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HIV-Resistant **CAR T Cells** by CRISPR/Cas-Mediated CAR Integration Into the *CCR5* Locus

Poster **403**

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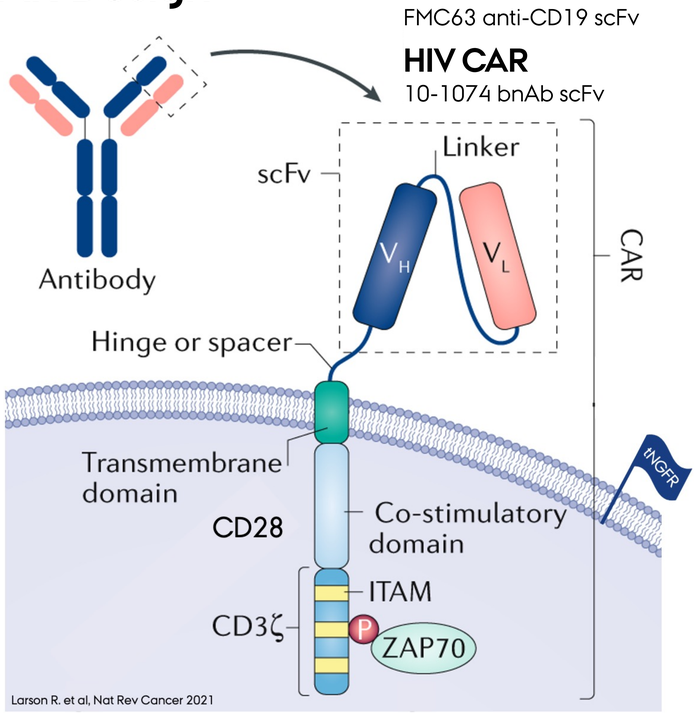
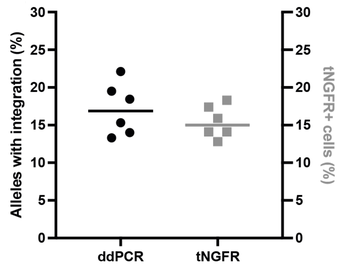
HIV-resistant anti-HIV CAR T cells

We have engineered **chimeric antigen receptor (CAR) T cells** using single-chain variable fragments (scFv) from the clinically potent anti-HIV broadly neutralizing antibody (bNAb) **10-1074** or anti-CD19 scFv FMC63. We used the **CRISPR/Cas9 system** to integrate the CAR expression cassette into either the safe harbor locus *AAVS1* or the ***CCR5*** locus leading to concurrent CAR gene integration and CCR5 disruption, making the engineered **CAR T cells resistant to HIV infection**. The CAR T cells can potentially persist in the patient life-long eliminating latently infected cells when reactivated, removing the need for ART and potentially leading to a **functional cure**.

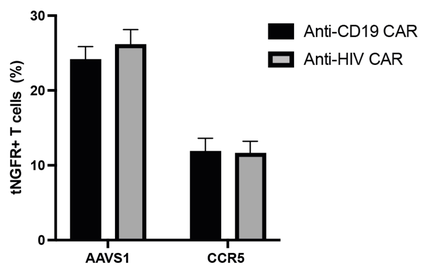
CAR Design



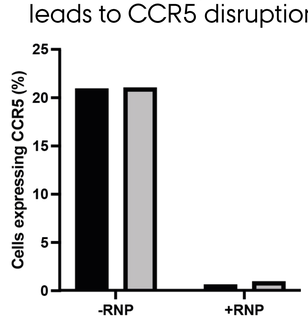
CARs are specifically integrated into *CCR5*



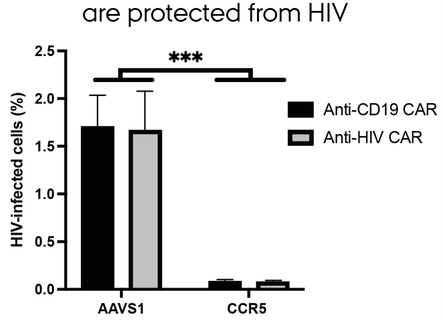
Expression of the reporter tNGFR on CAR T cells



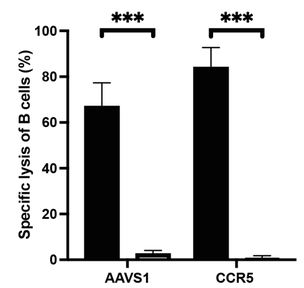
CRISPR editing of *CCR5* leads to CCR5 disruption



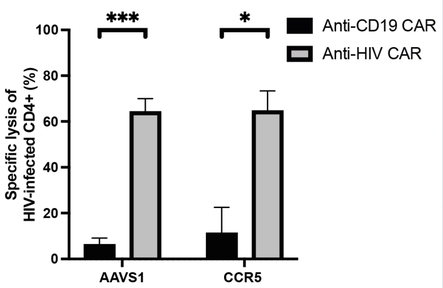
CCR5-integrated CARs are protected from HIV



CD19 CAR T cells kill autologous B cells



HIV-CAR T cells kill HIV-infected autologous CD4+

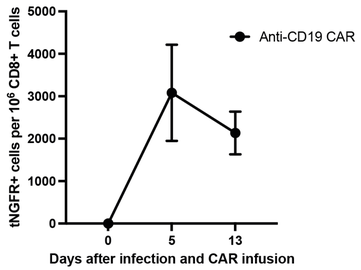


CAR T cells in vivo

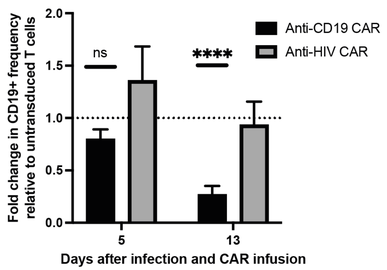
PBMC Humanized mice



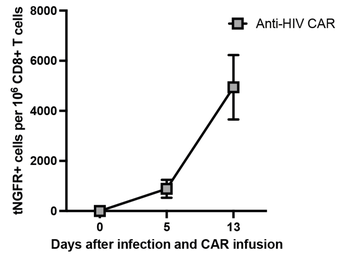
Expansion of anti-CD19 CAR T cells in vivo



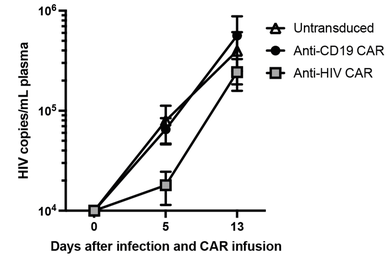
Anti-CD19 CAR T cells kill B cells in vivo



HIV-specific CAR T cells expand in vivo



HIV CAR T cells transiently limit HIV in vivo



Conclusion

We have developed **HIV-specific CAR T cells** with complete CCR5 disruption using the **CRISPR/Cas9** system leading to complete **protection from HIV infection** . Our engineered cells showed no off-target effects and high cytotoxic capacity against HIV-infected cells. Our anti-CD19 CAR T cells effectively killed B cells in **PBMC humanized mice** and the HIV-specific CAR T cells expanded and transiently limited HIV infection in vivo.

These data support the further development of gene edited cellular therapies to achieve a **functional cure for HIV**.

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