Polyreactivity of Broadly Neutralizing Antibodies Directed to the Membrane External Proximal Region of HIV-1 Envelope

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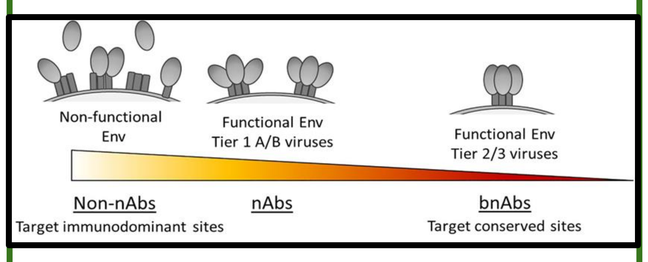
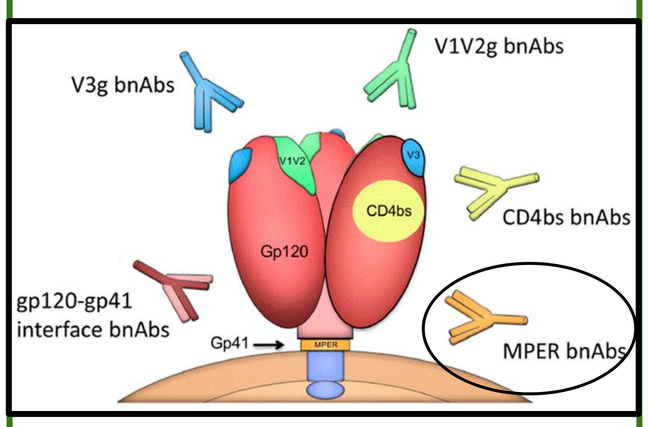
Introduction

The human immunodeficiency virus type 1 **(HIV-1)** remains a significant global health challenge, primarily due to its high mutation rate. While modern antiretroviral therapies (ART) have successfully made HIV into a manageable, chronic condition, these treatments have their own set of problems, including the need for strict daily adherence, potential for drug resistance, and various side effects.

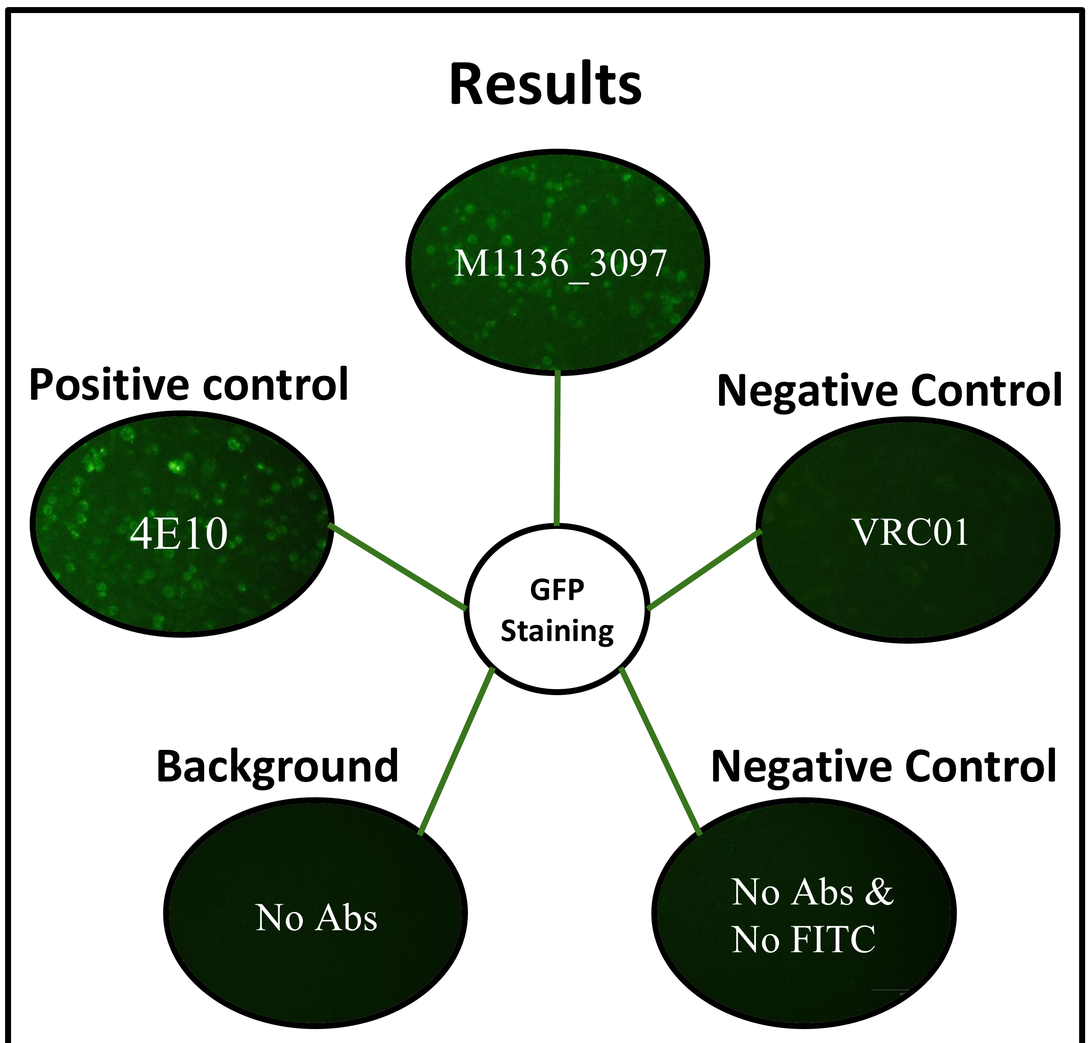


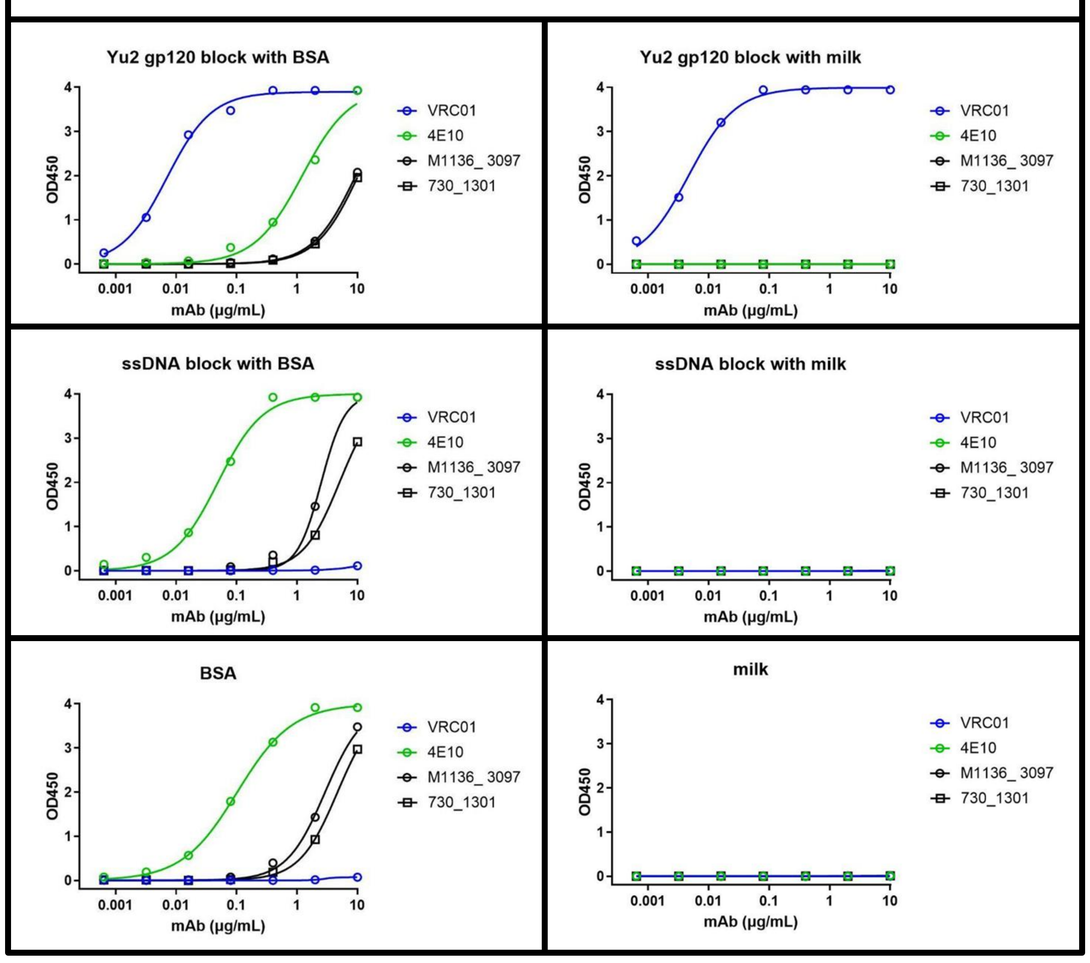
A promising alternative for therapeutic development is to target the virus's Envelope glycoprotein (Env) with broadly neutralizing antibodies **(bnAbs)**. Unlike conventional antibodies that target single strains and are easily evaded, bnAbs can neutralize a wide range of viral strains by targeting conserved regions on the Env protein that are essential for the virus's function. These unique antibodies possess special structural features, such as longer binding loops, that allow them to access these otherwise hard-to-reach sites, such as the membrane-proximal external region **(MPER)**.



The development of these therapies is complicated by the risk of **polyreactivity** or autoreactivity. In a clinical setting, polyreactivity in a therapeutic antibody can lead to dangerous off-target side effects by binding to healthy host tissues, potentially causing autoimmune-like reactions. Non-specific binding also reduces the effective concentration of the antibody available to fight the intended target, making the treatment less viability and potent. By assessing the specificity and polyreactivity of four different monoclonal antibodies directed to the MPER, this study's objective is to become more informed about the development of safer and more effective antibody-based therapies that could improve on current treatments.

Results





Discussion/Conclusions

VRC01 demonstrated high-affinity binding to Yu2 gp120 with negligible reactivity to ssDNA, BSA, and milk indicating high specificity. In contrast, 4E10, an MPER-targeting bNAb, exhibited strong binding to BSA, confirming its polyreactive characteristics. Both **M1136_3097 and 730_1301 mAbs displayed moderate reactivity** with BSA at high concentrations indicating **weak polyreactivity** of the MPER-directed antibodies.

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Future steps include analyzing these antibodies at a molecular level in comparison with highly specific antibodies such as VRC01 or highly polyreactive ones like 4E10 to determine what factors contribute most heavily to antibody polyreactivity.

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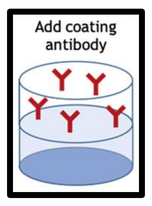
Removing such off-target reactivity might be required to develop the MPER-targeting bNAbs, including M1136_3097 and 730-1301, as safe and effective antibody-based therapies to combat HIV-1.

Methods

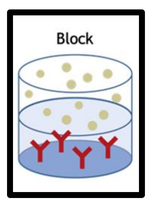
Indirect Immunofluorescence Assay

- Cultured **Hep-2 cells** were pre-seeded at 10^4 cells/well in a 96-well plate and incubated overnight
- Cells were fixed with **4% PFA in PBS** for 15 minutes to preserve their structure, and then the test antibodies (**mAbs**) were diluted to **50 µg/mL**, added to the wells, and incubated for one hour before washing.
- After washing the primary antibodies, an **FITC-conjugated mouse anti-human IgG** secondary antibody was added and incubated before a final wash to remove any unbound conjugate.
- The stained cells were then observed and imaged using a **fluorescence microscope (X40)** to visualize where the antibodies bound.

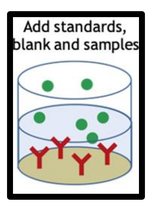
ELISA



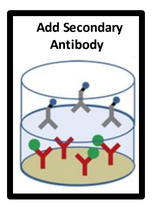
We coated the plate with our antigens, **Yu2 gp120 (at 2 µg/mL)** and **single-stranded DNA (ssDNA) (at 10 µg/mL)**, to assess antibody binding and test for polyreactivity.



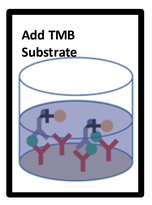
To prevent non-specific binding, we added a blocking buffer, running two separate trials with either **Bovine Serum Albumin (BSA)** or **2% fat-free milk.**



We then added our four test antibodies—**VRC01, 4E10, M1136_3097, and 730_1301**—at an initial concentration of **10 µg/mL**, performing a 5-fold serial dilution across the plate.



After incubating with the primary antibodies and washing, we added the **anti-human IgG-Fc-HRP** secondary antibody at **1:10K** dilution, which binds to our human antibodies and carries the detection enzyme



We added the **TMB** substrate solution, which reacts with the HRP enzyme to produce a **measurable blue color** wherever the antibody has bound.

Finally, we added **sulfuric acid** as a stop solution to halt the enzymatic reaction and turn the color from **blue to yellow** for quantitative measurement.

Optical Density Measurement of Well Plate at 405 nm

Reference

Acknowledgements

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