Vaccine xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Immune responses elicited by the GEN-003 candidate HSV-2 therapeutic vaccine in a randomized controlled dose-ranging phase 1/2a trial

Jessica Baker Flechtner *, Deborah Long, Shane Larson, Veronica Clemens, Amy Baccari, Lena Kien, Jason Chan, Mojca Skoberne, Matthew Brudner, Seth Hetherington

Genocea Biosciences, Inc., Cambridge, MA, USA

ARTICLE INFO

Article history:
Received 24 May 2016
Received in revised form 19 August 2016
Accepted 1 September 2016
Available online xxxx

Keywords:
Herpes simplex virus
Vaccine
Immunotherapy
Immune response

ABSTRACT

Purpose: GEN-003 is a candidate therapeutic HSV-2 vaccine containing a fragment of infected cell protein 4 (ICP4.2), a deletion mutant of glycoprotein D2 (gD2ΔTMR), and Matrix-M2 adjuvant. In a dose-ranging phase 1/2a clinical trial, immunization with GEN-003 reduced viral shedding and the percentage of reported herpetic lesion days. Here we examine the immune responses in the same trial, to characterize vaccine-related changes in antibody and cell-mediated immunity.

Methods: Participants with genital HSV-2 infection were randomized to 1 of 3 doses of GEN-003, antigens without adjuvant, or placebo. Subjects received 3 intramuscular doses, three weeks apart, and were monitored for viral shedding, lesions and immunogenicity. Antibody titers were measured by ELISA and neutralization assay in serum samples collected at baseline and 3 weeks post each dose. T cell responses were assessed pre-immunization and 1 week post each dose by IFN-γ ELISpot and intracellular cytokine staining. Blood was also collected at 6 and 12 months to monitor durability of immune responses.

Results: Antibody and T cell responses increased with vaccination and were potentiated by adjuvant. Among the doses tested, the rank order of reduction in viral shedding follows the ranking of fold change from baseline in T cell responses. Some immune responses persisted up to 12 months.

Conclusion: All measures of immunity are increased by vaccination with GEN-003; however, a correlate of protection is yet to be defined.

1. Introduction

Herpes Simplex Virus (HSV) is the etiologic agent of a lifelong, incurable sexually transmitted disease that establishes latency after infection and periodically reactivates. Genital infection is primarily caused by HSV-2, but the incidence of infection caused by HSV-1 is increasing [1]. Reactivation can either be symptomatic, resulting in genital ulcers that persist for several days, or asymptomatic. Acyclovir and valacyclovir are used episodically or chronically to treat infections [2,3], although protection is incomplete, with persistent asymptomatic shedding [4] and <50% reduction in transmission when administered daily.

Both the humoral and cell-mediated arms of the immune system are implicated in protective immunity against genital herpes. HIV-1 and HSV-2 co-infection studies have shown that decreases in $CD4^+$ T cell count are correlated with increased HSV-2 shedding [5]. In other studies, an influx of $CD8^+$ T cells has been proposed to control symptomatic HSV-2 reactivation in genital skin [6,7]. Antibody responses are important for virus neutralization, and recently, a statistically significant correlation between glycoprotein D (gD) antibody concentration and efficacy was demonstrated in a vaccine that prevented HSV-1 genital disease [8]. In addition, a prospective study demonstrated that the presence of HSV-2-specific maternal antibodies reduced the risk of neonatal transmission of HSV-2 [9].

Here we report the antibody and T cell responses observed in a clinical trial in HSV-2 positive subjects immunized with GEN-003, an adjuvanted protein subunit vaccine [10,11] designed to induce both antibody and T cell responses. The antibody target is a transmembrane deletion mutant of glycoprotein D (gD2ΔTMR), and the T cell antigen is a fragment of infected cell protein 4 (ICP4.2), identified by proteomic screens using T cells from HSV-2 infected or exposed human donors [12]. HSV-2-infected guinea pigs therapeutically immunized with GEN-003 had less disease and significantly reduced viral shedding frequency compared to controls [11]. In this

* Corresponding author at: Genocea Biosciences, Inc., Cambridge Discovery Park, 100 Acorn Park Drive, 5th Floor, Cambridge, MA 02140, USA.
E-mail address: jessica.flechtner@genocea.com (J.B. Flechtner).

http://dx.doi.org/10.1016/j.vaccine.2016.09.001
0264-410X/

clinical trial, immunization with GEN-003 resulted in up to a 52% reduction in viral shedding accompanied by a 65% reduction in percentage of days with reported herpetic lesions. At the most effective dose, the reduction in shedding was durable for at least six months, and the impact on disease persisted for at least one year [13,14].

2. Materials and methods

2.1. Study design

Subjects enrolled in this study were between 18 and 50 years of age, in general good health, with documented HSV-2 genital infection for at least one year and a history of three to nine recurrent episodes per year in the absence of antiviral suppression. Within each dose cohort of 10 μg, 30 μg and 100 μg per antigen, subjects were randomized in a 3:1:1 ratio to receive GEN-003 (antigens plus Matrix-M2 adjuvant), the proteins without adjuvant (antigens only) or placebo; the Matrix-M2 adjuvant (Novavax Inc., Baltimore, MD) was constant at 50 μg per dose. Unblinded pharmacists prepared and distributed treatment to participants. Blood was drawn for evaluation of T cell response seven days after each immunization, and for serum antibody, three weeks after each immunization. Blood was again collected at 6 and 12 months to monitor durability of immune responses. Institutional review boards approved all study procedures and the trial was registered with ClinicalTrials.gov, number NCT01667341. The study was conducted according to the 1964 Declaration of Helsinki, with written informed consent obtained from all participants. Safety was monitored by an independent Data Monitoring Committee.

2.2. Recombinant protein and adjuvant production

The two antigens included in GEN-003, ICP4.2 and gD2ΔTMR, were produced by Novasep (Brussels, Belgium) using a recombinant Baculovirus system as previously described [11]. Both proteins were concentrated to 0.6 mg/mL and stored at −70 °C. Matrix-M2 contained 92 parts of Matrix-A and 8 parts of Matrix-C and was prepared as previously described [10]. GEN-003, antigens only, and placebo control (Dulbecco's Phosphate-Buffered Saline (DPBS)) vaccinations were formulated at the study site just prior to administration and delivered intramuscularly in a 0.5 mL volume.

2.3. T cell responses

Leucosep tubes (Greiner Bio-One, Monroe, NC) were used to collect whole blood that was fractionated into peripheral blood mononuclear cells (PBMCs) by centrifugation within eight hours of phlebotomy. Washed PBMCs were frozen at a controlled rate and stored in the vapor phase of liquid nitrogen.

The IFN-γ ELISpot assays were performed as previously described with modifications [11]. PBMCs were thawed, counted, and then rested overnight in OpTmizer T cell serum-free medium (Life Technologies, Carlsbad, CA). Washed cells were plated at 4×10^5 cells/well and stimulated with 15-mer overlapping peptides (OLPs; JPT Peptide Technologies GmbH, Berlin, Germany) spanning the complete sequences of ICP4.2 or gD2 (including the transmembrane domain) at 1 μg/mL. Spot forming units (SFU) were quantified using an ELISpot reader system (ZellNet Consulting, Fort Lee, NJ).

For intracellular cytokine staining (ICS), thawed PBMCs were stimulated with OLPs as described for ELISpot. After 6–8 h incubation at 37 °C, 5% CO_2, 1.5 μL brefeldin A (Golgi Plug, BD Biosciences) was added and the plates incubated for an additional 9–12 h. Cells were washed and then labeled for 30 min at 4 °C with anti-CD3 PE (BD, Franklin Lakes, NJ), anti-CD4 APC and anti-CD8 PE-Cy5 (Biolegend, San Diego, CA). Fixed (BD Fix/Perm solution) and washed (BD Perm/Wash solution) cells were labeled with FITC-conjugated anti-IFN-γ antibody for 30 min at room temperature in the dark. Between 500,000 and 1,000,000 events were accrued (FACS Canto; BD) and data were analyzed with FlowJo Software (Ashland, OR).

2.4. Antibody responses

Blood was collected in SST tubes, and the serum processed by centrifugation then transferred into new polypropylene tubes and frozen at −20 °C. Vaccine antigen-specific IgG titers were determined by endpoint ELISA, as described previously [11]. HSV-2 neutralizing antibody (NAb) titers were determined via a β-galactosidase (β-Gal) colorimetric assay, as described previously [15].

2.5. Statistical analysis

For ELISpot analysis, the geometric mean and 95% confidence interval (CI) of log transformed SFU per 10^6 cells were calculated for each antigen and visit. Similar descriptive statistics were presented for the fold change from baseline. The proportion of subjects with a positive T cell response, defined as a >3.5-fold rise from baseline and absolute change from baseline of >37.5 SFUs/10^6 cells was determined by cohort and time point. Change from baseline was analyzed using a linear mixed model. ICS data are reported as the mean frequency of $CD4^+$ or $CD8^+$ IFN-γ-secreting cells ± standard deviation (SD). Data were normalized to the number of IFN-γ-secreting cells per 10^6 cells after media correction. Fold changes, and change from baseline were summarized as described for the other assays.

Antibody titers were logarithmically transformed and the geometric mean and 95% CI were calculated for each visit. Similar descriptive statistics were presented for the fold change from baseline. In addition, the proportion of subjects with a positive antibody response, defined as a ⩾ 4-fold rise in IgG titers or a ⩾ 2-fold rise in 50% NAb titer from baseline was analyzed by treatment group and time point. The 2-fold rise in NAb titer was determined based on results from a natural history study of a cohort of 26 subjects with the same disease history as the trial participants, who had a natural variability of 1.7-fold (mean + 2SD; unpublished data). Change from baseline was analyzed using a linear mixed model.

Correlations between immune responses and reduction in viral shedding were determined using linear regression analysis.

3. Results

3.1. T cell responses elicited by vaccination

Based on the definition of responder, ⩾96% of subjects in the adjuvanted dose groups were defined as responders to ICP4.2 and ⩾82% to gD2 at any one time point (Supplemental Table 1). There were more responders to ICP4.2 than to gD2. Response rates among the antigen only groups were lower than those to the adjuvanted doses.

The majority of subjects (71% and 92% to ICP4.2 and gD2, respectively) had baseline T cell responses, with a mean of 232 and 95 IFN-γ SFU/million cells in response to gD2 and ICP4.2, respectively. The peak T cell response occurred after the first immunization, and there was a non-statistically significant trend for an inverse dose response in the frequency of responding cells,

Please cite this article in press as: Flechtner JB et al. Immune responses elicited by the GEN-003 candidate HSV-2 therapeutic vaccine in a randomized controlled dose-ranging phase 1/2a trial. Vaccine (2016), http://dx.doi.org/10.1016/j.vaccine.2016.09.001