GEN-003 Immunotherapy Significantly Reduced the Viral Shedding Rate in a Phase 2b Genital Herpes Clinical Trial

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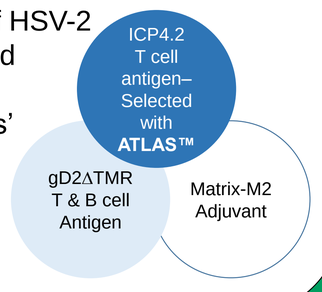
Introduction

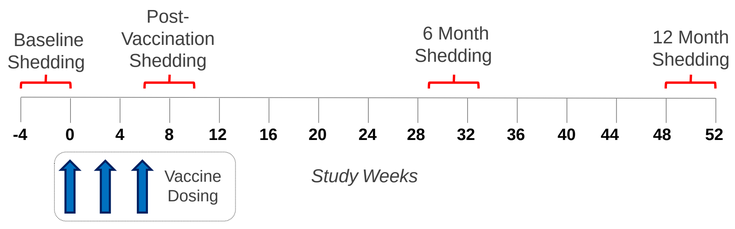
- Genital herpes, which is characterized by recurrent painful ulcers, is primarily caused by HSV-2 and affects more than 500 million people worldwide [1].
- HSV-2 infection increases the risk of HIV-1 transmission [2] and causes severe disease in infants and in immunocompromised individuals [3].
- Prior 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 (MM2) (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.



Study Design

- Subjects: 131 adults, aged 18-50, with genital HSV-2 infection and 3-9 lesion recurrences/year. Subjects on suppressive antivirals underwent washout period before dosing.
- Randomized to 1 of 3 treatments: 60µg antigen/50µg MM2, 60µg antigen/75µg MM2, or Placebo (saline). Three doses were administered at 21 day intervals.
- For viral shedding evaluations, subjects collected anogenital swabs twice daily for 28 day periods (56 swabs per period).
- Swabs were kept in 1mL of transport medium, selected because it reliably maintained DNA stability for as long as 12 weeks at room temperature.
- An HSV-2 real-time quantitative PCR assay was used to measure the presence of viral DNA in each swab sample. Viral shedding rates were determined as the number of swabs positive for HSV-2 DNA divided by the total number of swabs.
- Assay limit of detection (LOD) is 5 copies/reaction (1000 copies/mL). Assay lower limit of quantification (LLOQ) is 16 copies/reaction (3200 copies/mL).

See poster 8.04 for immunogenicity data and 8.25 for clinical data.



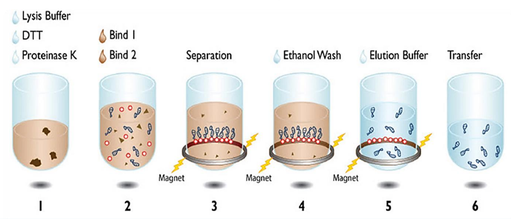
Materials and Methods

Step 1: Sample Preparation

Swab samples were arrayed into 96 well format. Swab transport media from subject swabs was then aliquoted into plates alongside viral controls and negative controls. Plate contents were then lysed.

Step 2: DNA Extraction

Viral DNA was extracted via an automated, high-throughput paramagnetic bead-based method using the Biomek NX^P Liquid Handling System (Beckman Coulter).



Step 3: qPCR

- Extracted viral DNA was quantified in duplicate using TaqMan™ quantitative real-time PCR for HSV-2 gG2 gene target.
- HSV-2 DNA quantity was extrapolated using a six-point standard curve prepared from commercially available HSV-2 genomic DNA (Vircell) and run in parallel with samples.

DNA Target	Oligo	Sequence
HSV-2 gG2	Forward 5'-3'	CGGAGACATTCGAGTACCAGATC
	Reverse 5'-3'	GCCCACCTCTACCCACAACA
	Probe 5' FAM, 3' TAMRA	ACCCACGTGCAGCTCGCCG

Cycling Profile			
	2 minutes	50ºC	
Initial Denaturation	10 minutes	95ºC	
Denaturation	15 seconds	95ºC	45 Cycles
Annealing	1 minute	60ºC	

Standard Curve Acceptance Criteria	
Slope	$-3.6 \leq x \leq -3.1$
R^2	≥0.98
% Efficiency	90-110

Rate of Viral Shedding Was Reduced by 40% in Subjects Treated with 60/50 Dose of GEN-003

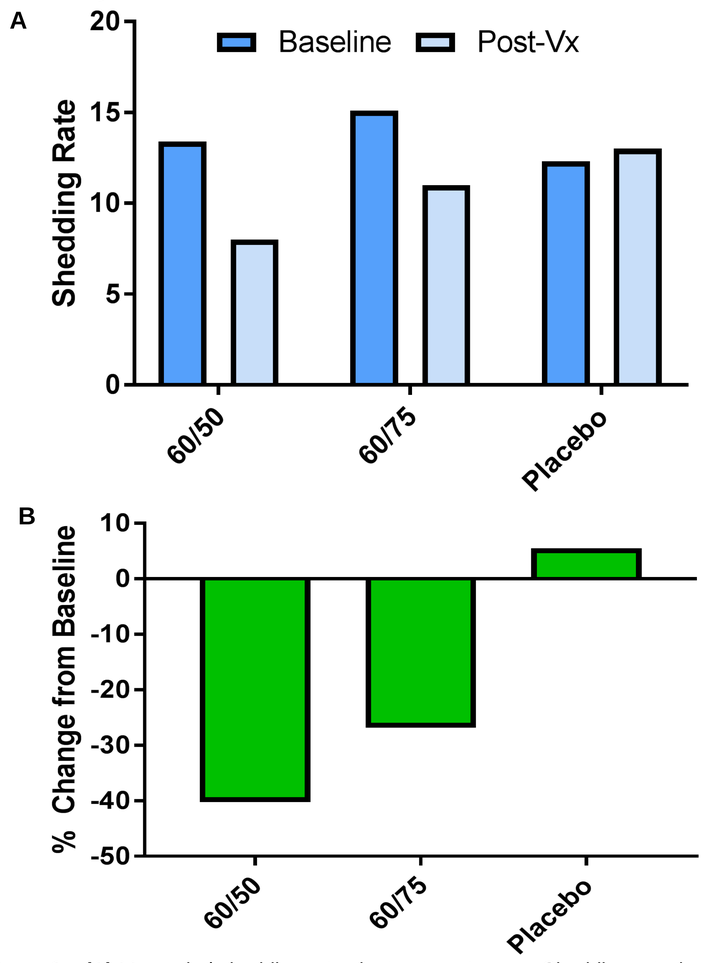


Figure 1 – **(A)** Mean viral shedding rates by treatment group. Shedding rate (swabs) is defined as the number of positive swabs divided by the total number of swabs. **(B)** Mean percent change from baseline in viral shedding rates by treatment group.

Rate Ratio of Viral Shedding is Significant for 60/50 Treatment Group

	GEN-003 Antigens/Matrix-M2 (µg)		
	60/50 (n=40)	**60/75 (n=42)**	**Placebo (n=42)**
Rate Ratio vs Baseline (95% CI)	0.58 (0.35, 0.95)	0.75 (0.50, 1.12)	1.06 (0.75, 1.49)
Within-Group *P* value	0.0302	0.1611	0.7579
Rate Ratio vs Placebo (95% CI)	0.55 (0.30, 1.00)	0.71 (0.41, 1.21)	-
Vs Placebo *P* value	0.0505	0.2045	-

Table 1 – Analysis of change from baseline in shedding rates (swabs), mixed effects model. Empirical method of variance estimation. Raw rates are presented. Rate ratios and p-values are based on a Poisson mixed model with treatment group, visit, treatment group by visit interaction (fixed effects), and subject (random effect). Abbreviations: CI = confidence interval.

Rate of Asymptomatic Viral Shedding Was Reduced by Over 38% in Subjects Treated with 60/50 Dose of GEN-003

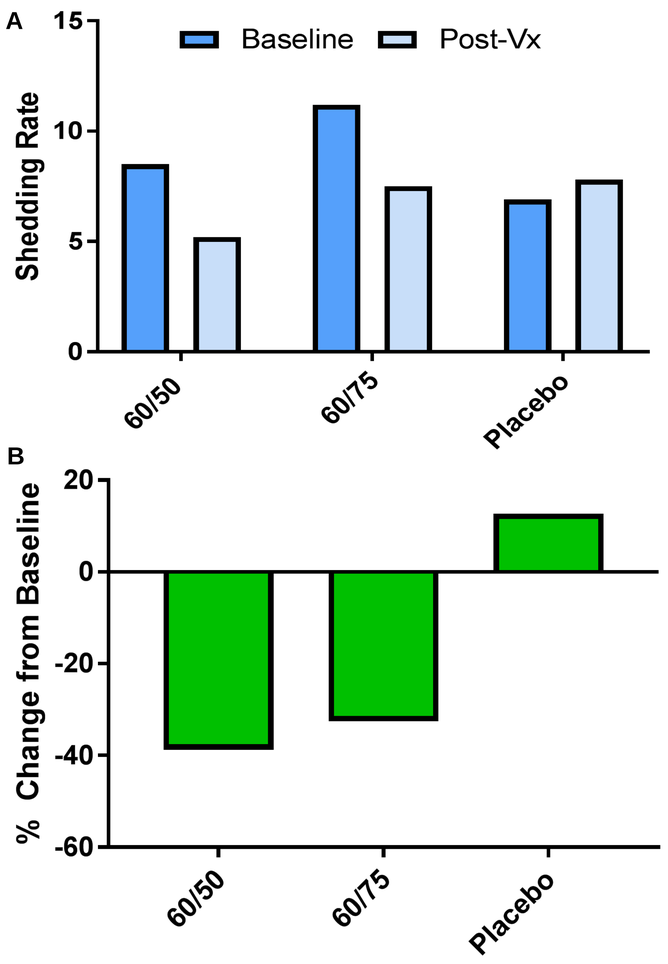


Figure 2 – (A) The 60/50 treatment group and 60/75 treatment group both had mean reductions in asymptomatic viral shedding rate compared to baseline, while the placebo group had a slight mean increase in asymptomatic viral shedding. **(B)** The 60/50 treatment group had the greatest percent change from baseline in asymptomatic viral shedding.

Descriptive Summary of Asymptomatic Shedding Rates by Treatment Group and Swab Period

	GEN-003 Antigens/Matrix-M2 (µg)		
	60/50 (n=43)	**60/75 (n=44)**	**Placebo (n=44)**
Baseline (%)	175/2056 (8.5)	233/2085 (11.2)	142/2052 (6.9)
Days 43-71 (Post-Vx) (%)	101/1940 (5.2)	140/1860 (7.5)	145/1860 (7.8)
% Change from Baseline	-38.8	-32.6	12.7

Table 2 – Asymptomatic shedding rates are calculated among swabs not associated with lesions. Numerator and denominator are summed across subjects. Swabs with no assay result available are not counted in the denominator.

60/50 Treatment Group Had Significant Reduction in HSV-2 Viral Shedding Rates Compared to Baseline

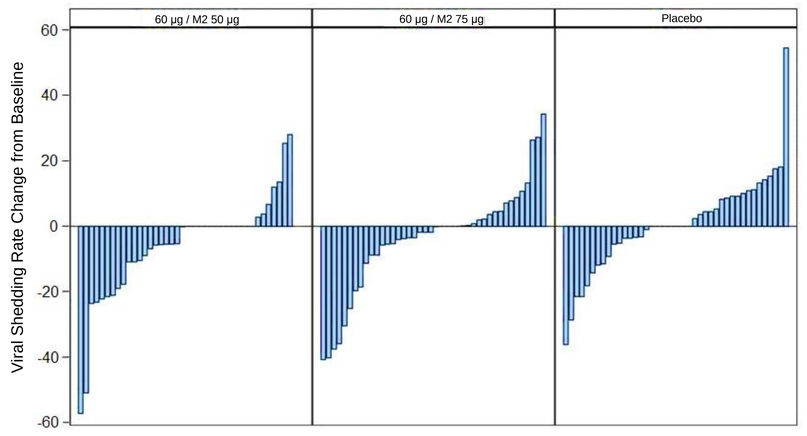


Figure 3 – Waterfall plot of absolute change in viral shedding rates from baseline to post-vaccination phase by treatment group. Each bar represents a subject's absolute change in HSV-2 shedding from baseline to post-vaccination phase (day 43-71); the absence of a bar indicates subjects with no change in viral shedding.

Dual Genital Infection with HSV-1 and HSV-2 Identified Using a Duplex qPCR Assay

- A duplex qPCR assay for HSV-1 and HSV-2 detection is being developed for use in Phase 3 clinical trials.
- The duplex assay will help assess dynamics of infection and co-infection with HSV; and will be a valuable tool in assessing infection and vaccine efficacy in future GEN-003 clinical trials.
- This assay is robust, linear, and accurately quantitates both HSV-2 (gG2 gene target) and HSV-1 (target downstream of gG1 gene) from subjects infected with HSV-1 and/or HSV-2.

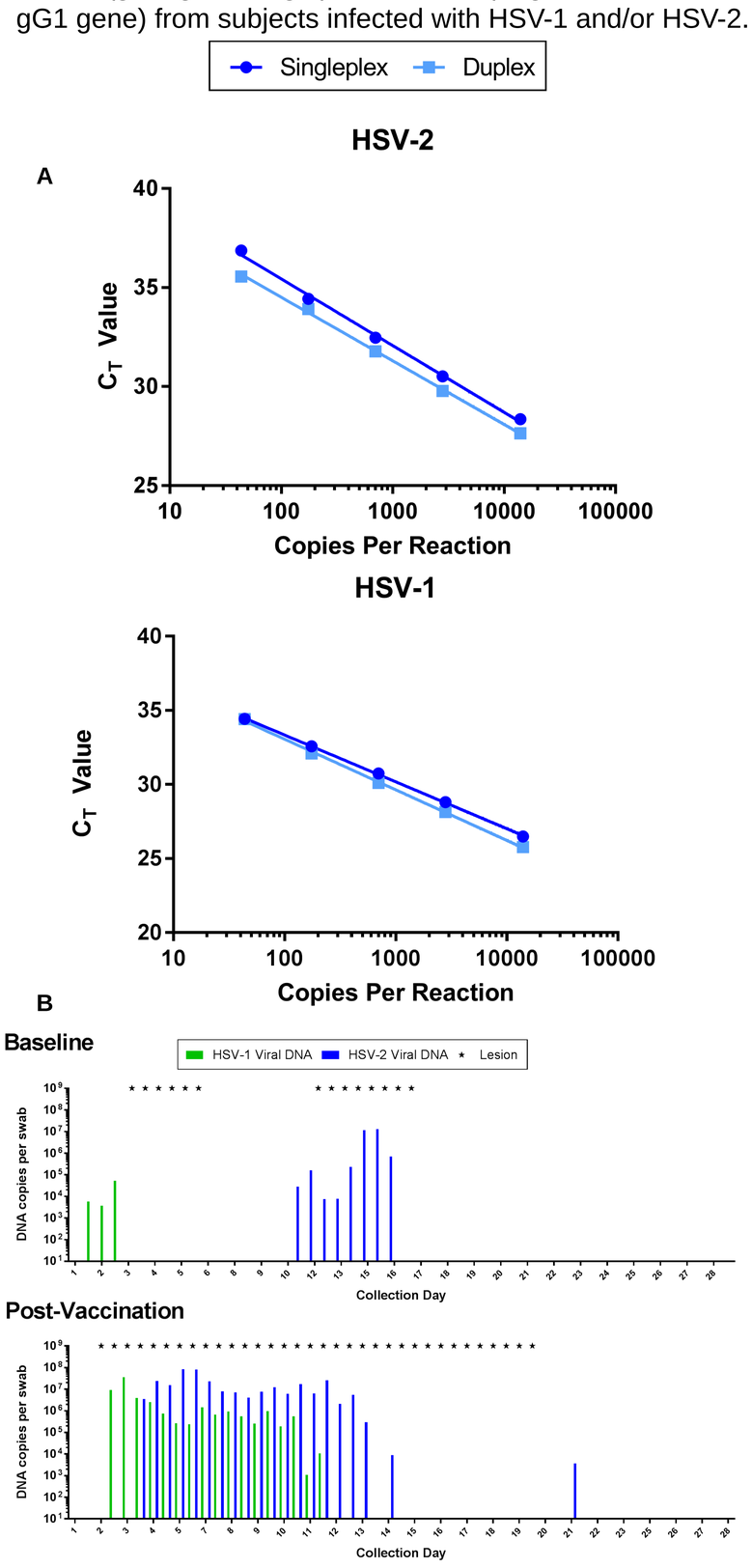


Figure 4 – (A) HSV-1 and HSV-2 standard curves prepared from commercially available gDNA (Vircell) were detected at similar levels in both singleplex and duplex qPCR formats. PCR efficiency values are reported as follows: HSV-2 singleplex: 98%, duplex: 104%; HSV-1 singleplex: 107%, duplex: 96%. **(B)** One case of co-infection was identified by the duplex assay, demonstrating multi-target detection of both HSV-1 and HSV-2 in clinical anogenital swab samples.

Conclusions

- The 60/50µg dose of GEN-003 demonstrated a statistically significant (vs placebo *p* value = 0.0505) 40.2% reduction from baseline in the viral shedding rate versus a marginal increase of 3.9% for placebo. The 60/75µg dose demonstrated a 24.7% reduction in the viral shedding rate.
- Asymptomatic shedding rates were also reduced among the 60/50 (38.8%) and 60/75 (32.6%) treatment groups.
- The 60/50µg dose of GEN-003 produced the more statistically significant reduction in viral shedding, supporting its selection for use in planned Phase 3 clinical trials.
- Dual genital infection of HSV-1 and HSV-2 is detectable using a duplex qPCR assay.
- Viral shedding will be evaluated for both HSV-1 and HSV-2 via the duplex qPCR assay in planned Phase 3 clinical trials.

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