Antibody-based CCR5 blockade protects Macaques from mucosal SHIV transmission

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In the absence of a prophylactic vaccine, the use of antiretroviral therapy (ART) as pre-exposure prophylaxis (PrEP) to prevent HIV acquisition by uninfected individuals is a promising approach to slowing the epidemic, but its efficacy is hampered by incomplete patient adherence and ART-resistant variants. Here, we report that competitive inhibition of HIV Env-CCR5 binding via the CCR5-specific antibody Leronlimab protects rhesus macaques against infection following repeated intrarectal challenges of CCR5-tropic SHIVSF162P3. Injection of Leronlimab weekly at 10 mg/kg provides significant but partial protection, while biweekly 50 mg/kg provides complete protection from SHIV acquisition. Tissue biopsies from protected macaques post challenge show complete CCR5 receptor occupancy and an absence of viral nucleic acids. After Leronlimab washout, protected macaques remain aviremic, and adoptive transfer of hematologic cells into naïve macaques does not transmit viral infection. These data identify CCR5 blockade with Leronlimab as a promising approach to HIV prophylaxis and support initiation of clinical trials.
Results of Leronlimab-based PrEP in the macaque model of HIV.

Potential broadly neutralizing antibodies (bNAb) represent a potential alternative to ART-based PrEP. Yet, bNAb also remain susceptible to antibody-resistant HIV strains, and alternate preventative modalities with complementary mechanisms of action are needed. To ART-based PrEP. Yet, bNAb also remain susceptible to antibody-resistant HIV strains, and alternate preventative modalities with complementary mechanisms of action are needed.2-10. HIV can utilize either the CCR5 or CXCR4 co-receptor for entry into CD4+ T cells, yet CCR5 is the primary co-receptor used during transmission of HIV11-13. Accordingly, individuals with a natural genetic deficiency in CCR5 via a homozygous 32-base pair deletion in ccr5 (CCR5Δ32/Δ32) are highly resistant to HIV infection14,15. Further underscoring the central role of CCR5 in viral spread in vivo, the only two documented cases of HIV cure occurred in the setting of allogenic stem cell transplantation using CCR5Δ32/Δ32 donor cells16,17. CCR5 therefore represents an ideal target for HIV prevention, yet small-molecule CCR5 inhibitors like Maraviroc have yielded disappointing results as PrEP agents18,19, and alternate CCR5-specific strategies are needed.

Leronlimab is an anti-CCR5 humanized IgG4 antibody currently in clinical trials for HIV therapy as a once weekly, subcutaneous injection with a favorable safety profile in over 1000 volunteers across multiple studies20. In contrast to Maraviroc, which interferes with HIV Env attachment to CCR5 by allosteric modulation, Leronlimab binds to the same CCR5 extracellular loop-2 and N-terminus domains used by HIV Env, thereby directly outcompeting HIV for binding to CCR5 (ref. 21). A single 10 mg/kg dose of Leronlimab lowered plasma viral loads by approximately 100-fold for 2 weeks in HIV-positive individuals22-24. When utilized as once weekly self-administered subcutaneous monotherapy, Leronlimab maintained undetectable plasma viral loads in HIV-infected individuals for over 2 years25, with the longest successful monotherapy patients now reaching over 6 years of continual use (Chang et al., manuscript in preparation). The ability to self-administer Leronlimab at home as a subcutaneous injection augurs well for its adherence profile as a PrEP agent. Finally, in both single dose and multiyear monotherapy studies, no viral co-receptor switching occurred, underscoring the high genetic barrier to developing Leronlimab resistance. Based on these antiviral, safety, and user-friendly characteristics, we hypothesized that Leronlimab could be utilized as an effective PrEP strategy with the potential for high patient usage and set out to establish the efficacy of Leronlimab-based PrEP in the macaque model of HIV.

Using a panel of 25 HIV isolates from multiple clades (Supplementary Table 1), we confirmed the ability of Leronlimab to mimic the resistance conferred by the CCR5Δ32/Δ32 phenotype onto CD4+ T cells from CCR5 wild-type donors, thereby protecting these cells from infection with CCR5-tropic isolates from multiple geographical origins (Fig. 1b).

CCR5 is highly conserved between humans and rhesus macaques30, and accordingly, Leronlimab specifically bound CCR5 on the surface of macrophage CD4+ T cells (Supplementary Fig. 2a, b). However, while the frequency of CCR5+ CD4+ T cells was similar between human and macaques in peripheral blood, macaque CD4+ T cells expressed a higher number of CCR5 molecules on a per-cell basis, with central memory CD4+ T cells expressing the highest levels overall (Supplementary Fig. 2c). This higher per-cell expression of CCR5 on macaque CD4+ T cells suggested that inhibition of SHIV infection in macaque CD4+ T cells may require higher concentrations of CCR5-targeted competitive inhibitors such as Leronlimab compared to inhibition of HIV infection in human CD4+ T cells. Indeed, a 10-fold higher concentration of Leronlimab was required to achieve full inhibition of SHIVSF162P3 infection in macaque cells compared to HIV in human cells in vitro (Fig. 1c and Supplementary Fig. 1a).

The primary co-receptor used during mucosal transmission is CCR5 (refs. 11–13). Thus, we explored whether Leronlimab could act as PrEP to protect macaques from repeated low-dose intrarectal (IR) SHIVSF162P3 challenges. The study design, shown in Fig. 2a, consisted of three groups of macaques (n = 6 per group, individual macaque information listed in Supplementary Table 2) that served as untreated controls (group 1), weekly 10 mg/kg Leronlimab-treated (group 2), or biweekly 50 mg/kg Leronlimab-treated animals (group 3). The 10 mg/kg dose in macaques is an allometrically scaled human clinical dose of 350 mg Leronlimab. Due to the emergence of antiretroviral antibodies (ADA), the allometrically scaled human clinical dose of 700 mg Leronlimab was adjusted to 50 mg/kg dose in macaques, a dose that does not elicit ADA likely due to high zone tolerance31, with dosing changed to biweekly to more closely approximate plasma levels from weekly doses in humans. Thus, these two macaque doses of Leronlimab represent the lowest and highest doses currently used in clinical trials. Animals received their first subcutaneous Leronlimab injection 1 week prior to the start of IR challenges. At study week zero, all animals received their first IR challenge with 3.2 TCID50 of SHIVSF162P3 that continued for eight consecutive weeks. We selected SHIVSF162P3 for the following two reasons: (1) the parental Env from HIV-1SF162 is CCR5-tropic, yet particularly resistant to CCR5-targeting agents27 and (2) SHIVSF162P3 can switch to CXCR4 co-receptor use in macaques via defined amino acid substitutions in Env V3 loop32. Uninfected animals received their last IR challenge and Leronlimab injection at study week 7. After this point, infected animals were euthanized at 10 weeks after confirmed infection while aviremic, protected animals were longitudinally monitored and euthanized 8 weeks after loss of Leronlimab receptor occupancy (RO) on peripheral blood CD4+ T cells.

In all animals, Leronlimab was well-tolerated and showed no adverse clinical effects, similar to the high safety profile reported previously in multiple clinical trials20,22-25. Serum chemistry and complete blood count parameters did not differ significantly between groups nor change significantly over the study period (Supplementary Fig. 3). Although no significant change in peripheral blood T cell counts or frequencies were found, we observed a dose-dependent increase in peripheral blood CCR5+ T cell frequencies and absolute counts during Leronlimab treatment that subsequently returned to baseline levels concomitant with Leronlimab washout, likely reflecting the previously...
described ability of Leronlimab to interfere with CCR5-mediated chemotaxis21 (Supplementary Fig. 4).

All untreated control macaques in group 1 became infected by the seventh IR challenge, while two of six macaques in group 2 (weekly 10 mg/kg) became infected ($p = 0.001$). In contrast, no macaques in group 3 (50 mg/kg biweekly) became infected ($p = 0.0005$), indicating that Leronlimab protected macaques from SHIV acquisition in a dose-dependent manner (Fig. 2b). To track CCR5 RO, we established and validated an ex vivo Leronlimab spreading assay (Supplementary Fig. 5). All six macaques in group 3 maintained full CCR5 RO on peripheral blood CD4+ T cells throughout the challenge phase (Fig. 2c). As expected with the high dose, these macaques did not develop ADA (Fig. 2d), and maintained sufficient plasma levels with a half-life of 7.5 weeks after the last Leronlimab injection at study week 7 (Fig. 2e). Following washout of plasma Leronlimab and loss of CCR5 RO, we observed earlier emergence of occult SHIV infection following Leronlimab treatment without ADA (Fig. 3a). Interestingly, following the final challenge this animal had the lowest Leronlimab levels in the colon of any group 3 animals (15 µg/mL Leronlimab on study week 7), indicating sterile protection during viral challenges. Following infection, 34487 experienced the lowest peak plasma viremia of all infected animals and was the only animal to control SHIVSF162P3 to undetectable levels, suggesting antiviral effects by Leronlimab similarly seen in clinical trials of monotherapy treatment of HIV-positive individuals20,22–25 (Fig. 2f and Supplementary Fig. 6). However, we cannot exclude the potential of spontaneous control of SHIV replication. We sequenced the V3 loop of SHIVV3–32 human CD4+ T cells treated with 100 µg/mL Leronlimab (top) or untreated CCR5 WT human CD4+ T cells (bottom). Each data point represents the mean Gag p24 levels of CCR5 WT human (n = 3) or CCR5Δ32/Δ32 human (n = 1; two replicates) CD4+ T cells. Each viral tropism group represents the mean Gag p24 levels of ten CCR5-tropic, ten CXCR4-tropic, and five dual-tropic viral infections. Gag p24 levels of CCR5 WT human CD4+ T cells were normalized to “no treatment”. (Right) Summary (mean ± SEM) of the longitudinal infection as measured by Gag p27 levels, with all values normalized to day 6 “no treatment”. Source data are provided as a Source Data file.

![Fig. 1 Leronlimab blocks the spreading of CCR5-utilizing strains in vitro.](image)

(a) Representative flow cytometry plots displaying intracellular Gag p24 staining of untreated CCR5 wild type (WT), Leronlimab-treated CCR5 WT, and untreated CCR5Δ32/Δ32 human CD4+ T cells in HIV-1 infection assay with CCR5-tropic (blue), CXCR4-tropic (red), and dual-tropic viruses (black). (b) Summary of the mean Gag p24 levels from HIV-1 spreading assays performed on CCR5 WT human CD4+ T cells treated with 100 µg/mL Leronlimab (top) or untreated CCR5Δ32/Δ32 human CD4+ T cells (bottom). Each data point represents the mean Gag p24 levels of CCR5 WT human (n = 3) or CCR5Δ32/Δ32 human (n = 1; two replicates) CD4+ T cells. Each viral tropism group represents the mean Gag p24 levels of ten CCR5-tropic, ten CXCR4-tropic, and five dual-tropic viral infections. Gag p24 levels of CCR5 WT human CD4+ T cells were normalized to “no treatment”. (c) Summary of the mean Gag p24 levels of CCR5Δ32/Δ32 human CD4+ T cells treated with increasing amounts of Leronlimab. (Right) Summary (mean ± SEM) of the longitudinal infection as measured by Gag p27 levels, with all values normalized to day 6 “no treatment”. Source data are provided as a Source Data file.
anatomical locations following the final SHIV challenge. As expected, higher levels of Leronlimab were present in group 3 versus group 2 animals, with the lowest colon levels found in 34487 (Fig. 3b). Finally, we examined tissues collected during necropsy and confirmed an absence of Leronlimab and CCR5 RO on CD4+ T cells from these tissues, demonstrating that the lack of plasma viremia in protected animals was not due to residual Leronlimab in tissue.

To assess if Leronlimab mediated sterile protection from challenge, we measured cell-associated SHIV DNA and RNA levels in multiple anatomical locations by tissue biopsy after the final challenge (study weeks 8–9) and at necropsy (Fig. 4a, b). As expected, SHIV nucleic acid was readily detected in macaques with plasma viremia, including in all untreated controls and the two infected group 2 animals, 34487 and 37032. However, many tissues from 34487 at necropsy were below the limit of quantification suggesting Leronlimab may have limited viral spread post acquisition. In contrast, we found no samples positive for SHIV DNA or RNA from the four aviremic group 2 animals and all six group 3 animals. Because Gag- and Vif-specific CD8+ T cells are a sensitive readout
of occult SIV infection\textsuperscript{33}, we longitudinally monitored for their emergence in all macaques. In line with longitudinal plasma and cell-associated viral load results (Figs. 2f and 4a, b), SHIV Gag- and Vif-specific CD8\textsuperscript{+} T cells responses developed in all viremic animals while it was absent in all aviremic animals, despite the presence of CMV-specific CD8\textsuperscript{+} T cells in all macaques (Fig. 4c and Supplementary Fig. 8). Finally, after confirming that Leronlimab and CCR5 RO was not present in tissues collected at necropsy (Fig. 3), we adoptively transferred pooled hematologic cells into one SHIV-naïve recipient macaque per group. Lymph node (axillary, inguinal, and mesenteric), bone marrow, and spleen cells from all six infected group 1 animals, the four protected group 2 animals, and three uninfected group 3 animals were used for the adoptive transfer.
and all six group 3 animals were pooled by group and infused at final cell counts of $7.8 \times 10^8$, $6.9 \times 10^8$, and $9.7 \times 10^8$ cells, respectively (Supplementary Table 3). Transfer of SHIVSF162P3 infection occurred with the infusion of cells from infected control animals, but not isolated cells from protected animals in group 2 or 3 (Fig. 4d). Together, these results indicate that Leronlimab mediated sterile protection against mucosal acquisition of SHIVSF162P3 in the aviremic animals.

**Discussion**

The efficiency of daily ART-based PrEP is heavily dependent on patient adherence\(^6\) and absence of drug-resistance variants\(^6\). Even new preventative approaches like long-acting injectable cabotegravir can lead to the emergence of drug-resistant variants, especially if initiated during undiagnosed acute infection\(^34\). Thus, alternative and cost-reducing methods are needed, including the use of antibody-based PrEP. Leronlimab is administered
subcutaneously at home, offering advantages over bNAb-based PrEP that requires IV infusion and intra-muscular injectables that are administered in a clinic by a health-care provider. Given the significantly longer Leronlimab plasma half-life in humans versus macaques, coupled with the lower per-cell CCR5 expression on CD4+ T cells in humans, it is possible that once monthly Leronlimab may be sufficient to prevent HIV acquisition. With advances in antibodyFc engineering to enhance plasma half-life, minor sequence modifications could extend Leronlimab to a once-quarterly injection to further improve adherence by lowering the frequency of administration.

Because CCR5 is the primary HIV co-receptor used by virions during transmission11-14, development of viral resistance is difficult, as demonstrated by the previously documented protection from infection in CCR5Δ32/A32 individuals1,15. Indeed, no escape mutants were observed with long-term Leronlimab monotherapy use in HIV+ individuals, in contrast to treatment with bNAbs16,17. This is likely due to targeting of the primary CCR5 co-receptor rather than epitopes on Env by bNAbs that permit selection for bNAB-resistant variants. Infection with CXCR4- or dual-tropic HIV isolates is possible, but such events represent a small minority of transmission due to multiple layers of restriction on CXCR4-tropic viruses in the mucosa16,18. Further, the ability of Leronlimab to fully protect animals from rectal transmission of SHIV is in contrast to a previous study where the small-molecule CCR5-inhibitor Maraviroc failed to protect animals challenged with 10 TCID50 SHIV_SF162P3 despite sufficiently high drug concentrations in rectal tissues19,20. Thus, competitive inhibition of the CCR5 Env interaction represents a powerful approach for PrEP. Finally, beyond targeting CCR5 for prophylaxis, significant effort is focused on genetic engineering approaches to knock out CCR5 in HIV cure strategies21,22. The results presented here show that the use of Leronlimab could mimic the same protection from acquisition seen in CCR5Δ32/A32 individuals via a reversible pharmacological therapy that does not require genetic modifications that risk off-target effects. Given that the CCR5Δ32/A32 phenotype carries no risk for life span23 and that multicyear Leronlimab use has been safely demonstrated, future studies are warranted to explore the utility of Leronlimab in HIV cure and prevention.

**Methods**

**HIV and SHIV stocks.** HIV-1 isolates were obtained from the NIH AIDS Reagent Program, with the majority from the HIV-1 International Isolate Panel (Cat #11412). The SHIVΔ162P3 stock (harvest 4, dated 9/18/2016, 173.3 ng/mL p27, 1 × 10^8 vRNA copies/mL, 2.67 × 10^6 TCID50/mL in TZM-bt cells, 1.28 × 10^6 TCID50/mL in rhesus PBMC (peripheral blood mononuclear cells)) used in the PrEP animal study was generated, characterized, and kindly provided by Nancy Miller.

**HIV and SHIV in vitro infection assays.** Human PBMC were first depleted of CD8+ T cells with Human CD8 Microbeads (Miltenyi) and then sequentially enriched for CD4+ T cells with Human CD4 Microbeads (Miltenyi) following the manufacturer’s instructions. The resulting CD4+ T cells were incubated at 2 × 10^6 cells/ml in R15-100 media (RPMI 1640 with antibiotic/mycotic, 15% FBS, and bovine serum (FBS), and 100 U/mL IL-2) and activated for 24 h with a stimulating cocktail containing CD3, CD49d, CD28 antibodies (BD Biosciences), and Staphylococcal enterotoxin B (Toxin Technologies, Inc.). After 24 h, cells were washed two times and incubated for 2–3 additional days in R15-100 before viral infection. At day of infection, 5 × 10^5 cells were incubated with or without the desired concentration of Leronlimab for 1 h at 37 °C. Next, cells were infected with the desired HIV isolate from Supplementary Table S1 by spinoculation for 2 h at 1200 × g at room temperature (RT). Cells were washed four times with R15-100 to remove free viruses and cultured with R15-100 media plus the same concentration of Leronlimab used during the pre-treatment step. An additional 5 × 10^5 cells were left uninfected and kept in culture as the uninfected control. Cultures were maintained by replacing 50% of the culture with new media containing the desired concentration of Leronlimab every other day for 5 days, when cells were harvested for intracellular p24 staining by flow cytometry.

HIV-1 or SHIV were depleted of CD8+ T cells by staining with NHP CD8-PE (Miltenyi) followed by anti-PE microbeads (Miltenyi) then subsequently enriched for CD4+ T cells with NHP CD4 microbeads (Miltenyi), following the manufacturer’s instructions. Purified RM CD4+ cells were activated and maintained in culture similarly to human CD4+ T cells, as described above. For the spreading assay with HIV-1 Ba-L, HIV-1 LAI, and SHIVGagΔ132, T cells were isolated, activated, infected, and cultured as described above but with the following changes. HIV-1 Ba-L, HIV-1 LAI, and SHIVGagΔ132 were used to infect at a multiplicity of infection (MOI) of 1 × 10^-3 (EFU/cell) and kept in culture for 6 days before p24 or p27 intracellular staining. For detection of intracellular HIV p24 or p27, cells were stained for CD45, CD3, CD4, and CD8 fluorescent dye for viability for 30 min at RT in the dark. Cells were washed once with phosphate-buffered saline (PBS), spun down at 830 × g for 4 min, and fixed with 2% paraformaldehyde (PFA) for 30 min in 4 °C. Afterwards, cells were washed once with FACS buffer (10% bovine growth serum in PBS) and stained with p24 or p27 antibodies in 100 μL of Permeabilization Medium B (Thermo Fisher) for 1 h at RT in the dark. Cells were washed once with FACS buffer and fixed again with 2% PFA for more than 30 min before collecting on LSR-II instrument and FACSDiVa version 6.1 (BD Biosciences, Franklin Lakes, NJ). Data were analyzed using FlowJo v10 (Tree Star) by gating on singlet, live, CD3+, CD8–, and p24+ or p27+.

**Human blood donors.** Healthy human donor whole blood was purchased from Innovative Research and processed in house to PBMC by density gradient centrifugation using Ficoll-Hypaque. Blood was collected with K2 EDTA anticoagulant and tested negative for the following viral markers: HIV-1 RNA, antibodies to HIV, antibodies to hepatitis C virus (HCV), HCV RNA, hepatitis B virus (HBV) DNA, and parvovirus B19 surface antisera. Phenotypic staining was confirmed via flow cytometry. Leukapheresis samples were collected from TRB following informed consent under the Research and Institutional Review Committee (IRRC) of the Queens Medical Center approval number RA-2014-307 of the H026 Study protocol.

ID individual consented to identification in this study.

**Quantitation of CCR5 expression levels.** To measure the frequency of CCR5-expressing cells, PBMCs were incubated with 5 μg/mL of unlabeled Leronlimab for 30 min at RT in the dark, and then washed once with PBS. Anti-human IgG4 was used as a secondary antibody to detect surface-bound Leronlimab for 30 min at RT in the dark. Cells were washed once with FACS buffer and once with PBS, and then stained for CD3, CD4, CD8, CCR5 (via antibody clone 3A9), which is specific to a distinct, non-competitive CCR5 epitope than Leronlimab), and amine-reactive dye for 30 min at RT in the dark. Cells were washed twice with PBS and fixed with 2% PFA before collecting through the LSR-II instrument and FACSDiVa version 6.1. Samples were analyzed by gating on singlet, live, CD3+, CD4+, CD8−, and CCR5+ (via clone 3A9) and/or human IgG4+ events. The number of CCR5 molecules on the cell surface was measured with quantitative cytometry using the Quantum Molecules of Equivalent Soluble Fluorochrome (MESF) kit (Bangs Laboratories, Inc). PE-conjugated Leronlimab used to quantify surface CCR5 expression and PE-labeled microspheres for standard curve generation were provided by InvicellX, Phenotypic staining was done using CD3+, CD4+, CD8−, and CD16− specific antibodies as used above. T cell memory subset determination was defined as central memory (human: CCR7+CD45RA−, RM: CD28+CD95+), effector memory (human: CCR7−CD45RA+, RM: CD28−CD95+), and naive (human: CCR7+CD45RA+, RM: CD28+CD95−). Gating was done using FlowJo v10. MFI was used to attain MESF according to the manufacturer’s protocol.

**Leronlimab CCR5 RO.** To measure the percentage of CCR5+ RO on the surface of CD4+ T cells, we developed the RO equation shown in Supplementary Fig. 5.

\[
\%\text{RO} = \frac{\text{MFI}_{\text{g/mL of unlabeled Leronlimab}}}{\text{MFI}_{\text{g/mL of unlabeled Leronlimab}} \times 100}
\]

The equation measures unoccupied CCR5 receptors by using Pacific Blue-conjugated Leronlimab (termed Leronlimab-PR). CCR5 RO is defined as the percentage of cells CCR5+ (measured by clone 3A9) and Leronlimab+ (measured by anti-human IgG4 and Leronlimab-PR) cells following incubation with a saturating concentration of Leronlimab-PR. This method is based on RO assays for anti-PD-1 antibodies in clinical trials23-25. PBMC or single cells from tissue homogenates (0.3–1 × 10^6) were stained with anti-human IgG4 for 30 min at RT in the dark. Next, cells were washed once with FACS buffer and three times with PBS and then stained with CD45, CD3, CD4, CD8, CD16, CD14, amine-reactive dye, CCR5 (via antibody clone 3A9), and Leronlimab-PR for 30 min at RT in the dark. Finally, cells were washed twice with PBS and fixed with 2% PFA for more than 30 min before collecting on LSR-II instrument and FACSDiVA version 6.1. Using FlowJo v10, cells were gated on singlet, live, CD3+, CD4+, and CCR5+ (via 3A9 staining) events. The number of CD4+ CCR5+ population was further gated on human IgG4+ or Leronlimab-PR+ events.

**Rhesus macaques.** All study RM were housed at the Oregon National Primate Research Center (ONPRC) in ABSL-2 rooms with automatically controlled temperature, humidity, and lighting. At assignment, all study RM were free of cCPRM herpesvirus 1, simian immunotrophic virus type 1, and Mycobacterium tuberculosis. RM were typed for the MHC alleles Mamu-A*01, Mamu-A*02, Mamu-B*17, and Mamu-B*08, with Mamu-B*17 and/
or 0-8° positive animals excluded when possible or placed into control groups when not possible to exclude biasing results. All attempts were made to pair housed RM during the study period. When RM died or had to be removed due to a 1:1 min beating and 1 min on ice. Supernatant from the tissue homogenate was transferred to a new tube and spun at 80 °C until assayed.

Viral nucleic acid detection. Nucleic acid from plasma and PBMC cell pellets were extracted using the Maxwell 16 instrument (Promega, Madison, WI) following the manufacturer’s protocol, which uses the LEV Viral Nucleic Acid Kit for RNA extraction. Three SHIV-naïve RMs served as adoptive transfer recipients for tissue homogenates from PrEP RM as described. All Leronlimab utilized in these studies were clinical-grade material provided by CytoDyn at a concentration of 175 mg/mL.

Processing of blood and tissue. Whole blood was collected into EDTA treated or non-anticoagulant tubes (BD Biosciences). Blood in EDTA-tubes was assessed for plasma Leronlimab and loss of Leronlimab CCR5 RO on CD4+ T-cells in blood. Three SHIV-naïve RMs served as adoptive transfer recipients for tissue homogenates from PrEP RM as described. All Leronlimab utilized in these studies were clinical-grade material provided by CytoDyn at a concentration of 175 mg/mL.

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Processing of blood and tissue. Whole blood was collected into EDTA treated or non-anticoagulant tubes (BD Biosciences). Blood in EDTA-tubes was assessed for plasma Leronlimab and loss of Leronlimab CCR5 RO on CD4+ T-cells in blood. Three SHIV-naïve RMs served as adoptive transfer recipients for tissue homogenates from PrEP RM as described. All Leronlimab utilized in these studies were clinical-grade material provided by CytoDyn at a concentration of 175 mg/mL.
rheus genomic DNA as carrier. Reactions were run with the Applied Biosystems QuantStudio 6 Flex instrument (Life Technologies) using the following thermal conditions: 95 °C for 20 s; 10 min for 1 s, 60 °C for 20 s × 45 cycles. The limit of quantification for this assay is 10 copies/million cells for cell pellets and 7 copies/million cells for whole tissue biopsies.

Leronlimab measurement in plasma and tissues. Enzyme-linked immunosorbent assay (ELISA) was used to detect free Leronlimab in plasma. Half-area 96-well Costar assay Plates (Corning) were coated with the anti-idiotypic antibody PA 22, provided by CytoDyn, at 1.5 μg/mL in carbonate-bicarbonate buffer (Thermo Fisher) and incubated overnight. Plates were washed three times with PBS-T (PBS + 0.1% Tween-20) and blocked with blocking buffer (PBS + 0.4% Tween-20 + 10% BSA) for at least 2 h at RT. Leronlimab concentration was calculated with a standard curve created with a serial titration of Leronlimab diluted in blocking buffer with a range of 4.7–300 μg/mL. Heat-inactivated plasma samples were also diluted with blocking buffer. After incubating for 30 min at RT, plates were washed three times with 0.5 M NaCl in PBS and incubated with 1:20,000 dilution of mouse anti-human IgG, FcεR1-horseradish peroxidase (HRP) (Southern Biotech) in blocking buffer for 30 min at RT. Plate was washed three times with PBS-T and developed for 2 min using 3,3’,5,5’-tetramethylbenzidine (TMB) substrate (Southern Biotech). Reaction was stopped with 1 N H2SO4. Plates were read on the Synergy HTX Multi-Mode Microplate Reader (BioTek) and data were collected using software Gen5 v3.09 at two absorbance wavelengths: 650 nm for the developing reaction and 430 nm for the developed reaction after the reaction was stopped. The OD430 nm was determined by OD650 nm. The limit of detection for the assay is 22.5 ng/mL. Leronlimab in tissue was quantified in supernatants prepared from tissue homogenates by ELISA as described above. Further, the mass of the total protein in the collected tissues was determined by the Pierce Coomassie Plus Broadassay Plus Assay Kit (Thermo Fisher) following the manufacturer’s instructions. Tissue concentration of Leronlimab is reported as the ng of Leronlimab per mg of total protein.

Measurement of Leronlimab antiviral antibodies (ADA). Half-area 96-well Costar assay Plates (Corning) were coated with 2 μg/mL Leronlimab (Cytodyn, Vancouver, WA). Plates were washed with PBS-T three times and blocked with blocking buffer for 2 h at RT. Plates were then washed three times with PBS-T. Heat-inactivated plasma samples were serially diluted in blocking buffer, added to the plates in duplicate, and incubated at RT for 30 min. Plates were then washed three times with 0.5 M NaCl in PBS. To determine ADAs from RM, a secondary antibody recognizing rhesus IgG (anti-rhesus IgG1/3 [IB]–HRP, NHP Reagent Resource) and conjugated to HRP was added. Plates were incubated at RT for 30 min, and then washed three times with PBS-T. TMB solution (Southern Biotech) was added at RT for 2 min and the reaction was stopped with 1 N H2SO4. Absorbance was read at 450 nm on a Synergy plate reader (BioTek). ADA titers are defined as the reciprocal of the highest dilution of the sample that yields a positive result (e.g. dilution of 1/2460 = titer of 2460). A positive result was defined as twice that of background values.

Env sequencing. Viral sequencing and analysis were adapted from previously published genre-wide SIVmac239 sequencing protocols. Viral RNA was isolated from virus stocks and plasma samples using QIAamp MinElute Virus Spin Kit following the manufacturer’s instructions. Complementary DNA was generated with the one-step RT-PCR with Platinum Taq (Thermo Fisher). SHIV env forward primer (GGCATAGCCTCATAAAATATCTG) and the SHIV env reverse primer (ACAGGCGAAGATTGAGTATTG) were used to amplify a ~4.5 kb amplicon spanning the env gene (Supplementary Table 4). RT-PCR reactions were performed on Eppendorf Mastercycler Pro S Thermal Cyclers using the following conditions: 50 °C for 20 min; 95 °C for 15 s, 60 °C for 1 min, 68 °C for 4 min × 2 cycles; [94 °C for 15 s, 55 °C for 1 min, 68 °C for 4 min × 2 cycles; [94 °C for 15 s, 60 °C for 1 min, 68 °C for 4 °C × 20 min] and 15 min, 6 °C for 10 min; and hold at 4 °C. The resulting 4.9 kb fragments were purified on a 1% agarose gel and purified using NucleoSpin Gel and PCR Clean-up Kit (Macherey-Nagel). Subsequent reactions were performed using the Nextera XT DNA Sample Prep Kit, purified with AMPure XP magnetic beads (Beckman Coulter). Libraries were analyzed on an Agilent 2100 Bioanalyzer using the HS DNA kit (Agilent), normalized to 2 nM, pooled at an equimolar ratio, and sequenced in parallel on the Illumina MiSeq.

T cell assays. SHIV-specific CD8+ T cell responses in PBMC were measured by flow cytometric intracellular cytokine staining (ICS)4,1 × 109 PBMCs were incubated with overlapping-15-mer peptide pools spanning SIVmac239 Gag or Vif open reading frame and co-stimulated with CD28 and CD49d antibodies (eBiosciences) for 1 h, followed by the incubation with Brefeldin A (Sigma-Aldrich) for an additional 8 h. Stimulation with rhinovirus CMV (RhCMV) lysis served as a positive control while incubation without antigen in the absence of background stimulation served as a negative control. SHIV-specific effector cells were stained for CD3, CD4, CD8, and CD107 on the basis of amine-reactive dye, fixed with 2% PFA, permeabilized with BD FACS Lysing Solution (BD Biosciences), and stained intracellularly for IFN-γ, TNF-α, and CD69. Samples were collected on LSR-II instrument and FACSDIVA software version 6.1 and analyzed with FlowJo v10 (Tree Star) by gating on singlet, live, CD3+, CD4+, and CD8+ cells. Responding CD8+ T cells were measured by Boolean gating on cells that are CD69+ /TNF-α+ and/or CD69+ /IFN-γ++. Adaptive transfer. To confirm sterilizing protection, cells from the infected control animals (all six animals) or uninfected Leronlimab-treated animals (four animals in the 10 mg/kg group and all six animals in the 50 mg/kg group) were adoptively transferred into one SHIV-naive RM per animal group, as detailed in Table S5. Cells were prepared an hour before infusion by resuspending in 1 mL of Hank’s Buffered Saline Solution (HBSS) with 15 U/mL heparin. Recipient RM were sedated with ketamine HCl (8–20 mg/kg) or Telazol (2–5 mg/kg) and prophylactically treated with Benzyl (5 mg/kg) prior to infusion of donor cells. Donor cells were slowly infused intravenously with an infusion pump at a maximum rate of 22 mL/kg/h. Animals were monitored for at least 2 h for post-procedural complications.

Antibodies. The following conjugated antibodies were used in these studies: (a) from BD Biosciences, D058-1283 (CD45; PE; Cy7; 1:100; cat# 561294), SP34-2 (CD3; Alexa 700; 1:100; cat# 557971), SP34-2 (CD3; PE; 1:20; cat# 552127), LP200 (CD4; PerCP-Cy5.5; 1:50; cat# 552835), RA-7A (CD8; PE-Cy7; 1:20; cat# 558234); (b) from BioLegend, OKT4 (CD4; APC-Cy7; 1:100; cat# 305612), RAPA-T4 (DC; APC; 1:100; cat# 300537); (c) from Beckman Coulter, RM052 (CD14; PE-Texas Red; 1:40; cat# 56267), SP34-2 (CD3; Pacific Blue; 1:100; cat# 558124); (f) from BioLegend, OKT4 (CD4; APC-Cy7; 1:100; cat# 305612), RAPA-T4 (DC; APC; 1:100; cat# 300537); (d) from Sigma, HP-6025 (IgG4; FITC; 1:100; cat# F9890); and (e) from SouthernBiotech, HPA023 (mouse anti-human IgG, FcεR–HRP; 1:2000; cat# 9190-05); (f) from NHP Reagent Resource, 1B3 (anti-rhesus IgG1/3; HRP; 1:5000). The following unconjugated antibodies were used: (a) 55-2F12 (SIV Gag p27; NIH AIDS Research and Reference Program, conjugated-in-house to FITC using Pierce™ FITC Antibody Labeling Kit (Thermo Fisher) and used at approximately 1:100 depending on the efficacy of conjugation; (b) PA-14 (Leronlimab; CytoDyn), conjugated-in-house to Pacific Blue™ Antibody labeling Kit (Thermo Fisher) and used at approximately 1:80 depending on the efficacy of conjugation; (c) anti-idiotypic antibody, PA-22 (CytoDyn). Live/dead Fixable Yellow Dead Cell Stain Kit and Near-IR Dead Cell Stain Kit (Thermo Fisher) were amine-reactive dyes used at 1:1000 dilution to assess cell viability.

Statistical analyses. Time to infection was assessed by log-rank test. Differences in CCR5 expression percentages were measured by nonparametric Kruskal–Wallis test, and differences in the number of CCR5 surface molecules were assessed by nonparametric Mann–Whitney test. Statistical significance was determined at the significant alpha level of 0.05. Statistical analyses were conducted using GraphPad Prism software version 6.0 (GraphPad Software, La Jolla, California).

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. SHIV erotrop3 sequence data that support the findings of this study have been deposited in GenBank with the accession code KF044063.1. All other relevant data that support the findings of this study are available with the corresponding authors upon reasonable request. Source data are provided with this paper.

Code availability. Leronlimab is available via MTA from CytoDyn.
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