An Enzyme-Based Antibody-Dependent Cell-Mediated Cytotoxicity Assay (ADCC): Measuring the Functional Antibody Responses to an HSV-2 Therapeutic Vaccine

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**Introduction**

- GEN-003 is an candidate therapeutic HSV-2 vaccine containing a fragment of ICP4 (ICP4.2) and a deletion mutant of gD2 (gD2/TMR), adjuvanted with Matrix M-2.
- As part of an investigation of the antibody immune responses we plan to measure antibody-dependent cell-mediated cytotoxicity (ADCC).
- ADCC is a natural killer (NK) cell-mediated killing of virus-infected cells that are targeted for destruction by antiviral antibodies.

- We developed a colorimetric ADCC assay using a recombinant HSV TMR protein coated ELISA plates.
- Serum collected from the remaining clinical trial subjects (N=143 total) will be used to determine if GEN-003 or HSV-2 immunization caused a significant change in ADCC activity.
- TMR antibodies ranging from 8% to 23%, approximately 2 to 6 fold higher than % Lysis using a HSV/TMR serum. (B) Lysis ranged from 10% to 23% when PBMCs from Donor 5 was used and the individual Donor's serum. The ADCC activity did not change significantly when the ADCC assay was performed using individual Donor's PBMC and their own serum (data not shown) or Donor 5 PBMC and individual Donor's serum.

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**Results**

- We developed a high-throughput assay to measure ADCC activity in HSV-2* human serum.
- CD56+CD16+ NK cells mediate ADCC activity.
- PBMCs from a single donor were selected to test HSV-2* serum for ADCC activity (A). (B) PBMCs from Donor 5 was selected to screen ADCC activity in HSV-2* serum samples.

- **Fig. 1.** CD56+CD16+ NK effector cells, not CD56+ alone, mediate ADCC activity.
- **Fig. 2.** A control PBMC donor was selected to test HSV-2* serum for ADCC activity.

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**Summary**

- We developed a high-throughput assay to measure ADCC activity in HSV-2* human serum.
- CD56+CD16+ NK cells mediate ADCC activity.
- PBMCs from a single donor were selected to test HSV-2* serum for ADCC activity.
- ADCC activity in HSV-2* human serum ranged from 5 to 25%, but an individual's ADCC activity did not change over a 10 week interval or during lesion outbreaks.
- 16 of 21 GEN-003 clinical trial subjects (blinded) showed an increase in gD2/TMR antibody titers post-immunization whereas their ADCC activity did not significantly change post immunization.
- Serum collected from 27 HSV-2* subjects every 21 days (4 visits) over a 10 week interval and during lesion outbreaks (OB) was tested for ADCC activity using PBMCs from Donor 5 and HSV-2* infected FS-4 cells as targets. Four subjects, G001, G029, G010 and G017, are shown in this figure as an example of the data observed for all 27 HSV-2* subject's serum samples.

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**Fig. 3.** ADCC activity in serum samples from HSV-2* subjects

Serum collected from 27 HSV-2* subjects every 21 days (4 visits) over a 10 week interval and during lesion outbreaks (OB) was tested for ADCC activity using PBMCs from Donor 5 and HSV-2* infected FS-4 cells as targets. Four subjects, G001, G029, G010 and G017, are shown in this figure as an example of the data observed for all 27 HSV-2* subject's serum samples.

**Fig. 4.** ADCC activity and gD2/TMR ELISA titers in serum samples from GEN-003 clinical trial HSV-2* subjects

Twenty-one HSV-2* subjects were tested for ADCC activity (A) and anti-gD2/TMR antibodies (B) in serum pre (D0) and post (D63) 3rd immunization. Each subject received 3 immunizations of either placebo, proteins without adjuvant or GEN-003. Serum was collected 21 days after each immunization. Anti-gD2/TMR IgG titers were performed by endpoint dilution on gD2/TMR protein coated ELISA plates.

**Results:** (A) No significant change in % Lysis was observed in post immunization (D63) compared to pre immunization (D0). ADCC activity: (B) 16 of 21 subjects showed an increase in gD2/TMR ELISA titers post immunization over pre-immunization (D0) antibody titers.