Poster 1655

Significant Neutralizing Antibody and Cytolytic T cell Responses to GEN-003, a Herpes Simplex Virus Immunotherapy, in a Phase 2b Study

Lisa K. McNeil, PhD[1], Amy Baccari, BS[1], Veronica Clemens, BA[1], David Dominguez, BS[1], Tyler Fenske, BS[1], Thomas Oliphant, MS, PhD[2], James Perry, BA[1], Nicolle Siddall, BS[1], Jin Yuan, PhD[1], Thomas Heineman, MD, PhD[1], Seth Hetherington, MD[1], Jessica Flechtner, PhD[1]

[1] Genocea Biosciences, Cambridge, MA; [2] Innovative Analytics, Kalamazoo, MI, USA

Lisa K. McNeil, PhD
Genocea Biosciences
100 Acorn Park Dr
Cambridge, MA 02140
Phone: 617-715-6630
e-mail: lisa.mcneil@genocea.com

Abstract

Background. Over 500 million people globally have genital ulcerative disease due to herpes simplex virus (HSV). Data suggest that both B- and T cell immune responses are critical for effective control of viral replication and disease symptoms. GEN-003 is a potential immunotherapy containing two recombinant HSV-2 proteins, ICP4.2 and gD2ΔTMR combined with adjuvant, Matrix-M2™ (MM2, Novavax). In a Phase 2 clinical trial, GEN-003 showed a reduction in viral shedding and lesion rates. Here we report immunogenicity responses to GEN-003 through 1 year of follow up.

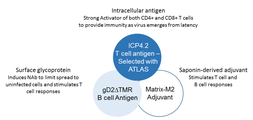
Methods. In total, 131 adults with clinically diagnosed HSV-2 infection were randomly assigned to one of two GEN-003 dose groups (60 µg of antigens with either 50 or 75 µg MM2 adjuvant) or placebo. Subjects were immunized three times at 21-day intervals. Serum was collected on days 1, 22, 43, and 71, and at 6 and 12 months. Heparinized whole blood for peripheral blood mononuclear cell enrichment was collected on days 1, 8, and 50, and at 6 and 12 months. Humoral responses were evaluated by indirect IgG ELISA and a cell-based colorimetric HSV-2 neutralizing antibody assay. Cellular responses were evaluated by an interferon-γ (IFN-γ)/Granzyme B (GrB) fluorescent enzyme-linked immunospot assay.

Results. Following the first immunization, mean IgG titers to ICP4.2 and gD2ΔTMR increased >100-fold and >10-fold from baseline, respectively, in both GEN-003 dose groups. Elevated antibody levels persisted above 10-fold through Day 71. Mean neutralizing antibody titers were >6-fold higher versus baseline at Day 71 for both dose groups. Increases in IFN-γ, GrB, and dual-secreting T cell responses to both vaccine antigens peaked at Day 8 post-first immunization and were sustained through Day 50 for the 60 µg GEN-003 antigens/50 µg MM2 group. T cell responses in the 60 µg GEN-003 antigens/75 µg MM2 group peaked at Day 8 and decreased thereafter. Placebo group values did not increase from baseline for all above parameters. Further evaluation of immunogenicity at the 6 and 12-month time points will be examined.

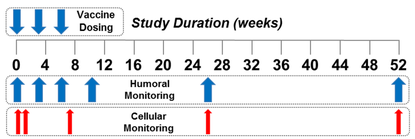
Conclusion. Overall, immunization of HSV-2 infected subjects with GEN-003 induced both cellular and humoral immune responses that may play a role in controlling both viral shedding and symptoms of genital disease.

Introduction

- Genital herpes is primarily caused by herpes simplex virus 2 (HSV-2) and affects more than 500 million people worldwide.[1] Primary infection occurs through direct contact with skin/mucosa and is characterized by recurrent, painful genital ulcers.
- HSV-2 has been associated with transmission of HIV-1[2] and causes severe disease in infants and immunocompromised individuals.[3]
- Prior attempts to develop prophylactic and therapeutic vaccines have failed as both T and B cell immunity are likely needed for immune control and prophylaxis.[4,5]
- GEN-003 is a subunit vaccine comprised of two viral antigens, ICP4.2 and gD2ΔTMR, and the adjuvant Matrix-M2 (Novavax, Gaithersburg, MD).[6,7]



GEN-003-003 Phase 2b Study Design



- 131 subjects with frequently recurrent HSV-2 genital herpes (3-9 outbreaks/year)
- 2 GEN-003 dose groups (60 µg of antigens with either 50 or 75 µg MM2 adjuvant) or placebo, ~44 subjects per group
- PBMCs for cellular responses were collected in a subset of subjects, ~15 per group.
- NCT02515175

Methods

Total IgG Antigen Specific Endpoint ELISA. Plates were coated with recombinant protein (0.75 µg/mL of ICP4.2, or 0.5 µg/mL gD2ΔTMR). Optical density readings were taken at 450 nm and antigen-specific IgG concentrations (units/mL) were calculated via interpolation of the reference serum-generated standard curve. The LOQ for this assay was 4700 units/mL for gD2ΔTMR and 15600 units/mL for ICP4.2.

Colorimetric Neutralization Assay. The colorimetric neutralization assay was performed as previously described [8]. Neutralizing antibody (NAb) titers were determined as the reciprocal of the serum dilution that produced a 50% reduction in the OD_{562} of the virus control. The LOQ for this assay was a titer of 20.

IFN-γ and GrB Fluorospot Assay. PBMCs isolated from clinical trial subjects were thawed and rested overnight at 37°C. PBMCs at a concentration of 2 x 10^5 cells/well were 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, then incubated for 20 ± 2 hours at 37°C. FluoroSpot plates were then developed. Fluorescent spots were analyzed on an Autoimmun Diagnostika (AID) iSpot Reader System. Spot forming cells (SFC) are averaged, background subtracted and normalized to 1x10^6 PBMCs.

GEN-003 Induces Antigen-specific and Neutralizing Antibodies Which are Durable for at Least 12 Months Post-Immunization

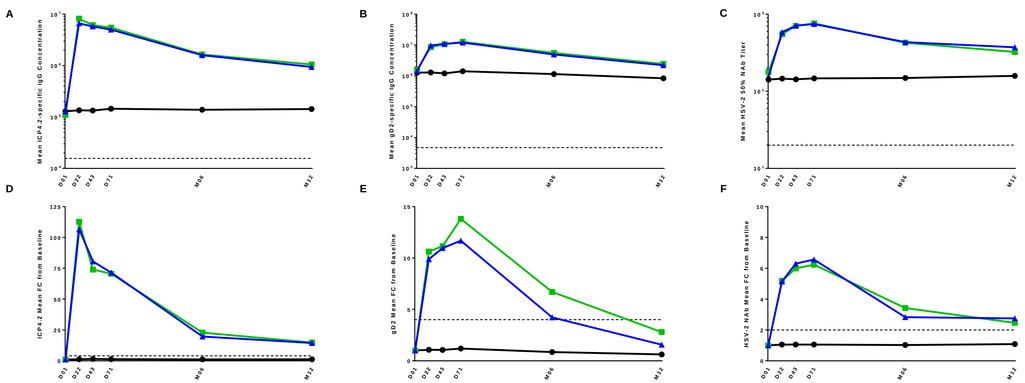


Figure 1. Antigen-specific IgG and NAb Titers and Fold Change (FC) From Baseline.
(**A-C**) IgG graphs. The dashed lines indicate the limit of quantification (LOQ) for each assay, a concentration of 15600 units/mL for ICP4.2 IgG ELISA, 4700 units/mL for gD2 IgG ELISA and a titer of 20 for the NAb titer. (**A**) ICP4.2-specific IgG concentration. (**B**) gD2-specific IgG concentration. (**C**) 50% HSV-2 Neutralizing Antibody Titer results. (**D-F**) Fold change from baseline graphs. At each time point the antigen-specific IgG concentration or NAb titers are compared to those obtained pre-dosing (baseline) by calculating the fold change. The dashed line represents the cutoff that defines a response, 4-fold increase over baseline for IgG and 2-fold for NAb titer. (**D**) ICP4.2-specific IgG Fold Change. (**E**) gD2-specific IgG Fold Change. (**F**) 50% HSV-2 NAb Fold Change.

Antibody Response Rates Were Increased by GEN-003 Vaccination; >40% of Participants Maintained ICP4.2-specific IgG and Neutralizing Antibody Responses at 12 months

	% ICP4.2-Specific Responders			% gD2-Specific Responders			% NAb Responders		
	60/50	60/75	PBO	60/50	60/75	PBO	60/50	60/75	PBO
D22	98	100	3	58	76	0	65	88	3
D43	100	98	5	73	85	0	88	93	3
D71	100	97	3	84	82	0	92	95	3
M06	94	92	0	36	32	0	69	55	3
M12	63	77	3	7	0	0	41	52	6

Table 1. Response Rates. A responder is defined as a measured fold change over baseline of ≥4 for IgG antibodies and ≥2 for neutralizing antibodies (NAb). The 60/50 µg and 60/75 µg designations refer to the amount of each antigen and adjuvant in each cohort, respectively. All % values are rounded for this table. PBO, placebo.

ICP4.2 and gD2-specific IFN-γ, Cytolytic and Polyfunctional T cells are Maintained through 12 Months Post-Immunization

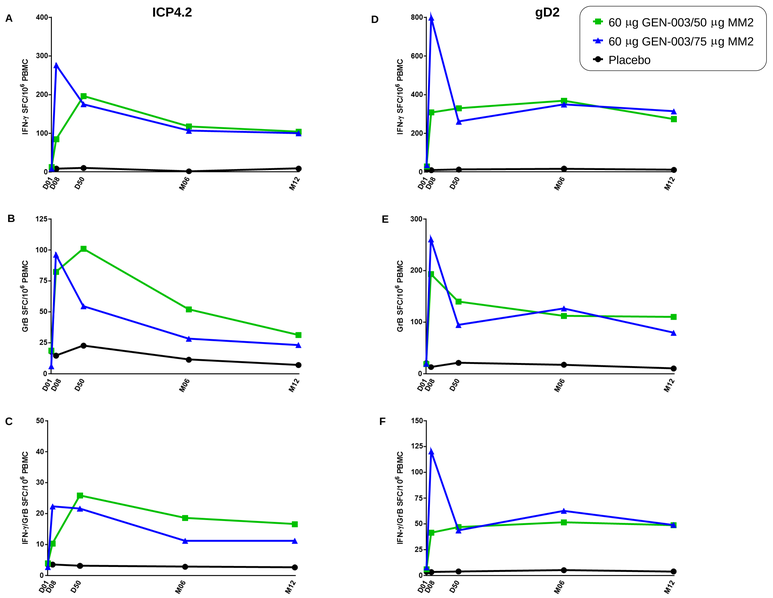


Figure 2. Antigen-specific polyfunctional T cells after immunization with GEN-003.
PBMCs were collected at D01, D08 D50, and months 6 and 12. The number of cells secreting IFN-γ and/or GrB in response to antigen stimulation was analyzed by Fluorospot assay. Double-secreting cells were determined as spots having the same position (center point) in an image overlay of FITC (IFN-γ) and Cy3 (GrB) images. Spot analysis was performed using an iSpot Spectrum reader from AID. Mean SFC/10^6 PBMCs are shown in each graph. **(A-C)** ICP4.2 stimulated T cell responses, **(D-F)** gD2 stimulated T cell responses. **(A, D)** IFN-γ secretion, **(B, E)** GrB secretion, **(C, F)** dual IFN-γ/GrB secretion.

T cell responses Correlated with Lesion Rates

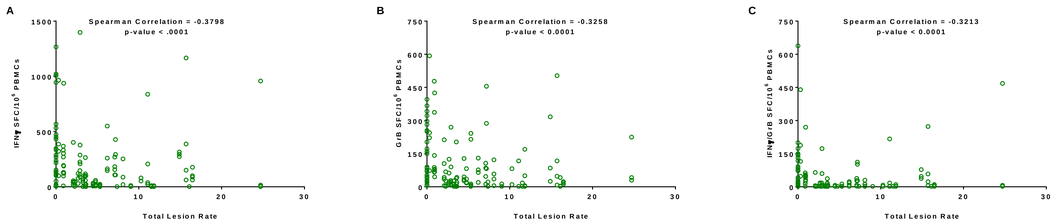


Figure 3. ICP4.2-specific T cell responses correlate significantly (p<0.0001) with lesion rate after vaccination.
(A) IFN-γ secretion, **(B)** GrB secretion and **(C)** Dual IFN-γ/GrB secretion from D08, D50, M06 and M12 and total lesion rate (through M12) were compared using Spearman's rank correlation. Not shown are gD2-specific T cells responses which also correlate with lesion rate.

Summary

- GEN-003 induced robust and durable IgG and HSV-2 neutralizing antibodies
  - Mean IgG titers increased greater than 100-fold and 10-fold to ICP4.2 and gD2, respectively, while neutralizing antibody titers increased greater than 6-fold.
  - These responses are very similar to humoral responses observed in the GEN-003-002 Phase 2 study.
- GEN-003 immunization induced robust and sustained IFN-γ, cytolytic and polyfunctional T cells. Polyfunctional T cells are cells that each produce multiple immune mediators. These types of cells are considered to deliver a more effective immune response than those that only secrete one mediator.
  - T cell responses generally peaked at Day 8 and were maintained at high levels through 12 months with minimal loss of responding T cells.
  - T cells maintain functionality (IFN-γ and GrB) through 12 months, with no evidence of exhaustion.
  - Maintenance of T cell response might be due to continual antigen stimulation from chronic HSV infection.
- Antigen-specific T cell responses significantly correlated with lesion rate after vaccination, suggesting T cell control of HSV reduces the frequency and duration of genital lesions.
  - Analysis of the correlation between humoral response and clinical efficacy is ongoing.

Acknowledgements

The authors would like to thank the research subjects and the study personnel who made this trial possible, including the trial investigators; David Bernstein, Anna Wald, Nicholas van Wagoner, Peter Leone, Kenneth Mayer, Gregg Lucksinger, Sandra Win, William Koltun, and Nisha Desai. This work was funded by Genocea Biosciences Inc.

References

1. Wald and Lawrence. Chapter 36 "Persistence in the population: epidemiology, transmission." Human Herpesviruses. Ed. Arvin A, et al., Cambridge: Cambridge University Press; 2007.

2. Corey *et al.* The Effects of Herpes Simplex Virus-2 on HIV-1 Acquisition and Transmission: a Review of Two Overlapping Epidemics. J AIDS 2004;35:435-445.

3. Corey and Handsfield. Genital herpes and public health: addressing a global problem. JAMA 2000;283:791-794.

4. Straus *et al.* Placebo-controlled Trial of Vaccination with Recombinant Glycoprotein D of Herpes Simplex Virus type 2 for Immunotherapy of Genital Herpes. The Lancet 1994;343:1460-1463.

5. Belshe *et al.* Efficacy Results of a Trial of Herpes Simplex Vaccine. N Engl J Med 2012;366:34-43.

6. Skoberne *et al.* An Adjuvanted HSV-2 Subunit Vaccine Elicits a T Cell Response in Mice and Is an Effective Therapeutic Vaccine in Guinea Pigs. J Virol 2013;87:3930–3942

7. Long *et al.* Identification of Novel Virus-Specific Antigens by CD4(+) and CD8(+) T Cells from Asymptomatic HSV-2 Seropositive and Seronegative Donors. Virology 2014;464–465:296–311.

8. Baccari *et al.* Development of a High-Throughput β-Gal-based Neutralization Assay for Quantitation of Herpes Simplex Virus-Neutralizing Antibodies in Human Samples. Vaccine 2016;34:3901-3906.