Conserved HIV Immunogen Broadens T-Cell Immunity and Enhances CD8⁺ Antiviral Activity

289

Bhawna Sharma[1], Jiani Li[1], Maria Gamez-Guerrero[1], Alivelu Irrinki[1], Ross Martin[1], Tariro Makadzange[2], Evguenia Svarovskaia[1], Jeffrey Murry[1]

[1]Gilead Sciences, Inc., Foster City, CA, USA; [2]Charles River Medical Group, San Mateo, CA, USA

Conclusions

- The conserved bivalent HIV-1 immunogens produced de novo T-cell responses and boosted preexisting ex vivo T-cell activity in samples from participants who were virally suppressed on antiretroviral therapy; additionally, in vitro CD8⁺ T-cell antiviral activity was improved, and Pol-specific responses were expanded in mice
- These findings demonstrate that vaccination with conserved HIV-1 immunogens has the potential to induce functional HIV-1–specific T cells, supporting continued investigation of this strategy for HIV cure–related research

Plain Language Summary

- HIV medicines can keep the virus under control but cannot remove all of the virus inside infected cells
- If HIV medicine is stopped, the virus can bounce back to high levels
- This study tested a new vaccine that targets areas of HIV that stay the same across many different forms of the virus, with the goal of getting a stronger immune response that would be harder for the virus to escape
- When the vaccine was tested in human immune cells, it caused certain immune cells, called T cells, to start new responses or strengthened the responses they already had
  - It also helped a specific type of T cells (CD8 “killer” T cells) destroy cells where HIV was hiding
- These results suggest that vaccines affecting areas of HIV that stay the same across many different forms of the virus could help the immune system better target and control the virus

References: **1.** Siliciano JD, et al. *Nat Med*. 2003;9:727-8. **2.** Henrich TJ, et al. *Ann Intern Med*. 2014;161:319-27. **3.** Chen Z, Julg B. *Transl Res*. 2020;223:61-75. **4.** Alrubayyi A, et al. *Front Immunol*. 2022;13:908697. **5.** Huang SSY, et al. Presented at: Conference on Retroviruses and Opportunistic Infections (CROI); March 9-12, 2025; San Francisco, CA, USA. Abstract 144. **6.** Miyahira Y, et al. *J Immunol Methods*. 1995;181:45-54. **7.** Bailón L, et al. *Nat Commun*. 2025;16:2146.

Acknowledgments: Medical writing and editorial support were provided by Katherine Townsend, PhD, of Lumanity Communications Inc., and were funded by Gilead Sciences, Inc.

Correspondence: Bhawna Sharma, bhawna.sharma1@gilead.com

Disclosures: **BS**, **JL**, **MG-G**, **AI**, **RM**, **ES**, and **JM** are stockholders and employees of Gilead Sciences, Inc. **TM** is a former employee of Gilead Sciences, Inc.

Introduction

- Despite antiretroviral therapy (ART), HIV can persist due to long-lived reservoirs in memory CD4⁺ T cells that trigger rapid viral rebound if treatment is stopped[1,2]
- Various therapeutic vaccine platforms evaluated in clinical trials have not achieved durable viral control, largely due to suboptimal immune responses directed toward immunodominant nonconserved regions within HIV, viral escape, and immune dysfunction during chronic infection[3]
- CD8⁺ T cells are a key component of an effective immune response to HIV, but responses in chronic infection are often insufficient[4]
- Targeting conserved regions of HIV offers a strategy to generate de novo immune responses that are less vulnerable to escape, with broader coverage across HIV quasispecies[3]

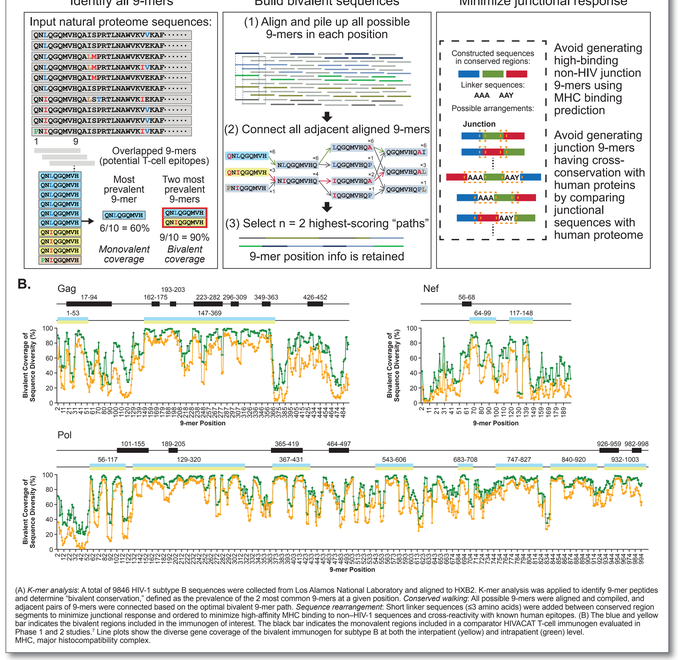
Objective

- To develop a conserved bivalent HIV-1 T-cell immunogen designed to refocus immunity toward conserved viral regions, with broad coverage and functional antiviral activity

Methods

- Conserved regions within HIV-1 clade B of Gag, Pol, and Nef were assembled into bivalent sequences using a conserved walking algorithm optimized for intra- and interpatient epitope coverage (**Figure 1**)
  - The resulting conserved bivalent immunogen has been evaluated in two Phase 1b clinical trials involving participants with HIV on suppressive ART (ClinicalTrials.gov Identifier: NCT06430905)[5]
- Immunogen processing and immunogenicity were assessed by monocyte-derived dendritic cell (Mo-DC)–peripheral blood mononuclear cell (PBMC) coculture assays
  - Mo-DCs from donor participants (people with HIV-1 on suppressive ART) were transduced with adenovirus serotype 5 (Ad5) vectors encoding the conserved HIV-1 immunogen (ie, vaccine), cocultured with autologous PBMCs, and assessed by an interferon gamma enzyme-linked immunosorbent spot assay as previously described[6] on Days 10 to 20 with overlapping peptides
- Vaccine-primed CD8⁺ T cells were tested for cytotoxicity against HIV-1 BaL-infected CD4⁺ T cells by flow cytometry
- In vivo immunogenicity was evaluated in mice receiving Ad5 single or bivalent immunogen sequences

Figure 1. (A) Workflow of Conserved Region Identification and Immunogen Construction and (B) Conserved Regions Included as Immunogens in the Vaccine



(A) *K-mer analysis*: A total of 9846 HIV-1 subtype B sequences were collected from Los Alamos National Laboratory and aligned to HXB2. K-mer analysis was applied to identify 9-mer peptides and determine “bivalent conservation,” defined as the prevalence of the 2 most common 9-mers at a given position. *Conserved walking*: All possible 9-mers were aligned and compiled, and adjacent pairs of 9-mers were connected based on the optimal bivalent 9-mer path. *Sequence rearrangement*: Short linker sequences (≤3 amino acids) were added between conserved region segments to minimize junctional response and ordered to minimize high-affinity MHC binding to non–HIV-1 sequences and cross-reactivity with known human epitopes. (B) The blue and yellow bar indicates the bivalent regions included in the immunogen of interest. The black bar indicates the monovalent regions included in a comparator HIVACAT T-cell immunogen evaluated in Phase 1 and 2 studies.[7] Line plots show the diverse gene coverage of the bivalent immunogen for subtype B at both the interpatient (yellow) and intrapatient (green) level.
MHC, major histocompatibility complex.

Results

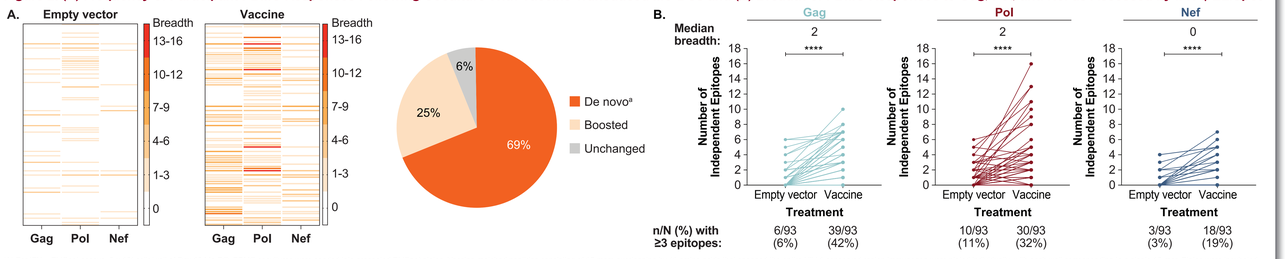
Ad5 Vector Expressing Vaccine Immunogen

- The bivalent vaccine design achieved >80% coverage of circulating intrapatient (n = 247) clade B virus across conserved epitopes

PBMC Responses

- Coculture of PBMCs with autologous Mo-DCs transduced with the vaccine induced de novo T-cell responses in 69% of participants’ PBMCs and boosted preexisting detectable responses in 25% of participants’ PBMCs (**Figure 2A**)
- Vaccination significantly enhanced the fraction of participant PBMCs that responded to ≥3 epitopes within Gag, Pol, and Nef (**Figure 2B**)

Figure 2. (A) Frequency of Participant PBMC Responses Following Coculture With Vaccine-Transduced Mo-DCs and (B) Breadth of PBMC Responses to Gag, Pol, and Nef as Assessed by IFN-γ ELISpot

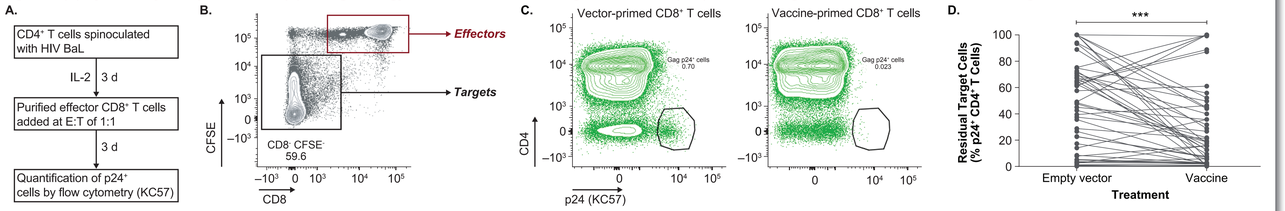


(A) For IFN-γ ELISpot assays, 3 × 10⁵ cells/well of Day 10 Mo-DC–PBMC cocultures were seeded into precoated ELISpot plates. Vaccine-matched peptides consisting of 15-mers overlapping by 11 amino acids that spanned the entire HIV-1 conserved region immunogen were plated individually, with each well corresponding to a single 15-mer. Plates were incubated for 24 hours, and SFUs were enumerated on an ImmunoSpot® reader following standard wash, stain, and developer methods.[7] Positive peptide responses were defined as SFUs >3-fold higher compared with the medium control. (B) The breadth of immune responses to Gag, Pol, and Nef was assessed by IFN-γ ELISpot assay post priming with Ad5 viral vectors expressing empty vector or conserved region constructs (vaccine). Each point represents 1 donor (n = 93 participants). *P* values were assessed by Wilcoxon matched pairs signed-rank test.
[a]De novo responses were directed at Gag and Pol.
****P <0.0001.
Ad5, adenovirus serotype 5; ELISpot, enzyme-linked immunosorbent spot; IFN-γ, interferon gamma; Mo-DC, monocyte-derived dendritic cell; PBMC, peripheral blood mononuclear cell; SFU, spot-forming unit.

CD8⁺ T-Cell Response

- Effector CD8⁺ T cells primed with vaccine showed enhanced cytotoxicity against HIV-1 BaL-infected target CD4⁺ T cells compared with CD8⁺ T cells primed with empty vector (P <0.001; **Figure 3**)

Figure 3. (A) Cytotoxicity Assay Schematic, (B) Gating Strategy to Define Effector and Target T Cells, and (C-D) Residual Target Cells Before and After Coculturing With Enriched Effector CD8⁺ T Cells

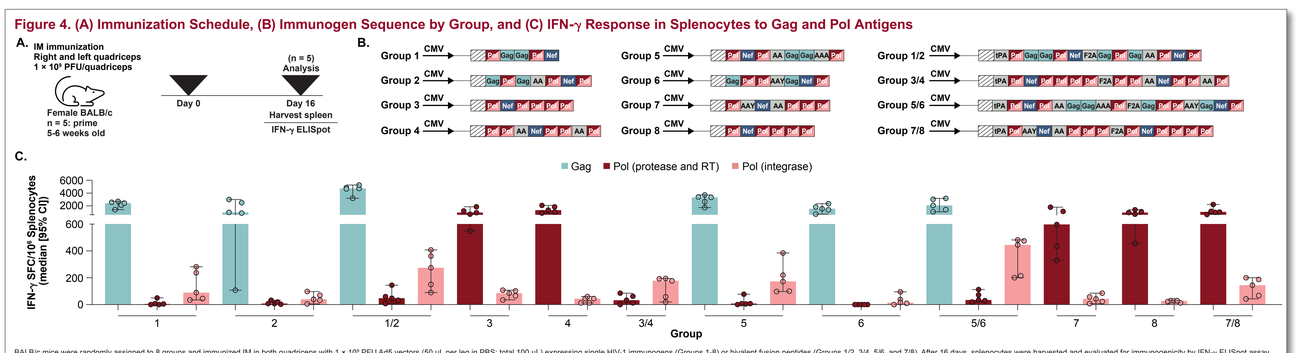


(A) Autologous CD4⁺ T cells were infected via spinoculation with HIV-1 BaL, cultured for 3 days alone or in the presence of CFSE-labeled CD8⁺ T cells (primed with empty vector or vaccine), and assessed by flow cytometry. (B) Representative flow cytometry plots illustrating the gating strategy to identify effector (CFSE⁻ CD8⁺ T cells) and target T cells (CFSE⁻ CD8⁻ T cells) after cells had been gated for live CD3⁺ cells. (C-D) Residual p24⁺ CD4⁺ T cells after coculture with enriched CD8⁺ T cells primed with empty vector or vaccine. Assays were completed in technical duplicate or triplicate depending on cell availability (n = 51 participants). *P* values were assessed by Wilcoxon matched pairs signed-rank test.
***P <0.001.
CFSE, carboxyfluorescein succinimidyl ester; E:T, effector-to-target ratio; IL-2, interleukin 2; KC57, anti-HIV Gag p24 antibody.

Immunogenicity

- In mice, most single-sequence immunogens induced responses, and joining immunogen sequences with an F2A sequence enhanced subdominant Pol responses; Gag responses were either enhanced or not inhibited (**Figure 4**)

Figure 4. (A) Immunization Schedule, (B) Immunogen Sequence by Group, and (C) IFN-γ Response in Splenocytes to Gag and Pol Antigens



BALB/c mice were randomly assigned to 8 groups and immunized IM in both quadriceps with 1 × 10⁵ PFU Ad5 vectors (50 µL per leg in PBS; total 100 µL) expressing single HIV-1 immunogens (Groups 1-8) or bivalent fusion peptides (Groups 1/2, 3/4, 5/6, and 7/8). After 16 days, splenocytes were harvested and evaluated for immunogenicity by IFN-γ ELISpot assay as previously described.[7]
Ad5, adenovirus serotype 5; CMV, cytomegalovirus; ELISpot, enzyme-linked immunosorbent spot; IFN-γ, interferon gamma; IM, intramuscular; PBS, phosphate-buffered saline; PFU, plaque-forming unit; RT, reverse transcriptase; SFC, spot-forming colony; tPA, tissue-type plasminogen activator.