GEN-003, a herpes simplex virus immunotherapy, elicits significant neutralizing antibody and cellular responses in HSV-2 seropositive subjects

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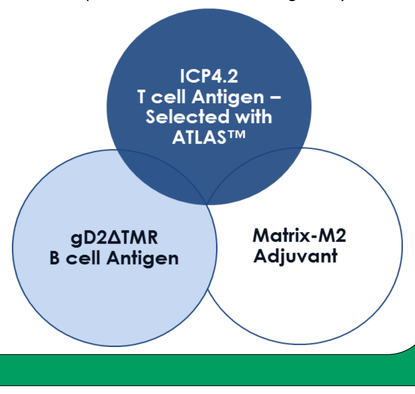
Abstract

Over 400 million people are infected with herpes simplex virus (HSV), the most common cause of genital ulcers worldwide. Data suggest that both B and T cell immune responses are critical for control of viral replication and disease symptoms. GEN-003 is an immunotherapy targeting HSV and is comprised of recombinant HSV-2 proteins ICP4.2 and gD2ΔTMR combined with adjuvant, Matrix-M2™ (MM2) (Novavax). A randomized, double-blind, placebo-controlled study, comparing two dose groups of GEN-003 antigen and adjuvant, was conducted in 131 adults with clinically diagnosed HSV-2 infection. Subjects were immunized three times at 21 day intervals and serum was collected on days 1, 22, 43, and 71, while heparinized whole blood for enrichment of peripheral blood mononuclear cells was collected on days 1, 8, and 50. Humoral responses were evaluated by indirect IgG ELISA and a cell-based colorimetric HSV-2 neutralizing antibody assay, while cellular responses were evaluated by an Interferon-γ (IFN-γ)/Granzyme B (GrB) fluorescent enzyme-linked immunospot assay in an interim analysis of this Phase 2b trial. Following the first immunization, both dose groups had greater than 60-fold and 8-fold increase above baseline in mean IgG titers to ICP4.2 and gD2ΔTMR, respectively, with elevated antibody levels persisting through Day 71. Mean neutralizing antibody titers increased greater than 4-fold 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. Overall, immunization of HSV-2 infected subjects with GEN-003 induces both cellular and humoral immunity, which may assist in controlling both viral shedding and symptoms of genital disease.

Introduction

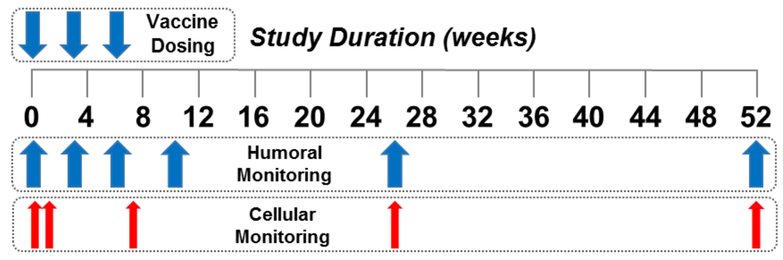
- Genital herpes, which is characterized by recurrent painful ulcers, is primarily caused by HSV-2 and affects more than 400 million people worldwide [1]
- HSV-2 infection increases the risk of HIV-1 transmission [2] and causes severe disease in infants and in immunocompromised individuals [3]
- Prior attempts to develop prophylactic and therapeutic HSV-2 vaccines have failed
- The effective control of primary and recurrent HSV-2 disease is likely to require T and B cell immunity [4,5]
- GEN-003 is a candidate subunit vaccine comprised of two viral antigens, ICP4.2 and gD2ΔTMR, and the adjuvant, Matrix-M2™ (Novavax, Gaithersburg, MD) [6,7]
  - ICP4.2 is an internal fragment of HSV-2 immediate early protein ICP4 and was identified as a target T cell antigen by Genocea Biosciences' ATLAS™ screening platform
  - gD2ΔTMR is HSV-2 gD lacking the transmembrane domain and is a T and B cell antigen



GEN-003-003 Phase 2b Study Design

Design
- A randomized, double blind, placebo-controlled clinical trial of a new formulation of GEN-003 for treatment of HSV-2 genital infection
  - Subjects (n=131) were aged between 18 and 50 years with documented diagnosis of genital HSV-2 infection for >1 year
  - Randomized to placebo or 1 of 2 active arms: 60 µg of each antigen and either 50 or 75 µg of Matrix-M2™ adjuvant

Objectives
- Quantify the reduction in HSV-2 viral shedding and evaluate clinical outcomes including safety and lesion rate
  - Please see posters #8.31 (M. Croshier) and #8.25 (T. Heineman)
- **Analyze humoral and cellular immune responses to ICP4.2 and gD2ΔTMR**



Methods

Humoral Responses

Indirect IgG ELISA: Plates were coated with ICP4.2 or gD2ΔTMR antigen. After blocking of non-specific binding, reference and subject sera were loaded, in duplicate, onto the antigen coated plate for antigen/antibody interaction. Specific binding detection and color development was facilitated by a series of incubations with goat anti-human HRP detection antibody and a TMB substrate system. Finally, 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.

Colorimetric Neutralizing Antibody Assay: Plates were seeded with Vero cells at 7.7 x 10^4 cells/mL and incubated for 18 – 24 hours at 37°C. Subject sera were incubated with recombinant HSV-2 virus (containing a lacZ reporter gene[8]) for one hour before being transferred to the prepared cell plate and incubated overnight for 18 – 20 hours at 37°C. Cells were lysed and infection was visualized via CPRG substrate hydrolysis by β-galactosidase. Optical density was measured at 562 nm and neutralizing antibody titers were calculated as the reciprocal of the serum dilution that produced a 50% reduction in the OD_{562} of the virus control.

Cellular Response

IFN-γ/GrB FluoroSpot Assay: PBMCs isolated from clinical trial subjects were thawed and rested 18-20 hours 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-IFN-γ and anti-GrB antibodies and stimulated with media alone or overlapping peptides spanning each antigen, then incubated for 20 ± 2 hours at 37°C. FluoroSpot plates were then developed via a series of incubations with detection antibody, conjugate antibody, and a fluorescence enhancer. Fluorescent spots were analyzed on an AID *i*Spot Reader System.

Based on IgG Antibody Titers, Response Rates Remained Level or Increased with each Subsequent GEN-003 Immunization

	ICP4.2-Specific Responders			gD2ΔTMR-Specific Responders			HSV-2 NAb Responders		
	60/50 µg	60/75 µg	Placebo	60/50 µg	60/75 µg	Placebo	60/50 µg	60/75 µg	Placebo
D22	97.5	100	2.6	57.5	75.6	0	65	87.8	2.6
D43	100	97.6	5.1	72.5	85.4	0	87.5	92.7	2.6
D71	100	97.4	2.6	83.8	81.6	0	91.9	94.7	2.6

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. The 60/50 µg and 60/75 µg designations refer to the amount of each antigen and adjuvant in each cohort, respectively.

GEN-003 Stimulated Increases in Immunogenic and Functional Antibody Responses: Fold Change from Baseline

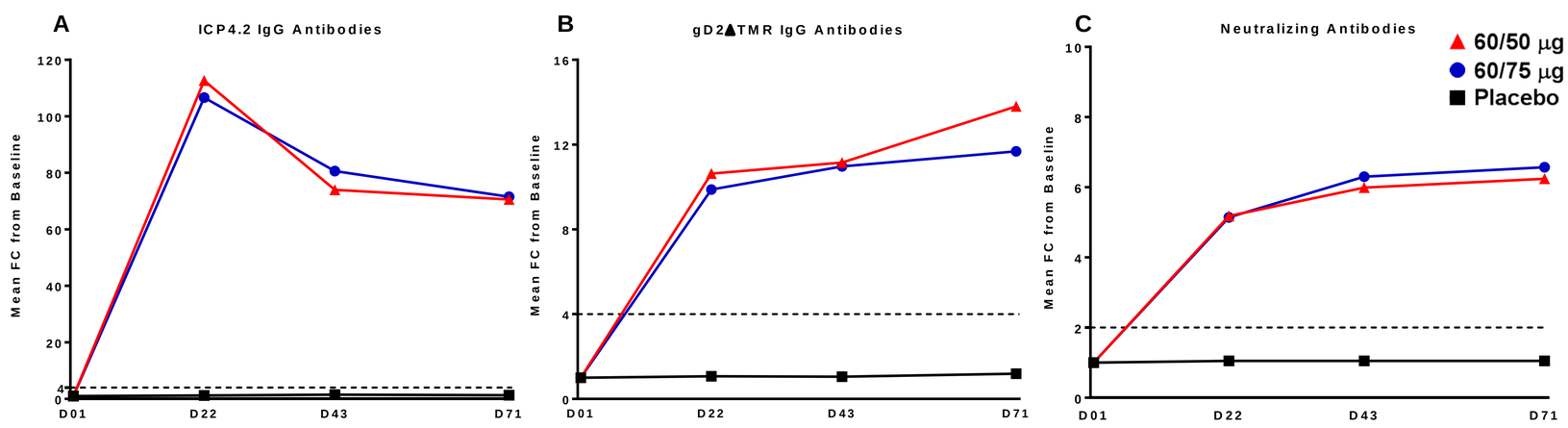


Figure 1. Mean Fold Change (FC) from Baseline. Antigen-specific mean IgG concentrations or 50% NAb titers at each sample collection point are compared to those collected at baseline (D01). The dashed lines represent the threshold defining a positive response, 4-fold increase for IgG and 2-fold increase in neutralizing titer. (A) ICP4.2 IgG Fold Change. (B) gD2ΔTMR IgG Fold Change. (C) HSV-2 50% Neutralizing Antibody Fold Change.

GEN-003 Stimulated Increases in Immunogenic and Functional Antibody Responses: Mean IgG Concentration (units/mL)

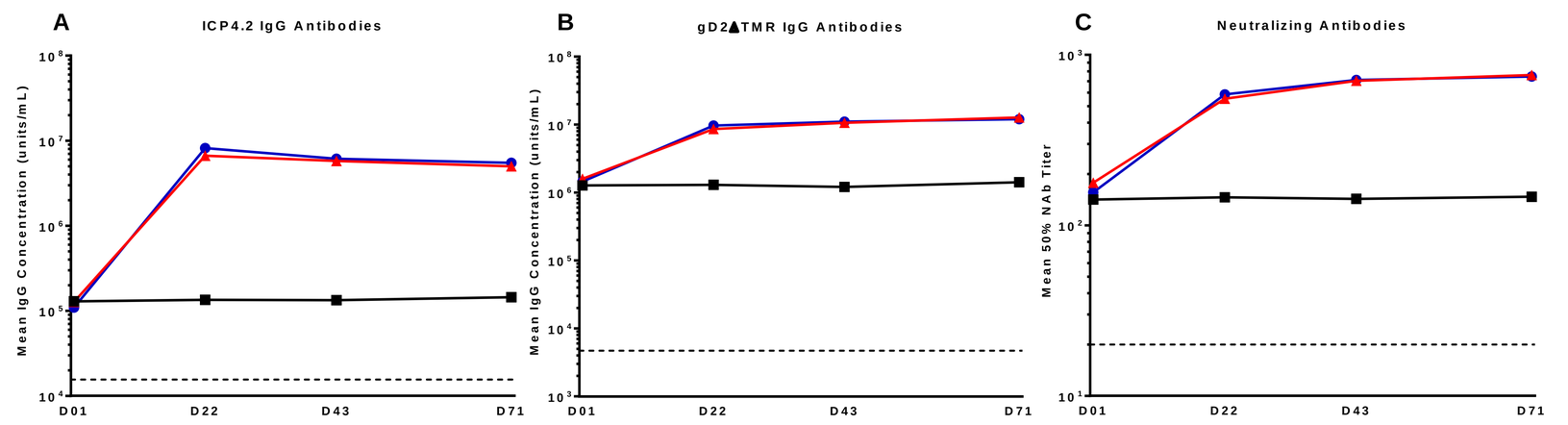


Figure 2. Mean IgG Concentration (units/mL). Interpolated antigen-specific mean IgG concentrations or 50% NAb titers are plotted at each sample collection point. The dashed lines represent the lower limit of quantitation; 15,600 for ICP4.2, 4,700 for gD2ΔTMR, and 20 for HSV-2 NAb. (A) ICP4.2 IgG Antibodies. (B) gD2ΔTMR IgG Antibodies. (C) HSV-2 50% Neutralizing Antibodies.

GEN-003 Induced Increases in Cytolytic T cells

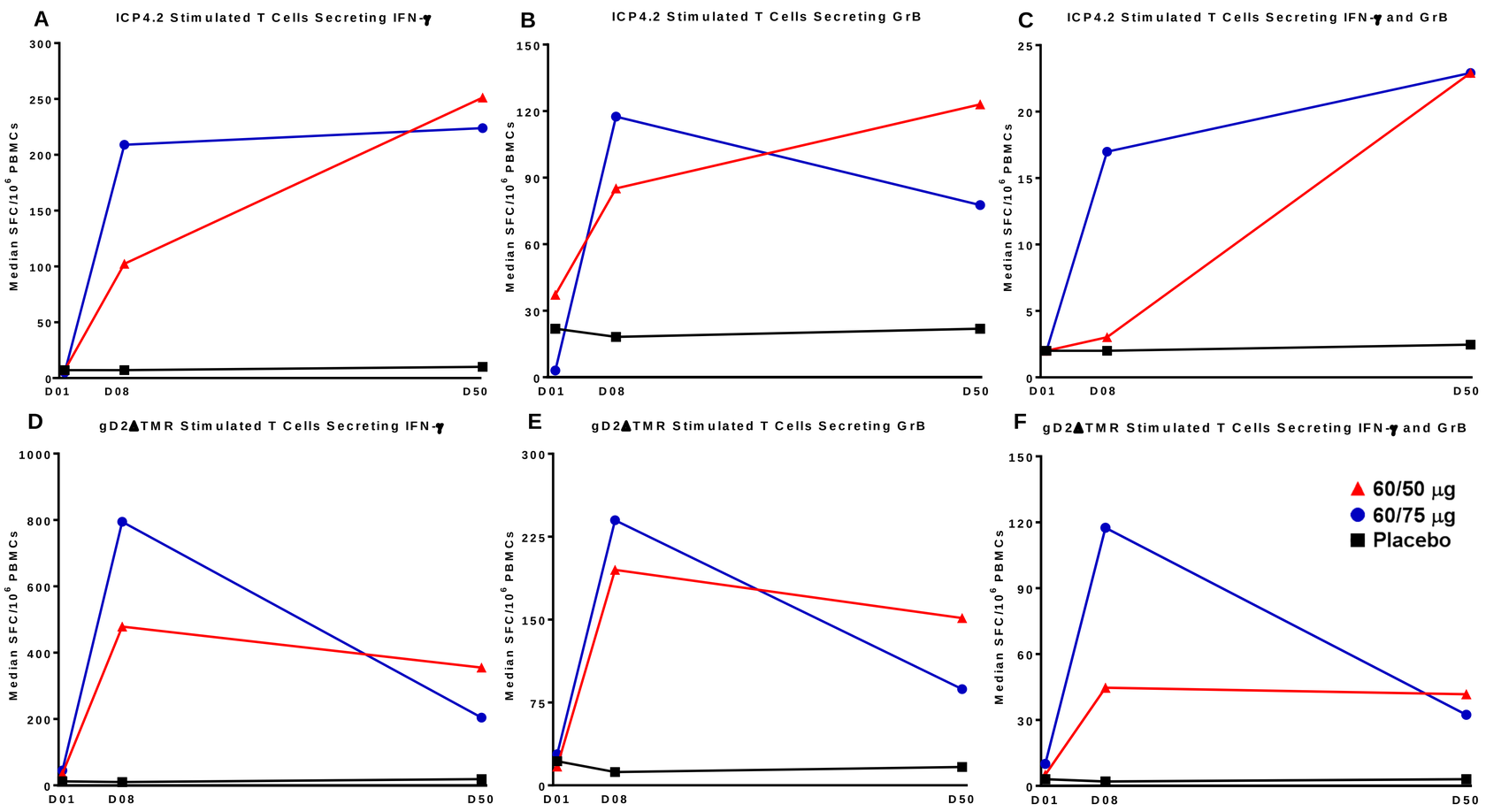


Figure 3. Antigen-specific Median Spot Forming Cells (SFC). Antigen-specific responses were measured by visualization of secreted cytokine/protease and are plotted by presence of interferon gamma (IFN-γ), Granzyme B (GrB), or both. (A) ICP4.2 Stimulated T Cells Secreting IFN-γ. (B) ICP4.2 Stimulated T Cells Secreting GrB. (C) ICP4.2 Stimulated T Cells Secreting IFN-γ and GrB. (D) gD2ΔTMR Stimulated T Cells Secreting IFN-γ. (E) gD2ΔTMR Stimulated T Cells Secreting GrB. (F) gD2ΔTMR Stimulated T Cells Secreting IFN-γ and GrB.

GEN-003 Induced Increases in Cytolytic T cells

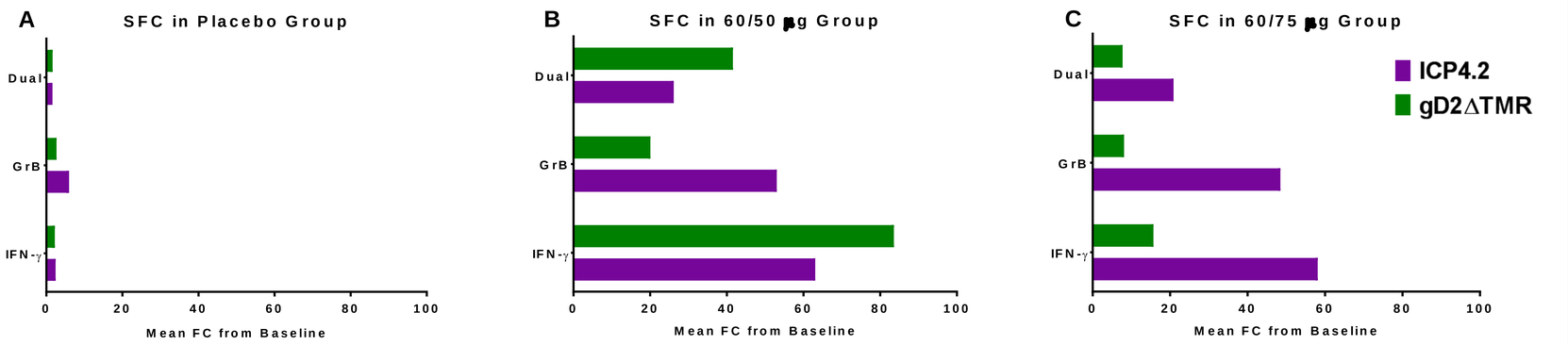


Figure 4. Mean Fold Change (FC) of Antigen-specific T cell Responses. Antigen-specific mean T cell responses of IFN-γ and GrB from day 50 are compared to those collected at baseline (D01). (A) Fold Change of SFC in Placebo Group. (B) Fold Change of SFC in 60/50 µg Group. (C) Fold Change of SFC in 60/75 µg Group.

Conclusions

- GEN-003 augmented pre-existing humoral immunity in subjects
  - Mean IgG titers increased greater than 60-fold and 8-fold to ICP4.2 and gD2ΔTMR, respectively, while neutralizing antibody titers increased greater than 4-fold
  - Humoral response rates were >80% at day 71, while placebo remained <3%, indicating a highly immunogenic vaccine
- Cytolytic T cells, believed to be essential for control of HSV-2 infection, were increased in subjects immunized with GEN-003
  - IFN-γ, GrB, and dual secreting T cells are increased in both dose groups with fold changes greater than 20-fold to ICP4.2 and 7-fold to gD2ΔTMR through day 50
  - A decrease in T cell responses from day 8 to day 50 was seen in the 60/75 µg dose group, suggesting increased adjuvant composition may lead to T cell exhaustion
- An overall increase in immunogenicity was observed in subjects immunized with GEN-003, though, the protective threshold for antiviral effect will need to be investigated further
- The immunogenicity data generated supports the selection of the 60/50 µg dose of GEN-003 for upcoming Phase 3 clinical trials

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