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SIV-Specific Immunogenicity of Replication-Competent Arenavirus Vectors in Rhesus Macaques

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Introduction

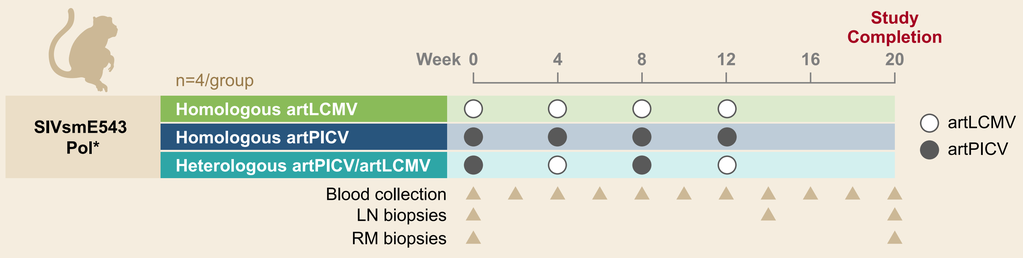
- Modern antiretroviral therapy has significantly improved HIV treatment options, but control during treatment interruption is rare; it may be possible to improve rates of posttreatment control by enhancing HIV-specific CD8 T-cell responses
- Effective prophylactic and therapeutic HIV vaccines will need to generate antiviral immunity in multiple tissue compartments, including rectal mucosa (RM), lymph nodes (LNs), and peripheral blood mononuclear cells (PBMCs)[1-3]
- Arenavirus-based vectors have demonstrated strong immunogenicity in clinical and preclinical studies for multiple indications[4-7]
- Lymphocytic choriomeningitis virus (LCMV) and Pichinde virus (PICV) are arenaviruses with low seroprevalence in humans, which reduces the risk of preexisting immunity for LCMV- and PICV-based vectors[7]
- Replicating arenavirus vectors with artificial genomic orientation (artLCMV/artPICV) have been shown to induce strong tumor-specific immunogenicity[5]
- Trisegmented arenaviral vectors artLCMV and artPICV encoding highly conserved simian immunodeficiency virus (SIV) immunogens have shown strong immunogenicity in preclinical nonhuman primate studies (Sharma B, et al, poster 2019)

Objectives

- To assess tissue-specific immunogenicity of artPICV and artLCMV vectors encoding SIVsmE543 polymerase (Pol) antigen dosed as homologous or heterologous prime/boost intramuscular immunizations in healthy rhesus macaques

Methods

Study Design

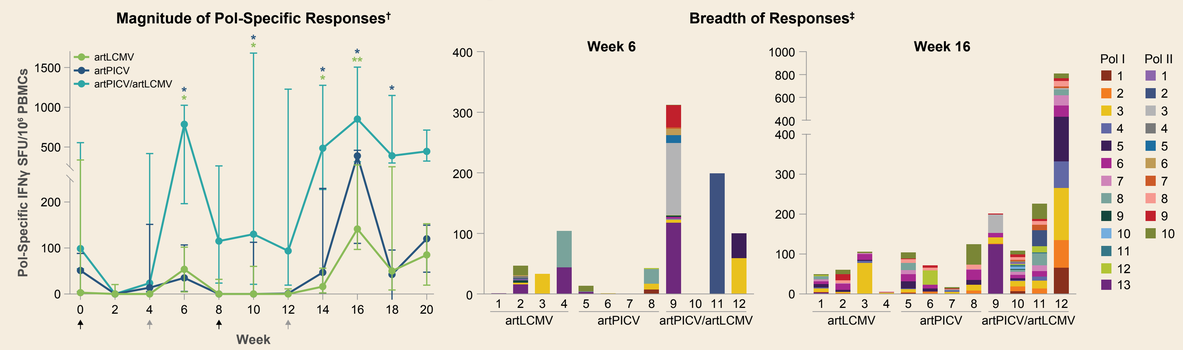


*Vector dose: 1x10⁸ replication competent virus particles/dose; route of administration: IM.

- Immunogenicity was analyzed in freshly isolated PBMCs by interferon-γ (IFNγ) enzyme-linked immunosorbent spot assay (ELISpot) every 2 wk; breadth of responses was evaluated with 23 subpools of overlapping SIV Pol 15-mer peptides (10 peptides/subpool) at Weeks 6 and 16
- LN and RM biopsies were done 10 d before the 1st immunization (baseline), and 2 and 8 wk after the last immunization
- Phenotyping and functionality of T cells were analyzed in freshly isolated PBMCs, and LN- and RM-tissue–isolated mononuclear cells by flow cytometry and intracellular cytokine staining (ICS)

Results

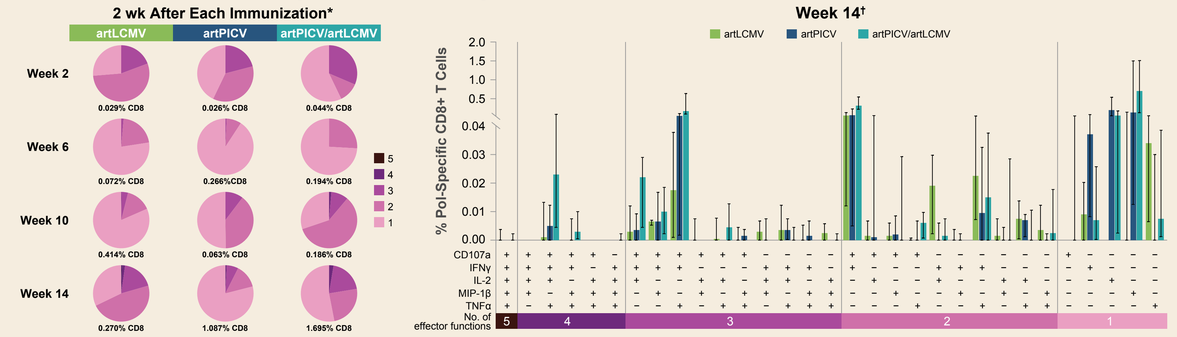
Heterologous Immunization With Replicating Arenavirus Vectors Induced Robust and Broad Immune Responses Against SIV Pol



*p<0.05; **p<0.01; significance determined by 2-way analysis of variance (ANOVA) plus Tukey's multiple comparison test; †*Arrows* indicate time of vaccine administration (*black* indicates artPICV and *grey* indicates artLCMV administration for heterologous regimen); magnitude of IFNγ ELISpot responses plotted after subtracting background; data plotted as median ± interquartile range (IQR); ‡Breadth analyzed with 23 subpools of SIV-Pol I and SIV-Pol II and calculated as number of Pol-specific responses minus 3x background.

- Heterologous immunization elicited a significantly higher magnitude of SIV Pol-specific responses ($p<0.05$) than either homologous regimen and a nonsignificant increase in breadth of responses
- IFNγ ELISpot responses were higher after each artLCMV boost

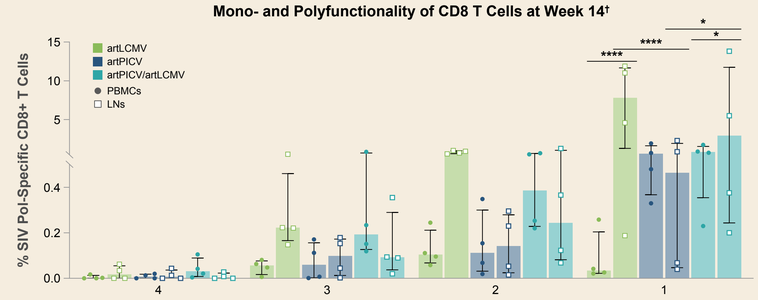
SIV Pol-Specific Polyfunctionality of CD8 T Cells in PBMCs



*Functional markers evaluated: IFNγ, tumor necrosis factor-α (TNFα), interleukin 2 (IL-2), CD107a, and macrophage inflammatory protein 1β (MIP-1β); % of total CD8 responding to SIV Pol antigen represented below each *pie chart*; †Data graphed as median ± IQR; 1 = expression of 1 functional marker; 2 = coexpression of any 2 functional markers; 3 = coexpression of any 3 functional markers; 4 = coexpression of any 4 functional markers; and 5 = expression of all 5 markers; significance determined by 2-way ANOVA plus Tukey's multiple comparison test.

- SIV Pol-specific polyfunctionality of CD8 T cells increased nonsignificantly after each vaccination dose for all groups

SIV Pol CD8 T Cell Polyfunctionality 2 wk After Last Immunization in PBMCs vs LNs



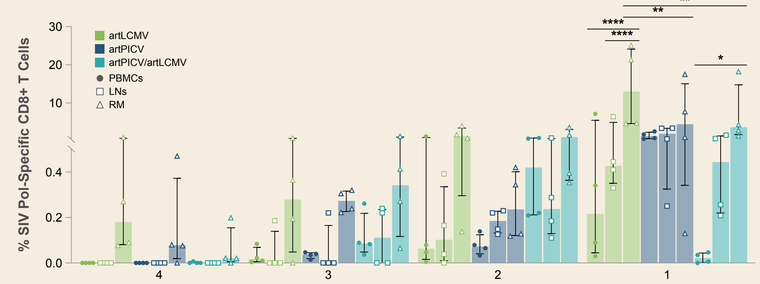
Magnitude of Responses‡

% CD8	artLCMV	artPICV	artPICV/ artLCMV
PBMCs	0.27	1.09	1.69
LNs	8.03	1.07	5.58

*p<0.05, ****p<0.0001; significance determined by 2-way ANOVA plus Tukey's multiple comparison test; †Coexpression of 5 phenotypic markers in CD8 T cells on SIV Pol antigen stimulation (background subtracted) by ICS, plotted as median ± IQR, and each *dot* and *square* depicts animal within group (n=4/group); ‡Magnitude of total (%) CD8 T-cell responses in PBMCs and LNs by ICS, calculated as sum of means within each group of % CD8 T cells expressing 1 or coexpressing 2–5 functional markers.

- Monofunctionality of Pol-specific CD8 T cells in LNs of artLCMV and artPICV/artLCMV was significantly higher than in PBMCs
- Polyfunctionality of Pol-specific CD8 T cells was nonsignificantly greater in LNs of artLCMV than in PBMCs
- Increased magnitude of Pol-specific responses of CD8 T cells was observed in LNs of artLCMV and artPICV/artLCMV compared with PBMCs

SIV Pol CD8 T Cell Polyfunctionality 8 wk After Last Immunization in PBMCs, LNs, and RM



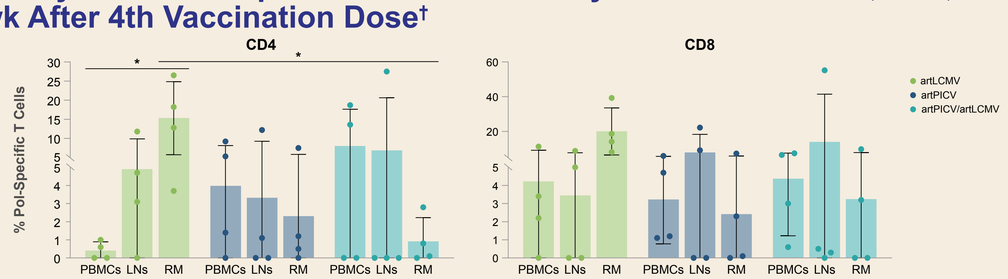
Magnitude of Responses‡

% CD8	artLCMV	artPICV	artPICV/ artLCMV
PBMCs	2.26	1.52	0.61
LNs	2.09	2.11	1.12
RM	16.23	7.28	8.70

*p<0.05, **p<0.01, ****p<0.0001; significance determined by 2-way ANOVA plus Tukey's multiple comparison test; †Coexpression of 5 markers in CD8 T cells on stimulation with SIV Pol antigen (background subtracted) by ICS; ‡Magnitude of total (%) CD8 responses in PBMCs, LNs, and RM by ICS, calculated as sum of means within each group expressing 1 or coexpressing 2–5 functional markers.

- In both artLCMV homologous and heterologous groups, monofunctional CD8 T cells were significantly higher in RM than in PBMCs

Frequency of SIV Pol-Specific Effector Memory T Cells in PBMCs, LNs, and RM at 8 wk After 4th Vaccination Dose†



*p<0.05; †Data graphed as median ± IQR; 2-way ANOVA plus Tukey's multiple comparisons test (comparison between groups) and Kruskal-Wallis test (comparison between tissues within each group); effector memory T cells gated as CD45RA−CCR7−CD27− within the CD4+ and CD8+ T-cell populations.

- A significantly higher frequency of effector memory CD4 T cells was observed in RM than in PBMCs with artLCMV prime/boost

Conclusions

- Immunization with replicating arenavirus vectors induced robust SIV Pol-specific T-cell responses in multiple tissues, as well as an enhancement of effector memory T-cell populations
- Homologous artLCMV generated strong T-cell responses in RM, but lower responses in PBMCs
- Heterologous artPICV/artLCMV generated the highest responses in PBMCs, as well as robust responses in lymph nodes and rectal mucosa
- This robust and site-specific immunogenicity supports further development of artPICV/artLCMV for HIV treatment and potential cure

References: 1. Buggert M, et al. Sci Immunol 2018;3Leaar4526; **2.** Fukazawa Y, et al. Nat Med 2012;18:1673-81; **3.** Hansen SG, et al. Nature 2011;473:523-7; **4.** Flatz L, et al. Nat Med 2010;16:339-45; **5.** Kallert SM, et al. Nat Commun 2017;8:15327; **6.** Penaloza-MacMaster P, et al. Vaccine 2017;35:1-9; **7.** Schwendinger M, et al. J Infect Dis 2022;225:1399-141.

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