Temsavir Treatment Improves Recognition of HIV-1 Infected CD4+ T Cells by Broadly Neutralizing Antibodies

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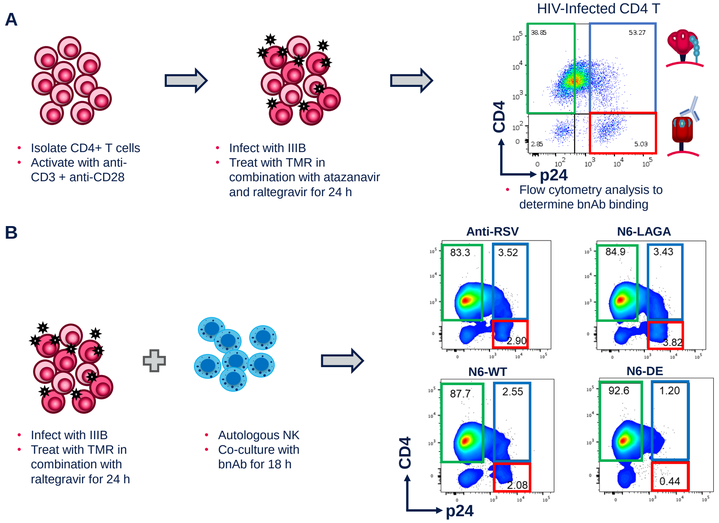
Introduction

- The HIV-1 envelope glycoprotein (Env) expressed on the surface of infected cells is a key target to elicit clearance of these cells.
- Antibodies that specifically recognize Env can result in elimination of the infected cells, through antibody-mediated immune activities, such as antibody-dependent cellular cytotoxicity (ADCC).[1,2]
- Treatment with temsavir (TMR), a small molecule, enhances the binding of the broadly neutralizing antibody (bnAb) N6, improving clearance of HIV-1 infected cells.[3]
- TMR, the active form of fostemsavir (FTR), approved for use in heavily treatment-experienced adults with multidrug-resistant HIV-1, binds and stabilizes Env in a 'closed' conformation, facilitating better recognition by N6 and potentially other bnAbs.
- We conducted studies to investigate whether TMR can modulate the binding of other bnAbs to Env on infected cells, besides N6.

Methods

- PBMCs were isolated from leukapheresis products of HIV seronegative donors.*
- CD4+ T cells were isolated and activated with Human T-Activator CD3/CD28 Dynabeads.
- Activated CD4+ T cells were infected with HIV-1 and treated with TMR for 24 hours prior to measurement of antibody binding by flow cytometry.
- Infected cells, identified by intracellular p24 staining, were categorized into CD4+p24+ and CD4-p24+ cells based on surface CD4 levels.
- The binding of bnAbs to these cell populations was measured by median fluorescence intensity (Figure 1A).
- ADCC experiments used HIV-1 infected primary CD4+ T cells as targets and autologous NK cells as effectors at a 3:1 ratio. After 24 hours, infected cells were quantified by flow cytometry using a fluorescently labeled anti-gag antibody, and the percentage of killing was calculated (Figure 1B).

Figure 1. Experiment Details of bnAb Binding and ADCC



Temsavir treatment enhances bnAb binding to HIV-1 infected cells that express CD4.

Results

TMR Treatment Increases N6 Recognition of HIV-1 Infected Cells

- TMR treatment increases N6 binding to the HIV-1 infected cells, especially cells that stain positive with anti-CD4 (Figure 2A).[3]
- TMR treatment expands the population of HIV-1 infected cells recognized by N6 (Figure 2B).

Figure 2. TMR Mediated N6 Recognition of HIV-1 Infected Cells

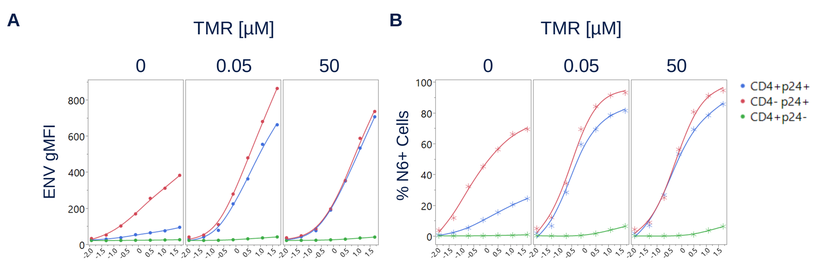
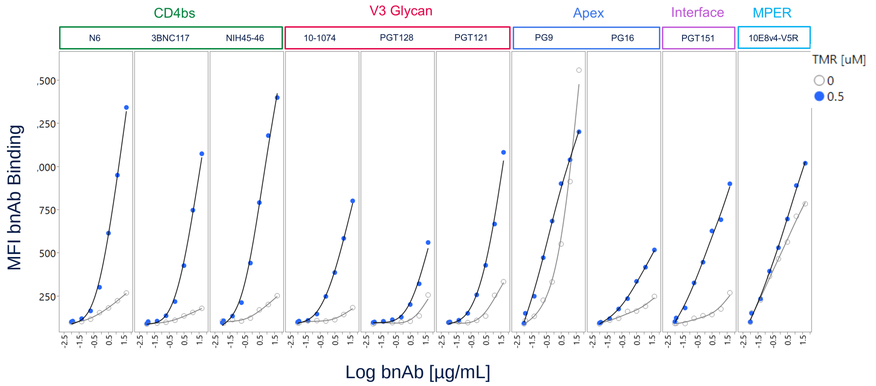


Figure 3. TMR Treatment Enhances Binding of bnAb From Multiple Classes to the HIV Infected CD4+ T Cells

- Enhanced binding was observed by most bnAb classes (excluding MPER) to HIV-1 infected cells expressing CD4 (CD4+p24+) post TMR treatment.



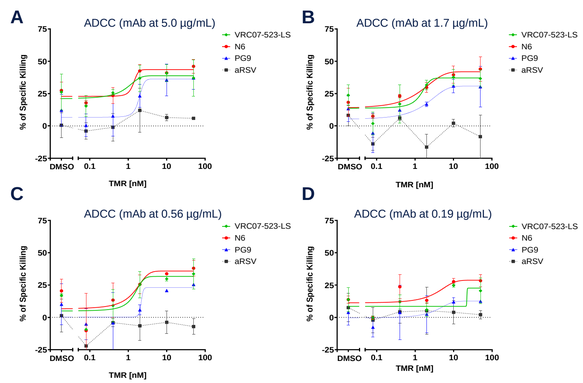
Primary CD4+ T cells were infected with IIIB and treated with TMR at 0.5 µM for 24 hours prior to bnAb binding and staining.
MFI of bnAb in CD4+p24+ cells were determined and plotted.

*Human biological samples were obtained from commercial vendors in accordance with institutional review board protocols at ViiV Healthcare. All participants provided written informed consent.

Enhanced bnAb Binding by TMR Treatment Leads to Better Killing of Infected Cells

- TMR treatment dose-responsively enhances ADCC activity of bnAbs against CD4+p24+ cells.
- Increased antibody concentrations promote max level of killing.

Figure 4. ADCC Activity of bnAb and TMR Combination Against HIV Infected Cells

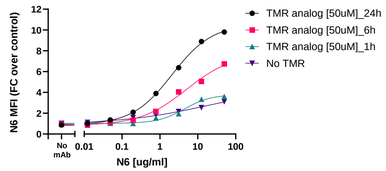


HIV-1 infected CD4+ T cells were co-cultured with autologous NK cells in the presence of titrated TMR and antibodies. Raltegravir was added to block spreading of virus infection. ADCC is calculated to reflect change of %CD4+p24+ cells at each concentration of TMR at a fixed concentration of mAbs for each figure.

Prolonged TMR Treatment Is Required to Enhance bnAb Binding

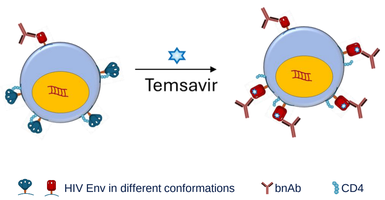
- 1 hour of TMR treatment is not sufficient to drive enhanced N6 binding to HIV infected cells (Figure 5).
- This result suggests that TMR may not be able to enhance bnAb binding on previously expressed envelopes, and may work through interactions with de novo synthesized Env. This needs to be further investigated.

Figure 5. N6 Binding Upon Various Amounts of TMR Analog Incubation



TMR analog was used in this experiment. The bnAb enhancement effect of TMR analog was comparable to TMR (Data not shown in this poster).

Figure 6. Model Depicting TMR-Enhanced bnAb Binding to CD4+p24+ Cells



Conclusions

- TMR treatment enhances antibody binding to the infected cells expressing CD4s with most classes of bnAbs.
- This suggests that combining bnAbs with TMR can broaden the range of HIV-1 infected cells susceptible to antibody-mediated clearance, potentially increasing the likelihood of reservoir reduction in clinical settings.

Acknowledgments: This study was funded by ViiV Healthcare. The authors would like to acknowledge the contributions of Mark Krystal, David Wensel, Tiancen Hu, Allan Tenorio, Jan Losos, Andrew Clark and Manyu Prakash to this work.

References: 1. Bruel et. *Nat Commun*. 2016;7:10844. **2.** Ren et al. *J Virol*. 2020;95:e01808-20. **3.** Ferris et al. CROI 2024; Denver, CO. Poster 374.

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