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Immunogenicity and safety of a live attenuated shingles (herpes zoster) vaccine (Zostavax®) in individuals aged ≥ 70 years

A randomized study of a single dose vs. two different two-dose schedules

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Abbreviations: AE, adverse event; CMI, cell-mediated immunity; EU, European Union; FAS, full analysis set; GMFR, geometric mean-fold rise; GMT, geometric mean titre; gpELISA, enzyme-linked immunosorbent assay using glycoprotein; HZ, herpes zoster; PCR, polymerase chain reaction; PHN, post-herpetic neuralgia; PPS, per protocol set; SPS, Shingles Prevention Study; VZV, varicella zoster virus; ZEST, Zostavax Efficacy and Safety Trial

Disease protection provided by herpes zoster (HZ) vaccination tends to reduce as age increases. This study was designed to ascertain whether a second dose of the HZ vaccine, Zostavax®, would increase varicella zoster virus (VZV)-specific immune response among individuals aged ≥ 70 y. Individuals aged ≥ 70 y were randomized to receive HZ vaccine in one of three schedules: a single dose (0.65 mL), two doses at 0 and 1 mo, or two doses at 0 and 3 mo. VZV antibody titers were measured at baseline, 4 weeks after each vaccine dose, and 12 mo after the last dose. In total, 759 participants (mean age 76.1 y) were randomized to receive vaccination. Antibody responses were similar after a single dose or two doses of HZ vaccine [post-dose 2/post-dose 1 geometric mean titer (GMT) ratios for the 1-mo or 3-mo schedules were 1.11, 95% confidence interval (CI) 1.02–1.22 and 0.78, 95% CI 0.73–0.85], respectively). The 12-mo post-dose 2/12-mo post-dose 1 GMT ratio was similar for the 1-mo schedule and for the 3-mo schedule (1.06, 95% CI 0.96–1.17 and 1.08, 95% CI 0.98–1.19, respectively). Similar immune responses were observed in participants aged 70–79 y and those aged ≥ 80 y. HZ vaccine was generally well tolerated, with no evidence of increased adverse event incidence after the second dose with either schedule. Compared with a single-dose regimen, two-dose vaccination did not increase VZV antibody responses among individuals aged ≥ 70 y. Antibody persistence after 12 mo was similar with all three schedules.

Introduction

Age is a major risk factor for herpes zoster (HZ), also known as shingles.[1-3] The most frequent and debilitating complication of HZ is post-herpetic neuralgia (PHN), a neuropathic pain syndrome that can persist for months, years, or even decades after the HZ rash has gone.[3-8] HZ, and particularly PHN, can have a devastating impact on an individual's quality of life.[9-12]

Increasing age is associated with immunosenescence, the natural decline of the innate and adaptive immune systems.[13] As a consequence of declining varicella zoster virus (VZV)-specific cell-mediated immunity (CMI), the elderly are more susceptible to HZ than younger individuals.[14] The severity and the risk of both HZ and PHN increase with age.[4,15,16] Thus, more than two-thirds of HZ cases occur in individuals aged > 50 y,[17] and 20–50% of adults with HZ aged ≥ 50 y develop PHN.[18,19] As the population ages, the number of cases of HZ and PHN is expected to rise.[20,21]

Zostavax® (Sanofi Pasteur MSD) is a live attenuated VZV vaccine developed specifically for the prevention of HZ and PHN in individuals aged ≥ 50 y.[22] It has been shown to boost VZV-specific CMI.[23-28] The efficacy of HZ vaccine is highest

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Table 1. Definition and description of analysis sets[a]

Set	Definition	Single-dose schedule, n (%)	Two-dose 1-mo schedule, n (%)	Two-dose 3-mo schedule, n (%)	All, n (%)
Randomized set		253	255	251	759
Full analysis set (FAS)	Randomized participants who received at least one dose of study vaccine and with any post-vaccination immunological evaluation	251 (99.2)	242 (94.9)	246 (98.0)	739 (97.4)
Per protocol set (PPS)	Participants without protocol deviations that may interfere with immune responses	243 (96.0)	203 (79.6)	198 (78.9)	644 (84.8)
12-mo per protocol set (12-mo PPS)	As for PPS, except for the 12-mo follow-up analysis	223 (88.1)	189 (74.1)	204 (81.3)	616 (81.2)
Post-dose 1 safety set[b]	Participants who received the first injection and who had safety follow-up data	252 (99.6)	249 (97.6)	248 (98.8)	749 (98.7)
Post-dose 2 safety set[b]	Participants who received the second injection and who had safety follow-up data		233 (91.4)	220 (87.6)	453 (59.7)

[a]Percentages are calculated based on the number of randomized participants. [b]For safety analyses, one patient randomized to the 1-mo schedule was analyzed as receiving the 3-mo schedule (i.e., as actually vaccinated).

among individuals aged 50–59 y and declines with increasing age. In the Zostavax Efficacy and Safety Trial (ZEST), subjects aged 50–59 y received a single dose of either HZ vaccine (n = 11,184) or placebo (n = 11,212). The vaccine significantly reduced the risk of developing HZ by 69.8% [95% confidence interval (CI) 54.1–80.6].[29] In the large-scale Shingles Prevention Study (SPS), vaccine efficacy (prevention of HZ incidence) was 63.9% (95% CI 55.5–70.9) in individuals aged 60–69 y, and 37.6% (95% CI 25.0–48.1) in those aged ≥ 70 y.[30,31] Reduced vaccine efficacy among individuals aged ≥ 70 y raises the question of whether a second dose given after either a shorter or longer interval might improve response to the vaccine among the elderly.

VZV-specific immune response to HZ vaccine has been shown to correlate with protection against HZ.[32] In a randomized, placebo-controlled study, two doses of Zostavax were given 6 weeks apart to individuals aged ≥ 60 y. VZV-specific CMI response, measured 6 weeks post-dose, was similar for both doses.[33] The second dose was generally well tolerated but did not boost VZV-specific immunity beyond levels achieved after dose 1. Therefore, there was no apparent immunological advantage of administering a second dose of HZ vaccine 6 weeks after an initial dose.

The current study was undertaken to evaluate whether VZV-specific immune response to HZ vaccine among elderly individuals (aged ≥ 70 y) is higher after a second dose than after the first dose, when the vaccine is administered according to a 0, 1-mo or 0, 3-mo schedule. The antibody persistence after receiving a one- or two-dose schedule was planned to be explored at 12 mo and, optionally, at 24- and 36 mo.

Results

Study population. Of the 779 individuals screened, 759 entered the study (randomization set), and 757 (99.7%) of those enrolled received at least one dose of HZ vaccine. Subsets of the randomization set were defined for the analysis of the data (**Table 1**).

Of the participants enrolled in the study, 509 (67.2%) were aged 70–79 y and 248 (32.8%) were aged ≥ 80 y; 421 participants (55.5%) were female.

Analysis of participants' medical histories indicated that 61.9% had vascular disorders (e.g., hypertension), 42.0% had metabolic and nutritional disorders (e.g., hypercholesterolemia), and 27.3% had cardiovascular disorders (e.g., coronary artery disease).

Immunogenicity. *Four weeks post-dose.* In the per protocol set (PPS), VZV antibody geometric mean titers (GMTs) at baseline and after the first vaccine dose were similar in the three study groups (**Table 2**).

The GMT 4 weeks after the second dose was higher in those receiving the 1-mo schedule [555.3 enzyme-linked immunosorbent assay using glycoprotein (gpELISA) units/mL] compared with those receiving the 3-mo schedule (410.5 gpELISA units/mL) with no overlap of the 95% CIs (**Table 2**). The second dose of HZ vaccine did not elicit superior VZV antibody responses compared with the first dose, with either the 1-mo or the 3-mo schedule. The lower bound of the 95% CI (including the Hochberg adjustment[34] to control the overall type 1 error) for the post-dose 2/post-dose 1 GMT ratio was < 1.2 for both two-dose schedules (post-dose 2/post-dose 1 GMT ratio: 1.11 and 0.78 for the 1- and the 3-mo schedule, respectively; **Table 2**). In addition, for the 3-mo schedule, GMT after the second dose was numerically lower than after the first dose, and the post-dose 2/post-dose 1 GMT ratio (together with the entirety of its 95% CI) was below 1.

Similar immune responses were observed in participants aged 70–79 y and those aged ≥ 80 y. Post-dose 2/post-dose 1 GMT ratios for the two age groups were 1.11 and 1.12 for the 1-mo schedule, and 0.79 and 0.77 for the 3-mo schedule, respectively. Similar results were also obtained from the full analysis set (FAS) analysis (data not shown).

Twelve months after the last dose (antibody persistence). The 12-mo post-dose 2/12-mo post-dose 1 GMT ratio was similar for

Table 2. Immunogenicity data at 4 weeks post-dose for participants receiving a single dose of HZ vaccine and those receiving one of two two-dose schedules [second dose 1 mo after the first (1-mo schedule), or 3 mo after (3-mo schedule)], measured using gpELISA, per protocol set

	Single-dose schedule (n = 243)	Two-dose 1-mo schedule (n = 203)	Two-dose 3-mo schedule (n = 198)
GMT pre-dose 1 (95% CI)	**233.7** (207.1–263.7)	**210.2** (185.7–238.0)	**228.4** (199.2–261.8)
GMT post-dose 1 [GMT1] (95% CI)	**550.0** (489.2–618.4)	**498.8** (438.9–566.9)	**523.3** (458.7–597.1)
GMFR pre-dose 1 to post-dose 1 (95% CI)	**2.35** (2.11–2.62)	**2.37** (2.11–2.66)	**2.29** (2.05–2.57)
GMT post-dose 2 [GMT2] (95% CI)		**555.3** (496.8–620.7)	**410.5** (363.3–463.9)
GMFR pre-dose 1 to post-dose 2 (95% CI)		**2.64** (2.37–2.95)	**1.80** (1.63–1.98)
GMT ratio [GMT2/GMT1] (95% CI)		**1.11** (1.02–1.22)	**0.78** (0.73–0.85)
Superiority[a]			**No**
(97.5% CI)		(1.00–1.24)	
Superiority[b]		**No**	
p value[c]		0.948	> 0.999

CI, confidence interval; GMFR, geometric mean-fold rise in VZV antibody titers; GMT, geometric mean titer for varicella zoster virus (VZV) antibodies in gpELISA units/mL; gpELISA, enzyme-linked immunosorbent assay using glycoprotein. [a]Hochberg adjustment procedure, first step: superiority is achieved if the lower bound of the two-sided 95% CI of the GMT ratio is > 1.2. [b]Hochberg adjustment procedure, second step: superiority is achieved if the lower bound of the two-sided 97.5% CI of the GMT ratio is > 1.2. [c]One-sided p value for testing superiority (GMT2/GMT1 > 1.2) to be compared with 0.025 in the first step of the Hochberg adjustment procedure or 0.0125 in the second step of the Hochberg adjustment procedure.

the 1-mo schedule as for the 3-mo schedule (1.06, 95% CI 0.96–1.17 and 1.08, 95% CI 0.98–1.19, respectively; 12-mo PPS), when adjusted for baseline values (data not shown). As the 95% CIs for these between-group ratios included 1, no significant differences were shown between the single-dose schedule and either of the two-dose schedules. The 12-mo GMT was 256.3 gpELISA units/mL for the single-dose schedule and the 12-mo post-dose 2 GMT was 251.1 and 265.2 gpELISA units/mL for the 1-mo and the 3-mo schedule, respectively (12-mo PPS; **Table 3**). Similar 12-mo data were observed in the FAS (data not shown). These results led to the study being stopped after the 12-mo analysis. Therefore, participants' follow-up was stopped and the 24- and 36-mo time points were not collected.

Safety. Overall, 57.8% of participants (n = 433) reported at least one adverse event (AE) within 28 d of their first dose of HZ vaccine, and 47.1% reported vaccine-related AEs (**Table 4**). A slightly lower proportion of participants reported AEs after the second dose: 53.0% with the 1-mo schedule (considered by the investigator to be vaccine-related: 43.1%), and 48.4% with the 3-mo schedule (considered by the investigator to be vaccine-related: 43.0%).

Seventeen participants withdrew from the study due to adverse events, of whom ten withdrew within 28 d after vaccination (**Table 4**). Ten of the withdrawals were due to serious AEs unrelated to vaccination, and seven were related to non-serious vaccine-related AEs.

Injection-site reactions. The overall incidence of injection-site reactions following the first dose of HZ vaccine was 45.5% (**Table 4**). A similar proportion was reported after the second dose. Almost all injection-site reactions were solicited (i.e., erythema, pain, or swelling). They were generally mild-to-moderate in intensity and resolved in 3–7 d. Injection site erythema, swelling and pain of severe intensity (diameter ≥ 10 cm or incapacitating, with inability to perform usual activities) were reported by 1.2%, 0.9%, and 0.7% of all participants, respectively.

Systemic AEs. Systemic AEs occurred within 28 d of the first HZ vaccination in 28.0% of recipients. Vaccine-related systemic AEs affected 6.4% of participants after the first dose. The most common vaccine-related systemic AE was headache, reported by 2.3% of participants. The rate of vaccine-related systemic AEs of severe intensity was low [0.9%: headache (n = 3), pain (n = 2), pruritus (n = 1), and rash (n = 1)].

The incidence of systemic AEs after the second dose of HZ vaccine was lower than after the first dose: 20.7% among recipients of the 1-mo regimen, and 15.4% with the 3-mo regimen. As with the first dose, headache was the most common vaccine-related systemic AE, affecting 0.7% of participants. No vaccine-related systemic AEs of severe intensity were reported after the second dose.

Serious AEs and deaths. Nineteen participants reported serious AEs between screening and 12 mo after the last vaccine dose. Two serious AEs were reported by one participant. None of the serious AEs was considered by the investigator to be vaccine-related. Serious AEs occurred within 28 d of the first vaccine dose in 1.2% of participants (n = 9), and within 28 d of the second dose in 0.9% of participants (n = 4). In 7 participants,

Table 3. Immunogenicity data at 12 mo post last dose for participants receiving a single dose of HZ vaccine or two doses on a 1-mo or 3-mo schedule, measured using gpELISA, 12-mo per protocol set

	Single-dose schedule (n = 223)	Two-dose 1-mo schedule (n = 189)	Two-dose 3-mo schedule (n = 204)
GMT pre-dose 1	**241.6**	**217.0**	**227.2**
(95% CI)	(213.6–273.2)	(190.9–246.7)	(197.9–260.9)
GMT 12 mo post last dose	**256.3**	**251.1**	**265.2**
(95% CI)	(229.4–286.4)	(223.5–282.3)	(235.7–298.5)
GMFR pre-dose 1 to post last dose	**1.06**	**1.16**	**1.17**
(95% CI)	(0.99–1.14)	(1.06–1.26)	(1.07–1.27)

CI, confidence interval; GMFR, geometric mean-fold rise in VZV antibody titers; GMT, geometric mean titer for varicella zoster virus (VZV) antibodies in gpELISA units/mL; gpELISA, enzyme-linked immunosorbent assay using glycoprotein.

serious AEs occurred between 28 d and 12 mo after the last dose.

Until the study was stopped, 12 participants died, seven within 12 mo of the last vaccination and five > 12 mo after the last vaccination. Causes of death were underlying cardiovascular disease (n = 5), pulmonary or gastrointestinal infarction disorders (n = 3), dehydration (n = 1), malignant neoplasm (n = 1).

Rashes of interest. Ten participants reported rashes of interest (HZ, zoster-like rash, varicella, varicella-like rash) between screening and the end of the study.

Two participants reported zoster-like rash within 28 d after the first dose of HZ vaccine [polymerase chain reaction (PCR) was negative for both], and two reported varicella-like rash within 28 d after the second dose (one was PCR-negative; no sample was collected for the other) (**Table 4**).

An additional six participants presented with rashes of interest > 28 d after vaccination. Zoster-like rash was reported by three of these participants (two were PCR-negative; no sample collected for the third case); two participants were diagnosed with HZ (one was wild-type VZV PCR-positive; no sample collected for the other); and one participant was hospitalized with severe trigeminal HZ (no sample collected).

Discussion

This study was undertaken to examine whether a second dose of the HZ vaccine Zostavax elicits superior VZV antibody titers compared with one dose, in individuals aged ≥ 70 y. The response to the first dose was not increased by a second dose, administered 4 weeks or 3 mo after the first dose. The result was similar when the analysis was undertaken in those aged 70–79 y and those aged ≥ 80 y.

In another study of HZ vaccine conducted in individuals aged ≥ 60 y, the immune responses to a second dose were similar to those following an initial dose.[33] The geometric mean-fold rise (GMFR) in VZV antibodies measured using gpELISA was lower in that study than in the present study: 1.6 (95% CI 1.4–1.8) after the first dose, and 1.5 (95% CI 1.3–1.7) after the second dose;[33] the difference could be related to the difference in vaccination schedule (0 and 6 weeks) and assessment of immune response 6 weeks after each dose, as opposed to 4 weeks in the current study.

In the present study, VZV antibody titers following the second dose of the 1-mo schedule appeared higher than those after the first dose. The post-dose 2/post-dose 1 GMT ratio and 95% CIs were > 1 for participants receiving this dosing schedule. Conversely, in the 3-mo schedule, the post-dose 2/post-dose 1 GMT ratio and 95% CIs were < 1. These results are consistent with the study performed by Vermeulen et al.[33] where participants had a similar level of antibodies after each dose given 6 weeks apart. These results seem to indicate a lower post-dose 2 response when increasing the interval between doses from 1 to 3 mo. In retrospect, it may have provided further helpful information on the post-dose 2 response, if additional samples were obtained before administration of dose 2 in the 3-mo schedule.

Twelve months after the last vaccine dose, all participants had similar GMTs for VZV antibodies, irrespective of which dosing schedule they received (single dose or either of the two-dose schedules). Similarly, 95% CIs for the 12-mo post-dose 2/12-mo post-dose 1 GMT ratios demonstrated no significant differences between either of the two-dose schedules and the single-dose schedule.

The HZ vaccine was generally well tolerated in this study, whether given as a single-dose or a two-dose schedule. The incidence of AEs was lower after the second dose than after the first dose. From the beginning of the study until cessation, ten rashes of interest were reported, three of which were diagnosed as HZ. No specimen was collected for four of the ten cases including two of those diagnosed as HZ, but wild-type VZV was confirmed as the cause of the third case of HZ. In the SPS, all VZV-positive rashes were due to wild-type VZV rather than the vaccine type.[30,35]

The results of this study demonstrate that there is no apparent advantage to administering a second dose of Zostavax on a 1-mo or 3-mo schedule among individuals aged ≥ 70 y. The safety profile of HZ vaccine in the current study after the first dose was similar to that reported in the SPS and other single-dose studies,[28,30,36] and there was no evidence of an increased risk of AEs in response to a second dose of the vaccine.

Materials and Methods

This study (ClinicalTrials.gov identifier: NCT00561080; EUCTR identifier 2007-000744-28) was undertaken in multiple

Table 4. Adverse events reported up to 28 d following vaccination with HZ vaccine in participants receiving a single dose or one of two two-dose schedules (post-dose 1 safety set, n = 749; post-dose 2 safety set, n = 453), safety set

	Post-dose 1, pooled data from single and two-dose schedules, n (%)	Post-dose 2, two-dose schedules, n (%)	
		1-mo schedule (n = 232)	3-mo schedule (n = 221)
Adverse event[a]	**433 (57.8)**	**123 (53.0)**	**107 (48.4)**
Vaccine related	353 (47.1)	100 (43.1)	95 (43.0)
Injection-site reaction	**341 (45.5)**	**98 (42.2)**	**94 (42.5)**
Solicited injection-site reaction[b]	338 (45.1)	98 (42.2)	93 (42.1)
Erythema	298 (39.8)	90 (38.8)	85 (38.5)
Pain	171 (22.8)	39 (16.8)	44 (19.9)
Swelling	162 (21.6)	54 (23.3)	49 (22.2)
Unsolicited injection-site reaction	28 (3.7)	3 (1.3)	7 (3.2)
Systemic adverse event	**210 (28.0)**	**48 (20.7)**	**34 (15.4)**
Vaccine-related	48 (6.4)	8 (3.4)	6 (2.7)
Rash of interest[c]	**2 (0.3)**	**1 (0.4)**	**1 (0.5)**
Varicella/varicella-like	0	1 (0.4)	1 (0.5)
Herpes zoster/zoster-like	2 (0.3)	0	0
Serious adverse event[d]	**9 (1.2)**	**2 (0.9)**	**2 (0.9)**
Withdrawal due to adverse event	**9 (1.2)**	**1 (0.4)**	**0 (0)**
Vaccine-related	7 (0.9)	0	0
Non-serious vaccine-related	7 (0.9)	0	0

n (%), Number and percentage of participants having presented the corresponding adverse event at least once. [a]Adverse events after the first dose pooled for all three dosing schedules (single dose and both two-dose schedules). [b]From day 0 to day 4. [c]All rashes of interest were non-injection-site rashes. [d]No vaccine-related serious adverse events.

centers across the European Union (EU): Finland, Germany, Italy, Spain, and The Netherlands. It was conducted in accordance with the Declaration of Helsinki and International Conference of Harmonization Good Clinical Practice Guidelines.[37,38] All study participants provided written, informed consent.

Participants. Individuals aged ≥ 70 y with either a history of varicella or > 30 y residency in a country with endemic VZV infection were enrolled. It was planned to recruit individuals aged 70–79 y and those aged ≥ 80 y in a 2:1 ratio to reflect EU demography. Individuals were excluded if they had: a history of HZ, previous varicella or HZ vaccination, exposure to varicella or HZ during the preceding 4 weeks, fever (oral temperature ≥ 38.3°C) during the preceding 72 h, live-virus vaccination during the preceding 4 weeks, and inactivated vaccination during the preceding 2 weeks.

Study design. This was a phase 3, open-label, randomized, comparative study. Individuals were screened up to 7 d before their first vaccination (visit 0). Participants were randomized during visit 1 to receive one of the following three dosing schedules in a 1:1:1 ratio: a single dose of live attenuated HZ vaccine (Zostavax, Sanofi Pasteur MSD, 0.65 mL) on day 0 (visit 1) only; one dose of HZ vaccine on day 0 (visit 1) and a second dose 1 mo later (day 28–35, visit 2; 1-mo schedule); or one dose of HZ vaccine on day 0 (visit 1) and a second dose 3 mo later (day 81–97, visit 4; 3-mo schedule). The allocation schedule was generated using balanced permuted blocks of randomization, with stratification by age (70–79 y and ≥ 80 y) and country. HZ vaccine was administered within 30 min of reconstitution by subcutaneous injection into the deltoid region. Serum samples were to be drawn before the first dose, 4 weeks after each dose received, and at 12, 24, and 36 mo after the last dose. If there was no statistical evidence or clinical trend for superiority of any two-dose schedule over the single-dose schedule at the 12-mo time point, it was planned to stop the study and to cancel visits at 24 and 36 mo after the last dose.

Objectives and assessments. *Immunogenicity.* The primary objective of the study was to demonstrate that a second dose of HZ vaccine, administered 1 mo or 3 mo after the first dose, elicits superior VZV antibody titers 4 weeks after vaccination compared with the first dose. The primary endpoint was the GMT of VZV antibodies 4 weeks post-dose 1 and 4 weeks post-dose 2, for each two-dose schedule.

Secondary objectives of the study were to compare VZV antibody titers 12 mo after completion of each two-dose schedule with those 12 mo after a single dose, and to describe the safety profile of all three HZ vaccination schedules. The secondary immunogenicity endpoint was the GMT of VZV antibodies 12 mo after completion of each vaccination schedule (visit 6).

During the development of Zostavax, VZV-specific immune response was evaluated using several assays. Both the gpELISA and VZV interferon-gamma enzyme-linked immunospot (IFN-γ ELISPOT) post-vaccination responses correlated with protection against HZ.[32] Since the Shingles Prevention Study, the gpELISA assay (Data from Merck and Co., Inc., USA) has been extensively used to assess the immune response to Zostavax and was therefore chosen for this study.

If there was statistical evidence or a clinical trend toward superiority of either two-dose schedule vs. the single dose, in terms of 12-mo VZV antibody response, it was planned to investigate VZV antibody titers 24 and 36 mo after completion of vaccination. VZV antibody titers were measured using a gpELISA assay.[39]

Safety. Participants were monitored by the investigator for at least 20 min following each vaccination for immediate AEs. Solicited injection-site reactions (erythema, swelling, and pain) occurring within 4 d of vaccination were recorded by participants in a diary card. Other injection-site reactions and systemic AEs were recorded in the diary card for up to 28 d following each vaccination. Any oral temperature ≥ 38.3°C was considered as an AE.

Vaccine-related serious AEs, deaths, and occurrences of HZ, varicella, or zoster-like and varicella-like rashes were recorded by the investigators until the study was stopped. Whenever possible, participants were examined within 72 h of rash onset, and lesion samples were collected for laboratory diagnosis and identification of the viral strain using PCR.[40]

Statistical methods. The primary immunogenicity analysis was undertaken in the PPS (excluding subjects with protocol deviations that may interfere with immunogenicity and subjects diagnosed with HZ), with supportive analysis in the FAS. The safety analysis was undertaken in the safety set (**Table 1**).

The hypothesis for the primary objective was that a second dose of HZ vaccine would be superior to the first dose in terms of VZV antibody GMT (gpELISA) at 4 weeks post-vaccination, within at least one of the two-dose schedules. The criterion for superiority was the lower bound of the 95% CI for the ratio of post-dose 2 GMT/post-dose 1 GMT being greater than 1.2 (the smallest ratio expected to be clinically relevant). Superiority was tested using an analysis of variance model on natural log-transformed data with repeated measurement on blood sample (i.e., post-dose 1 and post-dose 2), including country and age at first vaccination as independent variables. To control the overall two-sided type 1 error rate of 5% (one-sided type 1 error rate: 2.5%), the Hochberg adjustment step-up procedure was used to determine the overall study success.[34] For VZV antibody titers 12 mo after completing the vaccination schedule, GMT ratios and two-sided 95% CI were estimated using an analysis of covariance model on natural log-transformed data, including country, age at first vaccination, and dosing schedule as independent variables, and log-transformed baseline data as a covariate.

Sample size. For each dosing schedule, enrollment of 250 participants was anticipated to result in 200 evaluable participants, assuming 20% would be non-evaluable due to withdrawals and protocol deviations. With a true post-dose 2/post-dose 1 GMT ratio of 1.5 and standard deviation for the ratio of 0.93, the resulting power of each individual test to demonstrate that the within-group ratio was > 1.2 would be approximately 92.4% (one-sided 2.5% type 1 error rate: first step of the Hochberg procedure) or about 87.5% (one-sided 1.25% type 1 error rate: second step of the Hochberg procedure).

If the true post-dose 2/post-dose 1 GMT ratio were 1.5 in both groups, the overall power of the study to show superiority of a second dose in at least one of the two-dose schedules would be 98.7%, and the power to show superiority in both two-dose schedules would be 85.4%. If the post-dose 2/post-dose 1 GMT ratio was 1.5 in only one group, the overall power of the study would be 87.5%.

Disclosure of Potential Conflicts of Interest

R.H. has received financial support from Sanofi Pasteur MSD for travel and accommodation costs related to meetings for the study; he has also participated in a Zostavax advisory board in Germany. The institutions of T.V. and H.C.R. received a grant from Sanofi Pasteur MSD for participating in the study; H.C.R.'s institution has also received payment for lectures organized by several pharmaceutical companies and academic institutions. G.I. has previously participated at speaker's bureaus and advisory board meetings sponsored by GSK, Pfizer, Sanofi Pasteur and Sanofi Pasteur MSD and has received research funding as principal investigator from Crucell Berna, GSK, Pfizer, Sanofi Pasteur and Sanofi Pasteur MSD. J.M. has no conflicts of interest to declare. S.T. and C.S. are employees of Sanofi Pasteur MSD. A.F. was an employee of Sanofi Pasteur MSD when the study was performed but has since become an employee of Pfizer, a company which does not have any products relating to herpes zoster.

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Contribution of Authors

R.H., H.C.R., J.M., S.T. and A.F. were involved in the study conception and design. T.V., R.H., H.C.R. and G.I. contributed to patient enrollment for the study. R.H. participated in laboratory data acquisition. T.V., H.C.R., S.T., C.S. and A.F. contributed to the analysis and interpretation of the study data. C.S. and A.F. were Medical Officers of the study. S.T. was the study statistician. All authors critically reviewed the manuscript during its development and approved the final version.

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