GEN-003 Immunotherapy Shows Potential as a Prophylactic Vaccine Candidate for Genital Herpes in Guinea Pigs

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ABSTRACT

GEN-003 is a subunit vaccine candidate containing adjuvanted gD and ICp4 antigens that has shown durable therapeutic efficacy against genital herpes in Phase 2 clinical trials. Here we describe the potential benefit of GEN-003 as a prophylactic vaccine in a guinea pig model of genital herpes. Animals were immunized three times every two weeks with GEN-003 or control and intravaginally challenged with herpes simplex virus 2 (HSV-2, MS strain) 21 days later. GEN-003 significantly reduced frequency and severity of genital lesions in both acute and early stages of recurrent infection by over 85% and, within the 33 day follow-up period, 60% of vaccinated guinea pigs did not develop any lesions. During the acute phase, infectious virus titers recovered in vaginal swabs from GEN-003-immunized animals were 100-fold lower than from controls. Recurrent shedding frequencies were reduced by 27%. In addition, GEN-003 protected 40% of animals from the establishment of latency as shown by undetectable viral DNA in dorsal root ganglia (DRG). Furthermore, viral copy numbers normalized to β-actin in HSV-2-positive DRG from GEN-003-immunized animals were reduced by one log. GEN-003 vaccination induced antigen-specific antibody responses at least as high as those elicited by viral infection. Driven by gD-specific antibodies, HSV-2 neutralizing antibody titers followed the same pattern. After viral challenge, peripheral antigen-specific T cell responses in spleens of GEN-003-immunized animals were lower than those in controls as measured by interferon-γ ELISpot. This may have resulted from increased antigen-specific T cell recruitment to the genital muosa as a result of exposure to virus during acute and recurrent disease.

In summary, GEN-003 not only shows promise as a therapeutic, but also as a prophylactic vaccine candidate for genital herpes inducing robust immune responses that significantly impact primary disease, establishment of latency, and recurrences. Ongoing studies will determine how protection can be further enhanced.

INTRODUCTION

Genital herpes is primarily caused by herpes simplex virus 2 (HSV-2) and affects more than 500 million people worldwide1. The virus persists lifelong in infected individuals by establishing latency in dorsal root ganglia. From there, virus reactivates regularly resulting in viral shedding for transmission and occasionally recurrent genital lesions. In immunosuppressed patients, HSV-2 infection is associated with serious disseminated disease, illustrating the importance of the immune system in controlling viral reactivation.

GEN-003 is a subunit vaccine comprised of two viral antigens, ICp4.2 and gD2ΔTM, and the adjuvant Matrix-M2. In Genocea’s recent dose optimization trial for the treatment of genital herpes, GEN-003 was well tolerated by patients and reduced genital lesion rates by up to 65 % after 12 months as compared to pre-dosing baseline levels. Similarly, viral shedding rates were reduced by up to 62 %. These durable effects were comparable to those achieved with daily administration of oral antivirals with greatly improved convenience2. GEN-003’s efficacy as a prophylactic vaccine had not been studied.

Conceptually, antibody-mediated immune responses that can prevent infection will be highly important for prophylaxis and gD is a major neutralizing antibody target. In addition, the importance of T cell responses during primary infection is more and more appreciated3, and Genocea’s proprietary platform ATLAS has identified ICp4 as a highly important T cell antigen4. In this light, GEN-003 is an appealing candidate for HSV-2 prophylaxis.

The gold standard animal model for genital herpes uses guinea pigs, which closely mimics the course of human disease. Here, we are utilizing this model to evaluate the efficacy of GEN-003 as a prophylactic vaccine for HSV-2-induced genital herpes.

STUDY DESIGN

Figure 1: Study Design. Animals were immunized subcutaneously three times every two weeks and challenged intravaginally with 105 PFU HSV-2 MS strain three weeks later. Lesions were monitored during acute disease (day 15) and recurrent infection (day 35-36). Animal swabs were collected for quantification of viral genomes during both acute and recurrent infection. Serum was collected after the last immunization and at the end of the 33 day follow-up period. At termination of the study, spleens (T) and dorsal root ganglia (DRG) were collected.

Table 1: Experimental Groups. Groups 1 and 2 serve as negative control groups. GEN-003 is comprised of ICp4.2 and gD2ΔTM at a 1:1 ratio (15 μg each).

GEN-003 Reduced the Symptoms of Genital Herpes

Figure 2: GEN-003 Significantly Reduced Genital Lesions. Animals were assigned a daily lesion score based on severity of genital lesions, ranging from 0 (no lesion) to 4 (large ulcer with maceration). (A) Mean lesion scores are shown during the acute phase of infection and (B) in a cumulative fashion for the recurrent phase of infection. (C) The proportion of animals that did not develop any lesion during follow-up were calculated. Statistical analysis with Kruskal-Wallis test; ns, non-significant; *** p < 0.001, **** p < 0.0001.

GEN-003 Prevented Primary Infection and Reduced Viral Shedding

Figure 3: GEN-003 Decreased Primary Infection and Recurrent Viral Shedding. Vaginal swabs were collected throughout the course of the study and virus was quantified either by plaque titration or qPCR at the gD2 locus. (A) Infection was established in all groups by day 3. (B) Viral genome copies present in swabs collected during the recurrent phase of infection were measured by qPCR and the percentage of positive shedding days was calculated per animal (> 10 copies/ml). Statistical analysis with Kruskal-Wallis test; * p < 0.05, ** p < 0.01, *** p < 0.001.

GEN-003 Efficiently Induced Functional Antibody Responses

Figure 4: Immunization with GEN-003 Induced Antibodies at Least as Efficiently as Infection. Sera were collected one week after the last immunization (day 35, top row) and at the end of the study (day 83, bottom row) from four animals per group. Antigen-specific IgG antibodies (left and middle panels) for HSV-2 neutralizing antibodies (right panel) were quantified. Statistical analysis with Kruskal-Wallis test; ns, non-significant; p < 0.05.

Systemic T Cell Responses Are Reduced after GEN-003 Immunization

Figure 5: GEN-003-Immunized Animals Have Lower Systemic Interferon-γ T Cell Responses than Controls. At the end of the study, sera from five animals per group were collected for lymphocyte isolation. Interferon-γ production was quantified by ELISpot after stimulation with overlapping peptides spanning the sequences of the two GEN-003 antigens. Statistical analysis with Kruskal-Wallis test; * p < 0.05.

GEN-003 Reduced the Likelihood of Establishing Viral Latency

Figure 6: GEN-003 Can Protect from the Establishment of Viral Latency. At termination of the study, dorsal root ganglia were collected. DNA was extracted and viral genome copy numbers were quantified by qPCR. (A) The proportion of animals in which latent virus was detected was determined. (B) In virus-positive samples, viral copy numbers were normalized to the cellular housekeeping gene, β-actin. Statistical analysis with Kruskal-Wallis test; ns, non-significant; *** p < 0.001.

SUMMARY

In this guinea pig model, GEN-003 showed promise as a prophylactic vaccine for genital herpes by:

- reducing frequency and severity of genital lesions in both acute and early stages of recurrent infection by over 85 %.
- protecting 60 % of the animals from developing any lesions throughout the study.
- reducing infectious virus titers in genital tracts 100-fold during the acute phase, decreasing the likelihood of establishing latency.
- preventing establishment of latency in 40 % of animals, resulting in a 27 % reduction in recurrent shedding frequency.
- reducing systemic T cell responses, possibly a result of fewer natural boosts through recurrences or improved homing to the site of infection.

Ongoing studies will further characterize the location of GEN-003-specific T cells and will determine how protection can be further enhanced.

References

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