AN AI-REDESIGNED CONTEMPORARY HIV-1 ENV CONSENSUS ANTIGEN IS MORE IMMUNOGENIC THAN A NATIVE SEQUENCE

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Abstract

There is a need to design novel HIV-1 vaccine candidates that could elicit broadly neutralizing antibodies (bnAbs) and that reflect currently circulating viruses. Here we tested a subtype B envelope (Env) consensus designed *in silico* with shortened hypervariable loops and a native B Env to compare their Ab binding and neutralizing responses in rabbits.

Background

Elicitation of broadly neutralizing antibodies

- The elicitation of bnAbs remains the primary goal of HIV-1 vaccine design
- HIV-1 envelope (Env) mediates viral entry and is the target for neutralizing antibodies
- Interactions between B cells and the HIV founder envelope during acute infection may be critical to developing bnAbs (Townsley et al., 2022)
- Env hypervariable loops are adjacent to many bnAb epitopes and can occlude Ab contact

Global HIV-1 diversity

- Subtype B accounts for 11.3% of global HIV-1 infections (Nair et al., 2024)
- Subtype B Env diversity has increased 25% since 2000 and the consensus has gradually shifted (Lewitus, Li, Rolland, 2024)
- Nearly half of subtype B consensus Env changes have occurred in hypervariable regions
- A contemporary subtype B consensus Env best represents circulating HIV-1 subtype B diversity

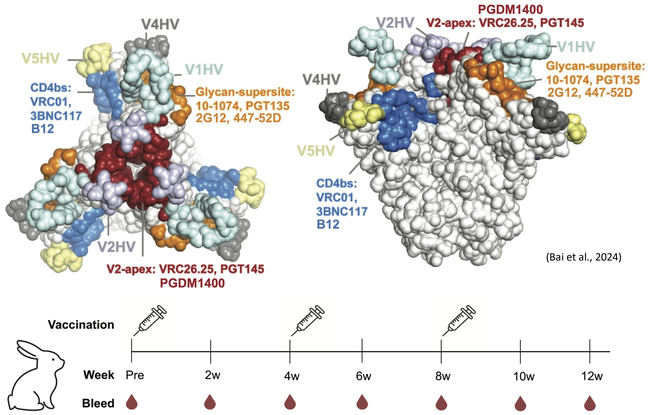
Hypothesis

An *in silico*-designed subtype B consensus Env will elicit stronger Ab binding and neutralizing responses than a native B Env.

Aim 1: Confirm membrane-bound stabilized consensus and native B trimers are expressed as mRNA encapsulated in lipid nanoparticles and recognized by bnAbs targeting Env epitopes.
Aim 2: Assess the Ab binding and neutralizing responses of rabbits immunized with each Env vaccine.

Methods

- Subtype B consensus Env sequence was derived from circulating B viruses sampled since 2011 (n=462)
- Native Env selected as the most sensitive to bnAb neutralization in a cohort of 34 participants sampled in early infection in the RV551 cohort (Wiezcorek et al., 2023)
- Env hypervariable loops were modeled (Bai et al., 2024) using AlphaFold2 by
  - Shortening hypervariable loops V1, V2, and V5 while maintaining Env structural integrity
  - Preserving anchor sites that form favorable interactions with the non-hypervariable region
  - Keeping at least one PNGS to maintain the glycan shield
- Sequences were designed as membrane-stabilized trimers and expressed as mRNA encapsulated in lipid nanoparticles (mRNA/LNP)
- Rabbits were immunized at weeks 0, 4, and 8 with 20ug of mRNA/LNP encoding a consensus or native Env (n=6/arm)
- Blood was collected every other week until week 12
- Binding Abs were measured with a multiplex Luminex assay (using fold-change over baseline)
- Neutralization was measured with a TZM-bl assay using tier 1 (n=5) and 2 (n=12) pseudoviruses



Results

Figure 1. Selection of subtype B consensus and native Env antigens

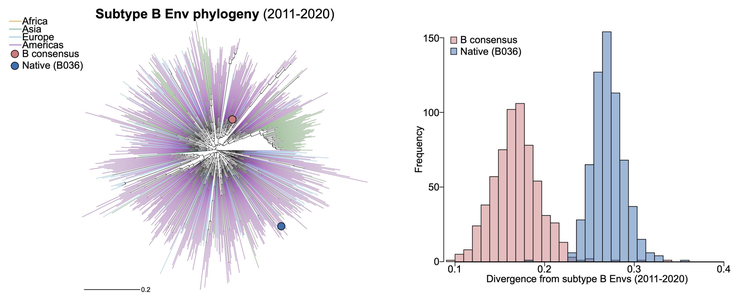
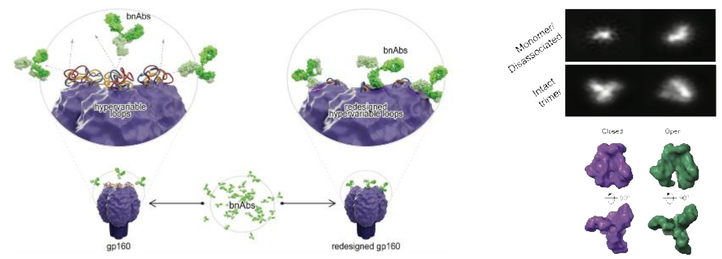


Figure 2. B consensus Env with AI-redesigned hypervariable loops V1, V2, V4, and V5 detected in closed trimeric conformation and promoted Ab recognition



(Bai et al., 2024)

Figure 3. B consensus and native Env proteins were well expressed and recognized by bnAbs targeting different Env epitopes

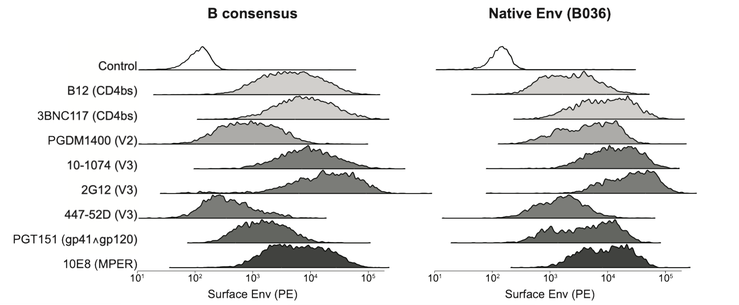


Figure 4. B consensus Env induced higher binding IgG responses than the native Env to heterologous SOSIPs

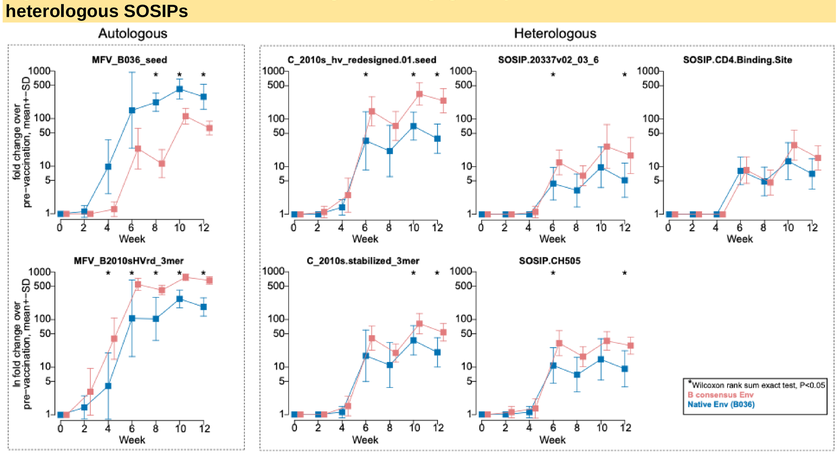


Figure 5. More rabbits in the B consensus than native Env vaccine group neutralized Tier 1a strains

Strain	Tier
93MW965	1a
GS015	1a
MN	1a
SF162	1a
TH023	1a
MuLV	Negative control

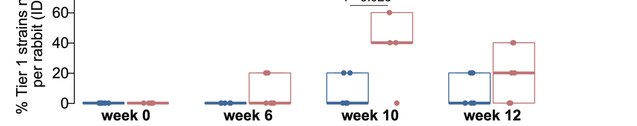
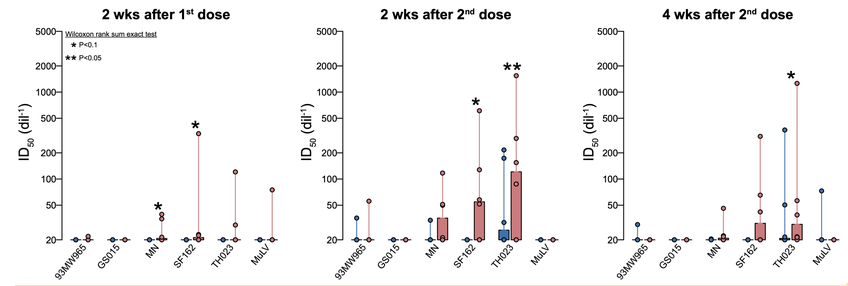


Figure 6. Neutralization titers tended to be higher in the B consensus than native Env vaccine group



Conclusions

- Contemporary HIV-1 Envs that are redesigned and optimized with AI tools are well expressed and immunogenic.
- AI-redesigned subtype B consensus Env elicited superior antibody responses compared to native Env, warranting further testing as a vaccine candidate.
- Similar results were obtained for a subtype C consensus Env.

References

Townsley, S. M., Donofrio, G. C., Jian, N., Leggat, D. J., Dussupt, V., Mendez-Rivera, L., ... & Krebs, S. J. (2021). B cell engagement with HIV-1 founder virus envelope predicts development of broadly neutralizing antibodies. *Cell host & microbe*, 29(4), 564-578.

Lewitus E, Townsley SM, Li Y, Donofrio GC, Dearlove BL, Bai H, Sanders-Buell E, O'Sullivan AM, Bose M, Kibuuka H, Maganga L, Nitayaphan S, Sawe FK, Eller LA, Michael NL, Polonis VR, Ake JA, Vasan S, Robb ML, Tovanabutra S, Krebs SJ, Rolland M. (2022) HIV-1 infections with multiple founders associate with the development of neutralization breadth. *PLoS Pathogens*, 18(3):e1010369.

Nair, M., Gettins, L., Fuller, M., Kirtley, S., & Hemelaar, J. (2024). Global and regional genetic diversity of HIV-1 in 2010–21: systematic review and analysis of prevalence. *The Lancet Microbe*, 5:100912.

Lewitus, E., Li, Y., Bai, H., Pham, P., & Rolland, M. (2024). HIV-1 Gag, Pol, and Env diversified with limited adaptation since the 1980s. *Mbio*, 15(3), e01749-23.

Wieczorek, L., Sanders-Buell, E., Zemil, M., Lewitus, E., Kavusak, E., Heller, J., ... & Polonis, V. R. (2023). Evolution of HIV-1 envelope towards reduced neutralization sensitivity, as demonstrated by contemporary HIV-1 subtype B from the United States. *PLoS Pathogens*, 19(12): e1011780.

Bai, H., Lewitus, E., Li, Y., Thomas, P. V., Zemil, M., Merbah, M., ... & Rolland, M. (2024). Contemporary HIV-1 consensus Env with AI-assisted redesigned hypervariable loops promote antibody binding. *Nature Communications*, 15(1), 3924.

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