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Therapeutic HSV-2 vaccine decreases recurrent virus shedding and recurrent genital herpes disease

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ABSTRACT

Background: Genital herpes simplex virus (HSV) type 2 is a common persistent infection that frequently reactivates to cause recurrent lesions and recurrent viral shedding which is incompletely controlled by antiviral therapy. GEN-003 is a candidate therapeutic vaccine containing 2 HSV-2 proteins, gD2 and ICP4, and Matrix-M2 adjuvant (M2).

Methods: HSV-2 seropositive persons with genital herpes were randomized into three dose cohorts of Gen-003 (60 μg antigen/50 μg M2, 60 μg/75 μg M2 or Placebo). Three intramuscular doses 21 days apart of GEN-003 or placebo were administered. Participants obtained genital area swabs twice-daily for HSV-2 detection and monitored genital lesions for 12 months. The rates of virus shedding and lesion rates before vaccination were compared to 3 defined periods after vaccination; Days 43–71, Month 6 and Month 12.

Results: GEN-003 at a dose of 60 μg each antigen/50 μg M2 reduced HSV shedding immediately after dosing with a rate ratio of 0.58, compared to 0.75 for the GEN-003 60 μg/75 μg M2 and 1.06 for placebo. Lesion rates, recurrence rates, and duration of recurrences were also reduced. Reactogenicity was higher with the 75 μg M2 dose than the 50 μg M2 dose, specifically for pain, tenderness, malaise and fatigue. Antibody and cellular immune responses were stimulated by both doses and persisted to 12 months.

Conclusions: GEN-003 vaccine manufactured with a scalable process gave results similar to those observed in prior clinical trials. GEN-003 had an acceptable safety profile and stimulated both humoral and cellular immune responses. The 60 μg antigen/50 μg M2 provided the maximal effect on virologic and clinical measures and warrants further development. (Funded by Genocea; ClinicalTrials.gov number *NCT02515175*).

1. Background

Herpes simplex viruses (HSVs) are the main cause of genital ulcers worldwide [1]. More than 400 million people worldwide are infected with HSV type 2 (HSV-2), which is primarily sexually transmitted while an estimated 140 million people have genital HSV type 1 (HSV-1) infection. There are no known curative treatments or therapeutic vaccines for HSV-2 infection. Current therapy includes episodic antiviral treatment to reduce the time to healing, and daily suppressive antiviral therapy to reduce the frequency of recurrences, recurrent virus shedding and the risk of transmission [2–4]. Therapeutic HSV vaccines are intended for people already infected with HSV with a goal to reduce clinical recurrences and recurrent virus shedding. Despite attempts to develop effective HSV therapeutic vaccines [5–9] none are currently available.

The antigens included in the GEN-003 therapeutic vaccine were selected by screening T-cell responses in asymptomatic HSV-2-positive or HSV-2-exposed but uninfected subjects to identify potential targets involved in controlling and limiting an active infection [10]. Those antigens identified were then evaluated in preclinical studies with different candidate adjuvants to select the components for inclusion in the vaccine [11]. The GEN-003 vaccine includes GB208, an internal fragment of the immediate early protein ICP4 (ICP4.2), and GB217, a deletion mutant of glycoprotein D2 (gD2) that lacks the transmembrane portion of the protein (gD2ΔTMR) combined with the adjuvant Matrix M2 (M2). M2 is a fixed combination of 2 defined fractions (A and C) from purified

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saponins derived from Quillaja saponin, incorporated into matrix particles containing phosphatidylcholine and cholesterol [12].

Previous studies of GEN-003-001, showed that it exhibited an acceptable safety and tolerability profile and elicited strong and durable antibody and T-cell immune responses to both vaccine-specific antigens. Further, the addition of M2 augmented these responses. In the first trial [13] a reduction in anogenital HSV-2 shedding that was maintained for at least 6 months after treatment was observed, with the greatest reduction observed with 30 μg GEN-003/50 μg M2 vaccine group. In the next study [14], a reduction in HSV-2 shedding and lesion rates was observed immediately after the last dose that persisted for 12 months. The most effective dose combinations in that trial was 60 μg GEN-003/50 μg M2 and 60 μg GEN-003/75 μg M2) [15]. In the trial reported here we further compared the virologic activity of these two doses manufactured by process suitable for large scale production and evaluated subjects for clinical disease over a 12-month period.

2. Methods

2.1. Study participants

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 were recruited at 9 sites. HSV-2 infection was documented by Western blot for HSV-2, HSV-2 HerpeSelect® 2 ELISA IgG (>3.5 index value), HSV-2 IgG LIAISON® assay, or type-specific viral culture or PCR. Exclusion criteria included: immunocompromised state, current infection with HIV or Hepatitis B or C, pregnant or nursing women, 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.

2.2. Study vaccine

GEN-003 is a purified protein subunit vaccine consisting of a transmembrane deletion mutant of gD (gD2ΔTMR, GB217) and a large fragment of infected cell protein 4 (ICP4.2, GB208) from HSV-2 (strain 333) [11]. The GEN-003 vaccine includes M2 adjuvant (Novavax, Gaithersburg, MD). Normal saline was used as the diluent and placebo. The process used to manufacture the vaccine for this trial were modified from that used in previous evaluations to improve purity and facilitate production in batches large enough to supply a phase 3 program. Of note, the affinity tags (histidine tags) used for purification of the antigens was removed and GEN-003 antigens were reformulated from a frozen liquid to a lyophilized form to allow storage at 2 °C to 8 °C.

We evaluated 60 μg of each GEN-003 protein with 50 μg of M2 (GEN/50 μg M2) or with 75 μg of M2 (GEN/75 μg M2).

2.3. Study procedures

As reported previously, after obtaining informed consent but prior to randomization and dosing, participants obtained genital swab samples twice daily for 28 days and maintained an electronic diary of genital lesions (baseline) [13,15]. To be eligible for randomization, subjects were required to provide a minimum of 45 baseline shedding swabs. Participants were then randomized into 3 cohorts (placebo, 60 μg GEN/50 μg M2, 60 μg GEN/75 μg M2 at a ratio of 1:1:1 and followed daily for recurrent lesions for 12 months. Vaccine was prepared and administered intramuscularly in the deltoid three times approximately 21 days apart by unblinded staff who did not participate in any subsequent evaluation of the participants. 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 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).

Anogenital swabs for measurement of viral shedding were collected twice daily for 28-day periods after Dose 3 (Days 43 to 71), during Months 5 to 6, and during Months 11 to 12 [16]. Swabs were stored frozen in PCR buffer [17]. Subjects also reported the first time they noted genital lesions, and were examined by study personnel to confirm the presence of genital lesions consistent with HSV. At this time a lesion swab was collected for detection of HSV-2 DNA. Subjects continued to report the presence or absence of genital lesions and the severity of genital herpes symptoms daily via electronic diary throughout the entire study period.

Initially, a pilot group of 12 subjects was randomized to receive GEN-003 with the higher amount of M2 adjuvant. Following a satisfactory review of the Day 7 safety data after the first dose by the Data Safety Monitoring Board (DSMB), randomization was opened to all groups.

2.4. Laboratory methods

HSV-2 DNA PCR. Genital swabs were tested for the presence of HSV-2 DNA using a paramagnetic, bead-based DNA extraction method (DNAdvance Kit, Beckman Coulter) and a quantitative real-time polymerase chain reaction (PCR) method [11] () . Each plate included four negative controls including two extractions and two PCR controls, and four 10-fold dilutions of HSV-2 strain 333 (positive controls). The limit of detection was 5 DNA copies per reaction (per 20 μl) or 1000 DNA copies per mL of sample with linearity ($R^2 = 0.9996$) over 5 logs of HSV-2 genomic DNA content. A positive swab was defined as one with mean HSV-2 DNA copies ≥1000 copies per mL.

Antibody assays. IgG antibody responses directed against each of the GEN-003 antigens were measured by endpoint ELISA titer, as published previously [11]. A positive IgG response was defined as ≥4-fold rise in ELISA antibody titers from Baseline. HSV-2 neutralizing antibody titers were determined via a β-galactosidase (β-gal) colorimetric assay [18]. A positive HSV-2 neutralizing antibody response was defined as ≥2-fold rise in antibody titers from Baseline.

At selected sites, whole blood samples were collected for evaluation of T cell responses. PBMCs were isolated from the blood within 8 h of sample collection and cryopreserved. GrB/IFN-γ FluoroSpot assays were performed to assess cellular immunity. After thawing, PBMCs were rested overnight at 37 °C. PBMCs at a concentration of 2×10^5 cells/well were then plated into 96-well PVDF plates that were pre-coated with anti-IFNγ and anti-GrB antibodies and stimulated in triplicate with media alone or overlapping peptides spanning each antigen. Plates were then incubated for 20 ± 2 h at 37 °C. FluoroSpot plates were then developed. Fluorescent spots were analyzed on an Autoimmun Diagnostika (AID) iSpot Reader System. Spot forming cells were averaged, background subtracted and normalized to 1×10^6 PBMCs.

Table 1
Demographic and clinical characteristics of study participants, by study arm.

	GEN-003 Antigens/Matrix-M2		
Variable	GEN/ 50 μg M2 (N = 43)	GEN/75 μg M2 (N = 44)	Placebo (N = 44)
Age (years)			
Mean (SD)	38.4 (7.97)	35.5 (7.4)	36.0 (7.78)
Range: Min, Max	25, 49	24, 50	21, 50
Sex n, (%)			
Female	28 (65.1%)	28 (63.6%)	23 (52.3%)
Male	15 (34.9%)	16 (36.4%)	21 (47.7%)
Race			
White	32 (74.4%)	29 (65.9%)	32 (72.7%)
Black	9 (20.9%)	14 (31.8%)	7 (15.9%)
Asian	2 (4.7%)	0	2 (4.5%)
Other	0	1 (2.3%)	3 (2.3%)
Ethnicity			
Not Hispanic or Latino	38 (88.4%)	38 (86.4%)	39 (88.6%)
Hispanic or Latino	5 (11.6%)	6 (13.6%)	5 (11.4%)
Disease Characteristics			
Time from initial diagnosis of HSV-2 to randomization (years)			
Mean (SD)	10.7 (8.7)	8.1 (6.7)	9.0 (9.8)
Range: Min, Max	1, 28	1, 31	1, 36
Number of episodes in last continuous 12-month period without suppression			
Mean (SD)	5.0 (2.0)	5.0 (1.7)	5.2 (1.7)
Range: Min, Max	3, 9	3, 9	3, 9
Previously treated with antiviral suppression therapy			
	25 (58.1%)	23 (52.3%)	19 (43.2%)
Currently taking antiviral suppression therapy			
	2 (4.7%)	8 (18.2%)	4 (9.1%)
Subject ever had oral lesions(s) or a diagnosis of HSV-1			
	10 (23.3%)	7 (15.9%)	10 (22.7%)
HSV-1 Serostatus			
Positive	17 (39.5%)	15 (34.1%)	16 (36.4%)

HSV-1 = herpes simplex virus type 1; HSV-2 = herpes simplex virus type 2; SD = standard deviation.

Table 2
Subject disposition by treatment group.

	GEN-003 Antigens/Matrix-M2		
Disposition	GEN/50 μg M2 (N = 43)	GEN/75 μg M2 (N = 44)	Placebo (N = 44)
Subject completed the study	34 (79.1%)	34 (77.3%)	36 (81.8%)
Subject completed all 3 doses	40 (93.0%)	41 (93.2%)	39 (88.6%)
Primary Reason for Discontinuation from Study			
Withdrew consent for reason other than AE	3 (7.0%)	3 (6.8%)	2 (4.5%)
Withdrew consent due to solicited or unsolicited AEs	0 (0.0%)	0 (0.0%)	1 (2.3%)
Lost to Follow-up	6 (14.0%)	7 (15.9%)	5 (11.4%)
Non-compliance	0 (0.0%)	0 (0.0%)	0 (0.0%)
Investigator discretion	0 (0.0%)	0 (0.0%)	0 (0.0%)
Sponsor request	0 (0.0%)	0 (0.0%)	0 (0.0%)
Primary Reason for Not Receiving All 3 Doses			
Safety, including solicited or unsolicited AEs	2 (4.7%)	2 (4.5%)	1 (2.3%)
Withdrew consent for reason other than AE	0 (0.0%)	1 (2.3%)	0 (0.0%)
Non-compliance	0 (0.0%)	0 (0.0%)	3 (6.8%)
Pregnancy	0 (0.0%)	0 (0.0%)	0 (0.0%)
Investigator discretion	1 (2.3%)	0 (0.0%)	1 (2.3%)
Sponsor request	0 (0.0%)	0 (0.0%)	0 (0.0%)

AE = Adverse event.

2.5. Statistical methods

The primary endpoint in this study was the reduction in viral shedding rate from baseline to days 43 to 71. Reduction in viral shedding rates from baseline to month 6 and month 12 was also assessed. A longitudinal Poisson mixed model with a random intercept using a log link was used to test for differences in virus shedding rates between baseline and each post-baseline swab collection period within treatment group as well as for a difference between each treatment group vs. placebo for each post-baseline swab collection period [13]. As a secondary supportive analysis, a Wilcoxon rank sum test, was used to compare shedding rates for each active treatment group vs. placebo by comparing the change from baseline to each post-baseline swab collection period,. The data presented is for the Modified Intent-to-Treat (mITT) population, which includes all randomized subjects who received at least 1 dose of vaccine.

Lesion rates was analyzed using the Wilcoxon rank sum test as lesions were evaluated daily. Individual participant's lesion rates were compared for treatment groups vs. placebo using Wilcoxon's rank sum test. Mean lesion duration was compared using the

Kruskal-Wallis test. Statistical significance was defined as two sided p-value <0.05 without correction for multiple testing.

Time to the first subject-reported recurrence after Dose 1 and Last Dose was estimated via Kaplan-Meier approach. The log rank test was used to compare time to first recurrence between active treatment groups and placebo. Additionally, Cox proportional hazards model was used to assess treatment effect after adjusting for baseline covariates (HSV-1 serostatus, sex, disease severity >6 vs ≤6 time outbreaks).. A Prentice, Williams, and Peterson gap time (PWP-GT) approach was employed, allowing for discontinuous intervals of risk.

The percentages of participants with a virologically confirmed recurrence (after Dose 1) were compared using a Kruskal-Wallis test. The proportion of participants in each treatment group who were recurrence-free from the last dose to Day 71, Month 6, and Month 12 was compared using a chi-square test for homogeneity of proportions.

Immune response comparisons were not included in the original analysis plan but were added post hoc. Means were compared by ANOVA followed by the Bonferroni-Sidak method to correct for the multiple comparisons

3. Results

3.1. Study population

After successful completion of the screening period (including provision of sufficient viral swab samples), 131 subjects were randomized. Demographic and HSV-2 disease characteristics were generally well balanced among the treatment groups (Table 1). Women outnumbered men in the study (60.3 vs 39.7%), a result that is consistent with the epidemiology of symptomatic HSV-2 disease and with previous trials of genital HSV-2 treatment. Most subjects were white (71.0%) or black/African-American (22.9%), and 12.2% of subjects were Hispanic or Latino. Few subjects discontinued dosing, and discontinuations were balanced among the treatment groups. At least 77% of subjects in each treatment group completed the study. In all treatment groups, at least 89% of randomized subjects received all 3 doses (Table 2).

3.2. Immunogenicity results

For each antigen, the IgG GMTs were similar in all the treatment groups before dosing, and the values in the placebo group subjects changed little over time (Peak responses shown in Fig. 1A). GMTs to GB208 peaked after dose 1 while for GB217) titers peaked after dose 3). Both then decreased at Months 6 and 12 but remained above baseline. Similarly, the HSV-2 neutralizing antibody titers were comparable in all the treatment groups before treatment and changed little over time in the placebo group (Fig. 1B). Neutralizing antibody titers in the vaccine groups peaked after dose 3then generally decreased at Months 6 and 12 but remained above a 2-fold increase through Month 12 in the 2 vaccine groups. There was no apparent difference between the 2 active treatment arms for IgG or neutralizing antibody titers.

For each antigen, the number of cells secreting IFN-γ, GrB and dual GrB/IFN-γ , was similar in all the treatment groups before dosing, and the values in the placebo group subjects changed little over time (peak γ responses shown in Fig. 1C). For the active treatment arms, IFN-γ, GrB and dual GrB/IFN-γ SFCs for each antigen peaked during the dosing period (Day 8 through 50) and then decreased at Month 6 but remained well above baseline through Month 12. The GEN/75 μg M2 treatment group peaked earlier and with a higher magnitude (at Day 8) than the GEN/50 μg M2 treatment group.

3.3. Efficacy results

3.3.1. Shedding results

Shedding rates are shown in Table 3 for each period of collection with comparison to baseline and to the placebo group and in Fig. 2 which shows the overall increase or decline in shedding rates compared to baseline. As measured by the primary endpoint, reduction in shedding rates immediately after Dose 3, vaccine groups had decreased shedding rates compared to placebo but the difference in rates was only significant for the GEN/50 μg M2 dose group ($P < 0.05$). During the Month 5 to 6 collection period, the shedding rate of the GEN/75 μg M2 treatment group remained decreased relative to baseline, but the shedding rate of the GEN/50 μg M2 group returned to the baseline rate. The placebo group shedding rate remained similar to baseline. During the Month 11 to 12 collection period, all groups including the placebo

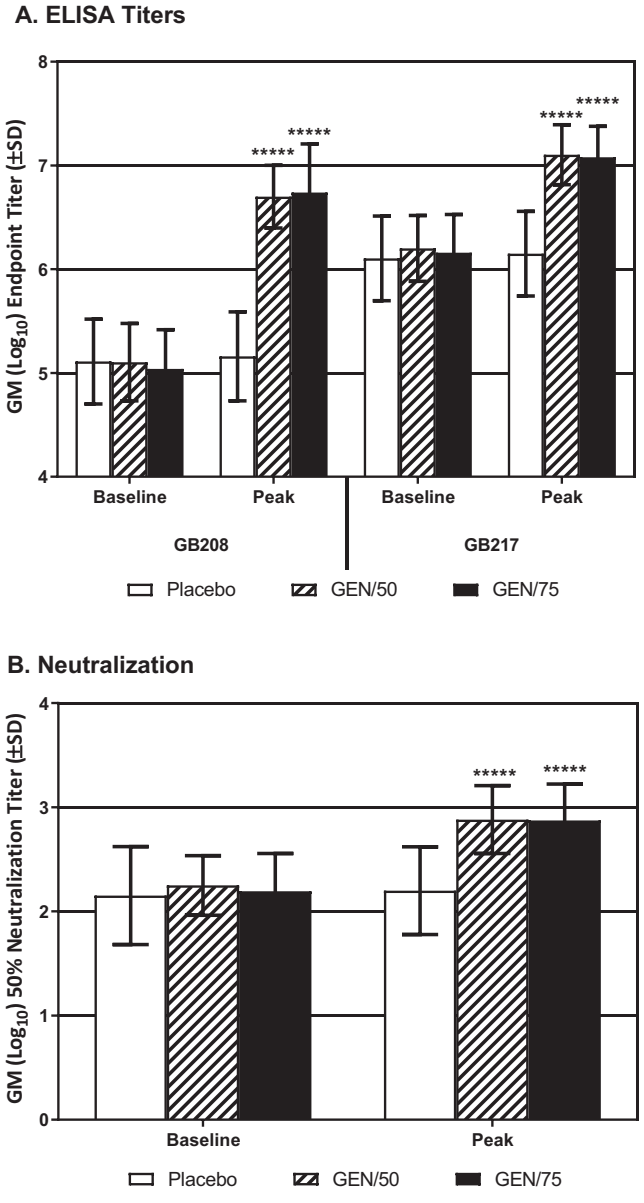

Fig. 1. Immune response to vaccine. A. Immunoglobulin G (IgG) response to GB208, an infected cell protein 4 (ICP4.2) and GB217 (glycoprotein gD2ΔTMR), a transmembrane deletion mutant of glycoprotein D. and. B. Neutralizing antibody response. C. Interferon γ (IFN-γ), granzyme B (GrB), or dual-secreting spot-forming T cells. GM, geometric mean; SD, standard deviation; ***** = $p < 0.0001$ by *t*-test using the Bonferroni-Sidak method comparing to baseline and placebo.

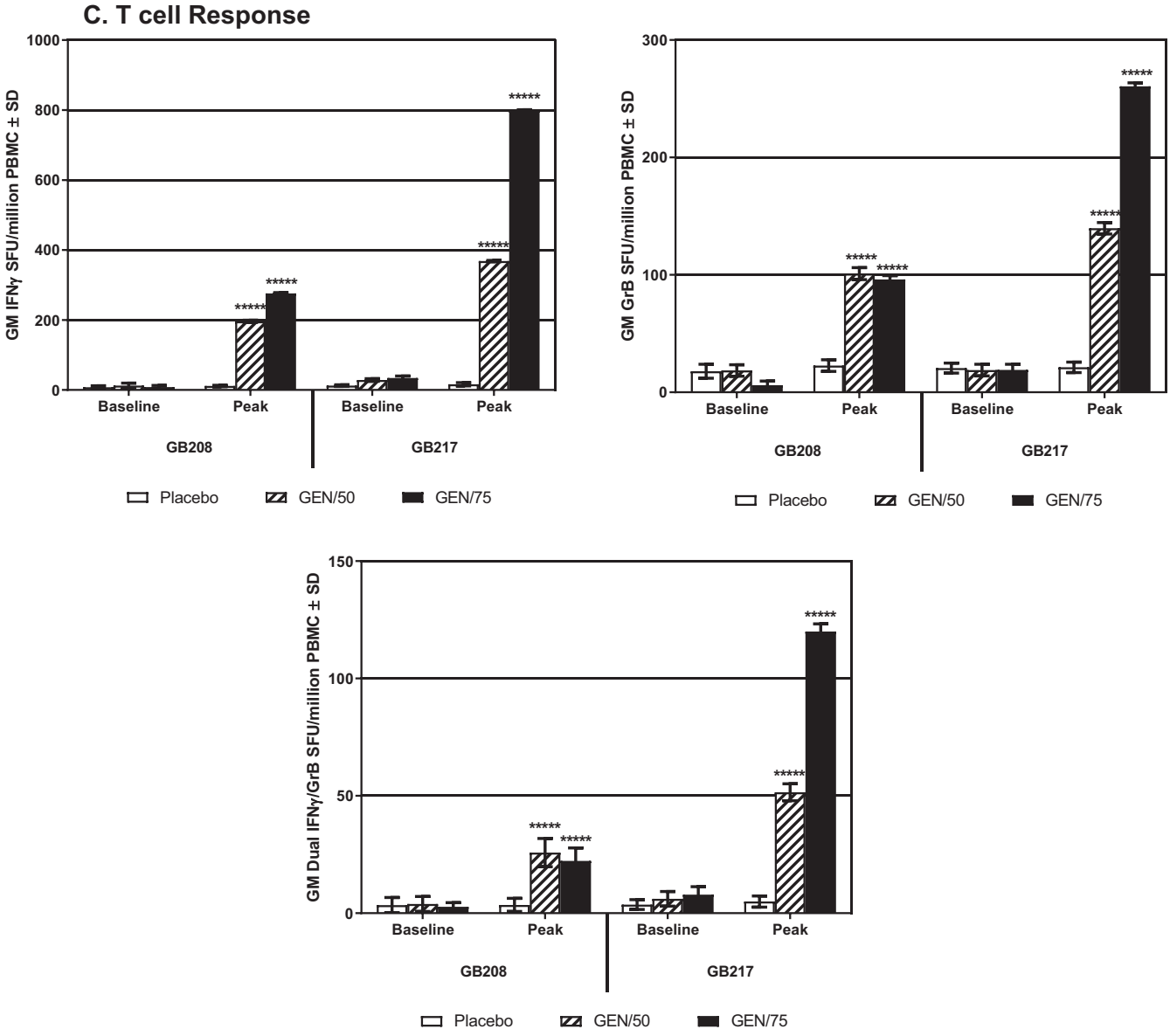

Fig. 1 (*continued*)

group exhibited decreased rates of viral shedding compared to baseline.

Lesion rates over the 6 months after the last dose were significantly lower for vaccine groups compared to placebo while over the entire 12-month follow up, the lesion rate was only significantly lower for the GEN/50 μg M2 dose group (mean lesion rate 3.60) versus the placebo lesion rate (7.33, P = 0.007 (Table 4). The median time to first recurrence after the first dose was 69 days for the GEN/50 μg M2 and 62 days for the GEN/75 μg M2 group compared to 42 days for the placebo group. The percent of recurrence free subjects 12 months after the first dose by Kaplan-Meier Estimate in the GEN/50 μg M2 dose group was 20% compared to 7% in the placebo recipients (P = 0.04), while the percent of recurrence free subjects for the GEN/75 μg M2 group (16%) was higher but not significantly different vs. the placebo group (p = 0.08) . There were no significant differences across the groups in proportion of recurrence free subjects 12 months after the last dose. Subjects in the GEN/50 μg M2 group also had significantly shorter durations of recurrences over the course of the study compared to those in the placebo group 3.2 ± 1.5 days vs. 4.4 ± 2.2 days.

3.3.2. Safety results

The incidences of solicited local reactions and systemic events were higher in the active vaccine groups than in the placebo group as expected (Fig. 3). Local reactions occurred in 100% and 96% of subject who received GEN/50 μg M2 or, GEN/75 μg M2, respectively, compared to 57% of subjects given placebo. Systemic reactions occurred in 86% and 98% of subject who received GEN/50 μg M2 or GEN/75 μg M2 respectively, compared to 66% of subjects given placebo. Pain and tenderness were the most common local reactions while muscle aches, fatigue, chills and headache occurred commonly in both vaccines groups. Fever occurred in 30% and 29% in the GEN/50 μg M2 group, GEN/75 μg group, respectively, compared to none in the placebo group. The higher dose of M2 appeared to be more reactogenic as seen in the higher incidences of chills, fatigue and muscle aches.

Grade 3 reactions were also more common among subjects who received GEN-003 than those who received placebo. Grade 3 local reactions occurred in 23% and 34% of GEN/50 μg M2 and GEN/75 μg M2 recipients respectively vs. 5% of placebo recipients while grade 3 systemic reactions occurred in 48% and 52% vs.

Table 3
HSV-2 shedding rates and change from baseline by treatment group.

	GEN-003 Antigens/Matrix-M2		
Visit Statistic	GEN/50 μg M2 (N = 43)	GEN/75 μg M2 (N = 44)	Placebo (N = 44)
Baseline			
n	43	44	44
Positive swabs/total swabs (%)	324/2409 (13.4)	367/2431 (15.1)	300/2440 (12.3)
Days 43–71			
n	40	42	42
Positive swabs/total swabs (%)	172/2137 (8.0)	241/2181 (11.0)	291/2248 (12.9)
Rate ratio vs Baseline (95% CI)	0.58 (0.35, 0.95)	0.75 (0.50, 1.13)	1.06 (0.75, 1.50)
Within-group *P* value	0.0295	0.1688	0.7281
Rate Ratio vs. Placebo (95% CI)	0.54 (0.30, 0.99)	0.71 (0.42, 1.20)	
Vs. Placebo P-value	0.0472	0.2022	
Month 6			
n	34	39	34
Positive swabs/total swabs (%)	247/1849 (13.4)	217/2022 (10.7)	216/1834 (11.8)
Rate ratio vs Baseline (95% CI)	1.00 (0.70, 1.42)	0.71 (0.48, 1.03)	1.09 (0.70, 1.69)
Within-group *P* value	0.9921	0.0741	0.7095
Rate Ratio vs. Placebo (95% CI)	0.92 (0.52, 1.61)	0.65 (0.36, 1.16)	
Vs. Placebo P-value	0.7663	0.1466	
Month 12			
n	30	32	31
Positive swabs/total swabs (%)	123/1582 (7.8)	155/1676 (9.2)	97/1659 (5.8)
Rate ratio vs Baseline (95% CI)	0.57 (0.35, 0.93)	0.63 (0.38, 1.03)	0.57 (0.34, 0.95)
Within-group *P* value	0.0249	0.0669	0.0312
Rate Ratio vs. Placebo (95% CI)	1.01 (0.50, 2.06)	1.11 (0.54, 2.28)	
Vs. Placebo P-value	0.9757	0.7653	

CI = Confidence interval.

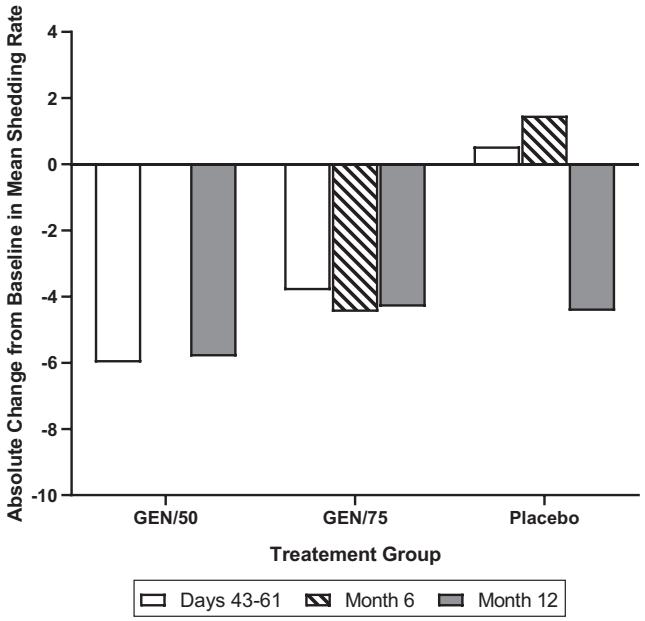

Fig. 2. Mean Absolute Change in HSV-2 Shedding Rate by Swab Collection Period and Treatment Group. Anogenital swabs for measurement of viral shedding were collected twice daily for 28-day periods after Dose 3 (Days 43 to 71), during Months 5 to 6, and during Months 11 to 12. Shedding was assessed by qPCR.

11% for GEN/50 μg M2, GEN/75 μg M2 and placebo recipients respectively. No increase in the incidence of local reaction occurred with repeated injections, while the incidence of systemic events generally decreased with repeated injections. The median duration of local reactions and systemic events ranged from 1.5 to 3.5 days. Use of antipyretic and analgesic medication was also more frequent in the active vaccine groups than in the placebo group (33.3% v 11.4%). Two subjects discontinued dosing due to solicited systemic events, 1 in each vaccine group (headache and chills in one and headache and fatigue in the other).

The incidence and severity of unsolicited AEs were similar across all treatment groups, including the placebo group. The most common AEs were spread across a number of system organ classes and preferred terms. Seven SAEs, including 1 death (drug overdose), were reported in 5 subjects. All was assessed as unrelated to the investigational product by the investigator or by the medical monitor. Three subjects (1 in each treatment group) discontinued dosing due to an unsolicited AE, one, panic attack, was deemed related to the investigational product by the investigator. The other unrelated AEs were intraductal proliferative breast cancer and a renal stone (placebo). No serious AEs or AESIs were reported.

No differences in laboratory results or vital signs were observed among treatment groups or with repeated injections. Decreases in hemoglobin and other hematology tests results from Baseline to Day 71 seen in all treatment groups, including the placebo group, may have been related to the blood sample collection for the study.

4. Discussion

The goals of a therapeutic vaccine are to decrease clinical disease either by reducing the number of recurrences, or the duration or severity of recurrences. We have previously demonstrated [13,15] that GEN-003 reduces both the days with recurrent lesions and days with virus shedding. These previous trials allowed us to select optimal doses that were then produced by methods that facilitate production in large batches suitable for a phase 3 program and/or commercial distribution.

In the trial presented here, we found that immediately after the third dose of vaccine, rates of shedding, as detected by qPCR, decreased in both vaccine groups with a rate ratio of 0.54 ($P \leq 0.05$) and 0.71 ($P = 0.20$) for the GEN/50 μg M2 and GEN/75 μg M2 groups, respectively. This decrease was detected again at the next 6 month evaluation for the GEN/ 75 μg M2 group only. The data for 12 months was confounded by an unexplained decrease in shedding for all groups including the placebo.

Table 4
Genital HSV-2 lesion rates by observation period and treatment group.

	GEN-003 Antigens/Matrix-M2		
Visit Statistic	GEN/50 μg M2 N = 43	GEN75 μg M2 (N = 44)	Placebo (N = 44)
Baseline			
Mean lesion rate (SD)	10.70 (13.241)	10.40 (13.759)	12.03 (11.034)
Median	5.19	4.39	10.06
Range: Min, Max	0.0, 58.0	0.0, 49.1	0.0, 42.2
Days 1–183 after Last Dose			
Mean lesion rate (SD)	4.55 (6.128)	4.68 (5.882)	7.88 (8.545)
Median	2.67	1.91	5.61
Range: Min, Max	0.0, 29.5	0.0, 25.7	0.0, 35.5
P-Value vs. Placebo[a]	0.0248	0.0341	
Days 1–365 after Last Dose			
Mean lesion rate (SD)	3.60 (4.737)	4.65 (5.285)	7.33 (8.503)
Median	2.27	2.82	4.48
Range: Min, Max	0.0, 24.7	0.0, 20.1	0.0, 36.0
P-Value vs. Placebo[a]	0.0072	0.0782	

[a] P-value based on Wilcoxon rank sum test.

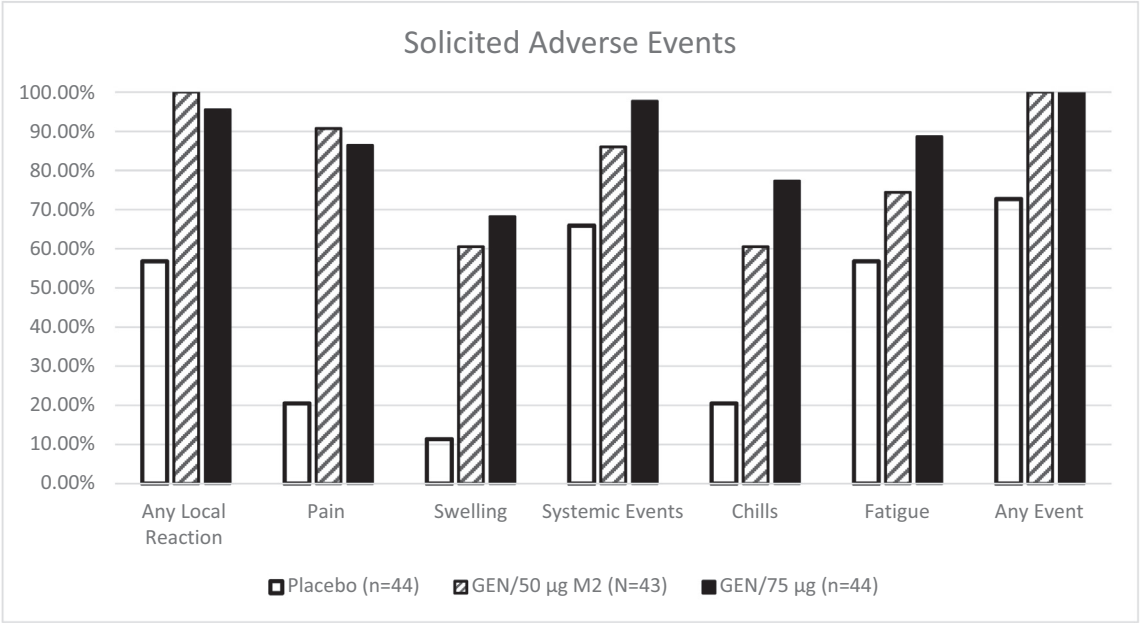

Fig. 3. Subjects completed an electronic diary card for 7 days following each vaccination. The per cent of subjects reporting the selected solicited AEs after any immunization are shown.

The effect on clinical disease (recurrent lesions) was more consistent and documented for several clinical outcome measures. During the 6 and 12-month observation periods both vaccine doses decreased the lesion rates compared to placebo by between 37% and 51% for the GEN/50 μg M2 and the GEN/75 μg M2 dose respectively. Further support for the clinical effectiveness was the reduction in time to first recurrences for both groups; 69 days for the GEN/50 μg M2 and 62 days for the GEN/75 μg M2) groups compared to 42 days for the placebo group from the time of first vaccination. These reductions were also reflected in an increase in the number of recurrence free subjects; (20% vs. 70%, P = 0.04) for the GEN/50 μg M2 group compared to placebo while the proportion of for the GEN/75 μg M2 group (16%) was higher but not significantly different vs. the placebo group. The final clinical benefit of vaccination with the GEN/50 μg M2 dose was a reduction in the duration of recurrences.

The clinical effectiveness of the vaccines is supported by the evaluations of the immune response. Peak mean IgG titers to GB208 and GB217 increased >100-fold and >10-fold from baseline, respectively, in both vaccine groups. These elevated antibody levels decreased but remained above baseline through 12 months. Similarly, mean neutralizing antibody titers peaked at >4-fold from baseline for both vaccine groups with titers remaining >2 fold elevated at month 12. T cell responses to the vaccine were also robust. IFN-γ, GrB and dual GrB/IFN-γ SFCs for each antigen peaked during the dosing period and then decreased but remained well above baseline through Month 12, with no evidence of T cell exhaustion. The maintenance of the T cell response in the vaccinated groups might be due to continual antigen stimulation from the chronic HSV-2 infection. Importantly, GEN-003 immunization induced robust and sustained dual GrB/ IFN-γ secreting T cells, indicating a polyfunctional T cell response.

Almost all of vaccine recipients developed short duration local and systemic solicited events compared to 57–66% of placebo recipients. Pain and tenderness were the most common local reactions while muscle aches, fatigue, chills and headache occurred commonly in both vaccines groups. Local reactions reached grade 3 levels in 23%, and 34% of GEN/50 μg M2 and GEN/75 μg M2

recipients respectively vs. 5% of placebo recipients. Grade 3 systemic reactions were also more common. No serious AEs or AESIs were reported among any subjects in this study and there were few discontinuations of dosing for solicited or unsolicited AEs (<5%). Thus, for the doses evaluated in this study, GEN-003 exhibited a higher than usual tolerability profile but one that is acceptable for use as a therapeutic vaccine. Comparison of the reactogenicity for the doses evaluated suggests that the GEN/50 μg M2 formulation may be preferred."

In summary, the third clinical trial of GEN-003 supported the clinical and virologic effectiveness of this therapeutic genital herpes vaccine and suggested that the dose of 60 μg of virus antigens and 50 μg of M2 adjuvant would be the preferred dose for future phase 3 trials. It remains to be determined if a vaccine strategy that reduces recurrent disease and recurrent virus shedding by about 50%, for about one year, is a viable alternative to antivirals that are more effective but must be taken daily. It is also possible that a combined approach using both a vaccine and antiviral would be the most effective.

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Potential conflicts of interest

D. I. B. receives funding from Genocea Biosciences for preclinical studies of vaccines and has been a consultant to Genocea Biosciences, Vical, GlaxoSmithKline, and Merck for herpes virus vaccines. J.B.F. and L.K. M are employees and stock owners of Genocea Biosciences. S. H. and T.H. were employees and stock owners of Genocea Biosciences. A. W. is a consultant for Aicuris and serves on DSMB for Merck; she has received travel reimbursement from Gilead, She has received funds for sponsored projects for Genocea Biosciences and Vical. 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.

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