Both HSV-2 and HSV-1 Neutralizing Antibody Titers are Boosted in HSV-1/2 Seropositive Subjects when Vaccinated with GEN-003, a Novel HSV-2 Immunotherapy

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Herpes simplex virus type 2 (HSV-2) is the main cause of genital herpes worldwide. However, HSV-1, usually associated with oral disease, has become a predominant cause of genital infections in some developed countries[1]. We are currently investigating the safety and efficacy of GEN-003, an investigational HSV-2 therapeutic vaccine in Phase 2 clinical trials. In addition to efficacy, we are examining immune responses induced by the vaccine, to determine if immune correlates of protection can be identified.

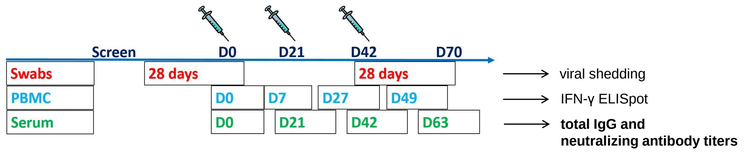
Recently, the HerpeVac prophylactic HSV-2 vaccine failed to meet clinical endpoints in a large Phase 3 trial. Rather than protection against HSV-2 acquisition, the sponsors reported protection against acquisition of HSV-1[2], which was attributed to the induction of higher neutralizing titers to HSV-1 than HSV-2 in response to vaccination[2]. As a result of this observation, we examined if there were differences in the induction of neutralizing antibody titers against HSV-1 and HSV-2 in response to GEN-003.

GEN-003-001

GEN-003 is comprised of HSV-2 glycoprotein D ($gD2\Delta TMR_{340\text{-}363}$) and a truncated form of infected cell polypeptide 4 ($ICP4_{383\text{-}766}$), formulated with Matrix-M2 (MM-2) adjuvant. gD2 shares 82% amino acid identity with gD from HSV-1. GEN-003-001 was a placebo controlled, dose escalating Phase 1/2a study in which 143 subjects with documented recurrent HSV-2 genital infection were enrolled. HSV-1 serostatus was not an exclusion criteria, but both HSV-1 and -2 serostatus were determined by HerpeSelect ELISA. Trial participants were vaccinated with either placebo or 10, 30, or 100µg of each antigen alone or in combination with 50µg MM-2. For every ten subjects enrolled, six received **GEN-003** (Antigens/Diluent/MM-2), two received **GEN-003NA** (Antigens/Diluent), and two received **Placebo** (Diluent only).

GEN-003-001 trial results summary

- The vaccine was safe and immunogenic, potentiated by adjuvant
- After the third immunization, the 30µg GEN-003 vaccine dose resulted in a 52% reduction of the mean viral shedding frequency from baseline which persisted at least six months (40% reduction). Participants also experienced up to a 65% reduction in lesion rates.
- The 100µg GEN-003 dose also demonstrated a durable reduction in viral shedding and lesion rates, at a lower magnitude
- The 10µg dose was immunogenic but not protective



As a result of the efficacy observed in the cohort of subjects receiving 30µg GEN-003, sera from this cohort were selected for the analyses described here.

Colorimetric Neutralization Assays

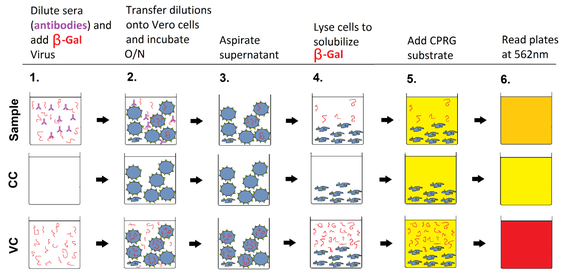


Fig. 1: Colorimetric neutralization assay. For the HSV-1 strain (KOS TK-12), the LacZ gene is under control of the ICP4 promotor[3]; for the HSV-2 strain (333), it is under control of the promotor between UL3 and UL4[4]. Neutralizing antibody titers are determined as the reciprocal of the serum dilution that produced a 50 % reduction in the OD_{562} of the virus control. Negative control wells contain virus without immune serum (virus control) and cells without virus (cell control); positive control wells contain dilutions of serum with known neutralizing activity.

Neutralizing antibody titers measured by the colorimetric neutralization assay correlate well with titers achieved in plaque reduction assays.

A

Serum Sample	HSV-1/HSV-2 serostatus	HSV-1 Neut Titers		HSV-2 Neut Titers	
assay		PR	β-Gal	PR	β-Gal
PC	NT	60	NT	120	76
1	+/+	320	430	640	542
2	+/+	160	162	240	334
3	+/+	1920	2069	2560	2158
4	-/-	<20	<20	<20	<30
5	-/+	80	106	120	243
6	-/+	60	90	160	226
7	-/+	160	157	240	325

B

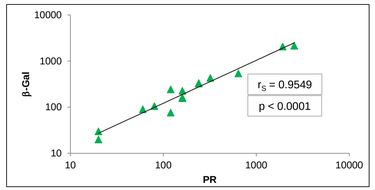


Fig. 2: Comparison of the colorimetric neutralization assay with the plaque reduction assay. Human sera were serially diluted and neutralization antibody titers were measured using both the colorimetric assay (β-gal) and the established plaque reduction assay (PR). Human AB serum from Valley Biomedical (VA, Lot# MI12678) was used as a positive control (PC). For each assay, 50% neutralization antibody titers were calculated and are listed in **(A)** together with the serostatus of the donors. Titers below 30 were considered negative, NT denotes not tested. **(B)** Spearman's Rank Correlation analysis demonstrates strong concordance between the two methods.

References

1. Roberts CM, *et al.* Increasing Proportion of Herpes Simplex Virus Type 1 as a Cause of Genital Herpes Infection in College Students. *Sexually Transmitted Diseases*, 30, 797–800, 2003.
2. Belshe RB, *et al.* Efficacy results of a trial of a herpes simplex vaccine. *New England Journal of Medicine*, 366, 34-43, 2012.
3. Nagel CH, *et al.* Nuclear Egress and Envelopment of Herpes Simplex Virus Capsids Analyzed with Dual-Color Fluorescence HSV1(17+). *Journal of Virology,* 82, 3109-3124, 2007.
4. Skoberne M, *et al.* An Adjuvanted Herpes Simplex Virus 2 Subunit Vaccine Elicits a T Cell Response in Mice and Is an Effective Therapeutic Vaccine in Guinea Pigs. *Journal of Virology*, 87, 3930-3942, 2013.

Neutralizing antibody titers against both viruses were increased after immunization with GEN-003; HSV-1 seronegative subjects had higher neutralizing antibody responses to HSV-2 than HSV-1.

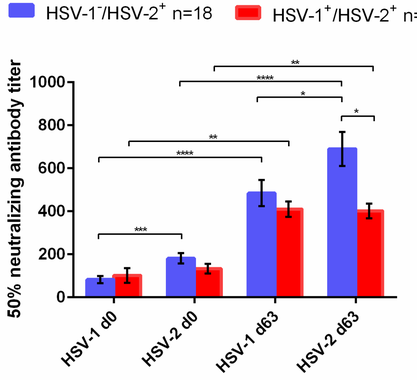


Fig. 3: HSV-1 and HSV-2 neutralization antibody titers in HSV-2 single-positive and HSV-1/2 double-positive subjects receiving 30µg of GEN-003. Titers are shown as mean ± SEM by subject HSV-1 serostatus (HSV-1- blue; HSV-1+ red), and were compared using Mann-Whitney tests (**** p < 0.0001, ** p < 0.01, * p < 0.05).

GEN-003 immunization resulted in greater neutralizing antibody titers against HSV-1 than HSV-2; adjuvant potentiated the responses.

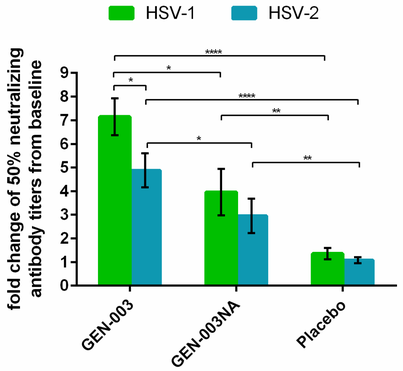


Fig. 4: Fold changes over baseline of HSV-1 and HSV-2 neutralizing antibody titers on day 63 post-vaccination with 30µg GEN-003 or GEN-003NA. Analysis was performed independently of HSV-1 serostatus. Data are represented as the mean fold change ± SEM, and groups were compared using Mann-Whitney tests (**** p < 0.0001, ** p < 0.01, * p < 0.05).

In subjects immunized with GEN-003 and GEN-003NA, neutralizing antibody responses to HSV-1 and HSV-2 strongly correlate with each other and with gD2-specific IgG, suggesting that the proportion of IgG antibodies capable of neutralizing virus is similar for both HSV-1 and HSV-2.

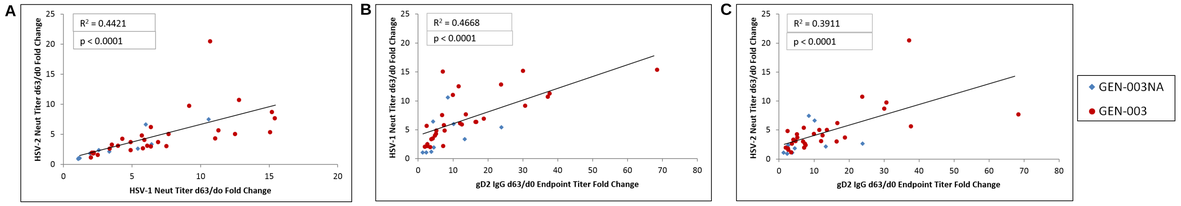


Fig. 5: Correlation of fold changes of HSV-1 and HSV-2 neutralization antibody titers and total IgG responses after vaccination with 30µg GEN-003 or GEN-003NA. Fold changes in titer on day 63 relative to baseline were calculated and plotted. Correlations were analyzed using a linear regression model and regression statistics are shown. **(A)** Correlation plot of HSV-1 vs. -2 neutralizing antibody titer fold changes. **(B)** Correlation plot of fold changes in HSV-1 neutralizing antibody and gD2 IgG endpoint titers. **(C)** Correlation plot of fold changes in HSV-2 neutralizing antibody titers compared with gD2 IgG endpoint titers.

Conclusions and Next Steps

- **Neutralizing antibody titers against both HSV-1 and HSV-2 were boosted in HSV-2 genitally infected subjects following vaccination with GEN-003 and GEN-003NA; increases in titer were greater in the presence of adjuvant.**
- **The neutralizing titers against HSV-2 in response to GEN-003 vaccination were higher in HSV-1 seronegative than seropositive subjects.**
- **These results suggest that the gD2 antigen included in our GEN-003 vaccine is capable of eliciting HSV-1 and HSV-2 cross-reactive neutralizing antibodies.**
- **Future studies will explore the therapeutic benefit of GEN-003 against HSV-1.**