Safety and Immunogenicity of Heat-Treated Zoster Vaccine (ZV_{HT}) in Immunocompromised Adults

Kathleen M. Mullane,[1] Drew J. Winston,[2] Michael S. Wertheim,[3] Robert F. Betts,[4] Donald M. Poretz,[5] Luis H. Camacho,[6] Steven A. Pergam,[7] Michael R. Mullane,[8] Jon E. Stek,[9] Tina M. Sterling,[9] Yanli Zhao,[9] Susan B. Manoff,[9] and Paula W. Annunziato[9]

[1]University of Chicago Medical Center, Illinois; [2]University of California Los Angeles Medical Center; [3]Hematology Oncology Associates of the Treasure Coast, Stuart, Florida; [4]University of Rochester Medical Center, New York; [5]Clinical Alliance for Research & Education Infectious Diseases, Annandale, Virginia; [6]Oncology Consultants, Houston, Texas; [7]Fred Hutchinson Cancer Research Center, Seattle, Washington; [8]Stroger Memorial Hospitals, Chicago, Illinois; and [9]Merck & Co., Inc., Whitehouse Station, New Jersey

Background. Safety and immunogenicity of heat-treated zoster vaccine (ZV_{HT}) were assessed in immunocompromised adults.

Methods. In a randomized, double-blind, placebo-controlled, multicenter study, 4 doses ZV_{HT} or placebo were administered approximately 30 days apart to adults with either solid tumor malignancy (STM); hematologic malignancy (HM); human immunodeficiency virus (HIV) with $CD4^+$ ≤200; autologous hematopoietic stem-cell transplant (HCT) or allogeneic-HCT recipients. Varicella-zoster virus (VZV) T-cell responses by interferon-γ enzyme-linked immunospot (IFN-γ ELISPOT) and VZV antibody concentrations by glycoprotein enzyme-linked immunosorbent assay (gpELISA) were measured at baseline and approximately 28 days after each dose.

Results. No safety signals were found in any group. IFN-γ ELISPOT geometric mean fold rises (GMFR) after dose 4 in STM, HM, HIV, and autologous-HCT patients were 3.00 ($P < .0001$), 2.23 ($P = .004$), 1.76 ($P = .026$), and 9.01 ($P = NA$), respectively. Similarly, antibody GMFR were 2.35 ($P < .0001$), 1.28 ($P = .003$), 1.37 ($P = .017$), and 0.90 ($P = NA$), respectively. T-cell and antibody responses were poor after 4 doses of ZV_{HT} in allogeneic-HCT patients.

Conclusion. ZV_{HT} was generally safe and immunogenic through 28 days post-dose 4 in adults with STM, HM, and HIV. Autologous-HCT but not allogeneic-HCT patients had a rise in T-cell response; antibody responses were not increased in either HCT population.

Study identification. V212-002

Clinical Trials Registration. NCT00535236

Keywords. herpes zoster; zoster vaccine; immunocompromised adults.

The incidence and severity of herpes zoster (HZ) are increased in immunocompromised populations with impaired cell-mediated immunity. Patients receiving chemotherapy for a hematologic malignancy (HM) or solid tumor malignancy (STM), undergoing a hematopoietic stem-cell transplant (HCT), or infected with human immunodeficiency virus (HIV) all have higher incidences of HZ than healthy persons [1]. Compared to the general population where the incidence of HZ is 2–5 cases/1000 person-years, the respective incidences of HZ in patients receiving treatment for a STM, given chemotherapy for a HM, infected with HIV, or undergoing HCT are 15–80, 25–100, 20–100, and 200 cases/1000 person-years [2–9]. These immunocompromised populations also experience significant morbidity and occasional mortality from complications associated

Received 18 December 2012; accepted 7 May 2013; electronically published 1 August 2013.

Data from this manuscript were presented in poster format at: Infectious Diseases Society of America 2010, Vancouver, Canada (abstract #3618). American Society of Hematology 2010, Orlando, FL (abstract 1394). American Society for Bone and Marrow Transplantation 2011, Honolulu, HI (abstract 70172). American Society for Clinical Oncology 2011, Chicago, IL (abstract 2551)

Correspondence: Dr Paula Annunziato, Merck & Co., Inc., PO Box 1000, UG3CD-28, North Wales, PA 19454-1099 (paula_annunziato@merck.com).

The Journal of Infectious Diseases 2013;208:1375–85

DOI: 10.1093/infdis/jit344

with reactivation of the varicella-zoster virus (VZV), including post-herpetic neuralgia (PHN), secondary bacterial infections, and disseminated VZV infection.

Currently, a live attenuated zoster vaccine (ZOSTAVAX™; Merck & Co., Inc., Whitehouse Station, NJ) is approved for prevention of HZ in immunocompetent individuals ≥50 years [10]. In large placebo-controlled trials, vaccination with the live attenuated zoster vaccine demonstrated an excellent safety profile and was associated with a reduction of the incidence of HZ by 51% to 70% and a reduction of the incidence of PHN by approximately 66% [11, 12]. Over 11 million doses of zoster vaccine have been administered; the vaccine has a favorable safety profile and rashes testing positive for the vaccine strain by polymerase chain reaction (PCR) occur very rarely. However, the live attenuated zoster vaccine is contraindicated in immunocompromised populations, which leave this high-risk population with no means of enhancing immunity against this virus.

Initial studies using a heat-treated inactivated Oka/Merck varicella vaccine showed the vaccine was generally safe, reconstituted T-cell immunity against VZV, and reduced morbidity from VZV reactivation in HCT recipients [13, 14]. Based on these favorable results, we undertook this study to further evaluate the safety and immunogenicity of a heat-treated VZV vaccine (ZV_{HT}) in a diverse population of immunocompromised patients, including patients receiving therapy for a STM or HM, HIV-infected patients, and individuals undergoing HCT.

METHODS

Study Design

This randomized, double-blind, placebo-controlled multicenter study to evaluate the safety and immunogenicity of ZV_{HT} in immunocompromised adults was conducted between November 2007 and January 2010 in accordance with the ethical principles of the Declaration of Helsinki and the principles of current Good Clinical Practices. The study protocol and amendments were approved by local or central institutional review boards. All patients provided written informed consent.

Randomization was stratified by the underlying disease (Table 1). Patients were randomized to receive either ZV_{HT} (n = 262) or placebo (n = 79), both of which were given as a 4-dose regimen (Figure 1). For HCT recipients, dose 1 was administered approximately 30 days prior to a scheduled autologous or allogeneic HCT and doses 2 through 4 were administered at 30, 60, and 90 days post-HCT. HIV-infected patients and those with HM not receiving chemotherapy received dose 1 at the time of enrollment and doses 2 through 4 at approximately 30-day intervals. For patients with STM or HM receiving chemotherapy, study vaccine was administered approximately 7 days before any chemotherapy cycle with approximately 30 days between doses.

The sizes of the underlying disease groups (STM, HM, and HIV) were chosen to provide initial immunogenicity and safety data prior to initiating larger phase III studies and provided 76% power to detect a VZV interferon-γ enzyme-linked immunospot (IFN-γ ELISPOT) geometric mean fold-rise (GMFR) (lower bound of 90% confidence interval [CI] > 1.0) if the true GMFR was 2.0-fold and 83% power to detect a glycoprotein enzyme-linked immunosorbent assay (gpELISA) GMFR if the true GMFR was 1.5-fold. Autologous and allogeneic HCT populations were included in this study to provide additional safety data and experience with immunogenicity assays not used in initial studies [13, 14].

Study Population

Immunocompromised patients ≥18 years with at least a 1-year life expectancy and a history of varicella or residence in a VZV-endemic area for ≥30 years were eligible for the study. Immunocompromised populations included in this study were (1) individuals with STM receiving chemotherapy, (2) individuals with HM, (3) HIV-infected individuals with a baseline $CD4^+$ cell count ≤200 cells/mm^3, and (4) autologous or allogeneic HCT recipients (Table 1).

Patients were excluded if they had a history of hypersensitivity to ZV_{HT} vaccine components; a history of HZ within 1 year of enrollment; a prior history of receipt of any VZV vaccine; were pregnant or expecting to conceive from the period of time of 2 weeks prior to vaccination through 6 months after the last vaccination; or had received, or were scheduled to receive, a live virus vaccine in the period from 4 weeks prior to dose 1 through 28 days post-dose 4.

Patients With STM Receiving Chemotherapy

Patients with STM diagnosed ≤2 years prior to enrollment were included if they were undergoing frontline cytotoxic chemotherapy (including biologic treatments) for primary breast, colorectal, lung, or ovarian cancer. Study candidates with STM were excluded if they had central nervous system (CNS) metastasis, recurrence of cancer, or if they required modification of planned chemotherapy regimen because of intolerance to a chemotherapeutic agent. Patients who received blood products, immunostimulants, or immunosuppressive monoclonal antibody biologic treatments were excluded.

Patients With HM

Patients with HM (leukemia, lymphoma, or multiple myeloma) were included if they were receiving a chemotherapeutic regimen without rituximab or received the last dose of rituximab ≥3 months prior to study enrollment. Individuals ≥50 years with chronic lymphocytic leukemia (CLL) or active lymphoma but not receiving chemotherapy and not likely to undergo HCT during the study period were also included. Patients with HM were excluded from the study if they had CNS metastasis, an underlying cancer (other than Hodgkin's

Table 1. Patient Demographics and Underlying Diseases by Patient Group

	ZV_{HT}		Placebo	
	no.	(%)	no.	(%)
Patients in population	262		79	
Gender				
Male	158	(60.3)	48	(60.8)
Female	104	(39.7)	31	(39.2)
Race				
White	183	(69.8)	57	(72.2)
Black	70	(26.7)	21	(26.6)
Asian	8	(3.1)	1	(1.3)
Other	1	(0.4)	0	(0.0)
Mean age (range) in years				
Autologous HCT recipients	40	47.4 (21–76)	10	47.0 (21–69)
Allogeneic HCT recipients	41	45.9 (19–72)	10	49.5 (19–71)
STM patients	59	55.8 (35–81)	20	64.0 (41–83)
HM patients	62	64.3 (19–85)	19	61.0 (28–91)
HIV-infected Patients	60	46.5 (32–64)	20	43.3 (27–56)
Total study population	262	52.9 (19–85)	79	54.1 (19–91)
STM patients	59		20	
Breast cancer	23	(39.0)	9	(45.0)
Colorectal cancer	20	(33.9)	5	(25.0)
Lung cancer	15	(25.4)	6	(30.0)
Ovarian	1	(1.7)	0	(0.0)
HM patients	61[a]		19	
ALL	4	(6.5)	1	(5.3)
AML	1	(1.6)	0	(0.0)
CLL	23	(37.1)	9	(47.4)
CML	4	(6.5)	0	(0.0)
Hairy cell leukemia	2	(3.2)	0	(0.0)
Non-Hodgkins lymphoma	14	(22.6)	5	(26.3)
Hodgkins lymphoma	3	(4.8)	1	(5.3)
Langerhan histiocytosis	0	(0.0)	1	(5.3)
Multiple myeloma	7	(11.3)	2	(10.5)
Myelodysplastic syndrome	1	(1.6)	0	(0.0)
Polycythemia vera	1	(1.6)	0	(0.0)
T-cell prolymphocytic leukemia	1	(1.6)	0	(0.0)
HIV-infected patients	60		20	
CD4 count ≤100 cells/mm^3	29	(48.3)	9	(45.0)
CD4 count >100 cells/mm^3	31	(51.7)	11	(55.0)
Autologous HCT patients	40		10	
AML	1	(2.5)	0	(0.0)
Non-Hodgkins lymphoma	16	(40.0)	5	(50.0)
Hodgkins lymphoma	6	(15.0)	2	(20.0)
Medulloblastoma	2	(5.0)	0	(0.0)
Testicular choriocarcinoma	1	(2.5)	0	(0.0)
Multiple myeloma	14	(35.0)	3	(30.0)
Allogeneic HCT patients	40[a]		10	
ALL	7	(17.1)	2	(20.0)
AML	11	(26.8)	2	(20.0)
CLL	5	(12.2)	0	(0.0)
CML	5	(12.2)	1	(10.0)
Non-Hodgkins lymphoma	6	(14.6)	1	(10.0)

Table 1 continued.

	ZV_{HT}		Placebo	
	no.	(%)	no.	(%)
Aplastic anemia	0	(0.0)	1	(10.0)
Multiple myeloma	1	(2.4)	0	(0.0)
Myelodysplastic syndrome	4	(9.8)	2	(20.0)
Myelofibrosis	1	(2.4)	0	(0.0)
RAEB Type II myelodysplasia	0	(0.0)	1	(10.0)

Abbreviations: HCT, hematopoietic stem-cell transplant; HIV, human immunodeficiency virus; HM, hematologic malignancy; STM, solid tumor malignancy.
[a] One patient was randomized into the vaccine group but was not vaccinated; that patient's diagnosis is not included in this table.

lymphoma) that had relapsed more than twice or had required chemotherapy modification due to intolerance.

HIV-Infected Patients

HIV-infected patients enrolled into the study were required to have a $CD4^+$ cell count $\leq$200 cells/mm^3 documented within 90 days prior to dose 1 and to be receiving highly active antiretroviral therapy (HAART) for at least 30 days prior to enrollment. Patients with opportunistic infections were to be on therapy for at least 30 days prior to dose 1. Patients who received blood products, immunostimulants, or immunosuppressive monoclonal antibody biologic treatments were excluded.

HCT Recipients

Patients undergoing HCT were included if they were scheduled to undergo an autologous HCT for the treatment of leukemia, lymphoma, or other cancer or receive an allogeneic HCT for any reason within 60 days of enrollment. Patients undergoing HCT were excluded if they had more than 2 relapses of their underlying cancer (excluding Hodgkin's lymphoma) or were expected to undergo a tandem transplant procedure.

Study Vaccine Description

The lyophilized ZV_{HT} vaccine (4.8 UAg/dose) and placebo (vaccine stabilizer for ZV_{HT} with no virus antigen) were supplied to the study centers in 3.0-mL single-dose vials and refrigerated at 2°C–8°C (or colder). Both study products were reconstituted with sterile diluent immediately prior to administration and were indistinguishable from each other. All patients received a total of 4 0.65-mL subcutaneous injections of either ZV_{HT} or placebo according to the predefined dosing schedule.

Four vaccine doses were administered in this study because of favorable safety and efficacy data obtained with a 4-dose vaccine regimen in a pilot study of patients undergoing autologous HCT [14]. The number of vaccine doses that may confer protection from HZ in the STM, HM, and HIV populations is not known.

Safety Surveillance

The safety and tolerability assessment in each of the immunocompromised populations was based on adverse experiences (AEs) reported from the first dose of vaccine through 28 days post-dose 4. Throughout this period, daily oral temperature readings, injection-site reactions, and systemic AEs were recorded on a vaccination report card (VRC). Patients recorded injection-site AEs (redness, swelling, and pain/tenderness) for 5 days after each vaccination on the VRC.

Patients who developed suspected varicella/varicella-like or HZ/HZ-like rashes during the follow-up period through 28 days

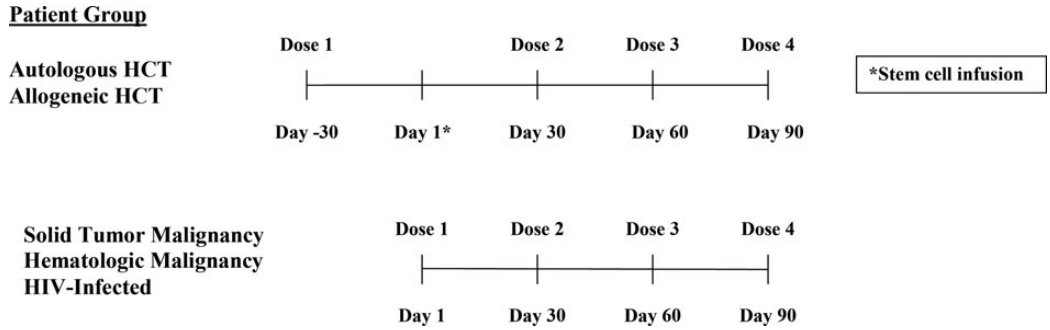

Figure 1. ZV_{HT} or placebo dosing schedule.

post-dose 4 were instructed to contact the investigator as soon as possible after rash onset for clinical evaluation and collection of clinical specimens from the lesion(s) for PCR analysis [15].

Immunogenicity Measurements

For the evaluation of immune responses to vaccination, blood samples were collected prior to each dose of study vaccine and at 28 days post-dose 4. VZV IFN-γ ELISPOT assay was performed to directly measure the presence of IFN-γ-secreting VZV-specific peripheral blood mononuclear cells (PBMCs) before and after vaccination [16] and VZV immunoglobulin G (IgG) antibodies to VZV glycoprotein were measured by gpELISA [17–19].

Statistical Methods

Safety

Safety assessment included proportions of patients with: (1) any AE, (2) any injection-site AE, (3) any systemic AE, (4) any serious AE, (5) any vaccine-related serious AE (as determined by the blinded investigator), and (6) any discontinuation from the study due to an AE occurring after any study vaccination through 28 days post-dose 4 in each population. For these measurements, 95% CI of the risk difference between vaccine and placebo groups were examined using the asymptotic methods proposed by Miettinen and Nurminen [20].

The primary safety endpoint of the study was based on the incidence of serious AEs observed during the 4 follow-up periods in each vaccination group in each population. Two-sided 95% CIs on the proportion of patients with any serious AEs were computed, based on the exact binomial distribution [21].

Immunogenicity

The primary immunologic endpoints were the GMFR from prevaccination to 28 days post-dose 4 of PBMC IFN-γ secretion measured by IFN-γ ELISPOT assay and of VZV antibody measured by gpELISA. The primary immunogenicity hypothesis was that ZV_{HT} would elicit significant VZV-specific immune responses measured by either VZV IFN-γ ELISPOT or gpELISA at 28 days post-dose 4. Hypothesis testing (applicable in the HM, STM, and HIV patient populations) and estimation for each assay (VZV IFN-γ ELISPOT or gpELISA) were based on a longitudinal model [22]. Success required that the lower bound of the 90% CI for GMFR be >1.0, which is equivalent to a 1-sided P value < .05. The model only used data from the vaccine recipients, with the log-transformed VZV responses at each visit being used as response variables and the visit variable as covariate. The immunogenicity analysis was exploratory in the HCT populations because confounding treatments such as blood products might interfere with immunogenicity measures, and because clinical efficacy and immunogenicity have already been shown in this group [13, 14].

RESULTS

Participant Accounting and Demographics

Overall, 341 patients were randomized. The ZV_{HT} and placebo groups in each immunocompromised population were similar in terms of age, gender, race, and underlying cancer diagnosis (Table 1). In all, 79.0% (207/262) of ZV_{HT} recipients and 84.8% (67/79) of placebo recipients completed all study visits (Table 2). Four (4) randomized patients who were not vaccinated are not included in the safety or immunogenicity analyses. Forty-eight (48) patients had protocol violations (some had more than 1 violation), whose immunogenicity results obtained subsequent to the violation were excluded from the immunogenicity analyses: 27 with prohibited vaccine or medication that could have interfered with immunity assessments, 13 with HZ or HZ-like rash, 9 reported exposure to VZV, and 3 did not undergo HCT (2 autologous, 1 allogeneic).

Safety

All randomized participants who received at least 1 dose of study vaccination were included in the safety analyses (Table 3). In the ZV_{HT} group, the proportion of patients reporting injection site AEs ranged from 3.3% in the HIV-infected group to 36.8% in the STM group. In the placebo group, injection site reactions ranged from 0% in the allogeneic HCT group to 15.0% in the HIV-infected placebo-treated group.

In the ZV_{HT} group, the proportion of patients reporting SAEs ranged from 12.3% for STM patients to 80.0% for allogeneic HCT patients. In the placebo group, the proportion of patients reporting SAEs ranged from 5.3% for the HM group to 70.0% for the allogeneic HCT population. Only 2 SAEs (motor sensory neuropathy [continuing at time of study discontinuation] and vomiting [resolved]), both in the HM population, were considered by the investigators to be possibly related to ZV_{HT}.

Two patients (5%) in the ZV_{HT} autologous HCT stratum developed auto-GVHD [23] following the first dose of the vaccine; no cases were detected in the autologous HCT placebo arm. Neither occurrence was deemed vaccine-related by the reporting investigator. GVHD occurrences were similarly distributed between the two allogeneic HCT vaccination groups (ZV_{HT}: 21 patients [52.5%]; placebo: 5 patients [50.0%]).

Immunogenicity

Patients With STM Receiving Chemotherapy

The primary IFN-γ ELISPOT hypothesis was met in this stratum, as post-dose 4 immune responses in ZV_{HT} recipients were significantly higher than those at baseline (GMFR = 3.0 [90% CI, 2.0–4.6], P value = < .0001; Table 4). Responses among ZV_{HT} recipients were generally higher than among placebo recipients (Table 5).

Table 2. Patient Accounting

	STM Patients		HM Patients		HIV-Infected Patients		Autologous HCT Recipients		Allogeneic HCT Recipients	
	ZV_{HT} (N = 59) no. (%)	Placebo (N = 20) no. (%)	ZV_{HT} (N = 62) no. (%)	Placebo (N = 19) no. (%)	ZV_{HT} (N = 60) no. (%)	Placebo (N = 20) no. (%)	ZV_{HT} (N = 40) no. (%)	Placebo (N = 10) no. (%)	ZV_{HT} (N = 41) no. (%)	Placebo (N = 10) no. (%)
Dose Administered										
Vaccination 1	58 (98.3)	19 (95.0)	61 (98.4)	19 (100)	60 (100)	20 (100)	40 (100)	10 (100)	40 (97.6)	10 (100)
Vaccination 2	52 (88.1)	19 (95.0)	58 (93.5)	19 (100)	57 (95.0)	19 (95.0)	35 (87.5)	10 (100)	37 (90.2)	8 (80.0)
Vaccination 3	48 (81.4)	19 (95.0)	51 (82.3)	18 (94.7)	53 (88.3)	18 (90.0)	34 (85.0)	10 (100)	35 (85.4)	8 (80.0)
Vaccination 4	47 (79.7)	19 (95.0)	51 (82.3)	18 (94.7)	50 (83.3)	17 (85.0)	34 (85.0)	10 (100)	32 (78.0)	6 (60.0)
Study Disposition										
Completed	47 (79.7)	19 (95.0)	51 (82.3)	18 (94.7)	47 (78.3)	16 (80.0)	33 (82.5)	8 (80.0)	29 (70.7)	6 (60.0)
Discontinued	12 (20.3)	1 (5.0)	11 (17.7)	1 (5.3)	13 (21.7)	4 (20.0)	7 (17.5)	2 (20.0)	12 (29.3)	4 (40.0)
Adverse event	3 (5.1)	0 (0.0)	5 (8.1)	1 (5.3)	2 (3.3)	1 (5.0)	1 (2.5)	0 (0.0)	9 (22.0)	3 (30.0)
Lost to follow-up	2 (3.4)	0 (0.0)	0 (0.0)	0 (0.0)	6 (10.0)	1 (5.0)	1 (2.5)	2 (20.0)	0 (0.0)	0 (0.0)
Physician decision	1 (1.7)	0 (0.0)	1 (1.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Protocol violation	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.7)	0 (0.0)	2 (5.0)	0 (0.0)	1 (2.4)	0 (0.0)
Withdrawal by patient	6 (10.2)	1 (5.0)	5 (8.1)	0 (0.0)	4 (6.7)	2 (10.0)	3 (7.5)	0 (0.0)	2 (4.9)	1 (10.0)

Abbreviations: HIV, human immunodeficiency virus; HM, hematologic malignancy; STM, solid tumor malignancy.

The primary gpELISA hypothesis was also met, as post-dose 4 antibody levels measured by gpELISA were significantly higher than those at baseline (GMFR = 2.4 [90% CI, 1.8–3.0], *P* value < .0001; Table 4). Antibody levels among ZV_{HT} recipients were generally higher than among placebo recipients post-dose 4 (Table 5).

Patients With HM

The primary IFN-γ ELISPOT hypothesis was met in this stratum, as post-dose 4 immune responses were significantly higher among ZV_{HT} recipients than those measured at baseline (GMFR = 2.2 [90% CI, 1.4–3.5], *P* value = .004; Table 4). Responses among those in the ZV_{HT} group were generally higher than among placebo recipients (Table 5).

Among ZV_{HT} recipients, the primary gpELISA hypothesis was met, as post-dose 4 antibody levels measured by gpELISA were significantly higher than those at baseline (GMFR = 1.3 [90% CI, 1.1–1.5], *P* value = .003; Table 4). The post-dose 4 antibody levels appeared similar among the ZV_{HT} and placebo recipients; however, baseline levels were numerically lower for the ZV_{HT} group compared with the placebo group (Table 5).

HIV-Infected Patients

The primary IFN-γ ELISPOT hypothesis was met, as post-dose 4 immune responses were significantly higher than those at baseline (GMFR = 1.8 [90% CI, 1.2–2.7], *P* value = .026; Table 4). Although the geometric mean concentrations (GMCs) for both the ZV_{HT} and placebo groups were very low, responses in ZV_{HT} group were generally higher than among placebo recipients (Table 5).

Among ZV_{HT} recipients in this stratum, the primary gpELISA hypothesis was met, as post-dose 4 antibody levels measured by gpELISA were significantly higher than those at baseline (GMFR = 1.4 [90% CI, 1.1–1.7], *P* value = .017; Table 4). Antibody levels were generally similar among both groups (Table 5).

Autologous HCT Recipients

The ZV_{HT} vaccine elicited significant VZV-specific immune responses measured by IFN-γ ELISPOT at 28 days post-dose 4 in this population (GMFR = 9.0 [90% CI, 4.4–18.4]; Table 4). Post-dose 4 GMCs among ZV_{HT} recipients were generally higher than those measured in the placebo recipients (Table 5).

Among ZV_{HT} recipients in this stratum, VZV-specific antibody responses measured by gpELISA at 28 days post-dose 4 were not statistically significant (GMFR = 0.9 [90% CI, 0.6–1.3]; Table 4). Antibody levels among ZV_{HT} recipients were generally higher than among placebo recipients post-dose 4 (Table 5).

Allogeneic HCT Recipients

Among ZV_{HT} recipients in this stratum, VZV-specific immune responses measured by IFN-γ ELISPOT were lower post-dose 4 than at baseline (GMFR = 0.2 [90% CI, .1–.4]), and responses measured by gpELISA were unchanged (GMFR = 1.0 [90% CI, 0.7–1.4]; Table 4).

Table 3. Adverse Event Summary by Patient Group

	ZV_{HT}		Placebo		Difference in % vs. Placebo Estimate (95% CI)
	N	(%)	N	(%)	
STM patients	57		19		
With ≥1 adverse events	40	(70.2)	13	(68.4)	1.8 (−19.6, 27.0)
With vaccine-related[a] adverse events	24	(42.1)	1	(5.3)	36.8 (14.8, 51.5)
Injection-site	21	(36.8)	1	(5.3)	31.6 (9.7, 46.2)
Noninjection-site	3	(5.3)	0	0.0	5.3 (−12.0, 14.5)
With serious adverse events	7	(12.3)	2	(10.5)	1.8 (−20.4, 15.7)
With serious vaccine-related[a] adverse events	0	0.0	0	0.0	0.0 (−17.0, 6.4)
Who died	1[b]	(1.8)	0	0.0	1.8 (−15.3, 9.4)
HM patients	61		19		
With ≥1 adverse events	53	(86.9)	10	(52.6)	34.3 (11.7, 56.8)
With vaccine-related[a] adverse events	24	(39.3)	5	(26.3)	13.0 (−12.5, 33.2)
Injection-site	19	(31.1)	2	(10.5)	20.6 (−2.6, 36.3)
Noninjection-site	11	(18.0)	3	(15.8)	2.2 (−21.2, 18.3)
With serious adverse events	12	(19.7)	1	(5.3)	14.4 (−6.5, 27.6)
With serious vaccine-related[a] adverse events	2[c]	(3.3)	0	0.0	3.3 (−13.9, 11.3)
Who died	4[d]	(6.6)	0	0.0	6.6 (−10.7, 15.8)
HIV-infected patients	60		20		
With ≥1 adverse events	34	(56.7)	12	(60.0)	−3.3 (−26.1, 21.6)
With vaccine-related[a] adverse events	6	(10.0)	4	(20.0)	−10.0 (−32.7, 5.8)
Injection-site	2	(3.3)	3	(15.0)	−11.7 (−33.2, 0.6)
Noninjection-site	4	(6.7)	1	(5.0)	1.7 (−17.6, 12.3)
With serious adverse events	8	(13.3)	2	(10.0)	3.3 (−18.1, 17.0)
With serious vaccine-related[a] adverse events	0	0.0	0	0.0	0.0 (−16.3, 6.1)
Who died	1[e]	(1.7)	0	0.0	1.7 (−14.7, 8.9)
Autologous HCT Recipients	40		10		
With ≥1 adverse events	40	(100.0)	10	(100.0)	0.0 (−8.9, 28.2)
With vaccine-related[a] adverse events	4	(10.0)	1	(10.0)	0.0 (−31.7, 16.3)
Injection-site	4	(10.0)	1	(10.0)	0.0 (−31.7, 16.3)
Noninjection-site	0	0.0	0	0.0	0.0 (−28.2, 8.9)
With serious adverse events	13	(32.5)	2	(20.0)	12.5 (−21.7, 35.3)
With serious vaccine-related[a] adverse events	0	0.0	0	0.0	0.0 (−28.2, 8.9)
Who died	1[f]	(2.5)	0	0.0	2.5 (−25.8, 13.0)
Allogeneic HCT Recipients	40		10		
With ≥1 adverse events	40	(100.0)	10	(100.0)	0.0 (−8.9, 28.2)
With vaccine-related[a] adverse events	6	(15.0)	1	(10.0)	5.0 (−27.2, 22.5)
Injection-site	6	(15.0)	0	0.0	15.0 (−14.1, 29.2)
Noninjection-site	0	0.0	1	(10.0)	−10.0 (−40.8, −0.4)
With serious adverse events	32	(80.0)	7	(70.0)	10.0 (−15.1, 42.9)
With serious vaccine-related[a] adverse events	0	0.0	0	0.0	0.0 (−28.2, 8.9)
Who died[g]	11	(27.5)	2	(20.0)	7.5 (−26.4, 30.0)

Abbreviations: CI, confidence interval; HCT, hematopoietic stem-cell transplant; HIV, human immunodeficiency virus; HM, hematologic malignancy; N, patients in population with follow-up.

[a] Determined by the investigator to be related to the vaccine.

[b] One ZV_{HT} recipient died of acute respiratory failure; assessed by the investigator as not related to vaccine.

[c] Two serious vaccine-related AEs in ZV_{HT} group: (1) vomiting on day 2 post-dose 1; (2) motor sensory polyneuropathy beginning on day 6 post-dose 2 (assessed as related to other suspect therapy [cyclophosphamide, doxorubicin, vincristine, cytarabine, and methotrexate] and possibly related to ZV_{HT}).

[d] Four ZV_{HT} recipients died (causes of death: arteriosclerosis of coronary artery, malignant neoplasm progression, leukemia, neutropenic sepsis); all assessed by the investigators as not related to vaccine.

[e] One ZV_{HT} recipient died of septic shock; assessed by the investigator as not related to vaccine.

[f] One ZV_{HT} recipient died of Non-Hodgkin's lymphoma, assessed as not related to vaccine by the investigator.

[g] Deaths occurred in 27.5% of ZV_{HT} recipients and 20.0% of placebo recipients; causes of death generally appeared related to underlying condition or treatment regimen, and all deaths were assessed by the investigator as not related to vaccine.

Table 4. GMFR from Baseline to Post-dose 4 in ZV_{HT} Recipients (Per Protocol Population)

Treatment Group	n[a]	Estimated GMFR[b]	90% CI	*P* Value
VZV-specific T-cell function by IFN-γ ELISPOT				
STM	56	3.0	(2.0, 4.6)	<.0001
HM	60	2.2	(1.4, 3.5)	.004
HIV-infected	60	1.8	(1.2, 2.7)	.026
Autologous HCT	38	9.0	(4.4, 18.4)	Exploratory only
Allogeneic HCT	40	0.2	(0.1, 0.4)	Exploratory only
VZV antibody response by gpELISA				
STM	55	2.4	(1.8, 3.0)	<.0001
HM	59	1.3	(1.1, 1.5)	.003
HIV-infected	60	1.4	(1.1, 1.7)	.017
Autologous HCT	38	0.9	(0.6, 1.3)	Exploratory only
Allogeneic HCT	36	1.0	(0.7, 1.4)	Exploratory only

Abbreviations: CI, confidence interval; ELISPOT, enzyme-linked immunospot; GMFR, geometric mean fold rises; gpELISA, glycoprotein enzyme-linked immunosorbent assay; HCT, hematopoietic stem-cell transplant; HIV, human immunodeficiency virus; HM, hematologic malignancy; IFN-γ, interferon γ; STM, solid tumor malignancy; VZV, Varicella zoster virus.

[a] n, no. of patients contributing to analysis (valid results at baseline or time points post-baseline).

[b] Calculated based on a single longitudinal regression model adjusting for prevaccination values.

VZV Infections

Two of 79 placebo patients (2.5%) and 11 of 262 vaccine patients (4.2%) presented with rashes suggestive of VZV infection during the study period and were given the diagnosis of varicella/varicella-like or HZ/HZ-like infection by a study investigator and/or primary physician (Table 6). PCR testing for VZV in a skin lesion was performed in lesion swabs from 8 of these 13 patients. PCR testing was ultimately positive for wild-type VZV in only 3 patients (2 placebo recipients, 1 ZV_{HT} recipient). No patient with PCR results had a rash positive for the ZV_{HT} strain.

DISCUSSION

The risk of reactivation of VZV, resulting in HZ, appears to inversely correlate with the level of VZV-specific cell-mediated immune responses [5, 24–26]. The greater the intensity of immunosuppression in a host, the greater the risk of HZ and attendant risk of more severe HZ presentations, including myelitis, encephalitis, or disseminated disease with visceral involvement. Profound and prolonged suppression of cell-mediated immunity occurs in individuals who are HIV-infected, have hematologic malignancies (especially those arising from the lymphatic system), are receiving long-term cytotoxic chemotherapy or immunosuppressant agents, and undergoing extensive radiation therapy. As expected, the patient groups enrolled in the present study had clear, even profound, suppression of cell-mediated immunity, as demonstrated by the observed low baseline IFN-γ ELISPOT GMCs. For context, baseline GMCs observed in this heterogeneous population of immunocompromised patients were generally lower than the baseline GMCs of immunocompetent adults observed in prior studies of the live zoster vaccine [27, 28].

Prevention and treatment of opportunistic infections, including VZV reactivation, in immunocompromised hosts remain formidable challenges and infections continue to be a major cause of morbidity and mortality in these patient populations, despite advancements in vaccines and other therapies. Live virus vaccines are typically contraindicated in immunocompromised patients due to the potential risk of unchecked replication of attenuated vaccine strains in the absence of a robust immune response. The zoster vaccine used in this study was heat-treated, which greatly limits the amount of live virus in the vaccine while preserving the quantity of viral antigen. In 2 proof-of-concept clinical trials involving both autologous HCT and allogeneic HCT recipients, there was an observed reduction in the incidence and severity of HZ among patients who received a heat-treated VZV-containing vaccine [13, 14].

In the current study, a 4-dose regimen ZV_{HT} was found to be generally safe in all 5 subpopulations of immunocompromised patients evaluated. Vaccine-related AEs were infrequent and consisted mostly of pain and erythema at the injection site. Two SAEs reported by 2 HM patients were deemed by the investigators to be possibly related to ZV_{HT}. These SAEs were confounded by concurrent conditions or therapies that may have caused the SAEs. The overall incidence of all AEs and SAEs were similar for both ZV_{HT} and placebo patients. For the HM patients, the frequencies of systemic AEs overall were increased in the vaccine group compared with the placebo group. There were no documented cases of skin lesions caused by the vaccine strain in the study.

Significant VZV-specific T-cell and antibody responses were elicited at 28 days post-dose 4 in the patients with STM, HM, and HIV infection with $CD4^+$ counts $\leq$200 cells/mm^3. Responses in all 3 of these populations met the protocol's prespecified success criteria. Immunogenicity responses observed for the STM and HM patient groups suggest that ZV_{HT} may reduce the incidence of HZ in these groups, although clinical protection conferred by this vaccine will need to be demonstrated in a controlled clinical efficacy trial.

Immunogenicity responses elicited in HIV-infected patients were of low magnitude and may be insufficient to translate into a reduction in HZ for this patient group. In HIV-infected individuals, the frequency of HZ is greater than that of the general

Table 5. Observed IFN-γ ELISPOT and gpELISA Results (Per Protocol Population)

	IFN-γ ELISPOT						gpELISA					
	Baseline			Post-dose 4			Baseline			Post-dose 4		
Treatment	n	GMC	(90% CI)	n	GMC	(90% CI)	n	GMT	(90% CI)	n	GMT	(90% CI)
STM patients												
ZV_{HT}	51	24	(15.6, 36.8)	32	88.8	(57.0, 138.3)	51	235	(163.3, 338.2)	33	452	(341.9, 597.4)
Placebo	18	18.7	(8.0, 43.9)	16	25.3	(12.2, 52.2)	19	262.6	(168.3, 409.6)	16	291.3	(178.0, 476.6)
HM patients												
ZV_{HT}	59	11.3	(7.2, 17.5)	38	26.2	(14.5, 47.3)	59	205.3	(158.1, 266.6)	38	252.9	(182.9, 349.6)
Placebo	19	21.2	(11.6, 38.8)	14	13.3	(4.4, 40.4)	17	258.9	(129.9, 516.2)	14	242.3	(133.4, 440.1)
HIV-infected patients												
ZV_{HT}	57	2.6	(1.8, 3.8)	42	5.3	(3.1, 8.9)	60	187.2	(127.4, 274.9)	43	229.9	(145.4, 363.3)
Placebo	20	2.3	(1.3, 4.1)	14	1.9	(1.0, 3.4)	19	196.9	(102.2, 379.5)	14	203.1	(111.1, 371.1)
Autologous HCT recipients												
ZV_{HT}	31	9.1	(5.2, 15.9)	24	92.2	(47.0, 180.9)	38	214.1	(147.3, 311.2)	30	196.2	(126.4, 304.5)
Placebo	7	8.6	(2.8, 26.1)	5	4.9	(0.8, 30.5)	10	132.4	(63.7, 274.9)	7	45.4	(13.3, 155.1)
Allogeneic HCT Recipients												
ZV_{HT}	28	13.7	(6.6, 28.4)	26	2.7	(1.5, 4.9)	35	151.6	(106.6, 215.6)	24	139.3	(102.7, 189.0)
Placebo	7	4.0	(0.7, 22.2)	5	0.5	(0.5, 0.5)	9	201.4	(85.4, 474.9)	5	69.9	(19.9, 245.6)

Abbreviations: CI, confidence interval; ELISPOT, enzyme-linked immunospot; GMC, geometric mean concentrations; GMT, geometric mean titer; gpELISA, glycoprotein enzyme-linked immunosorbent assay; HCT, hematopoietic stem-cell transplant; HIV, human immunodeficiency virus; IFN-γ, interferon-γ; n, no. of persons contributing to analysis (with valid result at stated time point); STM, solid tumor malignancy; VZV, Varicella-zoster virus.

population at all $CD4^+$ cell counts, moreover, the frequency increases as $CD4^+$ cell counts decline [29]. Although HZ is not associated with increased risk of death or faster progression to AIDS, HIV-infected patients with low $CD4^+$ counts are prone to HZ complications including ocular involvement, PHN, myelitis, meningitis, and chronic atypical skin lesions [30].

Table 6. PCR Results by Patient Group

Treatment	Age (years)	Rash Diagnosis	Onset in Days Postvaccination (dose)	Duration (days)	Total Lesions	PCR Results
STM patients						
ZV_{HT}	61	Rash varicelliform	8 (Dose 4)	8	26 to 175	Negative
ZV_{HT}	35	Rash zosteriform	10 (Dose 1)	14	1 to 25	Negative
HM patients						
ZV_{HT}	67	Rash varicelliform	2 (Dose 1)	11	1 to 25	Negative
ZV_{HT}	70	Rash varicelliform	14 (Dose 2)	24	1 to 50	Negative
ZV_{HT}	70	Rash varicelliform	19 (Dose 2)	5	1 to 25	Negative
HIV-infected patients						
ZV_{HT}	48	Rash varicelliform	7 (Dose 1)	1	Results Not Available	Sample Not Collected
ZV_{HT}	40	Rash varicelliform	24 (Dose 1)	4	26 to 100	Sample Not Collected
ZV_{HT}	43	Rash zosteriform	30 (Dose 1)	2	1 to 25	Sample Not Collected
Placebo	43	Herpes zoster	16 (Dose 4)	18	1 to 25	VZV-WT
		Rash varicelliform	16 (Dose 4)	18	1 to 25	Sample Not Collected
Autologous HCT recipients						
ZV_{HT}	34	Herpes zoster	34 (Dose 3)	13	26 to 100	VZV-WT
ZV_{HT}	48	Rash zosteriform	19 (Dose 1)	7	1 to 50	Sample Not Collected
Placebo	69	Herpes zoster	12 (Dose 2)	15	300 to 600 plus	VZV-WT
Allogeneic HCT recipients						
ZV_{HT}	65	Rash zosteriform	31 (Dose 1)	18	1 to 25	Sample Not Collected

Abbreviations: HCT, hematopoietic stem-cell transplant;HM, hematologic malignancy; Negative, negative for VZV-WT, VZV-OKA, and HSV DNA; PCR, polymerase chain reaction; STM, solid tumor malignancy.

No ZV_{HT} recipient had rash positive for vaccine strain.

Significant VZV-specific T-cell responses were elicited at 28 days post-dose 4 in autologous HCT recipients, whereas the response elicited in allogeneic HCT recipients was poor. After HCT, reconstitution of T cells may be derived from residual recipient T cells that survived conditioning regimens, donor T cells present in the graft, and stem cells that differentiate into T cells in the recipient. Poor immunologic response to ZV_{HT} in the allogeneic stem cell population may be related to complete or nearly complete ablation of VZV memory T cells during conditioning for allogeneic HCT and slow reconstitution of immunologically competent T cells, which may take more than a year following allogeneic transplant. [31–34]. Moreover, the suppression of T-cell function is greater in allogeneic HCT compared with autologous HCT due to prolonged use of graft-versus-host-disease prophylaxis with immunosuppressant agents; this may also contribute to the difference in immunogenicity of study vaccine seen between these 2 groups.

The limitations of this study included a small sample size and heterogeneous patient population and utilization of immunogenicity measures that have not been used in immunocompromised patients in previous studies. Therefore, the immunogenicity results for the HCT populations of this study cannot be directly compared to the results of earlier proof-of-concept trials that also assessed clinical efficacy [13, 14]. In addition, although both the IFN-γ ELISPOT and gpELISA have been shown to correlate with the clinical efficacy of the live zoster vaccine in healthy, older adults, these assays may not be relevant markers of vaccine efficacy in immunocompromised populations. Nonetheless, the results of this Phase I trial of ZV_{HT} in the immunocompromised populations examined in this study suggest a favorable safety and immunogenicity profile in certain immunocompromised populations, warranting further larger studies to evaluate the safety and efficacy of ZV_{HT} in selected populations.

Notes

Acknowledgments. The authors thank all of the patients, their families, and the entire study staff who participated in this study. A special thanks to the following study personnel for their exceptional level of support in protocol execution, patient coordination, and data collection/review: Cheryl Callais, Julie Carney, Cindy Coates, Doreen DeGray, Teo Franic, Christine Gerdes, Katherine Meneses, Brittany Pena-Alvarado, Maria Perez, Corey Rosmarin-DeStefano, Joanna Schaenman, Jennifer Theusch. V212 Protocol 002 Study Group: Luke Akard, Evan Alley, Huzefa Bahrain, Robert Betts, Mark Bloch, Ralph Boccia, Michael Boeckh, Janice Brown, Luis Camacho, Alan Cartmell, Terrence Cescon, Veena Charu, David Cooper, Matthew Greenwood, John Gibson, Peter Kadlecik, Divis Khaira, Myron Levin, Per Ljungman, Farid Marquez, Allison McGeer, Kathleen Mullane, Michael Mullane, R. Deva Nathan, Marcio Nucci, Constance Pachucki, Steven Papish, Ronald Poblete, Donald Poretz, John Richart, Rene Rothstein-Rubin, Shamel Sanani, Abraham Schwarzberg, Ronald Steis, Elizabeth Tan-Chiu, Karen Tashima, Michael Wertheim, Drew Winston, and Benny Wong.

Disclaimer. Merck's policy effective July 1, 2011, will allow JID to post on its website, at the time of publication, if it so desires, the critical sections of the protocol that are relevant to evaluating the study, specifically those sections describing the study objectives and hypotheses, the patient inclusion and exclusion criteria, the study design and procedures, the efficacy and safety measures, the statistical analysis plan, and any amendments relating to those sections. Merck reserves the right to redact proprietary information. Merck will also retain copyright of the protocol but allow JID to publish the critical sections described above on its website with a link to the published paper. Any further use of the protocol, including these critical sections, will require Merck's permission. If you wish to post this supplementary material with the final manuscript, please contact me to obtain the relevant protocol sections for posting

Author Contributions. K. M., D. W., M. W., R. B., D. P., L. C., S. P., M. M.: patient enrolment, data collection, data interpretation, and manuscript preparation. Y. Z., P. A.: study concept/design, data analysis/interpretation, and manuscript preparation. J. S., T. S., S. M.: data analysis/interpretation, and manuscript preparation.

Sponsor's Role. This study was funded by Merck & Co., Inc. (sponsor). In conjunction with the external investigators, this study was designed, executed, and analyzed by the sponsor. Although the sponsor formally reviewed a penultimate draft, the opinions expressed are those of the authorship and may not necessarily reflect those of the sponsor. All coauthors approved the final version of the manuscript.

Financial support. This work was supported by Merck & Co., Inc.

Potential conflicts of interest. Other than employees of Merck & Co., Inc. (as indicated on the title page), all authors have been investigators for the sponsor. Employees may hold stock and/or stock options in the company.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Cohen JI. HZ vaccine in immunocompromised patients. J Infect Dis **2008**; 197:S237–41.
2. Masci G, Magagnoli M, Gullo G, et al. Herpes infections in breast cancer patients treated with adjuvant chemotherapy. Oncology **2006**; 71:164–7.
3. Feld R, Evans WK, DeBoer G. Herpes zoster in patients with small-cell carcinoma of the lung receiving combined modality treatment. Ann Intern Med **1980**; 93:282–3.
4. Huberman M, Fossieck BE Jr, Bunn PA Jr, Cohen MH, Ihde DC, Minna J. Herpes zoster and small cell bronchogenic carcinoma. Am J Med **1980**; 68:214–8.13.
5. Schimpff S, Serpick A, Stoler B, et al. Varicella-zoster infection in patients with cancer. Ann Intern Med **1972**; 76:241–54.
6. Wade JC. Viral infections in patients with hematological malignancies. Hematology **2006**; 1:368–74.
7. Mattiuzzi GN, Cortes JE, Talpaz M, et al. Development of varicella-zoster virus infection in patients with chronic myelogenous leukemia treated with imatinib mesylate. Clin Cancer Res **2003**; 9:976–80.
8. Rusthoven JJ, Ahlgren P, Elhakim T, Pinfold P, Stewart L, Feld R. Varicella-zoster infection in adult cancer patients. Arch Intern Med **1988**; 148:1561–6.
9. Engels EA, Rosenberg PS, Biggar RJ. Zoster incidence in human immunodeficiency virus-infected hemophiliacs and homosexual men, 1984–1997. J Infect Dis **1999**; 180:1784–9.
10. U.S. Food and Drug Administration. Approved Products: Zostavax. Merck & Co., Inc., Whitehouse Station, NJ. http://www.fda.gov/downloads/biologicsbloodvaccines/vaccines/approvedproducts/ucm132831.pdf. Accessed 2 August 2012.
11. Oxman MN, Levin MJ, Johnson GR, et al. A vaccine to prevent herpes zoster and postherpetic neuralgia in older adults. N Engl J Med **2005**; 352:2271–84.
12. Schmader KE, Levin MJ, Gnann JW, et al. Efficacy, safety, and tolerability of herpes zoster vaccine in persons aged 50–59 years. Clin Infect Dis **2012**; 54:922–928.

13. Redman RL, Nader S, Zerboni L, et al. Early reconstitution of immunity and decreased severity of herpes zoster in bone marrow transplant recipients immunized with inactivated varicella vaccine. J Infect Dis **1997**; 176:578–85.
14. Hata A, Asanuma H, Rinki M, et al. Use of an inactivated varicella vaccine in recipients of hematopoietic-cell transplants. N Engl J Med **2002**; 347:26–34.
15. Harbecke R, Oxman MN, Arnold BA, et al. A real-time PCR assay to identify and discriminate among wild-type and vaccine strains of varicella-zoster virus and herpes simplex virus in clinical specimens, and comparison with the clinical diagnoses. J Med Virol **2009**; 81: 1310–22.
16. Smith JG, Liu X, Kaufhold RM, Clair J, Caulfield MJ. Development and validation of a gamma interferon ELISPOT assay for quantitation of cellular immune responses to varicella-zoster virus. Clin Diagn Lab Immunol **2001**; 8:871–9.
17. 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.
18. 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.
19. Provost PJ, Krah DL, Kuter BJ, et al. Antibody assays suitable for assessing immune responses to live varicella vaccine. Vaccine **1991**; 9:111–6.
20. Miettinen O, Nurminen M. Comparative analysis of two rates. Stat Med **1985**; 4:213–26.
21. Clopper CJ, Pearson ES. The use of confidence or fiducial limits illustrated in the case of the binomial. Biometrika **1934**; 26:404–13.
22. Liang K, Zeger S. Longitudinal data analysis of continuous and discrete responses for pre-post designs. Sankhya **2000**; 62:134–48.
23. Holmberg L, Kikuchi K, Gooley TA, et al. Gastrointestinal graft-versus-host disease in recipients of autologous hematopoietic stem cells: incidence, risk factors, and outcome. Biol Blood Marrow Transplant **2006**; 12:226–34.
24. Rand KH, Rasmussen LE, Pollard RB, Arvin A, Merigan TC. Cellular immunity and herpes virus infections in cardiac transplant patients. N Engl J Med **1976**; 296:1372–7.
25. Sokal JE, Firat D. Varicella-zoster infection in Hodgkin's disease. Am J Med **1965**; 39:452–63.
26. Antonelli MAS, Moreland LW, Brick JE. Herpes zoster in patients with rheumatoid arthritis treated with weekly low-dose methotrexate. Am J Med **1991**; 90:295–8.
27. Levin MJ, Oxman MN, Zhang JH, et al. Varicella zoster virus-specific immune responses in elderly recipients of a herpes zoster vaccine. J Infect Dis **2008**; 197:825–35.
28. Vermeulen JN, Lange JMA, Tyring SK, et al. Safety, tolerability, and immunogenicity after 1 and 2 doses of zoster vaccine in healthy adults ≥60 years of age. Vaccine **2012**; 30:904–10.
29. Talmadge JE, Reed E, Ino K, et al. Rapid immunologic reconstitution following transplantation with mobilized peripheral blood stem cells as compared to bone marrow. Bone Marrow Transplant **1997**; 19:161–72.
30. Diviné M, Boutolleau D, Delfau-Larue MH, et al. Poor lymphocyte recovery following CD34-selected autologous peripheral blood stem cell transplantation for non-Hodgkin's lymphoma. Br J Haematol **1999**; 105:349–60.
31. Glesby MJ, Moore RD, Chaisson RE. Zidovudine Epidemiology Study Group. Herpes zoster in patients with advanced human immunodeficiency virus infection treated with zidovudine. J Infect Dis **1993**; 168:1264–8.
32. Vafai A, Berger M. Zoster in patients infected with HIV: a review. Am J Med Sci **2001**; 321:372–80.
33. Gebo KA, Kalyani R, Moore RD, Polydefkis MJ. The incidence of, risk factors for, and sequelae of herpes zoster among HIV patients in the highly active antiretroviral therapy era. J Acquir Immune Defic Syndr **2005**; 40:169–74.
34. Moir S, Fauci AS. B cells in HIV infection and disease. Nat Rev Immunol **2009**; 9:235–45.