Long-term immunogenicity and safety of an investigational herpes zoster subunit vaccine in older adults

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ABSTRACT

Background: An investigational subunit vaccine containing the varicella-zoster virus (VZV) glycoprotein E (gE) and the AS01â adjuvant system is being evaluated for the prevention of herpes zoster (HZ) in older adults. A phase II trial evaluating different formulations of this vaccine (containing 25 μg, 50 μg, or 100 μg gE) was conducted in adults ≥60 years of age and showed that all formulations elicited robust cellular and humoral immune responses for up to 3 years after vaccination. In this follow-up study in subjects who received two doses of the 50 μg gE/AS01â formulation (HZ/su), we assessed the persistence of the immune responses for up to 6 years after vaccination.

Methods: This phase II, open-label, multicenter, single-group trial conducted in the Czech Republic, Germany, Sweden, and the Netherlands followed 129 subjects who had received two doses (2 months apart) of HZ/su during the initial trial. Vaccine-induced immune responses (frequencies of gE-specific CD4+ T cells expressing ≥2 activation markers and serum anti-gE antibody concentrations) were evaluated at 48, 60, and 72 months after the first HZ/su dose.

Results: Six years after vaccination with HZ/su, gE-specific cell-mediated immune responses and anti-gE antibody concentrations had decreased by 20–25% from month 36, but remained higher than the prevaccination values. At month 72, the gE-specific cell-mediated immune response was 3.8 times higher than the prevaccination value (477.3 vs. 119.4 activated gE-specific CD4+ T cells per 10^6 cells), and the anti-gE antibody concentration was 7.3 times higher than the prevaccination value (8159.0 vs. 1121.3 mIU/mL). No vaccine-related serious adverse events were reported between months 36 and 72.

Conclusions: gE-specific cellular and humoral immune responses persisted for 6 years after two-dose vaccination with HZ/su in healthy older adults. No safety concerns were identified.

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1. Introduction

Herpes zoster (HZ), or shingles, results from the reactivation of latent varicella-zoster virus (VZV), usually many years after primary VZV infection (chickenpox) that typically occurs during childhood [1]. HZ is characterized by a painful unilateral dermatomal vesicular rash. The most frequent complication is postherpetic neuralgia (persistent pain after resolution of the rash), which can last for months or years [1,2]. The incidence of HZ increases with age, and HZ is most frequent in adults aged ≥50 years [1,2]. Similarly, the incidence of postherpetic neuralgia increases with age [3]. HZ is also more frequent in persons with immunocompromising conditions [2]. Reactivation of latent VZV is believed to occur when VZV-specific cell-mediated immunity (CMI) falls below

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a critical threshold, either because of aging or immunosuppression [1,2,4,5]. A live attenuated vaccine against HZ (Zostavax®, Merck & Co, Inc.), containing a high concentration of the live Oka VZV vaccine strain, is licensed for adults aged ≥50 years [1,6]. However, it is contraindicated for immunocompromised persons and its efficacy against HZ decreases with age [3,7]. Efficacy was 63.9% in adults aged 60–69 years but decreased to 37.6% in adults aged ≥70 years [3]. Moreover, efficacy of Zostavax® against HZ decreases gradually after vaccination, from 62.0% at year 1 to 43.1% at year 5 in adults ≥60 years of age [8], and remained statistically significant only through year 8 after vaccination [9]. Recombinant subunit vaccines are alternatives to live attenuated vaccines, notably because of their high immunogenicity when administered with an adjuvant [10]. VZV glycoprotein E (gE) is an attractive candidate antigen because it is a prominent target of VZV-specific CD4+ T-cell responses [11–13]. An investigational recombinant subunit vaccine containing VZV gE and the AS01B adjuvant system (GSK Vaccines) is currently being evaluated for the prevention of HZ in older adults and in patients with immunocompromising conditions. A phase II clinical trial was conducted in adults ≥60 years of age to evaluate different formulations of this candidate vaccine (containing 25 µg, 50 µg, or 100 µg gE combined with AS01B or saline) using different schedules (one or two doses). This trial showed that two doses of all the adjuvanted vaccine formulations in older adults had clinically acceptable safety profiles and elicited robust cellular and humoral immune responses that persisted for up to 3 years after vaccination [14]. Furthermore, immunogenicity changed little with increasing age [14,15]. Based on the results of this and other clinical trials [14,15], the 50 µg gE/AS01B formulation (herein referred to as HZ/su) was selected for further clinical development. Recently, a randomized, observer-blind, placebo-controlled phase III study demonstrated that HZ/su efficacy against herpes zoster was 97.2% (95% confidence interval, 93.7–99.0) in adults ≥50 years of age after a mean follow-up of 3.2 years, and that vaccine efficacy did not decrease with increasing age [16].

To investigate the potential of this candidate vaccine to provide long-term protection against HZ, we assessed the persistence of vaccine-induced immune responses between years 4 and 6 after vaccination in subjects who received two doses of HZ/su.

2. Patients and methods

2.1. Study design and subjects

This follow-up study was a phase II, open-label, multicenter, single-group trial conducted in the Czech Republic, Germany, Sweden, and the Netherlands (ClinicalTrials.gov, NCT01295320) between February 28, 2011 and June 20, 2013. This trial followed subjects who had received two doses of HZ/su 2 months apart during a single-blind, randomized, controlled trial that was completed in July 2010 (ClinicalTrials.gov, NCT00434577) [14]. The study protocol was approved by the national independent ethics committees of the participating countries and was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. Written informed consent was obtained from all subjects before study entry. All the subjects who had received two doses of HZ/su 2 months apart (i.e., the 50 µg gE/AS01B group) in the initial trial were eligible for inclusion in the follow-up trial. Subjects were excluded if they had participated (or planned to participate) in another trial in which they were exposed to an investigational or non-investigational product (pharmaceutical product or device) after the end of the initial study; had received immunoglobulins or any blood products within the 3 months preceding the first blood draw; had received a vaccine containing 3'-desacyl-4'-monophosphoryl lipid A (MPL) or Quililja saponaria Molina, fraction 21 (QS21; Antigenics Inc., a wholly owned subsidiary of Agenus Inc., Lexington, MA) after the end of the initial study.

2.2. Study vaccine

No vaccine was administered in this study. In the initial trial, the subjects were vaccinated with two doses of HZ/su (GSK Vaccines) at months 0 and 2 [14]. HZ/su contains 50 µg of VZV gE and the liposome-based adjuvant system AS01B, which contains the immunoenhancers MPL and QS21 (50 µg each). The vaccine was administered intramuscularly (0.5 mL) in the deltoid region.

2.3. Assessment of immunogenicity

The cellular and humoral immune responses induced by the vaccine were evaluated in blood samples collected 48, 60, and 72 months after the first dose of HZ/su. The frequencies of antigen-specific CD4+ T cells expressing at least two activation markers among interferon-γ, interleukin-2, tumor necrosis factor-α, and CD40 ligand (herein referred to as CD4[2+] T cells) per 106 cells were measured by intracellular cytokine staining after in vitro stimulation with gE or with VZV and detection by flow cytometry as previously described [14]. Serum anti-gE antibody concentration (mIU/mL) was measured by a GSK in-house enzyme-linked immunosorbent assay (ELISA) with an assay cut-off of 18 mIU/mL.

2.4. Assessment of safety

Fatal serious adverse events (SAEs), SAEs related to study participation or study vaccine, potential immune-mediated inflammatory diseases, and suspected HZ episodes were recorded between months 36 and 72. Subjects were asked to contact the investigator immediately if they manifested any signs or symptoms that they believed to be serious or if a suspected HZ rash occurred. In addition, the investigator asked about the occurrence of AEs at each visit or contact during the whole study period.

2.5. Statistical analysis

The primary objective of the study was to evaluate cell-mediated and humoral immune responses to HZ/su in healthy older adults overall and for each age cohort (60–69 years and ≥70 years of age) at 48, 60, and 72 months after the first dose of HZ/su. The secondary objectives were to evaluate the safety of HZ/su in healthy older adults (60–69 years and ≥70 years of age) at months 48, 60, and 72 and to collect clinical data on suspected HZ cases. Only descriptive analyses were performed. Unless otherwise specified, data are presented as medians with the first and third quartiles (Q1–Q3).

Immunogenicity was analyzed on the according-to-protocol (ATP) cohort for immunogenicity, which included all evaluable subjects excluding those who reported an HZ episode during the study. The frequency of gE-specific CD4[2+] T cells was calculated as the frequency of CD4[2+] T cells upon in vitro stimulation with gE minus the frequency of CD4[2+] T cells upon stimulation with medium alone (background). Safety was analyzed on the total cohort for persistence, which included all subjects.

3. Results

3.1. Subjects

Among the 714 subjects enrolled in the initial trial, 166 subjects were vaccinated with HZ/su (i.e., the 50 µg gE/AS01B vaccine group)
and 147 completed the study to month 36 [14]. Of the 146 subjects eligible for the current trial, 17 subjects were not willing or able to participate. Thus, 129 subjects who had received two doses of HZ/su during the initial trial were enrolled in the current trial (Fig. 1). Of these, 57 were from Germany, 32 from Sweden, 26 from the Czech Republic, and 14 from The Netherlands, and 119 (92.2%) completed the study. Reasons for withdrawal were lost to follow-up (n = 4), an SAE (death; n = 2), consent withdrawal (n = 2), or inability to attend a visit (n = 2). Three subjects were excluded from the ATP cohort for immunogenicity: two subjects received interfering concomitant medications and one subject reported an HZ episode.

Demographic characteristics of subjects enrolled in this long-term follow-up study were not different from those of subjects enrolled in the initial study (Table 1). The mean age of the subjects at first vaccination in the total cohort for persistence was 72.8 years (range, 60–84 years). Most subjects were ≥70 years of age (n = 103, 79.8%). The overall proportion of women was 60.5%, and all but one subject was Caucasian (99.2%).

### 3.2. Cell-mediated immune responses

In the initial study, the frequency of gE-specific CD4[2+] T cells peaked at month 3 (i.e., one month after the second HZ/su vaccination) and decreased by approximately 50% at month 12 [14]. During the course of the current study, the CMI response began to plateau beginning approximately 48 months after the first vaccine dose. The median CD4[2+] T cell frequencies (per 10⁶ cells) decreased by approximately 25% between month 36 (640.0; Q₁–Q₃, 403.0–1405.4) and month 72 (477.3; Q₁–Q₃, 231.4–1037.0), but they remained higher than the prevaccination level (119.4; Q₁–Q₃, 67.8–286.9) (Fig. 2A). At month 72, the median gE-specific CMI response was still 3.8 times higher than the prevaccination value. The frequencies of gE-specific CD4[2+] T cells decreased in both age groups in parallel but they were generally lower in subjects ≥70 years of age than in those 60–69 years of age (Fig. 2B).

Similarly, the median frequencies of VZV-specific CD4[2+] T cells decreased by 42% between month 36 (555.5; Q₁–Q₃, 266.7–998.2) and month 72 (322.7; Q₁–Q₃, 180.1–667.0) (data not shown).

### 3.3. Humoral immune responses

All subjects were seropositive for anti-gE antibodies before vaccination [14] and remained positive at all time points after vaccination up to month 72. Median anti-gE antibody concentrations were highest at month 3 and appeared to level off starting
at about month 24, with a gradual decrease by approximately 20% between months 36 and 72 (Fig. 3A). Antibody concentrations remained above prevaccination values for up to 6 years after the first vaccination. At month 72, the anti-gE antibody concentration (8159.0 mIU/mL; Q₁–Q₃, 5451.2–12212.4) was 7.3 times higher than the prevaccination value (1121.3 mIU/mL; Q₁–Q₃, 624.2–2309.0). Anti-gE concentrations in both age groups (60–69 and ≥70 years of age) were similar (Fig. 3B).

3.4. Safety

Four SAEs were reported in three subjects between months 36 and 72. One subject had anemia and a concomitant acute flare of Crohn’s disease, both of which resolved. The other two SAEs were fatal: one subject died from a cardiovascular event and the other subject died of an unknown cause. None of the SAEs were considered related to vaccination by the investigators.

Two subjects, both ≥70 years of age, developed a potential immune-mediated disease following vaccination with HZ/su: one case of polymyalgia rheumatica and the case of Crohn’s disease described above. The former occurred more than 4 years after vaccination and the latter more than 5 years after vaccination. Both SAEs were considered unrelated to vaccination by the investigators. One 89-year-old subject reported a suspected HZ episode during this long-term follow-up, more than 5 years after vaccination. The episode lasted for 15 days and the patient recovered without sequelae.

4. Discussion

This study adds an additional 36 months of immunopersistence data following vaccination with HZ/su to the previously reported results [14]. Together, the initial and current studies provide a 72-month follow-up assessment of the persistence of the immune responses induced by two doses of HZ/su and show that these immune responses persist for up to 6 years in healthy older adults.

Both cell-mediated and humoral immune responses were highest at one-month following the second vaccine dose and then declined until they began to level off at about month 24 (for the humoral response) or month 48 (for the cell-mediated response). Most of the decline in both cellular and humoral responses occurred during the first year following vaccination. The cell-mediated and humoral gE-specific immune responses remained above the prevaccination levels through month 72. These are currently the longest immunopersistence data available for HZ/su. Given the results of this study, even longer follow-up will be required to experimentally determine the durability of humoral and cellular immune responses to HZ/su. Additionally, the available data may
be used to model the long-term persistence of HZ/su immune responses.

The immune responses to the HZ/su vaccine in subjects vaccinated at age 60–69 years and ≥70 years were comparable at all time points, indicating little impact of age on either the peak immune response levels or the persistence of cellular and humoral immune responses to HZ/su. These findings are consistent with those of previous studies using different schedules or formulations of the adjuvanted gE subunit vaccine candidate [15,17]. This also suggests that HZ/su is able to overcome the effects of immune senescence, which is believed to contribute to reduced immune responses to some vaccines in older adults [18,19]. However, in the absence of an established immunological threshold of protection for HZ [20], a correlation between the immune response levels described here and clinical protection against HZ should not be inferred. Although the first phase III study of HZ/su showed that the vaccine had 97.2% efficacy against herpes zoster in adults ≥50 years of age approximately after 3 years of follow up [16], long-term follow-up studies will be needed to determine the duration of the protection induced by HZ/su, especially in older adults who respond less well to vaccination in general and for whom the medical need is the greatest [5,21].

The HZ/su vaccine had a clinically acceptable safety profile in this population of older adults. Over the whole study period, reported SAEs were consistent with expectations for this population of adults aged 60 years or older at the time of vaccination, such as cardiovascular disorders and cancers [22]. No SAEs were considered related to vaccination. Also, no safety concerns related to immune-mediated diseases were identified between month 36 and month 72. These results are consistent with those observed during the 36 month follow-up period of the initial study [14].

This study has some limitations. First, the study was open and only subjects vaccinated with HZ/su were included. Thus, the persistence of the immune responses could be compared only to subjects' prevaccination levels and not to a control group. Second, this study was conducted in healthy immunocompetent adults, which may not fully represent the overall older population. It is worth noting, however, that HZ/su has demonstrated its ability to stimulate robust immune responses in two different immunocompromised populations: HIV-infected adults and adult autologous hematopoietic stem-cell transplant recipients [23,24]. In addition, this study was conducted in four European countries with similar socio-economic and ethnic characteristics, and other populations may thus respond differently to this vaccine [25]. Finally, although the number of subjects was sufficient to provide robust immunogenicity results, the evaluation of the incidence of SAEs, potential immune-mediated diseases, and HZ cases was limited by the small number of subjects. Larger studies would be needed to detect rare AEs.

In conclusion, although the cellular and humoral immune responses induced by two doses of HZ/su decreased over time, they remained substantially above prevaccination levels for 6 years. These results suggest that the vaccine may have the potential to provide long-term protection against HZ in older adults, although this needs to be established in clinical efficacy studies.

**Contributors**

R.C., K.P., L.R., G.V.R., J.H.R., G.P., and T.F.S. contributed to the data collection, data interpretation, and critical review of the manuscript. G.C. did the statistical analyses and contributed to data interpretation and critical review of the manuscript. H.L. and T.C.H. contributed to the study design, data analysis, data interpretation, and critical review of the manuscript. All authors read and approved the final manuscript.

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**Conflict of interest statement**

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