Temsavir Treatment Enhances bNAb Recognition and Subsequent Clearance of HIV-1–Infected Cells

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Introduction

- HIV-1 gp160 is a viral envelope glycoprotein (Env) expressed on the surface of infected cells, which renders it an attractive mechanism for the clearance of infected cells. However, Env is metastable and presents in multiple conformations, making it challenging for antibody-mediated clearance.
- Temsavir (TMR), the active form of the HIV-1 attachment inhibitor fostemsavir, binds to and stabilizes Env in a 'closed' conformation that may allow better targeting by broadly neutralizing antibodies (bNAbs).

Methods

- To understand whether TMR can modulate bNAb binding to Env on the infected cells, we conducted experiments with primary CD4+ T cells isolated from HIV-negative donors infected with a broad panel of viruses that included 26 clinical HIV isolates.
- Antibody binding to Env on the infected cell surface was determined after 24-h treatment with a serial titration of TMR ranging from 0 to 25 µM.

Figure 1. Temsavir (TMR) Treatment Enhances N6 Binding and Clearance of HIV-Infected Cells

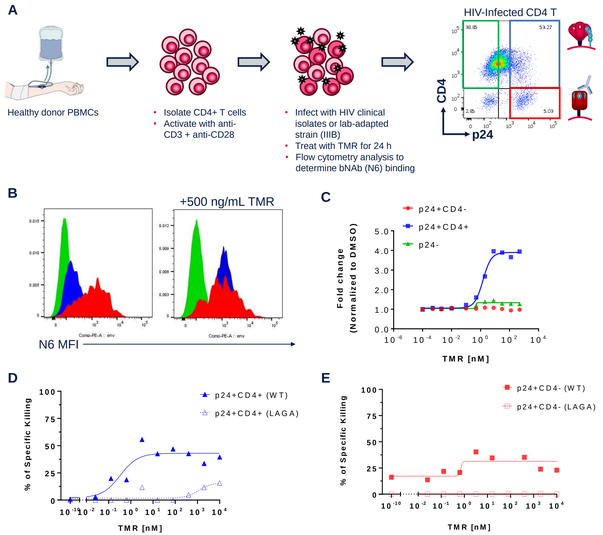


Figure 1. TMR treatment enhances N6 binding and clearance of HIV-1 (IIIB) infected cells.
(A) Schematic picture of experiment procedures. The cells were stained with N6 and gated based on levels of intracellular p24 and CD4 on the cell surface. **(B)** TMR treatment enhances N6 binding to CD4+ infected cells but does not change the binding to uninfected (p24−) and infected cells with CD4 downregulated (p24+CD4−). **(C)** Dose dependent improvement of TMR on N6 binding to infected CD4+ cells. **(D)** TMR treatment leads to better killing of CD4+ infected cells in ADCC but not against infected cells that are CD4 downregulated **(E)**.

TMR treatment leads to enhanced N6 binding and subsequent clearance of infected cells that maintain CD4 expression, suggesting potential benefit of improved reservoir reduction if TMR and N6 are co-dosed.

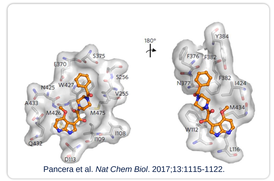
Results

- TMR treatment enhances binding of N6 to HIV-1–infected cells and leads to increased clearance of infected cells through antibody-dependent cellular cytotoxicity (ADCC).
- This synergistic effect of N6 and TMR is most prominently observed on infected cells that maintain CD4 expression, the cells that are otherwise difficult to target by N6 alone.
- The enhanced N6 binding is diminished when tested against strains with reduced TMR sensitivity (clade AE), suggesting that it is an on-target MOA.
- TMR has additional effects on Env biology of infected cells with some viruses showing decreased N6 binding to infected CD4− cells, but at much higher concentration than required for the primary MOA.
- The altered N6 binding is not attributed to the modified gp160/gp120 processing by TMR.

Figure 2. Mutations With Reduced TMR Sensitivity Lead to Reduced Enhancement in N6 Binding by TMR Treatment

Mutations	FC (EC_{50})
WT ($NL_{4\text{-}3}$)	1
M434I	2
S375M	320
M426L	210
S375M/M434I	>25,000
M426L/M475I	>50,000

FC: fold change of EC_{50} compared with WT $NL_{4\text{-}3}$.



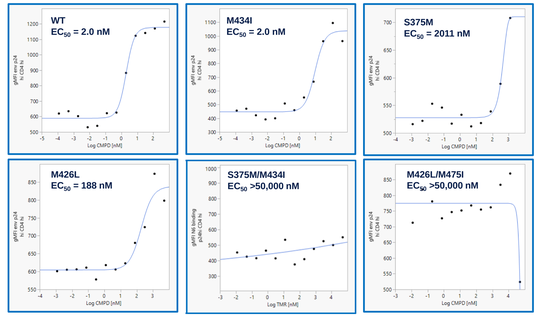


Figure 2. The N6 binding enhancement is through TMR binding to gp120.
Table: Mutations result in weaker binding to TMR and reduced sensitivity. **(A)** TMR binding sites on gp120. **(B)** Effect of mutations on TMR-enhanced N6 binding to HIV-1–infected cells.

Figure 3. TMR Enhances N6 Binding to Infected Cells at Antiviral Concentrations Across a Diverse Panel of Clinical Isolates

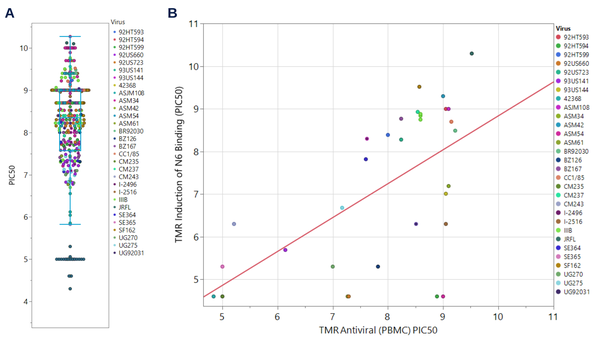


Figure 3. TMR is effective in enhancing N6 binding to a diverse panel of clinical isolates. The potency of TMR enhancement of N6 binding correlates with antiviral potency of TMR.
(A) Potency of TMR antiviral effect against clinical isolates. **(B)** Correlation of TMR antiviral potency and enhancement of N6 binding to infected cells.

Figure 4. TMR Effects on CD4− Infected Cells Are Diverse Among Different Viruses

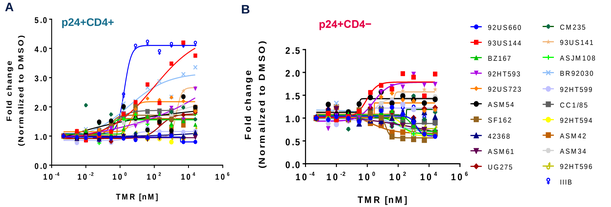


Figure 4. Diverse effect of TMR response in late-stage infected cells (p24+CD4−) among different clinical isolates.
(A) N6 binding of p24+CD4+ cells responding to TMR treatment. **(B)** N6 binding of p24+CD4− cells responding to TMR treatment.

Figure 5. Inhibitory Effect of TMR on N6 Binding to CD4− Infected Cells Occurs at Higher Concentrations Than the Corresponding Antiviral Effect

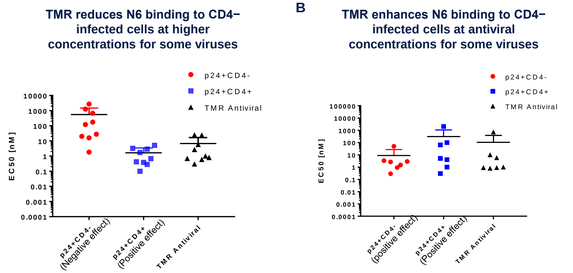


Figure 5. Quantitation of different TMR effects on N6 binding to CD4− infected cells. **(A)** Comparison of TMR inhibitory EC_{50} on N6 binding to the TMR antiviral EC_{50} against same viruses. **(B)** Comparison of TMR enhancement EC_{50} on N6 binding to the antiviral EC_{50} against the same viruses.

Figure 6. TMR Affects gp160 Processing Into gp120 and gp41 at High Concentrations

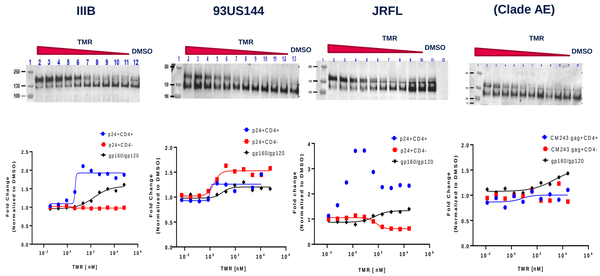


Figure 6. Characterizing effect of TMR on gp160 processing by Western blot. Infected primary CD4 T cells were treated with increased concentrations of TMR for 24 h and then subjected to Western blot analysis. Intensity of gp120 and gp160 bands were quantified and ratio of gp160/gp120 was determined and overlayed with fold change of N6 binding to the two infected populations.

Conclusions

- We have demonstrated for the majority of viruses tested that TMR treatment leads to enhanced N6 binding to the infected cells that maintain CD4 expression, and the reduced N6 binding to the CD4 downregulated infected cells occurs at much higher concentrations than the neutralization effect of TMR.
- These results suggest that combination of N6 and TMR can expand the population of HIV-1–infected cells susceptible to bNAb-mediated clearance and may increase the likelihood of reservoir reduction in the clinical setting.

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References: 1. Zhou et al. *J Antimicrob Chemother*. 2014;69:573-581. **2.** Pancera et al. *Nat Chem Biol*. 2017;13:1115-1122. **3.** Boutin et al. *mBio*. 2022;13:e0057722.

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