VH3810109 (N6LS) Administration Dose-Responsively Enhances Anti-HIV Antibody-Dependent Cellular Cytotoxicity (ADCC) and Antibody-Dependent Cellular Phagocytosis (ADCP) Activity in Ex Vivo Models

P208

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Key Takeaways

- An exploratory analysis among participants naive to antiretroviral therapy (ART) in the phase 2a BANNER study was conducted to measure the effect of the broadly neutralizing antibody (bNAb) VH3810109 on antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP) activity and its relation to virologic response
- An observable dose-response increase in ADCP activity corresponded to VH3810109 exposure
- Viral load decline corresponded to increasing VH3810109 exposure; no independent effect of ADCP or ADCC on viral load was observed
- These results highlight the important immunologic effects of bNAbs and support their further development as direct-acting antiviral agents and as components of remission/cure regimens

Introduction

- HIV-specific bNAbs are being investigated for their antiviral activities through neutralization of the HIV Env protein, preventing interaction with cellular receptors and infection of new cells[1,2]
- Antibodies also mediate important immunologic effects, including directing effector cells of the innate immune system to clear infected cells producing viral proteins through ADCC or ADCP, which may contribute to reducing the viral reservoir[1,3]
- VH3810109 (also known as N6LS) is a CD4-binding site bNAb in phase 2b clinical trials for the maintenance of virologic suppression
- In this exploratory analysis among participants naive to ART in the phase 2a BANNER study, we measured the capacity of VH3810109 to change ADCP and ADCC activity in serum after single-dose monotherapy and related this to virologic response

Methods

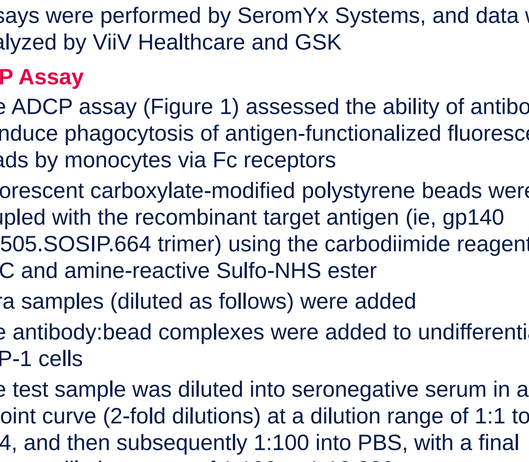
Samples and Analysis

- BANNER study participants (N=62) received the following VH3810109 doses: 70 mg intravenously (IV; n=16), 280 mg IV (n=6), 700 mg IV (n=16), 700 mg subcutaneously (SC; n=16), and 40 mg/kg IV (n=8)
- Serum samples (N=182) were taken at the following time points: Day 1/pre-dose (n=61), Day 3 (n=59), Day 28 (n=20), and time of standard-of-care (SOC) ART initiation (n=42)
  - Samples taken at time of SOC initiation were used when SOC initiation (after viral rebound) occurred before Day 28 of monotherapy
- For this analysis, samples from Day 28 and time of SOC initiation were pooled into a single time point
- Each sample was tested in an 8-point dilution curve, alongside HIV-positive (high, medium, and low) and HIV-negative control sera
- Area under the concentration-time curve (AUC) for ADCP and ADCC was determined and compared across study time points
- Assays were performed by SeromYx Systems, and data were analyzed by ViiV Healthcare and GSK

ADCP Assay

- The ADCP assay (Figure 1) assessed the ability of antibodies to induce phagocytosis of antigen-functionalized fluorescent beads by monocytes via Fc receptors
- Fluorescent carboxylate-modified polystyrene beads were coupled with the recombinant target antigen (ie, gp140 BG505.SOSIP.664 trimer) using the carbodiimide reagent EDC and amine-reactive Sulfo-NHS ester
- Sera samples (diluted as follows) were added
- The antibody:bead complexes were added to undifferentiated THP-1 cells
- The test sample was diluted into seronegative serum in an 8-point curve (2-fold dilutions) at a dilution range of 1:1 to 1:64, and then subsequently 1:100 into PBS, with a final in-assay dilution range of 1:100 to 1:12,800
- Phagocytosis was allowed to proceed overnight. The cells were washed and fixed, and the extent of phagocytosis was measured by flow cytometry. Data were reported as the average (n=2 replicates) phagocytic score, which accounts for the proportion of effector cells that were phagocytosed and the degree of phagocytosis
- 2 HIV-seropositive control samples previously determined to have pre-existing high and low ADCP activity were tested in 2 replicates against gp140 trimer. The AUC across all 8 dilution points was calculated for each control across all plates. The average AUC of the high and low controls across each plate was within the pre-specified coefficient of variability (CV) ≤30%

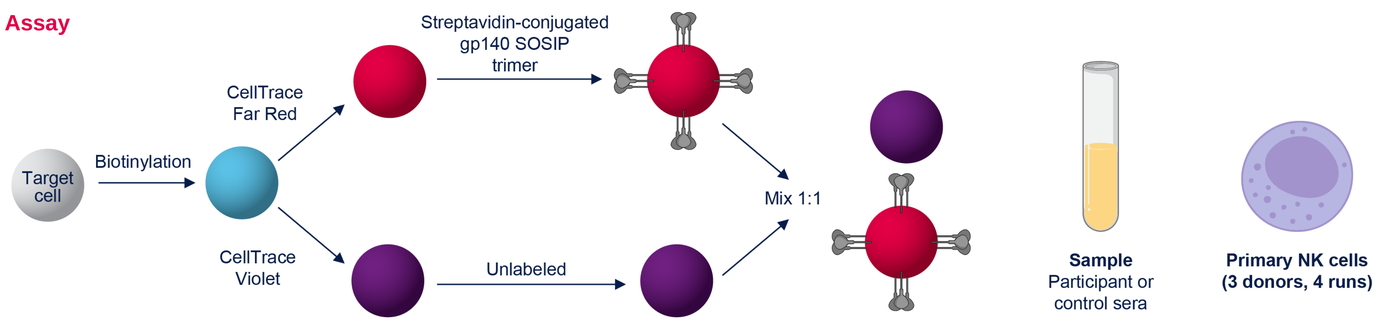
Figure 1. ADCP Assay



ADCC Assay

- The ADCC assay (Figure 2) assessed the ability of antigen-specific antibodies to recruit natural killer (NK) cell lytic activity
- Anti-CD4 fAb-blocked CEM.NKR target cells were biotinylated (EZ-Link Sulfo-NHS-LC-Biotin) and stained with 1 of 2 dyes (half with CellTrace™ Violet and half with CellTrace Far Red [Thermo Fisher Scientific Inc, Waltham, MA])
- Stained cells were pulsed with either streptavidin-conjugated recombinant antigen (ie, gp140 BG505.SOSIP.664 trimer) or left unpulsed
- Sera samples (diluted as follows) were added to a 1:1 mixture of the target cells stained with each dye

Figure 2. ADCC Assay



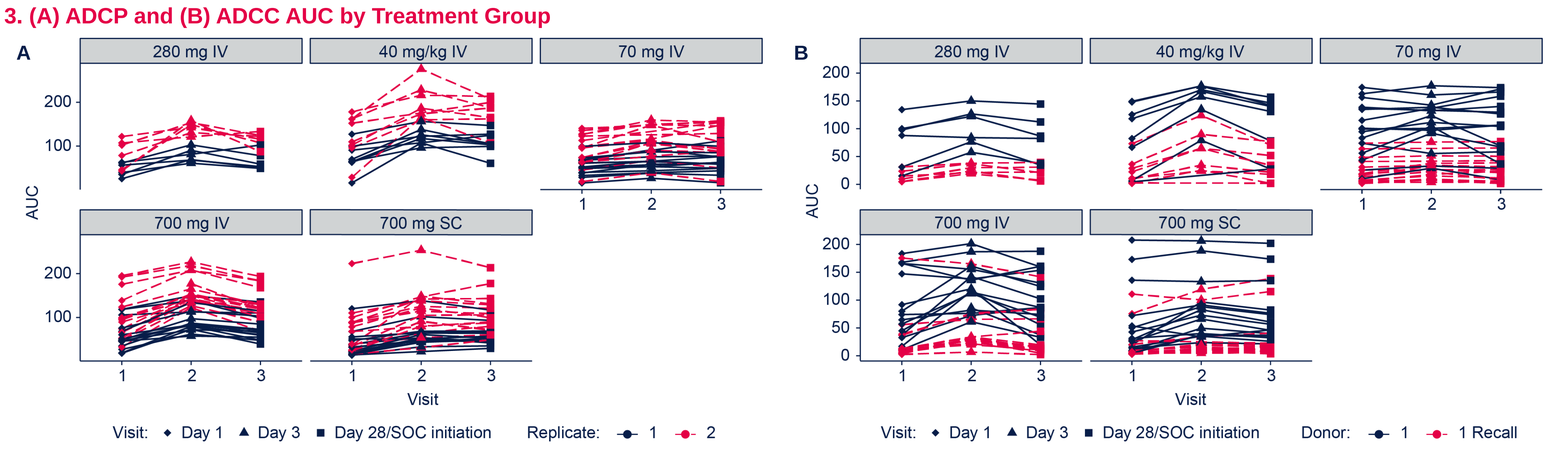
- NK cells, purified from seronegative donor buffy coats using the EasySep™ Human NK Cell Isolation Kit (STEMCELL Technologies, Vancouver, BC), were added and incubated for 4 hours; cells were then washed and stained with a viability dye using the Zombie Green™ Fixable Viability Kit (BioLegend, San Diego, CA)
- The test sample was diluted 1:8 into seronegative serum, and then 1:100 (total, 1:800) into media with subsequent dilution in an 8-point curve (1 half-log-fold dilutions), for a final in-assay dilution range of 1:800 to 1:2,517,095
- ADCC was measured as depletion of BG505.SOSIP.664-coated, anti-CD4 fAb-blocked CEM.NKR targets by primary NK cells isolated from a donor without HIV in the presence of an 8-point serial dilution of study serum (final, 1:800 to 1:2,517,095)
- Lysis was measured by flow cytometry and reported as the average (n=2 replicates) percent specific lysis, as determined by the percent lysis of antigen-coated (CellTrace Far Red–positive) cells vs that of the uncoated (CellTrace Violet–positive) cells using NK cells isolated from 1 donor on 2 different occasions
  - Note: Initially, 3 donors were used; however, assays using NK cells from Donors 2 and 3 failed to demonstrate any significant activity. Donor 1 was recalled and only results using NK cells from Donor 1 are presented
- 2 HIV-seropositive control samples previously determined to have pre-existing high and low ADCC activity were tested for ADCC activity using donor NK cells against gp140 trimer. The AUC across all 8 dilution points was calculated for each control across all plates. The average AUC of the high controls across all replicates was within the pre-specified CV ≤30%

Results

ADCP and ADCC

- A range of baseline ADCP and ADCC activity was observed, with some individuals having nearly no activity and others having near maximum activity even with dilutions >1:100,000 and >1:1000, respectively
- After VH3810109 administration, an increase in ADCP and ADCC activity was observed in most participants (Figure 3)

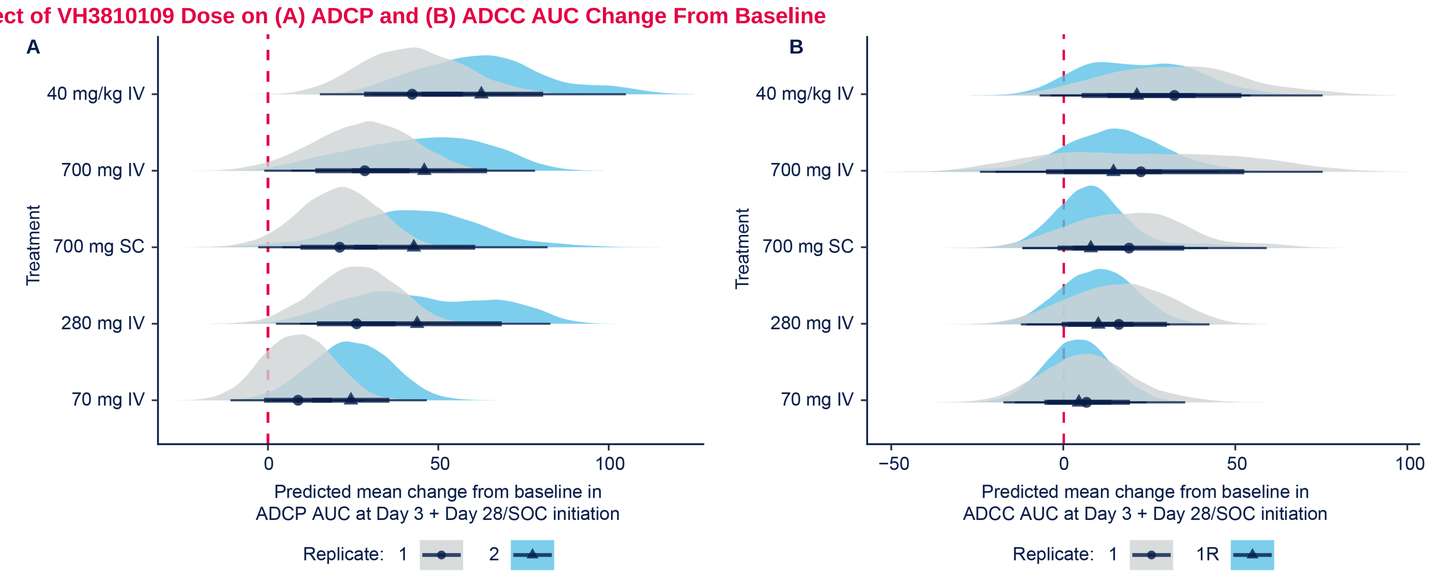
Figure 3. (A) ADCP and (B) ADCC AUC by Treatment Group



Effect of Treatment Dose on ADCP and ADCC Change From Baseline

- All doses of VH3810109 were able to elicit ADCP and ADCC activity, with a clear dose-response relationship observed for ADCP (Figure 4A); a weaker trend was observed for ADCC (Figure 4B)

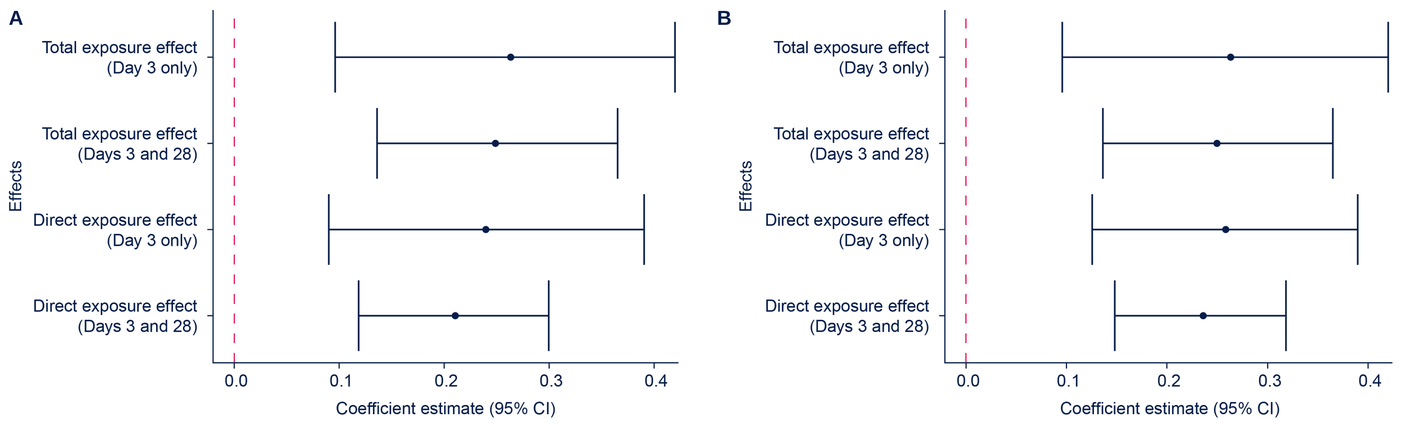
Figure 4. Effect of VH3810109 Dose on (A) ADCP and (B) ADCC AUC Change From Baseline



Effects of Exposure on Viral Load

- The observed decline in viral load across treatment groups in the BANNER study corresponded to both increasing VH3810109 exposure and ADCP AUC
- No independent effect of ADCP or ADCC on viral load was observed (Figure 5)

Figure 5. Total (VH3810109 and (A) ADCP or (B) ADCC) and Direct (VH3810109 Only) Effects of Exposure on Viral Load



Conclusions

- Results demonstrate that VH3810109 has immunologic activities ex vivo that correlate with clinically relevant virologic outcomes
- These data underscore the importance of the immunologic activities of bNAbs and support their continued development as direct-acting antiviral agents, as well as components of remission/cure regimens

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