Therapeutic Vaccine for Genital Herpes Simplex Virus-2 Infection: Findings from a Randomized Trial

Running title: Therapeutic Vaccine for Genital Herpes

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ABSTRACT

Background. Genital herpes simplex virus (HSV) infection causes recurrent lesions and frequent viral shedding. GEN-003 is a candidate therapeutic vaccine containing HSV-2 gD2ΔTMR and ICP4.2, and Matrix-M2 adjuvant.

Methods. Persons with genital herpes were randomized into three dose cohorts to receive three intramuscular doses 21 days apart of 10 µg, 30 µg, or 100 µg of GEN-003, antigens without adjuvant, or placebo. Participants obtained genital area swabs twice-daily for HSV-2 detection and monitored genital lesions for 28-day periods at baseline and at intervals after the last dose.

Results. One hundred and thirty-four persons received all three doses. Reactogenicity was associated with adjuvant, but not antigen dose or dose number. No serious adverse events were attributed to GEN-003. Compared to baseline, genital HSV-2 shedding rates immediately after dosing were reduced with GEN-003 (30 µg, from 13.4% to 6.4% [$p<0.001$]; 100 µg, from 15.0% to 10.3% [$p<0.001$]). Lesion rates were also significantly ($p<0.01$) reduced immediately following immunization with 30µg or 100µg GEN-003. GEN-003 elicited increases in antigen binding, virus neutralizing antibody, and T cell responses.

Conclusions. GEN-003 had an acceptable safety profile and stimulated humoral and cellular immune responses. GEN-003, 30 µg and 100 µg, reduced genital HSV shedding and lesion rates.

Clinical Trials Registration. www.ClinicalTrials.gov number: NCT01667341 (funded by Genocea).

Keywords: genital herpes; herpes simplex virus type 2 infection; therapeutic vaccine; GEN-003; immunotherapy

BACKGROUND

Herpes simplex virus type 2 (HSV-2) is a common sexually transmitted infection that often results in recurrent genital lesions. Transmission occurs largely from asymptomatic genital tract virus shedding, making this a key target for a therapeutic HSV vaccine. The management of genital herpes currently includes episodic or daily suppressive therapy with nucleoside analogues which abrogates most recurrences but only partly reduces viral shedding and transmission [1, 2].

GEN-003 contains a transmembrane deletion mutant of glycoprotein D (gD2ΔTMR), a primary target antigen for neutralizing antibody and T cells, combined with a large fragment of infected cell protein 4 (ICP4.2), an HSV-2 T cell antigen prioritized through human T cell screens. This antigen was selected by comparing T cell responses of HSV-2-seropositive but asymptomatic and HSV-2-exposed seronegative individuals to individuals with recurrent disease using high-throughput HSV-2 proteomic screens [3]. GEN-003, is a combination of these two proteins with a novel adjuvant, Matrix-M2 [4]. Preclinical studies have demonstrated that GEN-003 elicits antibody and T cell responses in mice and is protective in a guinea pig model of recurrent HSV-2 infection [5].

We conducted a double-blind, placebo-controlled, dose-escalation Phase 1/2 study of GEN-003 to evaluate the safety, immunogenicity and effect on viral shedding of this candidate immunotherapy (ClinicalTrials.gov number, NCT01667341).

METHODS

Study Participants

We recruited healthy, HSV-2 seropositive adults 18 to 50 years of age with a history of recurrent genital herpes for at least 1 year and 3 to 9 recurrences per year, in the absence of antiviral suppressive therapy. HSV-2 infection was documented by Western blot, HSV-2 HerpeSelect® 2 ELISA IgG (>3.5 index value),

HSV-2 IgG LIAISON® assay, or type-specific culture or PCR and confirmed for all subjects during screening by HSV-2 IgG LIAISON® assay. Exclusion criteria included: immunocompromised state, antibody to HIV or Hepatitis C, presence of hepatitis B surface antigen, pregnant or nursing, history of genital HSV-1 infection, ocular HSV infection, HSV-related erythema multiforme, HSV meningitis or encephalitis, or prior receipt of HSV-2 vaccine. Effective contraception was required throughout the study.

Study Vaccine

GEN-003 is a purified protein subunit vaccine consisting of a transmembrane deletion mutant of gD (gD2ΔTMR) and a large fragment of infected cell protein 4 (ICP4.2) from HSV-2 (strain G) [5]. GEN-003 also contained Matrix-M2 (Novavax, Gaithersburg, MD), 50 µg per dose. Dulbecco's phosphate buffered saline (DPBS), was used as diluent (or as placebo) to achieve a volume of 0.5 mL for intramuscular administration. For the antigen only assigned subjects, Matrix-M2 was not added to the antigens.

Study Procedures

After obtaining informed consent but prior to randomization and dosing, participants obtained genital swab samples twice daily for 28 days and maintained a diary of genital lesions (baseline) [6]. To be eligible for randomization, subjects were required to provide a minimum of 44 baseline shedding swabs. The swabbing procedure was repeated immediately after the third dose of vaccine and from weeks 29 to 33, and weeks 53 to 57 (referred to as 6 and 12 months after the last dose of vaccine, respectively). The initial protocol specified only the first post-dosing shedding evaluation and was later amended to include 6 and 12-month collection periods.

Participants were randomized into 3 cohorts sequentially, defined by antigen dose (10, 30, or 100 µg of each antigen, Figure 1). Within each dose cohort, participants were randomized at a 3:1:1 ratio to receive either complete GEN-003, HSV-2 antigens without the adjuvant (antigens only), or placebo. Subjects were randomized in blocks of 10 to assure an assignment to GEN-003, antigens only, and placebo at a ratio of 3:1:1 by a computer-based system. The study product was administered intramuscularly in the deltoid approximately 21 days apart. Study vaccine was prepared and administered by unblinded staff not involved in subsequent study procedures. Investigators and other clinic personnel, as well as subjects were blinded to treatment assignment. Clinical laboratory evaluation was performed prior to treatment and at intervals thereafter. Blood was drawn for assessment of cellular immune response at baseline, 7 days after each dose, and 6 and 12 months after the last dose. Antibody responses to each antigen were evaluated on the day of each dose, and 6 and 12 months after the last dose.

Subjects were followed for safety until 12 months after the last scheduled dose. Adverse events (AEs) were captured from the first immunization until 28 days after the last dose. Solicited AEs including those generally associated with immunization were recorded by subjects for 7 days after each immunization. Serious AEs (SAEs), and Adverse Events of Special Interest (AESI), consisting of a pre-defined list of autoimmune disorders, were recorded through the end of the study. All AEs were graded by severity according to specified criteria (http://www.fda.gov/biologicsbloodvaccines/guidancecomplianceregulatoryinformation/guidances/vaccines/ucm074775.htm).

Study Oversight

The study was designed collaboratively by investigators at Genocea Biosciences and University of Washington in accordance with the ethical principles of the Declaration of Helsinki and the principles of

current Good Clinical Practices. An independent Data and Safety Monitoring Board reviewed the cumulative data by dose cohort at specified intervals. The efficacy analyses reported were conducted by external statisticians and independently at the University of Washington, according to a previously developed statistical plan. Both academic and industry authors contributed to manuscript drafts and approved the submission of the final manuscript. The study authors had full access to all data, verified their accuracy, and vouch for the fidelity of the study to the protocol and the completeness of the data presented. The study was approved by the institutional review board at each study center and each participant provided written informed consent.

Laboratory Methods

HSV-2 DNA PCR. Genital swabs were tested for the presence of HSV-2 DNA using a quantitative real time polymerase chain reaction (PCR) method [5]. Each plate included four negative controls including two extraction and two PCR controls, and four 10-fold dilutions of HSV-2 strain 333 (positive controls). The limit of detection was 2 DNA copies per 20 µL reaction with linearity ($R^2 = 0.98$) over 5 logs of HSV-2 genomic DNA content.

Antibody assays. IgG antibody responses directed against each of the GEN-003 antigens were measured by endpoint ELISA titer, as published previously [5]. HSV-2 neutralizing antibody titers were determined via a β-galactosidase (β-gal) colorimetric assay [5].

Cellular immunity assays. Gamma interferon enzyme-linked immunospot assays (ELISPOTs) were performed as previously described with modifications for assays with human peripheral blood mononuclear cells [5].

Statistical Methods

The primary endpoint of the study was safety and tolerability, assessed by the frequency, nature, duration and severity of adverse events by the study group. The secondary endpoints included the reduction in HSV shedding rate in participants following immunization, compared to baseline. Other secondary endpoints included the humoral and cellular immune responses to GEN-003 and the ability of Matrix-M2 adjuvant to promote T cell responses. Reduction in genital lesions was an exploratory endpoint.

Efficacy and safety analyses were conducted on a modified intent-to-treat population that included all participants who received at least one dose of study vaccine. Because of the small number (approximately 10 each) assigned to the unadjuvanted dose and placebo groups within each dose cohort, results from these groups were reported as combined for all analyses except immune response analyses.

Shedding rate was calculated as the number of positive swabs divided by the total number of swabs tested. An analysis of change from baseline in shedding rate (rate ratio) was performed using a previously described longitudinal Poisson mixed model with a random intercept to test for differences from baseline to each post-baseline time period within treatment group [6]. The model has the total positive swabs as the dependent variable and includes terms for treatment group, visit, treatment group by visit interaction, log of total swabs collected (offset) and a random intercept. With this model, the sample size of 30 subjects per treatment group was selected to detect a 30% reduction in the shedding rate of HSV-2 compared to baseline, with a power of 80% and a 2-sided α of 0.05, and assuming a 20% shedding rate at baseline. More recently, the statistical model was modified to include empirical variance estimate, as type I error was found to be greater than 5% using the previous method. A recent study by Magaret comparing available methods for evaluating crossover studies for viral detection indicated that up to a 28% type I error was possible with previous methods but that type I error was controlled at 5% and power maximized using the empirical variance structure [7]. Since these simulation results were not available either at the

time of the writing of the statistical analysis plan or when the study data became available for analysis, we have included the primary analysis as well as updated analysis (see Supplementary Results and Table S1). Because few subjects contributed data at 12 months, and the Poisson model failed to converge, this time-point was not included in the model and results are presented as descriptive only.

Change from baseline to each of the follow-up visits was analyzed for antibody titers and T cell responses using a linear mixed model. The model included terms for treatment group, baseline log titer, visit, treatment by visit interaction, and subject ID (random effect).

RESULTS

Study Population

The trial was conducted at 7 sites in the United States between July 2012 and April 2014. Of 204 persons screened, 143 (70%) were randomized and received at least one vaccination. The median age of participants was 37 (range 20–50); 62% were women (Table 1). One hundred and thirty-four (94%) participants received all 3 doses of the study product (Figure 1) and 130 provided genital swabs during the 28 days immediately following the scheduled third immunization and were included in the efficacy analyses. Some participants, including all participants in the 10 µg dose group, had completed the original protocol prior to an amendment to collect swab and blood samples 6 and 12 months after the last dose. Thus, 114 participants provided swabs at 6 months and 68 at 12 months following vaccination. Overall, the percent of expected swabs collected was 96% (per-person median 98%, range 71 to 100).

Safety and Tolerability of GEN-003

Local and systemic reactions within the first hour after immunization were infrequent, and the most common were pain, tenderness, induration and redness at the injection site. These were most frequent

among subjects receiving GEN-003, but all were graded as mild or moderate (data not shown). In the first 7 days following any of the 3 administered doses, 75% of placebo recipients, 100% of antigen only recipients, and 96 to 100% of GEN-003 recipients reported local reactogenicity at the site of administration (Figure 2; Table 2). The most common reported systemic AEs for any of the 3 administered doses were myalgia and fatigue: 43% and 46% for placebo, 54% and 61% for antigen alone and 82% and 71% for GEN-003, respectively, without significant differences across antigen dose levels. Neither local nor systemic reactogenicity increased with subsequent doses of the vaccine (Figure 2), or with the dose of the antigen (Table 2). Grade 3 or 4 solicited AEs were experienced by 11% of placebo recipients, 7% of participants in the antigen only groups and in 42%, 21%, and 22% of participants in the 10, 30, and 100 µg GEN-003 groups, respectively. The most common Grade 3 and 4 local AEs were pain, tenderness and induration, and for systemic AEs were nausea, fatigue and myalgias (Table 2). Participants who received GEN-003 had longer lasting adverse events, and reported using more analgesics following immunization than the participants in the antigen only or placebo groups. For example, pain was reported as a cumulative duration of 1.5 days (range: 1-2 days), 2 days (range: 1-7 days) and 3 days (range: 1-10 days) in the subjects receiving placebo, antigen only, and GEN-003, respectively. Three of 31 participants in the GEN-003 10 µg dose group, but none in the other GEN-003 groups, discontinued dosing because of systemic reactogenicity. Five unrelated SAEs were observed: femur fracture (GEN-003 10 µg, Day 185); suicide attempt (GEN-003 10 µg, Day 62); spontaneous miscarriage (placebo, Day 115); complex migraine (GEN-003 100 µg, Day 230) and myocardial infarction (GEN-003 100 µg, Day 370). No deaths or new onset immune-mediated diseases were noted.

Immunogenicity of GEN-003

Immunization with GEN-003 elicited IgG and T cell responses to gD2ΔTMR and ICP4.2 at all dose levels. IgG antibody responses were greatest with the 30 µg dose, while neutralizing antibody titers were the highest with the 100 µg dose. For each antigen dose level, the addition of Matrix-M2 increased the responses (Figure 3). T cell responses were highest for the lowest doses of GEN-003 (Figure 4). The IgG and T cell responses observed for both antigens in the GEN-003 groups after the third immunization were significantly higher than those in the same groups on Day 0 and those in the placebo group ($p<0.001$). A detailed description of immune responses to GEN-003, including the data collected at 6 and 12 months post immunization, has been published [8].

Effect of GEN-003 on Viral Shedding

Among all subjects, HSV was detected in 11.9% (933 of 7824) of swabs collected at baseline, and ranged from 7.4% in the antigen only group to 15.0% in the GEN-003 100 µg dose group (Table 3). In the placebo group, the rate ratio for viral shedding was unchanged immediately after immunization but appeared to increase at 6 months (RR=1.30, $p=0.012$). In the antigen only group, the shedding rate increased from baseline (7.4%) to 10.0% immediately after immunization (RR=1.59, $p=0.017$), and was 8.6% at 6 months (RR=2.22, $p<0.001$).

The shedding rate for participants who received GEN-003 10 µg was unchanged immediately following the immunizations, but increased at 6 months (RR=1.58, $p<0.001$; Table 3). Data at 12 months is not available for this dose group (see methods). In the GEN-003 30 µg group, the shedding rate was reduced 52% immediately following immunization (RR=0.47, $p<0.001$), and remained lower at 6 months (RR=0.58, $p<0.001$), returning close to baseline at 12 months. Immunization with GEN-003 100 µg was also followed by a reduction in shedding immediately after immunizations (RR=0.68, $p<0.001$) that was not significant at 6 months (RR=0.88, $p=0.224$), and returned close to baseline at 12 months. As observed

in similar studies of viral shedding, [9, 10] some subjects did not shed at baseline. This may explain the apparent increase in shedding after immunization among a few subjects (see Supplementary Figure S1).

Effect of GEN-003 on Genital Lesions

Lesion rates were similar before and after immunization for the placebo, antigen only and GEN-003 10 µg groups (Table 3). In the placebo group, a slight increase in the lesion rates at baseline was observed immediately after immunization where it remained at 6 months. Lesion rates then decreased at 12 months, but it should be noted that only a limited number of participants in the control group provided 12-month data (n=13). A similar pattern was observed in the antigen only group where lesion rates remained stable at 3 and 6 months but decreased at 12 months, possibly due to the smaller, more limited number of participants providing data (n=15). The lesion rates were reduced for the GEN-003 30 µg group from 9.7% to 5.0%, (RR=0.52, $p<0.001$), immediately after immunization and remained lower 6 and 12 months after immunizations (3.4% and 5.6%, respectively; RR=0.33, $p<0.001$ for the 6-month timepoint). The lesion rates in the GEN-003 100 µg dose group were reduced immediately after immunization from 6.8% to 3.7% (RR=0.53, $p=0.009$), but not thereafter.

As described in the methods, an alternative analysis of lesion and shedding rates using a Poisson regression method with empirical variance estimation can be found in Supplementary Table S1.

DISCUSSION

Our results of immunotherapy with GEN-003, a novel vaccine for genital herpes composed of HSV-2 gD2ΔTMR and a fragment of ICP4 protein (ICP4.2) adjuvanted with Matrix-M2, demonstrate that it had an acceptable safety profile, was immunogenic, and reduced both recurrent viral shedding and days with lesions at doses of 30 µg and 100 µg for each antigen. Despite the frequent mild to moderate AEs

associated with injections, and the percentage of Grade 3 AEs, only 3 participants discontinued from further dosing. The addition of the Matrix-M2 adjuvant was critical for boosting the cell-mediated response and was dose-sparing for the antibody response to the vaccine.

Most of the reactogenicity of GEN-003 was likely due to the addition of the adjuvant, Matrix-M2, to the viral antigens, and appeared acceptable for a therapeutic vaccine. Reassuringly, no events of special interest emerged during the study. While a few participants missed a day of work as a result of adverse events, study discontinuation caused by these events was rare, further indicating that the safety profile, as observed in this trial, was acceptable to participants.

Prior attempts to develop a therapeutic vaccine for HSV-2 were unsuccessful. Some of the prior candidate vaccines focused on eliciting IgG and neutralizing antibody responses to surface antigens [11]. It had been hypothesized that effective T cell responses are likely to be required to control intracellular pathogens such as HSV. However, in this study the level of T cells, at least as measured by gamma interferon ELISPOT did not correlate to the clinical reductions; the frequencies of responding T cells were highest in the 10 μg group which had little to no impact on viral shedding or lesion rates. Additionally, a replication defective (gH deletion) mutant virus failed in a double-blind, randomized study to reduce recurrence rates or viral shedding in subjects with genital HSV-2 although T cell immune responses were identified in animals [12, 13]. The specific T cell target may be critical to eliciting an effective immune response. We previously identified ICP4 as a potential T cell target from HSV-2 using a high throughput antigen-screening methodology [3].

GEN-003 elicited both humoral and cellular immune responses. Antibody titers directed against gD2ΔTMR and ICP4.2 increased after each dose [8] and peaked at 30 μg. Titers of neutralizing antibody were the highest in subjects who received the 100 μg dose. By contrast, T cell responses, measured by gamma interferon ELISPOT were maximal after the first dose, and appeared to be inversely related to

antigen dose [8]. A similar effect was identified in pre-clinical studies of immunogenicity in mice [5]. Chronic infections may induce high or chronic loads of antigen, and after vaccination, T cells may become anergised, exhausted or die of apoptosis [14]. Additional analyses are underway to explore the correlate of activity.

Viral shedding was used as the main efficacy outcome because it is an objective measure of mucosal HSV replication, its measurement has been previously used to demonstrate differences in efficacy between doses of antiviral therapies [6], and it is the primary mode for transmission [15]. Consequently, for dose selection of a therapeutic agent, shedding rates are most efficient and quantitative, keeping in mind that shedding was measured by QPCR and thus infectious virus was not measured directly. There are inherent limitations of comparing results from across trials due to differences in study design, patient populations, exclusion/inclusion criteria and endpoints measured. In the current study, the 6.4% of positive swabs detected in the 30 µg group after the last dose is similar to that observed in a study with valacyclovir (500 mg daily) of 4–6% [6].

Immunization with GEN-003 at 30 and 100 µg significantly reduced viral shedding and clinical disease (lesion rate) immediately after vaccination. At 6 months after immunization, significant reductions in viral shedding and lesion rates continued to be observed in the 30 µg group while only a reduction in lesion rate was observed in the 100 µg dose group. However, no such conclusion can be stated for the results at 12 months due to the limited number of subjects who provided data at this time. Changes between 6- and 12-month measurements may simply reflect this reduction in number of subjects, and further studies are required to assess the durability of response. Furthermore, although shedding is most certainly a requirement for transmission, no quantitative data exists to allow extrapolation from the observed reduction in shedding to a possible benefit on transmission. The durability of response is encouraging although the number of participants in the study at the 12-month evaluation period was small

and may explain the apparent fall in lesion rates observed in the control groups. Currently, the data indicate a durability of 6 months, an interval likely to be practical to implement for maintenance dosing of a therapeutic vaccine. Further studies are being conducted to better estimate true durability of effect. The importance of an effective HSV-2 vaccine has been reviewed recently in an article prepared for the WHO Product Development for Vaccines Advisory Committee (PDVAC) [16]. HSV and the complications associated with infections are such that an effective HSV vaccine will have significant clinical and economic impacts.

In conclusion, GEN-003, a novel therapeutic vaccine for HSV-2 currently in clinical development, was shown to have an acceptable safety profile, reduce viral shedding, reduce the frequency of genital lesions and boost the humoral and cellular immune responses to HSV-2. The optimal dose of the proteins and adjuvant, and the durability of the effect will require further study.

Notes

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Conflicts of interest statement: David I. Bernstein receives funding from Genocea for preclinical studies of vaccines. He has been a consultant to Genocea Biosciences, Vical, GlaxoSmithKline and Merck for herpes virus vaccines. Anna Wald is a consultant for Aicuris, Amgen, GlaxoSmithKline and received travel reimbursement from Admedus. She has received funds for sponsored projects for Genocea Biosciences and Vical. Terri Warren has served as the principal investigator on clinical trials related to

herpes vaccines with Vical, Genocea Biosciences, GlaxoSmithKline and Merck. Kenneth Fife receives research funding from Genocea Biosciences and Vical. Stephen K. Tyring has received research funding from Genocea for clinical studies on herpes simplex virus vaccines. Patricia Lee has received research funding from Genocea Biosciences for clinical studies on herpes simplex virus vaccines. Nick Van Wagoner has been a consultant for Genocea Biosciences for herpes virus vaccines. Amalia Magaret consults for Immune Design Corporation and for AiCuris. Jessica B. Flechtner is an employee and stock owner of Genocea Biosciences. Sybil Tasker was an employee of Genocea Biosciences at the time of the study. Jason Chan was an employee of Genocea Biosciences at the time of the study. Amy Morris is owner of IND2Results. Seth V. Hetherington is an employee and stock owner of Genocea Biosciences.

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Table 1. Demographic and Clinical Characteristics of Study Participants, by Study Arm

Characteristic	Placebo N=28	Antigen only N=28	GEN–003, 10 µg N=31	GEN–003, 30 µg N=29	GEN–003, 100 µg N=27
Median age, year (range)	36.5 (23–50)	37 (20–47)	37 (24–49)	38 (22–50)	34 (24–50)
Female sex, n (%)	17 (61)	17 (61)	21 (68)	18 (62)	15 (56)
Caucasian, n (%)	17 (61)	15 (54)	23 (74)	16 (55)	17 (63)
African American, (%)	10 (36)	8 (29)	5 (16)	10 (35)	8 (30)
HSV-1+/2+, n (%)	11/25 (44)	16/25 (64)	15/26 (56)	5/22 (22)	14/25 (56)
Median duration of genital herpes, years (range)	6.5 (1–26)	6.0 (0–24)[a]	6.0 (1–33)	8.0 (1–25)	6.0 (2–33)
Frequency of recurrences prior to study entry, [b] median per year (range)	5 (3–8)	5 (3–9)	5 (3–8)	5 (3–9)	5 (3–9)
Ever on suppressive therapy prior to study entry, n (%)	21 (75)	19 (68)	18 (58)	18 (62)	11 (41)
Swabs prior to immunization, total n (per person median, range)	1527 (55, 50–56)	1531 (56, 51–56)	1707 (56, 50–56)	1576 (56, 49–56)	1483 (56, 50–56)
Swabs post immunization, total n (per person median, range)	1373 (56, 37–56)	1365 (56, 31–56)	1361 (55, 13–56)	1414 (54, 26–56)	1346 (55, 27–56)
Swabs at 6 months, total n (per person median, range)	1287 (56, 45–69)	1183 (55, 41–64)	1414 (56, 40–68)	1017 (55, 46–57)	1323 (56, 36–69)

Swabs at 12 months, n (per person median, range)	730 (56, 50–62)	834 (56, 49–60)	–	1080 (56, 33–59)	1108 (56, 51–58)

[a]One subject had a formal diagnosis of genital HSV-2 infection only 6 months before randomization rather than 1 year as required by the inclusion criteria but had been symptomatic for much longer. Enrollment of this subject was approved by the Sponsor

[b]During year prior to initiation of suppressive therapy for those on suppressive therapy

Abbreviations: HSV, Herpes simplex virus; n, number

Table 2: Adverse Events Reported Following any Dose of Administered Study Treatment[a]

Event, n (%)	Placebo N=28	Antigen only N=28	GEN-003, 10 µg N=31	GEN-003, 30 µg N=29	GEN-003, 100 µg N=27
Total solicited adverse events	21 (75)	28 (100)	31 (100)	28 (97)	26 (96)
Grade 3 or 4 solicited adverse events, total	3 (11)	2 (7)	13 (42)	6 (21)	6 (22)
Injection-site reactions, Grade 3 or 4					
Pain	0	0	5 (16)	0	3 (11)
Tenderness	0	1 (4)	5 (16)	3 (10)	3 (11)
Induration	0	0	1 (3)	2 (7)	3 (11)
Redness	0	0	1 (3)	1 (3)	0
Systemic reactions, Grade 3 or 4					
Nausea	1 (4)	0	6 (19)	2 (7)	1 (4)
Vomiting	0	0	3 (10)	1 (3)	0
Diarrhea	0	0	0	0	0
Fatigue	3 (11)	2 (7)	7 (23)	2 (7)	4 (15)
Myalgia	1 (4)	0	8 (26)	3 (10)	4 (15)
Fever	0	0	3 (10)	0	1 (4)
Other adverse events					
Any adverse event	20 (71)	21 (75)	27 (87)	18 (62)	16 (59)
Grade 3 or 4	3 (11)	1 (4)	7 (23)	3 (11)	3 (11)

Serious adverse event	1 (4)	0 (0)	2 (6)	0 (0)	2 (7)
Adverse event leading to discontinuation	0 (0)	0 (0)	3 (10)	0 (0)	0 (0)

[a]All AEs were graded by severity according to specified criteria (http://www.fda.gov/biologicsbloodvaccines/guidancecomplianceregulatoryinformation/guidances/vaccines/ucm074775.htm)

Table 3. Genital HSV-2 Shedding and Lesions Rates Before and After Immunization

Characteristic	Placebo N=28	Antigen only N=28	GEN-003, 10 µg N=31	GEN-003, 30 µg N=29	GEN-003, 100 µg N=27
HSV shedding rate per swab, n/n (%)					
Pre-vaccine	190/1527 (12.4)	114/1531 (7.4)	185/1707 (10.8)	221/1576 (13.4)	223/1483 (15.0)
First period post immunization	176/1373 (12.8)	136/1365 (10.0)	147/1361 (10.8)	91/1414 (6.4)	138/1346 (10.3)
At 6 months	214/1287 (16.6)	102/1183 (8.6)	244/1414 (17.3)	81/1017 (8.0)	164/1323 (12.4)
At 12 months	90/730 (12.3)	120/834 (14.4)	NA	133/1080 (12.3)	123/1108 (11.1)
Rate ratio for HSV shedding relative to pre-immunization[a]					
First period post immunization	0.95	1.59[b]	1.06	0.47[c]	0.68[c]
At 6 months	1.30[b]	2.22[c]	1.58[c]	0.58[c]	0.88
Lesion rate, n/n (%)					
Pre-vaccine	59/820 (7.2)	76/804 (9.5)	128/873 (14.7)	79/816 (9.7)	52/761 (6.8)
First period post immunization	67/737 (9.1)	48/721 (6.7)	64/711 (9.0)	38/760 (5.0)	26/708 (3.7)
At 6 months	61/665 (9.2)	41/611 (6.7)	82/733 (11.2)	18/532 (3.4)	32/691 (4.6)
At 12 months	15/372	11/427	NA	31/556	33/569

	(4.0)	(2.6)		(5.6)	(5.8)
Rate ratio for lesions relative to pre-immunization[a]					
First period post immunization	1.21	0.90	0.76	0.52[d]	0.53[d]
At 6 months	1.32	1.23	0.91	0.33[c]	0.85

[a]A limited number of participants collected swabs and reported lesions in the Weeks 53 to 57 period, so the Poisson mixed model failed to converge for this period, thus, 12-month comparisons are not presented.

[b]$p<0.05$; [c]$p<0.001$; [d]$p<0.01$. An analysis of change from baseline in shedding rate was performed using a previously described longitudinal Poisson mixed model with a random intercept to test for differences from baseline to each post-baseline time period within treatment group [6]. The model has the total positive swabs as the dependent variable and includes terms for treatment group, visit, treatment group by visit interaction, log of total swabs collected (offset) and a random intercept.

Abbreviations: HSV, Herpes simplex virus.

Figure Legends

Figure 1. Consort diagram of the study.

Figure 2. Rates of local and systemic solicited adverse events within 7 days following vaccination by treatment assignment and dose number

Figure 3. Humoral immune responses following the third immunization measured on Day 63, 3 weeks post the third immunization. Values represent the geometric mean titer (GMT) with 95% confidence intervals (CI). A. IgG response to glycoprotein gD2ΔTMR; B. IgG response to ICP4.2; C. NAb response

* $p<0.001$ compared with placebo and compared with Day 0 in the same group

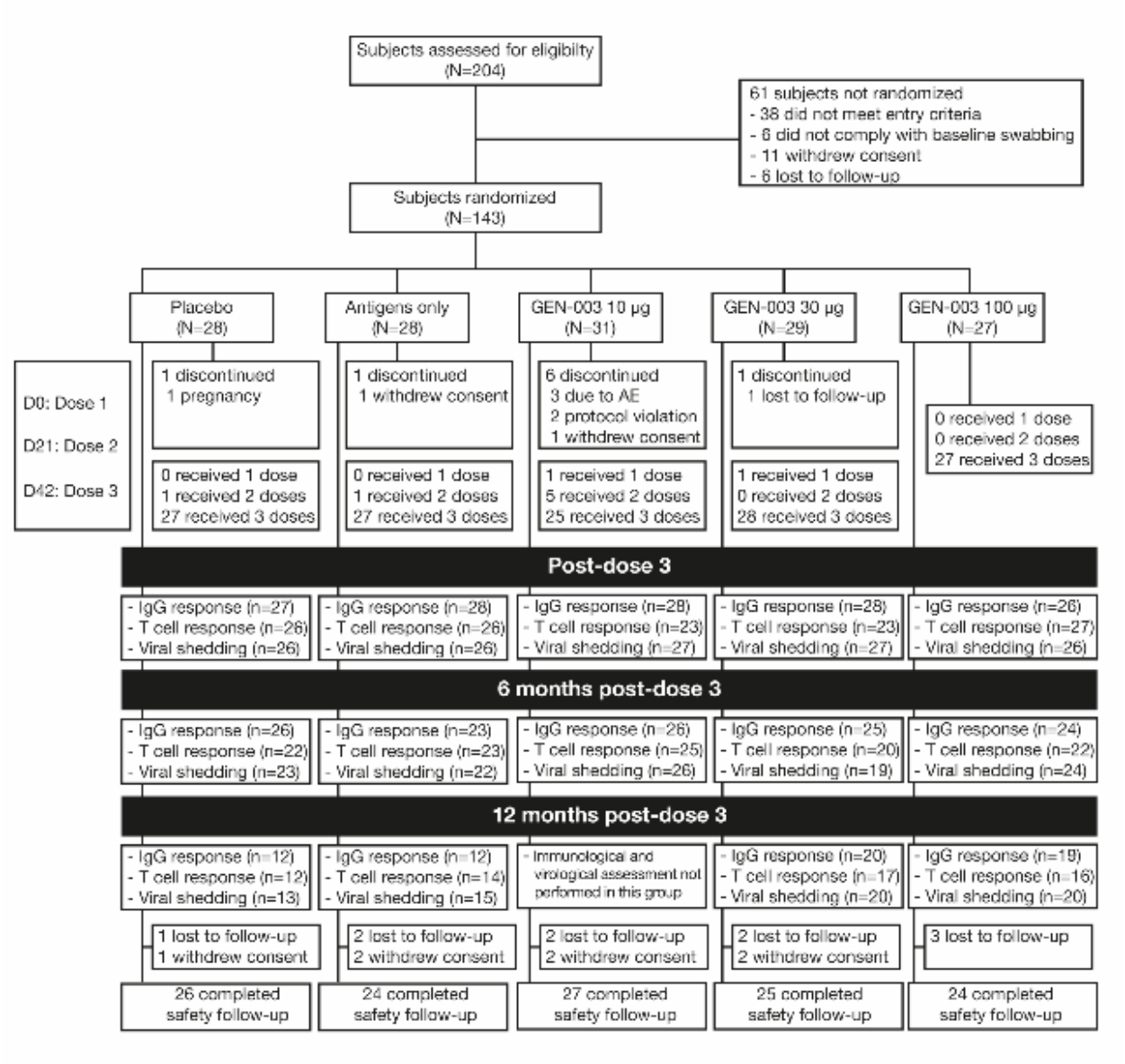
Abbreviations: CI, confidence interval; gD2ΔTMR, a transmembrane deletion mutant of glycoprotein D; GMT, geometric mean titer; ICP4.2, infected cell protein 4; Nab, neutralizing antibody.

Figure 4. Cellular immune responses measured on Day 49, 1 week post the third immunization. The mean spot-forming units (SFU)/10^6 cells with 95% CI are shown. A. Interferon-γ release to glycoprotein D2; B. Interferon-γ release to ICP4.2

* $p<0.001$ compared with placebo and compared with Day 0 in the same group

Abbreviations: CI, confidence interval; gD2, glycoprotein D2; ICP4.2, infected cell protein 4; IFN-γ, interferon-γ; SFU, spot-forming units.

Figure 1



Abbreviations: CI, confidence interval; gD2, glycoprotein D2; ICP4.2, infected cell protein 4; IFN-γ, interferon-γ; SFU, spot-forming units.

Figure 2

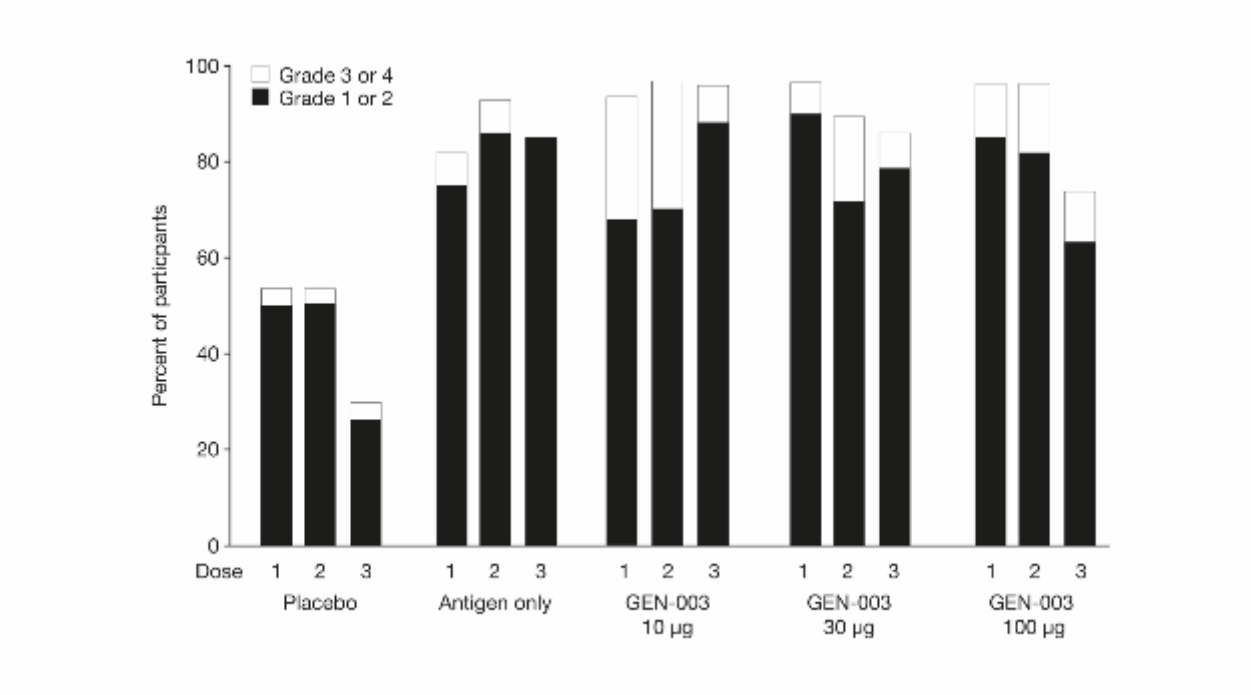
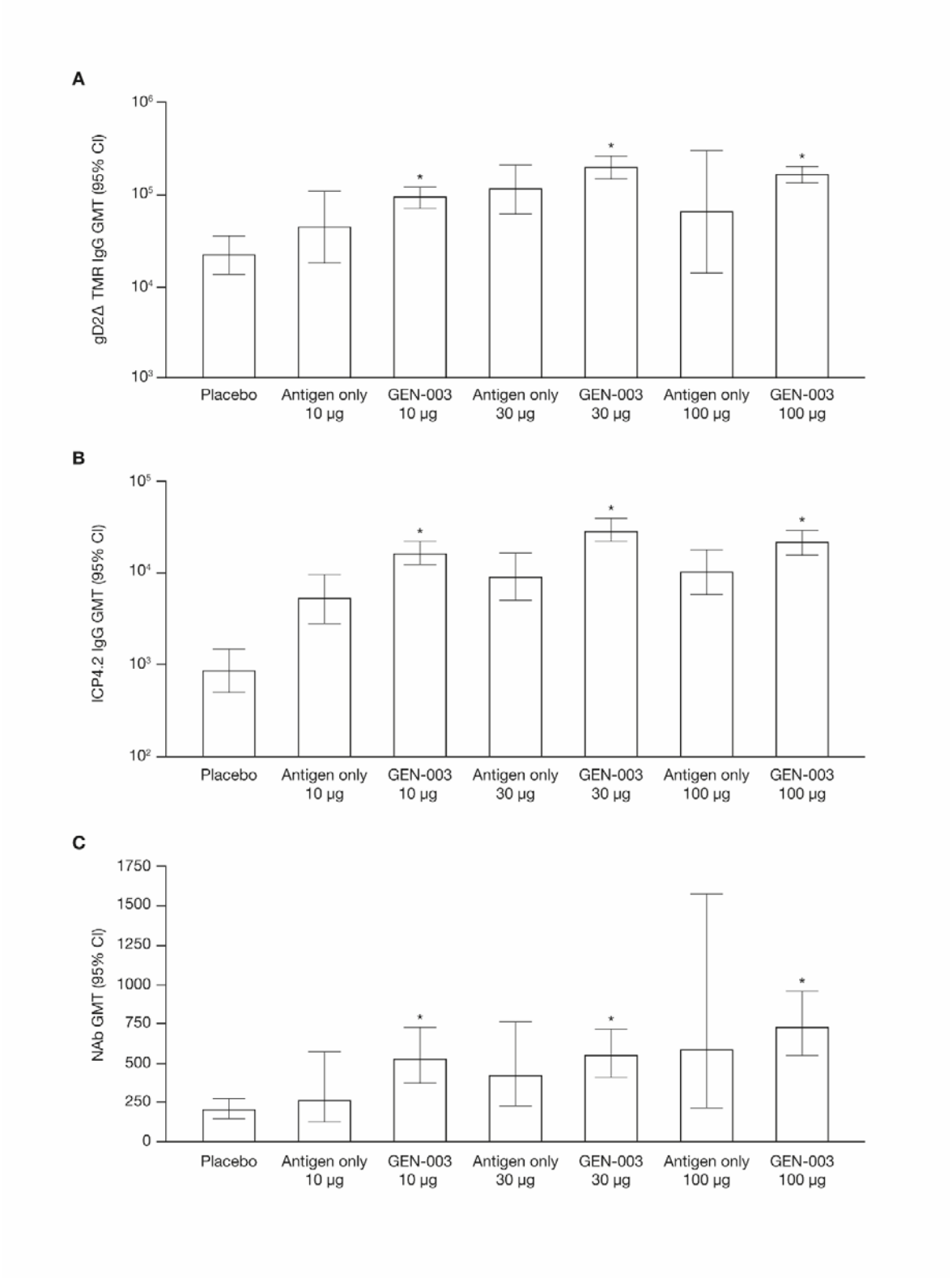
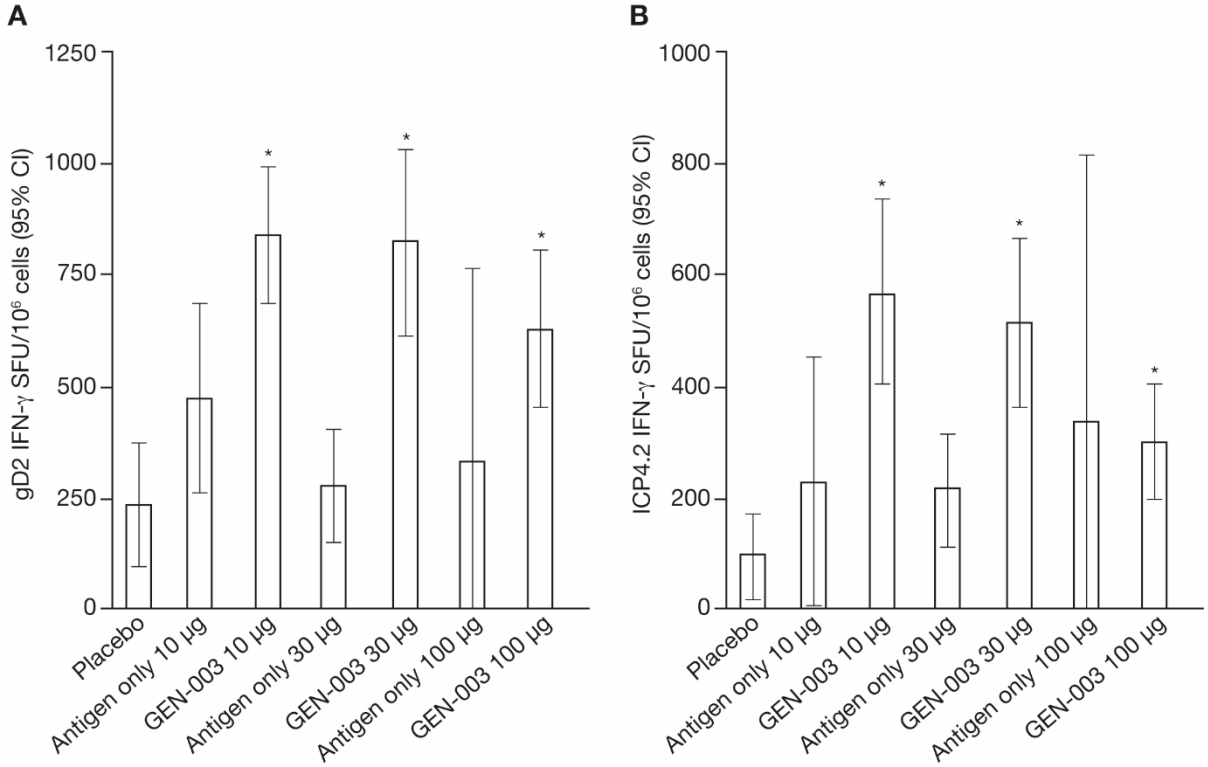

Grade 3 or 4
Grade 1 or 2
Percent of participants
100
80
60
40
20
0
Dose 1 2 3
Placebo
Antigen only
GEN-003 10 µg
GEN-003 30 µg
GEN-003 100 µg

Figure 3



* p<0.001 compared with placebo and compared with Day 0 in the same group

Figure 4



* p<0.001 compared with placebo and compared with Day 0 in the same group