·4)	44·5% (41·0–48·1)	0·4% (0·1–1·1)	0·3% (0·0–0·9)	0·0% (0·0–0·5)	7·3% (5·6–9·3)
MMR	256	256	14·1% (10·0–18·9)	7·8% (4·8–11·8)	2·3% (0·9–5·0)	39·8% (33·8–46·1)	0·0% (0·0–1·4)	0·0% (0·0–1·4)	0·0% (0·0–1·4)	6·3% (3·6–10·0)
Dose 2										
MMRV	758	758	24·8% (21·8–28·0)	12·0% (9·8–14·5)	9·5% (7·5–11·8)	24·9% (21·9–28·2)	3·4% (2·3–5·0)	0·0% (0·0–0·5)	0·5% (0·1–1·3)	3·7% (2·5–5·3)
MMR+V*	774	773	13·7% (11·4–16·3)	8·1% (6·3–10·3)	4·0% (2·7–5·6)	26·0% (22·9–29·2)	0·4% (0·1–1·1)	0·1% (0·0–0·7)	0·0% (0·0–0·5)	4·5% (3·2–6·2)
MMR	247	247	9·3% (6·0–13·6)	6·5% (3·7–10·3)	1·2% (0·3–3·5)	22·3% (17·2–28·0)	0·0% (0·0–1·5)	0·4% (0·0–2·2)	0·0% (0·0–1·5)	5·7% (3·1–9·3)

Reactogenicity subset (N) consists of participants with at least one documented dose. Redness (up to 25% of participants in any group) was the most common solicited injection-site symptom in all three groups and after both doses. Occurrence of redness and swelling only increased after the second dose in the MMRV group. MMRV=measles-mumps-rubella-varicella combined vaccine. MMR+V=measles-mumps-rubella combined vaccine plus monovalent varicella vaccine. MMR=measles-mumps-rubella combined vaccine only. *In the MMR+V group, reactions after dose 1 were to MMR and reactions after dose two were to V.

Table 3: **Participants reporting solicited injection-site (local) symptoms up to 4 days after each dose and of fever up to 15 days after each dose in the reactogenicity subset**

two of MMRV, the anti-VZV antibody GMC was about 18 times higher (1839 *vs* 97 mIU/mL) and the seroresponse rate was higher (99·6% *vs* 79·2%) than 42 days after dose one (see figure 4B). Although reduced at 2 years, the apparent improved immunogenicity of two live varicella-vaccine doses compared with one dose persisted, such that the occurrence of seroresponse for two doses versus one dose was 96·1% (95% CI 95·1–96·9) versus 78·2% (76·2–80·0) and the anti-VZV antibody GMCs were 414 mIU/mL (95% CI 392–438) versus 169 mIU/mL (157–182). After 2 years, the percentage of seropositive participants in the control group (who were seronegative before vaccination) was 26·6% (95% CI 23·2–30·2).

Redness was the most frequently reported solicited injection-site symptom in each group and after each dose (table 3). After dose two, 24·8% of participants who received MMRV reported redness by contrast with 13·7% of those who received monovalent varicella vaccine and 9·3% of those who received MMR. Percentages of participants reporting fever (≥38°C) in all groups peaked at day 9 after dose one; 31% (248 of 807) after receiving MMRV and 23% (184 of 812) and 17% (45 of 268) and after receiving MMR in the other two groups (appendix p 7). During the 15 day period after dose one, fever was reported by 57·4% of participants after MMRV, by contrast with 44·5% and 39·8% after MMR (table 3). Grade 3 fever was reported by 12·9% of participants after MMRV, by contrast with 6·3% and 7·3% after MMR. During 15 days after dose two, fever was reported by 24·9% (MMRV), 26·0% (movovalent varicella vaccine), and 22·3% (MMR) of participants. The percentage of participants reporting at least one unsolicited symptom after the first or second dose ranged between 31 and 37% across the three groups. The nature of these unsolicited symptoms seemed much the same in each group (data not shown).

	MedDRA system organ class[21]	Age (months)	Sex	Dose	Onset (days after dose)	Duration (days)	Intensity (grade)
MMRV							
Febrile convulsion	Nervous system disorders	14	M	1	8	1	3
Febrile convulsion	Nervous system disorders	17	F	1	9	1	3
Febrile convulsion	Nervous system disorders	13	M	1	8	1	2
MMR+V							
Herpes zoster*	Infections and infestations	17	F	1	24	36	1
Rash papular	Skin and subcutaneous tissue disorders	15	F	1	16	10	1
Bacterial infection	Infections and infestations	17	F	2	3	13	2
Peritonsillar abscess	Infections and infestations	18	M	2	6	21	3
MMR							
Febrile convulsion	Nervous system disorders	15	M	1	8	1	3

Serious adverse events were recorded during the entire study period. MMRV=measles-mumps-rubella-varicella combined vaccine. MMR+V=measles-mumps-rubella combined vaccine plus monovalent varicella vaccine. MMR=measles-mumps-rubella combined vaccine only. *This case was considered by IDMC but was not confirmed as herpes zoster.

Table 4: **Serious adverse events deemed by the investigator to be related to vaccination in the total vaccinated cohort**

The occurrence of serious adverse events was 17·9% (148 of 827) in the MMR group, 19·3% (481 of 2487) in the MMR+V, and 19·0% (474 of 2489) in the MMRV group. Occurrence of serious adverse events attributed a causal link to vaccination were 0·1% in the MMR (one of 827), 0·2% (four of 2487), and 0·1% (three of 2489) in the MMRV group. All these serious adverse events were reported as recovered or resolved (table 4). 13 febrile seizures were reported as serious adverse events up to day 84 in the total vaccination cohort. Four were attributed as having a causal link to vaccination, and occurred 8–9 days after dose one; three were reported after MMRV and one was reported after MMR (table 4). These 13 febrile seizures included two that were solicited

Panel: **Research in context**

Systematic review

We searched PubMed with the terms "varicella", "vaccine", "efficacy", and "effectiveness" for articles published in English up to June 12, 2012. The relevance of papers was further considered (with the additional search term "clinical trial") on the basis that they reported a clinical efficacy or effectiveness trial of currently licensed live-attenuated varicella vaccines, or a meta-analysis or review of these trials.

Interpretation

As far as we are aware, this study is the first randomised concurrently controlled trial in healthy children aged 12–22 months, assessing the multiyear efficacy of a live varicella vaccine, formulated either as a monovalent vaccine or combined with MMR vaccine, on the basis of active surveillance for rash illness of any intensity with rigorous case confirmation. The results from this study confirm the highly efficacious protection afforded by two doses of MMRV given over 6 weeks against varicella of any intensity. They also support the highly efficacious protection afforded by one dose of monovalent varicella vaccine against typical varicella disease (moderate to severe), although prevention of mild breakthrough varicella was not as high as that afforded by two doses of MMRV.

from the reactogenicity subset; one from a participant 9 days after the first dose of MMRV (and related to vaccination), and another from a participant 20 days after monovalent varicella vaccine (at dose two and not related to vaccination).

Discussion

In this study of more than 5000 children, efficacy of two-dose MMRV against all varicella was almost 95%, and against moderate to severe varicella was more than 99%. This protection was greater than for one-dose varicella vaccine, which had efficacy against all varicella of about 65%, and against moderate to severe varicella of roughly 91%. Redness was the most frequently reported solicited injection-site symptom in each group and after each dose (up to 25% of participants). More participants in the MMRV group (about 57%) reported fever of 38°C or more within 15 days of dose one than in the other groups (40–45%). Occurrence of serious adverse events deemed related to vaccination was three in the MMRV group, four in the MMR+V group, and one in the MMR group.

As far as we are aware, this is the first study to assess vaccine efficacy of one and two doses of live varicella vaccines in comparison with a control vaccine, and in a uniform age cohort living in a setting of endemic VZV transmission (panel).[7] We used a randomised, observer-blind, parallel group design with active case ascertainment that deemed even mild rash to be illnesses. These results describe the first 3 years of follow-up, but more data for longer term protection are anticipated with the analysis of 10 years of follow-up in the same study cohort.

One dose of live varicella vaccine was 90·7% efficacious at prevention of moderate to severe disease but only 65·4% efficacious at prevention of all breakthrough varicella. Most breakthrough varicella was mild in line with other findings.[3,14] Because mild breakthrough disease is associated with less VZV transmission than more severe disease,[22] one dose of live varicella vaccine is likely to reduce the risk of VZV transmission. It is also likely to be effective in prevention of varicella complications and many of the associated costs (eg, admissions to hospital),[1,3] especially with a rapid implementation of universal vaccination, as noted in Uruguay.[2] The 10 year analysis of the MMR+V cohort might address whether a one-dose schedule offers long-term protection or defers the appearance of moderate or severe disease to a later age. In Uruguay, 8 years after implementation of universal vaccination, rates of varicella-related admissions to hospital are estimated to have remained at about 90% lower than before implementation.[2,23]

The short two-dose schedule of live varicella vaccination was 94·9% efficacious at prevention of breakthrough varicella. This two-dose schedule is in line with recommendations for combining monovalent varicella vaccine with MMR vaccination, with the first dose being administered in the second year of life and with the second dose shortly after (at least one month).[5,7] The short schedule could also be implemented with MMR+V as first dose and MMRV as second dose to minimise the risk of febrile seizures.[24] Administration of the second dose soon after the first and before the child enters into day care or school should also minimise the risk of breakthrough varicella. By preventing even mild disease, two-dose live varicella vaccination is also likely to be better than one dose in further reducing the risk of VZV transmission.[22]

We identified no evidence that the protection afforded by one or two doses of live varicella vaccine was diminished over 3 years in the face of highly endemic disease. The relatively greater protection against even mild varicella afforded by the short two-dose (MMRV) schedule compared with the one-dose (monovalent varicella vaccine) schedule was associated with an increased anti-VZV GMC and seropositivity rate at all timepoints after full vaccination, in line with earlier studies.[8,11,25–29] VZV exposure (including subclinical infections) after day 84 might have boosted the anti-VZV antibody responses at later timepoints, but this effect, if substantial, should have narrowed the difference between MMRV and MMR+V groups and that was not noted.

Reactogenicity and safety variables were in line with other studies,[8,11,25–27,29–33] including the findings that in MMRV recipients compared with MMR recipients, fever within 15 days after the first dose was more frequent, especially at day 9; and injection-site redness within 4 days after the second dose was more frequent.[8,11,25,31,33] An increased risk of febrile seizure 7–10 days after the first dose of MMRV (ProQuad, Merck & Co, Whitehouse Station, NJ, USA) has been reported elsewhere in an analysis of vaccine surveillance data from 459 000 infants.[24] Although three of the four febrile seizures related to vaccination in our study were reported after the first dose of MMRV, a substantially larger study would be required to establish whether the relative risk of febrile seizure differs

from that assessed for ProQuad. Overall, no new clinically important safety signals were identified and the results are supportive of an acceptable safety profile of the MMRV, monovalent varicella vaccine, and MMR schedules in healthy children during their second year of life.

The strengths of this study compared with previous assessments of VZV vaccine efficacy were its use of a randomised control group, active surveillance, and stringent procedures for case confirmation. All these measures were designed to achieve a robust estimate of vaccine efficacy, including against even mild breakthrough disease. Case confirmation by epidemiological link afforded increased sensitivity for case detection in situations where the VZV-PCR result was negative or unobtainable. The greater reliance on an epidemiological link for confirming cases in the MMRV group most probably resulted from the mildness of the disease. In mild disease, failure to detect VZV-DNA might have been attributable to its true absence, implying another cause, or to difficulties in sampling such as inadequate unroofing of the papule, or because of VZV being cleared before sampling. Some cases might have been falsely confirmed by epidemiological link; if this occurred more often among varicella vaccine recipients than controls, then vaccine efficacies could have been underestimated.

The one-dose efficacy of 65·4% for monovalent varicella vaccine was less than the previous estimate of 88% (95% CI 72–96) for GSK's vaccine of similar potency.[13] In the previous study, done in Finland, case confirmation might have been less rigorous and biased in favour of detecting cases in the control group. Case confirmation required a four-times difference between anti-VZV titres in acute and convalescence samples, which might have been less likely with seropositive vaccine recipients than with seronegative control participants. Furthermore, case identification through epidemiological link to index cases was not considered. Moreover, the measures used in the present study to avoid overestimation of vaccine efficacy might explain differences in comparison with other previous long-term studies done in the USA. In those studies, one-dose vaccine efficacy was estimated in the range of 88·9%–94·4%[12,14,15] and two-dose vaccine efficacy was estimated at 98·3%[14] (in children aged up to 6[12] or 12[14,15] years). These studies did not include control groups and parents were aware that their children were vaccinated against VZV: this knowledge might have biased parents against reporting rashes. Moreover, vaccine efficacies were potentially overestimated because they were based on historic attack rates from the pre-1995 universal live varicella vaccination era in the USA,[12,14,15] and yet the surveillance periods extended well beyond 1995–2003.

Although vaccine effectiveness studies are not as rigorously controlled as efficacy studies and would not use systematic procedures for case follow-up, the 65·4% efficacy estimate of one-dose monovalent varicella vaccine is in line with the effectiveness estimate of 72% (95% CI 68–76) from a meta-analysis of 14 studies of varicella outbreaks.[34] However, this efficacy estimate seems lower than the median effectiveness estimate of 84·5% from numerous effectiveness studies of monovalent varicella vaccine (Varivax) or MMRV (ProQuad) in the USA,[35] and lower than effectiveness estimates in case controlled studies, such as 88% (95% CI 77–94) in Israel,[36] 86% (73–93) in China,[37] and 85% (95% CI 78–90) in the USA.[19] The 94·9% efficacy of two doses of MMRV is in line with the study of outbreaks at German daycare centres, in which MMRV (PriorixTetra) effectiveness was estimated at 91% (95% CI 65–98) and in which two doses were estimated to be more effective than one.[38] The efficacy of two doses of MMRV is also in line with estimates of two doses of monovalent varicella vaccine, such as 98% (95% CI 84–100).[39]

The design and multinational nature of the present study provide a robust estimate of vaccine efficacy and support the generalisability of the study conclusions to regions or countries with high rates of VZV transmission. During 3 years of active follow-up in settings where varicella is endemic, one dose of live varicella vaccine provided strong protection against moderate to severe forms of varicella, whereas two doses of live varicella vaccine provided high rates of protection against all forms of varicella disease. These results support the implementation of two-dose varicella vaccination on a short course, to ensure optimum protection from all forms of varicella disease.

Contributors

PW and BI conceived the trial, designed the trial, and obtained research funding. MD provided statistical advice on trial design and drafted the analysis plan which was approved by PW and BI. VV designed the laboratory testing for case confirmation. All authors contributed to the acquisition and review of the data. RP, MRB, SE, LG, SM, NS, MS, VU, and JW recruited patients. MD analysed the data. 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.

Conflicts of interest

MD, BI, ON, and VV are employees of GlaxoSmithKline [GSK] Vaccines. PW was an employee of GSK Vaccines when the trial was ongoing. BI and PW have stock options in GSK Vaccines. RP, VU, SM, and SE have received grants from GSK Vaccines. RP, VU, and LG have received consulting fees from GSK Vaccines. VU, SM, and JW have received travel grants from GSK Vaccines to attend scientific meetings. SM has received fees for lectures including service on speakers' bureaus from GSK Vaccines. MRB, NS, and MS have not received any consulting fees and declare that they have no conflicts of interest.

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