Enhanced recognition of V3 bnAb epitopes induced by Fostemsavir on cell-associated primary HIV-1 Env

304

Raymond A. Alvarez[1], Eric Acosta[1], Svenja Weiss[1], Dania Figueroa Acosta[1], and Benjamin K. Chen[1]

[1]Division of Infectious Diseases, Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, USA

BACKGROUND & AIMS

Fostemsavir (FTR), approved by the FDA in July 2020, is a first-in-class small molecule for treating multi-drug resistant HIV-1 infection. It is the prodrug of its active form, temsavir (TMR). TMR is classified as an attachment inhibitor and is thought to stabilize a native state of HIV Env, the state recognized by most broadly neutralizing antibodies (bnAbs)[1], while also influencing Env glycosylation and proteolytic cleavage[2,3]. However, the precise mechanism(s) by which TMR affects Env processing and conformation, particularly on cell-associated versus virus-incorporated forms of Env, remains unclear.

In this study, we aimed to expand on previous findings by examining the effects of TMR on the binding of a set of bnAbs targeting different epitopes of the HIV Env (CD4bs, V1, V2, V3). We also utilized an extended panel of lab-adapted and primary transmitted founder (T/F) strains (NL4-3, JRFL, YU2, T/F RHPA, and T/F QH06942).

MATERIAL AND METHODS

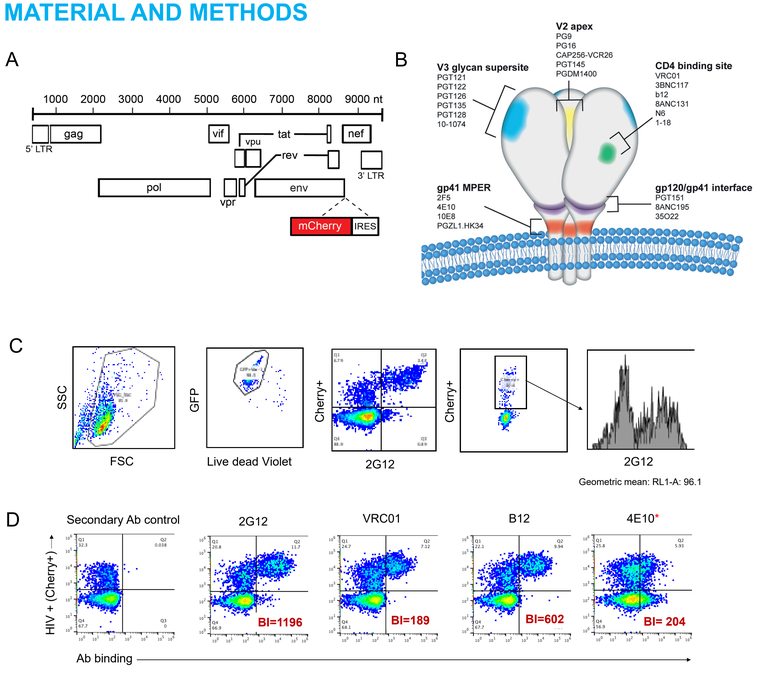


Figure 1 (A) HIV-1 mCherry reporter virus used in experiments. **(B)** HIV envelope and major epitopes targeted by broadly neutralizing antibodies (bnAbs)[4]. **(C)** FACS plots depicting the levels of HIV-specific Ab binding to infected cells using the bnAb 2G12. **(D)** Sample binding plots for 2G12, VRC01, b12 and 4E10. Binding index (BI) values are shown.

RESULTS

Temsavir differentially modulates bnAb binding in cell-associated vs virus particle forms of HIV Env

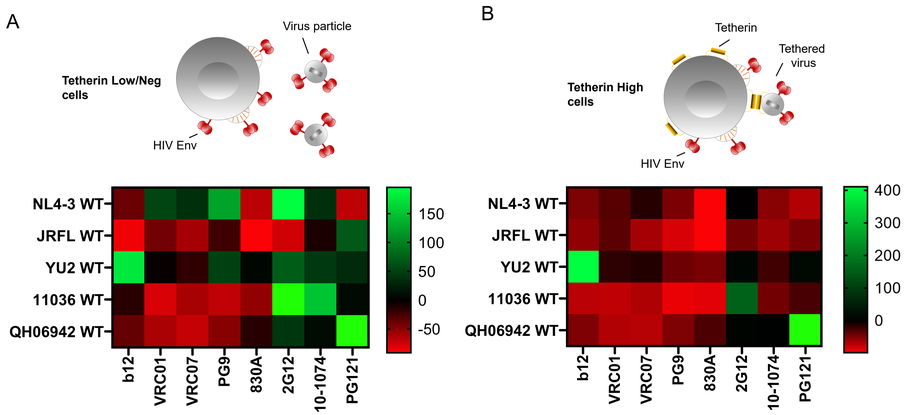


Figure 2. TMR modulation of IgG cell surface binding differs between cell-surface and virus-incorporated forms of HIV-1 Env. (A) Heatmap depicting the percent modulation of bnAb binding in the tetherin-low system, which primarily displays cell-surface expressed forms of the HIV-1 Env. (B) Heatmap depicting the percent modulation of bnAb binding in the tetherin-high system, which primarily displays virus particle incorporated forms of HIV-1 Env. Both panels compare the effect of 10 μM TMR for 24 hrs across different HIV-1 strains and bnAbs. Green indicates increased binding, and red indicates decreased binding relative to untreated controls.

Temsavir treatment preferentially enhances V3 region bnAb binding in uncleaved forms (gp160) of T/F Env QH0692

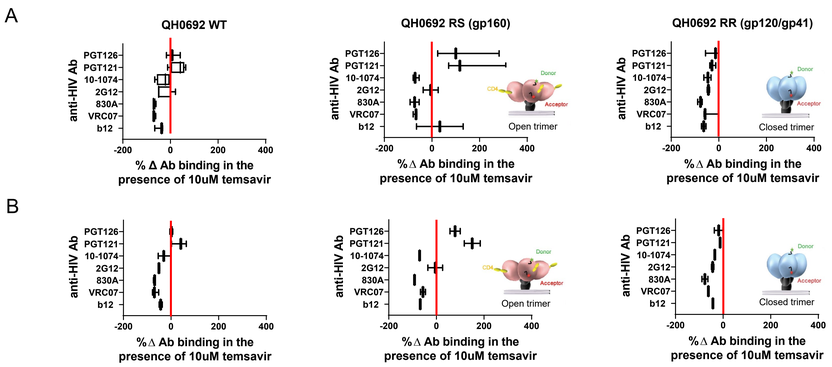


Figure 3. Effect of Env processing on QH06942 Ab binding. To examine the effects of Env processing on bnAb binding in the presence of TMR, we examined two furin cleavage site mutants: a hyper-cleavable (RR) and an uncleavable (RS) mutant. (A-B) Depicts how HIV-1 Env processing effects the levels of bnAb binding in the (A) tetherin-low or (B) high system in the presence of 10 μM of TMR following 24 hrs of treatment.

Temsavir treatment preferentially enhances V3 region bnAb binding on uncleaved forms (gp160) of T/F Env QH0692

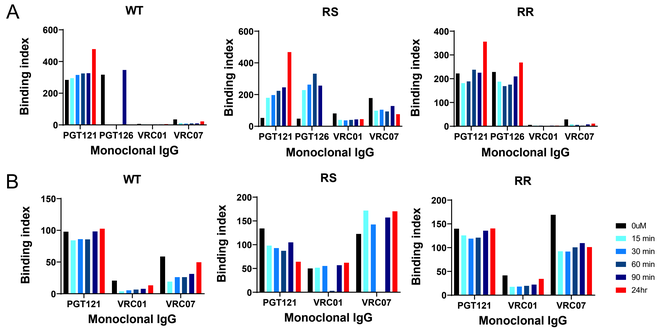


Figure 4. Time course treatment of TMR using T/F QH06942 WT and cleavage mutants. Using 10 μM of TMR, cell surface binding of bnAbs was affected within 15 mins of treatment and continued to have effects up to 24 hrs post treatment. (A) Time course in the tetherin-low system showed a more pronounced and immediate effect following treatment compared to (B) the tetherin-high system. In the tetherin-high system, temsavir continued to display immediate effects on the binding levels of certain bnAbs.

CONCLUSION

TMR increased V3 region recognition of bnAbs for wildtype viruses (YU2, T/F QH0692), with more pronounced effects on cell-associated versus virus-incorporated forms of Env. Additionally, enhancement of V3 directed bnAb binding after 15 mins of TMR treatment with the RS mutant (gp160), suggests that TMR may directly influence Env conformation rather than indirectly affecting it through processing or glycosylation inhibition.

These data indicate that FTR may be used in combination with certain V3 bnAb treatments to potentially enhance the effectiveness of bnAb-based therapies.

REFERENCES

1. Zou S., et al., Long-acting BMS-378806 analogues stabilize the state-1 conformation of the human immunodeficiency virus type 1 envelope glycoproteins. J Virol. 2020.
2. Boutin M., et al., Temsavir modulates HIV-1 envelope conformation by decreasing its proteolytic cleavage. Viruses. 2023.
3. Boutin M., et al., Temsavir treatment of HIV-1-infected cells decreases envelope glycoprotein recognition by broadly neutralizing antibodies. mBio. 2022.
4. Spencer D., et al., Advancing HIV broadly neutralizing antibodies: from discovery to the clinic. Front Public Health. 2021.