Bispecific Biologics That Target and Activate the HIV Latent Reservoir Ex Vivo and In Vivo

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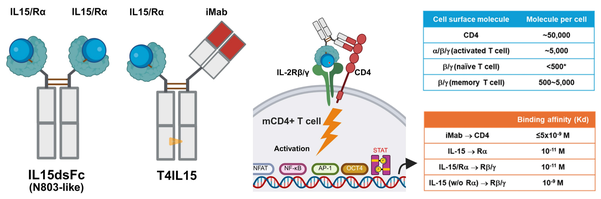
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BACKGROUND

- HIV latent reservoirs appear to be persistent but in a quasi-equilibrium (Ho 1998).
- Many purported latency reversal agents failed to reactivate the virus in ex vivo samples (Bullen 2014).
- N-803 (Anktiva) is an IL-15 superagonist that reactivated latent HIV and SIV when CD8 cells were depleted (McBrien 2020) and slightly reduced the reservoir size in a Phase-1 study but had significant dose-limiting toxicity (Miller 2022). At high doses, it induced SHIV reactivation in NHP (Lim 2024)
- We hypothesized that targeted delivery of IL-15 to CD4+ T cells will be more effective in activating resting memory CD4 T-cells (mCD4+) harboring latent HIV.

METHODS

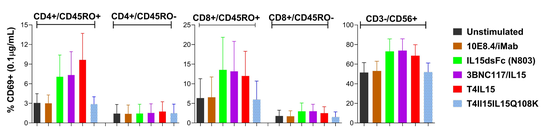


Design & construction of CD4-targeted bispecific biologic T4IL15

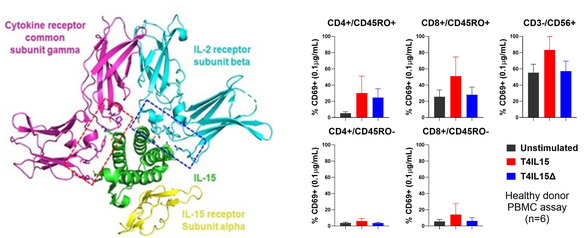
RESULTS

In vitro characterization of T4IL15

- T4IL15 improved activation of mCD4+ T-cell but retained activation of mCD8+ T-cell and NK cells in PBMC from healthy donors (n=12).

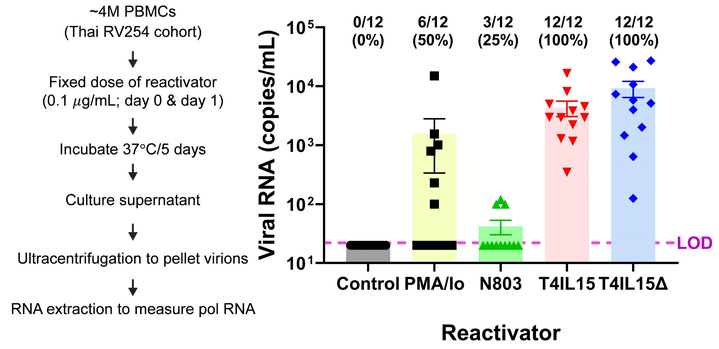


- Structure-assisted mutagenesis identified variant **T4IL15Δ** that abrogated off-target activation of IL-15 to mCD8+ T-cell and NK cells.



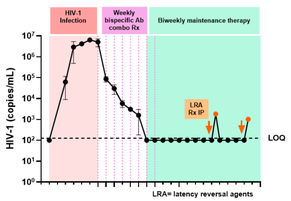
Targeting IL-15 to memory CD4+ cells activated latent HIV in PBMCs ex vivo and humanized mice in vivo with high frequency

Ex vivo reactivation of latent HIV-1 in PBMC from participants on suppressive ART



- In comparison to N803, T4IL15 and T4IL15Δ achieved high frequencies of HIV reactivation in a PBMC-based reactivation assay.

In vivo reactivation of latent HIV-1 in humanized mice model of ART

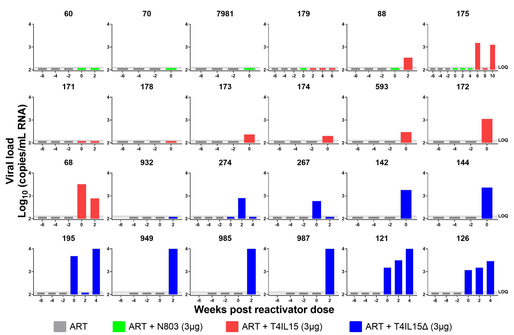


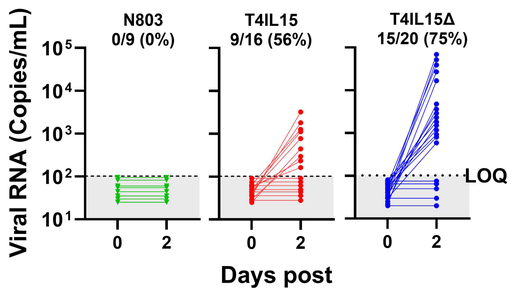
Humanized mice were infected with HIV-1 AD8

Antibody Rx QW: 10E8.4/iMab97A (0.25mg)+ VRC07/PGT121 (0.25mg)

Maintenance ART Q2W: Cabotegravir (120 mg/kg)+ Rilpivirine (180 mg/kg)

3 μg of N803, T4IL15 or T4IL15Δ were evaluated as latency reactivators

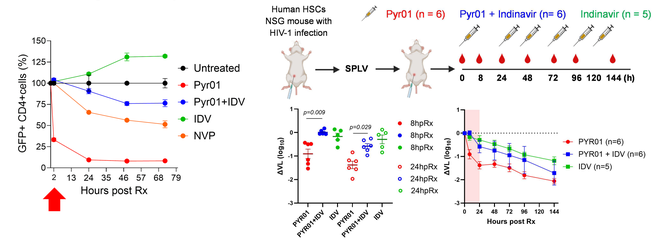




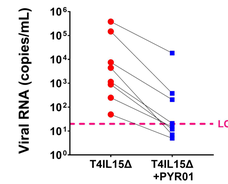
- N803 failed to reactivate HIV whereas T4IL15 or T4IL15Δ reactivated HIV at high frequencies.

What could eliminate latently infected cells once HIV reactivation occurs ?

- Merck scientists identified novel NNRTIs capable of targeted activator of cell killing (TACK) (Balibar 2023) by premature activation of the viral protease that cleaves CARD8 leading to pyroptosis.
- Prototypic compound is Pyr01, which eliminated 75% HIV-GFP-positive cells within 2 hours of treatment, and this cytotoxic activity is largely abrogated by a protease inhibitor, Indinavir.
- The in vivo killing of infected cells by Pyr01 was evident within 8 hours post treatment, and this activity was abrogated by the co-administration of Indinavir.



- Pyr01 substantially/completely blunted virion production by T4IL15Δ reactivation of latently infected cells from ART-suppressed patient PBMC (n=8).



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SUMMARY

- ❖ Our constructs T4IL15 and T4IL15Δ can reactivate latent HIV with high frequencies.
- ❖ Novel NNRTI molecules like Pyr01 can kill latently infected cells once they are activated by T4IL15Δ.