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A double-blind, randomized, controlled, multi-center safety and immunogenicity study of a refrigerator-stable formulation of VARIVAX® ☆

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ABSTRACT

Objective: VARIVAX® (varicella virus vaccine, live Oka/Merck, Merck & Co., Inc., Kenilworth, NJ, USA) was originally licensed as a frozen formulation. A refrigerator-stable formulation of VARIVAX was subsequently developed to allow for increased availability of the product around the world. The objective of this study (V210-051) was to demonstrate that the safety, tolerability and immunogenicity profile of the refrigerator-stable formulation of VARIVAX was similar to the frozen formulation.

Methods: In this double-blind, randomized, multicenter study, healthy 12- to 23-month-old children with negative vaccination and clinical histories for measles, mumps, rubella, varicella, and zoster were vaccinated with either a refrigerator-stable formulation of VARIVAX (at two dosage levels; 8000 PFU [$N = 320$] or 25,000 PFU [$N = 315$]) or the frozen formulation of VARIVAX (10,000 PFU, $N = 323$) given concomitantly with M-M-RII® (measles, mumps, and rubella virus vaccine live, Merck & Co., Inc., Kenilworth, NJ, USA). Children were followed for 42 days after vaccination for adverse experiences. Immunogenicity was evaluated 6 weeks after vaccination.

Results: The refrigerator-stable formulation of VARIVAX was generally well tolerated. The incidence of adverse experiences was similar between all three groups. No vaccine-related serious adverse experiences were reported with any of the vaccine formulations. The immune response (percentage of subjects with varicella antibody titers $\geq$5 gpELISA units) for both refrigerator-stable formulations of VARIVAX at 6 weeks postvaccination was similar to that of the frozen formulation. Administration of either refrigerator-stable formulation of VARIVAX with M-M-RII yielded seroconversion rates and GMTs for measles, mumps and rubella that were comparable to those achieved after administration of the frozen formulation of VARIVAX with M-M-RII.

Conclusion: The safety, tolerability, and immunogenicity profile of the refrigerator-stable varicella vaccine was similar to that of the frozen formulation.

1. Introduction

Two important elements in the development and implementation of any vaccine are the temperature sensitivity of the pathogen of interest and the available cold chain where the vaccine will be used. Varicella zoster virus (VZV) is a cell associated virus that is heat labile [1]. The first varicella vaccine developed in the United States, VARIVAX was developed as a formulation that required storage at −20 °C or colder. This limited its ability to be used in parts of the world where the cold chain for vaccine storage consists only of refrigeration at 2 °C to 8 °C.

In 1998, several improvements were made to the originally licensed manufacturing process for varicella vaccine. These changes, known collectively as Process Upgrade, were designed to improve the yield of Oka/Merck VZV from MRC-5 cells in order to supply a higher potency vaccine for future refrigerator-stable

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formulations of varicella vaccine and for a quadrivalent measles, mumps, rubella, and varicella vaccine which includes a higher dose of varicella virus. Vaccine manufactured by the Process Upgrade is referred to as 1998 Production Lots Process Upgrade Varicella Vaccine (PUVV).

In previous clinical trials, over 1870 healthy, varicella history-negative children 12 months to 12 years of age received a single immunization with PUVV at doses ranging from 1450 to 78,000 plaque-forming units (PFU)/0.5-mL dose; at least 700 of these children received PUVV at doses ≥50,000 PFU [2]. The varicella antibody response to PUVV was similar to that induced by vaccine manufactured using earlier processes, both in terms of the immune response (percentage of subjects with a 6-week postvaccination varicella antibody titer ≥5 glycoprotein enzyme-linked immunosorbent assay [gpELISA] units) and the geometric mean titer (GMT) [2]. No PUVV-related serious adverse experiences were reported in these studies [2]. The rates of systemic adverse experiences, non-injection-site varicella-like rashes, and elevated temperatures (≥102°F [38.9 °C] oral equivalent or abnormal) were similar across the dose range of PUVV that was tested, and was consistent with rates reported in previous studies with vaccine manufactured by earlier processes. Only the rate of local injection-site reactions appeared to show a dose-related increase, although more than 90% of reactions were rated as mild by the parent/guardian [2]. PUVV was licensed as a frozen formulation in the United States in March 2000.

The manufacture of a refrigerator-stable varicella vaccine required two additional changes. The varicella potency release range was increased and the stabilizer containing phosphate, gelatin, and sucrose was supplemented with urea to help stabilize the vaccine virus at temperatures between 2 °C and 8 °C. These adjustments to the vaccine formulation maintained the minimum claimed potency of 1350 PFU/0.5-mL dose after 24 months of storage at 2 °C to 8 °C. This formulation is henceforth referred to as the refrigerator-stable formulation of varicella vaccine.

The purpose of this study (V210-051) was to compare the safety, tolerability, and immunogenicity of the refrigerator-stable formulation to the frozen formulation of varicella vaccine when each was administered concomitantly with M-M-RII (henceforth referred to as MMR) to healthy, varicella-history negative children 12 to 23 months of age. Two dose levels of the refrigerator-stable formulation (8000 PFU/dose [3.9 $\log_{10}$] and 25,000 PFU/dose [4.4 $\log_{10}$]) were compared with one dose level of the frozen formulation (10,000 PFU/dose [4.0 $\log_{10}$]). The doses for the refrigerator-stable formulation of varicella vaccine were selected as representative of the average release-dose range for this formulation. A lot of the frozen formulation of varicella vaccine that contained a similar PFU/dose as the lower dose of the refrigerator formulation of varicella vaccine was selected as a comparator.

2. Methods

2.1. Study design and population

This double-blind, randomized, controlled, multi-center clinical trial operating under in-house (Sponsor) blinding procedures evaluated the safety, tolerability, and immunogenicity of two dosage levels of the refrigerator-stable formulation and one dosage level of the frozen formulation when administered concomitantly with MMR. The study was conducted at 19 centers in the United States between September 1999 and October 2000. The ethical review committee of each site approved the protocol, which was conducted in conformance with applicable requirements.

Healthy children, 12–23 months of age, with no previous clinical history of measles, mumps, rubella, varicella, or zoster were eligible for the study. Subjects were excluded for any of the following reasons: previous vaccination with any type of varicella, measles, mumps, or rubella vaccine; a history of anaphylactoid reactions; hypersensitivity to gelatin, neomycin, or any component of the vaccines; any immune impairment; any neoplastic disease; a recent (<72 h) febrile illness (≥101°F [38.3 °C] oral); or receipt of an inactivated vaccine (within 14 days) or live virus vaccine (within 30 days) prior to enrollment.

Subjects were randomized 1:1:1 to receive a 0.5-mL injection of 1 of the 3 formulations of varicella vaccine (refrigerator-stable 8000 PFU [Group 1], refrigerator-stable 25,000 PFU [Group 2] or frozen 10,000 PFU [Group 3]). Each dose was reconstituted with sterile diluent prior to administration. All doses of varicella vaccine were given concomitantly with a 0.5-mL injection of MMR at separate injection sites in different arms. The 3 treatment groups are henceforth referred to as Groups 1, 2, and 3, respectively.

Subjects, parents/legal guardians, study personnel administering the study vaccines and handling all clinical and serology follow-up, and Sponsor personnel performing the laboratory testing were blinded to group assignment for each subject. Separate study personnel reconstituted, withdrew, and verified the study vaccine dose and were not blinded to the group assignment. All subjects were followed for 42 days postvaccination and had serum samples obtained for serologic analysis prior to vaccination and at 6 weeks postvaccination.

All serum samples collected were tested for levels of anti-VZV immunoglobulin G (IgG) using a glycoprotein enzyme-linked immunosorbent assay (gpELISA) [3,4,5]. All samples were also tested for measles, mumps, and rubella IgG antibodies using an enzyme-linked immunosorbent assay (ELISA) [6,7].

2.2. Study objectives

The primary objectives of this study in subjects 12 to 23 months of age were to determine: (1) the safety and tolerability of the refrigerator-stable formulation of varicella vaccine (Groups 1 and 2) when administered concomitantly with MMR at separate injection sites; and (2) if the refrigerator-stable formulation (Group 1) would yield a similar immune response (defined as the percent of subjects with VZV-specific antibody titer ≥5 gpELISA units) as the frozen formulation (Group 3).

Secondary objectives of the study were to: (1) determine if Group 2 would yield a similar varicella immune response as Group 3 at 6 weeks postvaccination; (2) determine if Group 1 would yield a similar varicella immune response as Group 2; (3) determine if Group 1 would yield a similar varicella GMT as Group 3; (4) summarize the seroconversion rates (SCRs) and GMTs for varicella, measles, mumps, and rubella at 6 weeks postvaccination for Groups 1, 2, and 3; (5) summarize the percent of subjects with VZV-specific antibody titer ≥10 and ≥20 gpELISA units in Groups 1, 2 and 3 at 6 weeks postvaccination; and (6) assess the safety profiles in terms of varicella-like rash, measles-like rash, rubella-like rash, mumps-like symptoms, fever (≥102°F [38.9 °C] oral or equivalent) within 6 weeks postvaccination, and injection-site adverse experiences (AEs) in Groups 1, 2, and 3 within Days 0 to 4 postvaccination.

2.3. Vaccine description

MMR was administered under open label conditions from clinical material sent to each study center. All varicella vaccine was administered under blinded conditions. The subject, investigator, study staff, and Sponsor did not have access to the randomization code while the study was being conducted. Separate study personnel reconstituted, withdrew, and verified the study vaccine dose and were not blinded to the subject group assignment.

All clinical materials were supplied in 0.7-mL single-dose vials. All vaccines were lyophilized. Study personnel were asked to monitor storage temperatures of the clinical materials and to inform the Sponsor if temperature excursions outside of the recommended storage limits occurred.

2.4. Immunogenicity measurements and endpoints

The primary endpoint for the evaluation of the immune response was the percentage of subjects with a VZV-specific antibody titer ≥5 gpELISA units at 6 weeks postvaccination. The protocol originally planned to include all subjects with a prevaccination varicella antibody titer of <5 gpELISA units in the primary analysis; however, after discussions with the United States regulatory authority (CBER) the primary analysis population was changed to include only those subjects with a prevaccination VZV-specific antibody titer of <1.25 gpELISA units.

The secondary endpoints for the evaluation of the immune response were SCRs, GMTs, and the percent of subjects with VZV-specific antibody titers ≥10 gpELISA units and ≥20 gpELISA units at 6 weeks postvaccination. A subject who was seronegative (VZV-specific varicella antibody titer generally <0.6 gpELISA units) at baseline and seropositive (≥5 gpELISA units) postvaccination was considered to have seroconverted.

2.5. Safety measurements and endpoints

The primary safety endpoint was the incidence of vaccine-related serious AEs reported Days 0 to 42 postvaccination in subjects receiving either refrigerator-stable formulation.

The secondary safety endpoints were the incidence of injection-site AEs (Days 0–4), temperatures (≥102°F [≥38.9 °C], oral equivalent), generalized (noninjection-site) varicella-like, measles-like, and rubella-like rashes, and mumps-like symptoms as well as other local and systemic AEs reported Days 0 to 42 postvaccination as per the subject Vaccination Report Card (VRC).

2.6. Statistical methods

2.6.1. Immunogenicity

For the primary analyses, with 300 subjects enrolled in each treatment group, approximately 270 evaluable subjects per group (90% evaluability) were expected to be included in the primary immunogenicity analysis. Assuming the expected response rates between Group 1 and Group 3 were identical (90%), the study had 96% power to rule out a 10-percentage-point difference between the 2 treatment groups. The power calculations were based on testing a null hypothesis of non-zero difference at a level of 0.025 (one-sided) using the method described by Farrington and Manning [8].

2.6.2. Safety

For safety comparisons, all subjects were expected to be evaluable. No power statements were made for the safety hypothesis, since comparisons were limited to examining trends between the treatment groups.

The probability of observing at least one serious AE in this study depended on the number of subjects enrolled and the incidence rate of serious AEs in the general population. For Groups 1 and 2, there were 50% and 80% probabilities of observing at least one serious AE if the true underlying incidence rates were 0.23% (1 in 433 subjects) and 0.54% (1 in 187 subjects), respectively. If no serious AEs were observed in 300 subjects, this study provided 97.5% confidence that the true rate was <1.3% for either refrigerator-stable formulation.

3. Results

3.1. Participant accounting and demographics

A total of 959 subjects were randomized into the study with 320 subjects vaccinated in Group 1, 315 subjects vaccinated in Group 2, and 323 subjects vaccinated in Group 3 (Fig. 1). One

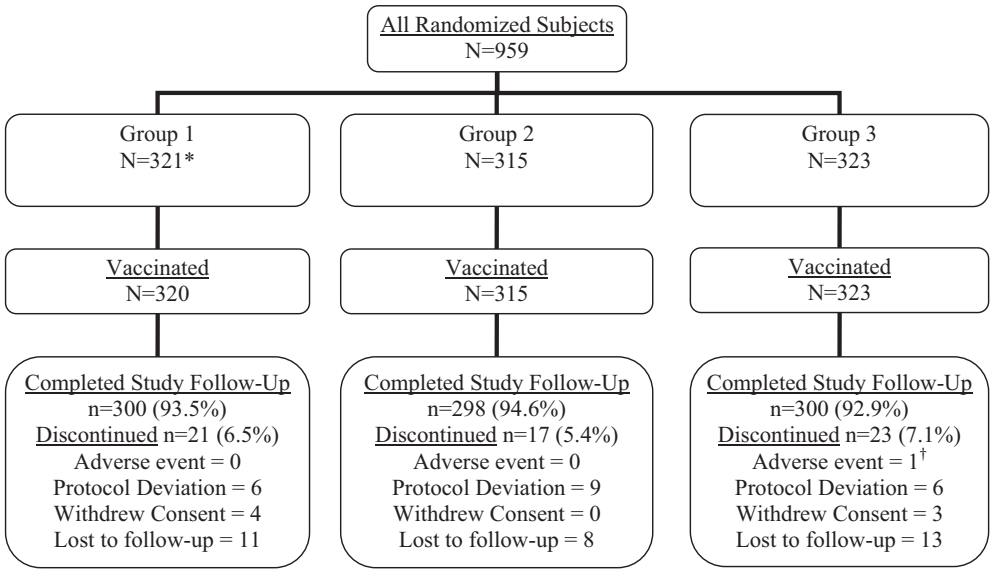

Fig. 1. Subject disposition.

subject was randomized to Group 1 but not vaccinated. Subjects in all 3 vaccination groups were generally comparable with respect to age, race, and initial serostatus, with slightly more males enrolled (Table 1). The proportion of subjects with underlying medical conditions and/or prior or concomitant therapies were comparable between vaccination groups (data not shown).

3.2. Immunogenicity

The immunogenicity analyses on the primary analysis population (per-protocol subjects with prevaccination VZV-specific antibody titers <1.25 gpELISA units) are shown in Table 2. The varicella responses were similar in each of the treatment groups

Table 1
Summary of subject characteristics by group.

	Group 1* N = 320		Group 2 N = 315		Group 3 N = 323	
	n	(%)	n	(%)	n	(%)
Gender						
Male	169	(52.8)	173	(54.9)	174	(53.9)
Female	151	(47.2)	142	(45.1)	149	(46.1)
Age (Months)						
Mean (SD)	12.8 (1.6)		12.9 (1.8)		12.8 (1.7)	
Range	12 to 23		12 to 22		11 to 22	
Race/ethnicity						
Caucasian	178	(55.6)	173	(54.9)	179	(55.4)
Black	68	(21.3)	61	(19.4)	75	(23.2)
Hispanic	61	(19.1)	64	(20.3)	58	(18.0)
Other†	13	(4.1)	17	(5.4)	11	(3.4)
Varicella Initial serostatus						
Negative‡	241	(75.3%)	257	(81.6%)	249	(77.1%)
Positive‡, <1.25 gpELISA units	51	(15.9%)	38	(12.1%)	46	(14.2%)
≥1.25 and <5 gpELISA units	22	(6.9%)	16	(5.1%)	23	(7.1%)
≥5 gpELISA units	2	(0.6%)	2	(0.6%)	2	(0.6%)
Unknown	4	(1.3%)	2	(0.6%)	3	(0.9%)
Measles Initial Serostatus						
Negative‡	307	(95.9%)	305	(96.8%)	312	(96.6%)
Positive‡	9	(2.8%)	8	(2.5%)	8	(2.5%)
Unknown	4	(1.3%)	2	(0.6%)	3	(0.9%)
Mumps Initial Serostatus						
Negative‡	305	(95.3%)	305	(96.8%)	307	(95.0%)
Positive‡	11	(3.4%)	8	(2.5%)	13	(4.0%)
Unknown	4	(1.3%)	2	(0.6%)	3	(0.9%)
Rubella Initial Serostatus						
Negative‡	316	(98.8%)	312	(99.0%)	317	(98.1%)
Positive‡	0	(0.0%)	1	(0.3%)	3	(0.9%)
Unknown	4	(1.3%)	2	(0.6%)	3	(0.9%)

gpELISA = Glycoprotein enzyme-linked immunosorbent assay.
N = Number of subjects vaccinated in each treatment group.
n = Number of subjects in each category.
Group 1 – Refrigerator-stable varicella vaccine (8000 PFU) + MMR.
Group 2 – Refrigerator-stable varicella vaccine (25,000 PFU) + MMR.
Group 3 – Frozen varicella vaccine (10,000 PFU) + MMR.
* One subject was randomized but was not vaccinated.
† Other category includes Asian/Pacific, Black/Caucasian, Caucasian/Asian/Pacific, Indian, Mixed Race, Native American, and Other.
‡ As defined by the optical density cutoff for each assay. For varicella, this cutoff generally corresponds to 0.6 gpELISA units.

Table 2
Statistical analysis of varicella antibody responses at 6 weeks postvaccination for subjects with prevaccination varicella antibody titers <1.25 gpELISA units.

Immunogenicity Endpoint - 6 weeks postvaccination	Comparison	Group 1		Group 2		Group 3		Estimated difference/fold difference (95% CI)*
		n	Estimated responses*	n	Estimated responses*	n	Estimated responses*	
% of subjects with VZV-specific antibody titers ≥5 gpELISA units	Group 1 vs 3	268	93.2%	N/A		267	95.4%	−2.2 (−6.3, 1.9)†
	Group 2 vs 3	N/A		276	93.8%	267	95.4%	−1.6 (−5.5, 2.4)†
	Group 1 vs 2	268	93.2%	276	93.8%	N/A		−0.6 (−5.0, 3.7)†
VZV-specific GMTs	Group 1 vs 3	268	14.6	N/A		267	15.3	1.0 (0.8, 1.1)†
	Group 2 vs 3	N/A		276	16.0	267	15.3	1.0 (0.9, 1.2)‡
	Group 1 vs 2	268	14.6	276	16.0	N/A		0.9 (0.8, 1.0)

n = Numbers of subjects with prevaccination VZV-specific antibody titers <1.25 gpELISA units contributing to the per-protocol analysis.
CI = Confidence interval.
N/A = Not applicable.
Group 1 – Refrigerator-stable varicella vaccine (8000 PFU) + MMR.
Group 2 – Refrigerator-stable varicella vaccine (25,000 PFU) + MMR.
Group 3 – Frozen varicella vaccine (10,000 PFU) + MMR.
* Estimated responses, estimated differences, and CIs were computed from a statistical analysis model adjusting for study center.
† Because the lower bound of the 95% CI on the difference/or the fold difference excludes a 10-percentage-point decrease/or 1.5-fold difference or more, the two groups compared are similar with respect to the respective immunogenicity endpoint.
‡ No hypothesis test was planned.

and the primary and secondary immunogenicity hypotheses were satisfied. The immune responses for Groups 1, 2, and 3 were 93.2%, 93.8% and 95.4% and the GMTs were 14.6, 16.0 and 15.3 gpELISA units, respectively. The immunogenicity results were generally consistent whether the analyses were performed on subjects with prevaccination VZV-specific antibody titers <1.25 gpELISA units or on subjects with prevaccination VZV-specific antibody titers <5 gpELISA units (data not shown). While no statistical hypothesis testing was performed on the 6-week postvaccination SCRs or GMTs to measles, mumps, or rubella for initially seronegative subjects, the immune responses appeared generally similar across the 3 treatment groups (Table 3). The distribution of varicella antibody immune responses were similar across the 3 treatment groups when measuring percentage of subjects at ≥ 5, ≥10, and ≥ 20 gpELISA units at 6 weeks postvaccination (Table 4).

3.3. Safety

The proportion of subjects with injection-site AEs/vaccine-related injection-site AEs in Groups 1, 2, and 3 were 44.0%, 39.4%, and 43.1%, respectively (Table 5). The corresponding 95% CIs included zero suggesting no statistically significant differences. Although the injection-site AE and vaccine-related injection-site AE rates were higher than those that had been historically reported, they appeared to be associated with items that had been prompted for on a newly introduced VRC such as pain/tenderness/-soreness, redness, rash, and swelling [9]. The majority of injection-site AEs were reported as mild with no severe episodes (defined as extremely distressed or unable to do usual activities) reported.

The incidence of systemic reactions was comparable across the 3 groups. The incidence rates of varicella-like rash for Groups 1, 2, and 3 were 0.6%, 1.3%, and 0.6%, and the rates of measles/rubella-like rash were 0.6%, 1.3%, and 1.9%, respectively. Comparison of the varicella-like and measles/rubella-like rash rates between Groups 1 and 3 (p = 0.995 and p = 0.158, respectively), and between Groups 2 and 3 (p = 0.410 and p = 0.527, respectively) indicated no statistically significant differences in rash rates between groups. There were no zoster, zoster-like, measles, or rubella rashes or mumps-like symptoms reported in any of the 3 treatment groups.

There was one laboratory-confirmed case of non-injection-site varicella rash in a subject in Group 1. The child was reported to have 96 lesions 4 days postvaccination following a household exposure (exact date unknown) to a non-vaccine recipient. A sample for polymerase chain reaction (PCR) testing was obtained and confirmed the virus to be wild-type varicella virus. Another subject in Group 3 reported a varicella rash with 15 lesions 19 days postvaccination. The sample obtained for PCR was inadequate for virus strain evaluation.

The percentages of subjects with elevated temperature (≥102°F [≥38.9 °C] oral equivalent or abnormal) within 6 weeks postvaccination for Groups 1, 2, and 3 were 27.0%, 29.0%, and 29.2%, respectively. The test of risk differences between Groups 1 and 3 (p = 0.537) and Groups 2 and 3 (p = 0.950) indicated no statistically significant risk differences.

Six subjects reported non-vaccine-related serious AEs (including one or more of the following: bronchiolitis, dehydration, esophageal tear, gastroenteritis, hypokalemia, otitis media, rotavirus infection, respiratory syncytial virus infection, pharyngitis, and pneumonia). No subjects reported vaccine-related serious AEs, satisfying the primary safety hypothesis concerning the incidence of serious adverse experiences related to the refrigerator-stable formulation. No enrolled subjects died during the study period.

4. Discussion

This clinical trial demonstrated that the refrigerator-stable formulation of varicella vaccine (8000 PFU and 25,000 PFU) was highly immunogenic and generally well tolerated in children 12 to 23 months of age. Varicella immune responses, as defined by the percentage of subjects with varicella antibody titers ≥5 gpELISA units at 6 weeks postvaccination, were similar between the refrigerator-stable formulation and the frozen formulation. The GMT for the refrigerator-stable formulation containing 8000 PFU was similar to that of the frozen formulation. Although not a formal hypothesis, the GMT for the refrigerator-stable formulation containing 25,000 PFU appeared to be similar to that of the frozen formulation. The seroconversion rates and GMTs for measles, mumps, and rubella were comparable regardless of whether MMR was administered with either the refrigerator-stable or the frozen formulation of varicella vaccine. The similar immune responses between the frozen and refrigerator-stable formulations suggest that the effectiveness of the two formulations should be comparable in clinical practice.

There were no reports of vaccine-related serious AEs in any of the 3 treatment groups, satisfying the primary safety hypothesis concerning the incidence of serious adverse events in the refrigerator-stable formulations. Based on the sample size evaluated, this study provides 97.5% confidence that the true rate of serious adverse events when either refrigerator-stable formulation of varicella vaccine was administered concomitantly with MMR was <1.3%.

Immunogenicity bridging studies such as the current study are widely accepted by regulatory agencies to assess changes in formulations and have been used to support similar changes for other vaccines. The results of this study with the monovalent varicella vaccine are similar to the results of another study in which the refrigerator-stable formulation of measles, mumps, rubella and varicella (MMRV) vaccine was found to have a safety and immunogenicity profile similar to the earlier frozen formulation of MMRV [10]. All vaccines were developed by the same manufacturer and contained the same Oka/Merck varicella strain.

Table 3
Summary of seroconversion rates (SCR) and geometric mean titers (GMT) to measles, mumps, and rubella at 6 weeks postvaccination for initially seronegative subjects (per-protocol analysis).

Antibody (ELISA)	Group 1 N = 320				Group 2 N = 315				Group 3 N = 323			
	n	SCR	(95% CI)	GMT	n	SCR	(95% CI)	GMT	n	SCR	(95% CI)	GMT
Measles	283	98.9%	(96.9, 99.8)	195.6	284	99.3%	(97.5, 99.9)	195.4	281	97.9%	(95.4, 99.2)	194.5
Mumps	282	100.0%	(98.7, 100)	75.8	284	98.9%	(96.9, 99.8)	66.4	279	100.0%	(98.7, 100)	72.0
Rubella	291	100.0%	(98.7, 100)	161.3	291	99.7%	(98.1, 100)	160.4	288	100.0%	(98.7, 100)	167.9

Cutoffs for seroconversion: Measles (≥21.3 measles Ab units/mL), Mumps (≥2 mumps Ab units/mL), and Rubella (≥10 rubella Ab units/mL).
CI = Confidence interval.
Group 1 – Refrigerator-stable varicella vaccine (8000 PFU) + MMR.
Group 2 – Refrigerator-stable varicella vaccine (25,000 PFU) + MMR.
Group 3 – Frozen varicella vaccine (10,000 PFU) + MMR.

Table 4
Summary of varicella antibody responses at 6 weeks postvaccination (per-protocol analysis).

Population	Parameter	Group 1 (N = 320*)			Group 2 (N = 315)			Group 3 (N = 323)		
		n	Observed response	95% CI	n	Observed response	95% CI	n	Observed response	95% CI
Subjects with baseline Varicella antibody Titers <1.25 gpELISA Units	% ≥5 gpELISA units	268	93.3% (250/268)	(89.6%, 96.0%)	276	93.8% (259/276)	(90.3%, 96.4%)	267	95.1% (254/267)	(91.8%, 97.4%)
	% ≥10 gpELISA units	268	72.4% (194/268)	(66.6%, 77.7%)	276	72.5% (200/276)	(66.8%, 77.6%)	267	71.2% (190/267)	(65.3%, 76.5%)
	% ≥20 gpELISA units	268	22.4% (60/268)	(17.5%, 27.9%)	276	26.1% (72/276)	(21.0%, 31.7%)	267	21.7% (58/267)	(16.9%, 27.2%)
	GMT	268	14.7	(13.3, 16.2)	276	16.1	(14.5, 17.9)	267	15.2	(13.8, 16.7)
Subjects with baseline Varicella antibody Titers <5 gpELISA Units	% ≥5 gpELISA units	289	93.1% (269/289)	(89.5%, 95.7%)	290	93.8% (272/290)	(90.4%, 96.3%)	288	94.8% (273/288)	(91.6%, 97.1%)
	% ≥10 gpELISA units	289	70.2% (203/289)	(64.6%, 75.5%)	290	72.4% (210/290)	(66.9%, 77.5%)	288	71.9% (207/288)	(66.3%, 77.0%)
	% ≥20 gpELISA units	289	21.1% (61/289)	(16.5%, 26.3%)	290	25.9% (75/290)	(20.9%, 31.3%)	288	21.9% (63/288)	(17.2%, 27.1%)
	GMT	289	14.3	(13.0, 15.7)	290	16.0	(14.5, 17.7)	288	15.1	(13.8, 16.6)
Subjects who were Seronegative to Varicella at baseline	Seroconversion Rate	221	99.1% (219/221)	(96.8%, 99.9%)	238	99.6% (237/238)	(97.7%, 100%)	225	100% (225/225)	(98.4%, 100%)
	%≥5 gpELISA units	221	92.8% (205/221)	(88.5%, 95.8%)	238	92.9% (221/238)	(88.8%, 95.8%)	225	95.1% (214/225)	(91.4%, 97.5%)
	%≥10 gpELISA units	221	70.6% (156/221)	(64.1%, 76.5%)	238	72.3% (172/238)	(66.1%, 77.9%)	225	72.0% (162/225)	(65.6%, 77.8%)
	%≥20 gpELISA units	221	21.7% (48/221)	(16.5%, 27.7%)	238	26.1% (62/238)	(20.6%, 32.1%)	225	23.6% (53/225)	(18.2%, 29.7%)
	GMT	221	14.2	(12.7, 15.8)	238	15.7	(14.0, 17.5)	225	15.6	(14.0, 17.4)

N = Number of subjects vaccinated in each treatment group.
n = Number of per-protocol subjects in the respective population.
gpELISA = Glycoprotein enzyme-linked immunosorbent assay.
CI = Confidence interval.
GMT = Geometric mean titer.
Percentages are calculated based on the number of subjects with follow-up after each visit.
Group 1 – Refrigerator-stable varicella vaccine (8000 PFU) + MMR.
Group 2 – Refrigerator-stable varicella vaccine (25,000 PFU) + MMR.
Group 3 – Frozen varicella vaccine (10,000 PFU) + MMR.
* One subject was randomized to Group 1 treatment group but was not vaccinated. This subject is not counted in this table.

Table 5
Summary of clinical adverse experiences (days 0 to 42 postvaccination).

	Treatment groups					
	Group 1		Group 2		Group 3	
	n	%	n	%	n	%
Number of subjects vaccinated	320*		315		323	
Number of subjects without follow-up	11		5		12	
Number of subjects with follow-up	309	(96.6)	310	(98.4)	311	(96.3)
Number (%) of subjects:						
with no AEs	50	(16.2)	40	(12.9)	48	(15.4)
with one or more AEs	259	(83.8)	270	(87.1)	263	(84.6)
injection-site AEs	136	(44.0)	122	(39.4)	134	(43.1)
systemic AEs	233	(75.4)	256	(82.6)	248	(79.7)
with vaccine-related AEs†	181	(58.6)	172	(55.5)	179	(57.6)
injection-site AEs	136	(44.0)	122	(39.4)	134	(43.1)
systemic AEs	87	(28.2)	97	(31.3)	86	(27.7)
with serious AEs	2	(0.6)	3	(1.0)	1	(0.3)
discontinued study due to an AE	0	(0.0)	0	(0.0)	1‡	(0.3)

n = Number of subjects in each category.
AE = Adverse experience.
No subjects reported a serious vaccine-related AE†, died, or discontinued test vaccine due to an AE.
Percentages are calculated based on the number of subjects with follow-up after each visit.
Group 1 – Refrigerator-stable varicella vaccine (8000 PFU) + MMR.
Group 2 – Refrigerator-stable varicella vaccine (25,000 PFU) + MMR.
Group 3 – Frozen varicella vaccine (10,000 PFU) + MMR.
* One subject was randomized to Group 1 but was not vaccinated. This subject is not counted in this table.
† Determined by the investigator to be possibly, probably, or definitely related to the vaccine.
‡ One subject in Group 3 discontinued the study 49 days postvaccination. The parent/guardian refused to return with the subject for the 42-day follow-up visit. It was reported by the investigator that fever and irritability on Days 1 to 6 postvaccination were the reasons for the refusal to return.

The strengths of this study are the head-to-head nature of the study, adequate sample size to detect small differences, and use of the same laboratory for all assays. The limitations of this study are that most adverse events were based on parental reporting and the sample size did not allow for the detection of rare adverse events.

The results of this study are particularly important as more countries around the world decide to include varicella vaccine in their routine immunization program. Many countries lack sufficient capacity to routinely store vaccines in a freezer. For many developing and remote areas as well as during catastrophes and disasters anywhere in the world, successful implementation of a vaccine program necessitates the use of a product that does not require frozen storage [11–13]. Compatibility with a refrigerated cold chain (2–8 °C), as is done with the refrigerated formulation of this varicella vaccine, allows for increased feasibility in a much larger part of the world [13].

5. Conclusions

This study demonstrated that the safety, tolerability and immunogenicity profile of a refrigerator-stable formulation of varicella vaccine was similar to the frozen formulation. Use of a refrigerator-stable formulation of varicella vaccine will allow for increased availability of the product throughout the world and may help to increase vaccination rates against varicella.

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References

[1] Paul William E. Fundamental Immunology. 7th ed. Philadelphia: Lippincott Williams & Wilkins, a Wolters Kluwer business; 2013.
[2] Merck internal data.
[3] Keller PM, Lonergan K, Neff BJ, Morton DA, Ellis RW. Purification of individual varicella-zoster virus (VZV) glycoproteins gpI, gpII, and gpIII and their use in ELISA for detection of VZV glycoprotein-specific antibodies. J Virol Methods 1986;14:177–88.
[4] Wasmuth EH, Miller WJ. Sensitive enzyme-linked immunosorbent assay for antibody to varicella-zoster virus using purified VZV glycoprotein antigen. J Med Virol 1990;32:189–93.
[5] Provost PJ, Krah DL, Kuter BJ, Morton DH, Schofield TL, Wasmuth EH, et al. Antibody assays suitable for assessing immune responses to live varicella vaccine. Vaccine 1991;9:111–6.
[6] Shehab ZM, Brunell PA, Cobb E. Epidemiological standardization of a test for susceptibility to mumps. J Infect Dis 1984;149:810–2.
[7] Johnson CE, Kumar ML, Whitwell JK, Staehle BO, Rome LP, Dinakar C, et al. Antibody persistence after primary measles-mumps-rubella vaccine and response to a second dose given at four to six vs. eleven to thirteen years. Pediatr Infect Dis J 1996 Aug;15:687–92.
[8] Farrington CP, Manning G. Test statistics and sample size formulae for comparative binomial trials with null hypothesis of non-zero risk difference or non-unity relative risk. Stat Med 1990;9:1447–54.

[9] VARIVAX package insert. Kenilworth, NJ: Merck & Co., Inc.; 2017. Available at: http://www.merck.com/product/usa/pi_circulars/v/varivax/varivax_pi.pd. [accessed October 19, 2017].
[10] Bernstein HH, Eves K, Campbell K, Black SB, Twiggs JD, Reisinger KS, et al. Comparison of the safety and immunogenicity of a refrigerator-stable versus a frozen formulation of ProQuad (measles, mumps, rubella, and varicella virus vaccine live). Pediatrics 2007;119(6):e1299–305.
[11] Heymann DL, Smith EL, Nakano JH, Jato JG, Martin GE, Maben GK. Further field testing of the more heat-stable measles vaccine in Cameroon. Br Med J (Clin Res Ed). 1982;285:531–53.
[12] Galazka A, Milstien J, Zaffran M. Thermostability of vaccines: global programme for vaccines and immunization (GPV). Geneva, Switzerland: World Health Organization; 1998.
[13] Bernstein HH, Kuter BJ, Schodel F. Refrigerator-stable and frozen formulation of ProQuad: In reply. Pediatrics 2007;120:924.