Preexposure Prophylaxis with Albumin-Conjugated C34 Peptide
HIV-1 Fusion Inhibitor in SCID-hu Thy/Liv Mice

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Running title: PrEP with Albumin-Conjugated C34 HIV Fusion Inhibitor

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PC-1505 is a C34 peptide derived from the heptad repeat 2 region of HIV-1 gp41 conjugated to human serum albumin for sustained in vivo activity. One single preexposure dose of PC-1505 reduced viral RNA in HIV-1-infected SCID-hu Thy/Liv mice by 3.3 log$\text{_{10}}$ and protected T cells from virus-mediated depletion. In contrast, a single preexposure dose of Truvada reduced viral RNA by only 0.8 log$\text{_{10}}$ and was substantially less effective in preventing T-cell depletion.
The entry of human immunodeficiency virus type 1 (HIV-1) into CD4-bearing target cells involves three main steps, the binding of gp120 to the CD4 receptor, the subsequent binding to coreceptor CXCR4 or CCR5, followed by the conformational changes of the ectodomain of HIV-1 gp41 critical to membrane fusion. T-20 (DP-178, enfuvirtide, Fuzeon, Trimeris/Roche Applied Sciences), a synthetic peptide based on the C-terminal helical region (CHR) heptad repeat 2 (HR2) sequence of HIV-1 gp41, remains the only compound marketed to date that targets the conformational rearrangements of gp41. Another C-peptide, C34, composed of a peptide sequence which overlaps with T-20 but contains the gp41 coiled-coil cavity binding residues, WMEW631, competes with the CHR of gp41 for the hydrophobic grooves of the NHR region yet is incapable of functioning at a post-lipid mixing stage (10). Despite the successes of T-20, its commercial utility has been restricted to salvage therapy because of cost and injection site reactions (3, 11). The challenge in developing therapeutic peptides is further complicated primarily by their rapid renal clearance, poor distribution, and susceptibility to peptidase degradation.

C34 peptide was engineered into a 1:1 human serum albumin conjugate through stable covalent attachment of a maleimido-C34 analog onto cysteine-34 of albumin. This bioconjugate, PC-1505, was designed to require less frequent dosing and less peptide than T-20 as a direct result of the significantly improved pharmacokinetic profile of the C34 peptide following albumin conjugation. The bond linking maleimide to cysteine 34 is highly stable in vivo, and the C34 peptide is rendered more stable against rapid renal clearance and against peptidase degradation (16). In previous work in HIV-1-infected SCID-hu Thy/Liv mice, we found that T-20 lost activity with infrequent dosing whereas the antiviral potency of PC-
1505 was sustained, even for a single preexposure dose (16). Contrary to previous reports that the gp41 NHR trimer is poorly accessible to C34 fused to protein cargoes of increasing size (5), these results were the first demonstration of the capacity for a large, endogenous serum protein to gain unobstructed access to the transient gp41 intermediates that exist during the HIV-1 fusion process. The superior pharmacokinetics and preexposure prophylaxis (PrEP) activity of PC-1505 as compared to T-20 (16) prompted us to evaluate its efficacy for PrEP compared to Truvada (tenofovir disoproxil fumarate and emtricitabine), a prevention strategy shown to be effective for men who have sex with men in the iPrEx trial (4).

We compared the activity of a single preexposure dose of PC-1505 and Truvada in SCID-hu Thy/Liv mice inoculated with HIV-1 24 h after subcutaneous (PC-1505) or oral (Truvada) administration. The human thymus implant in these mice supports long-term differentiation of human T cells, and the model has been standardized and validated with four classes of licensed antiretrovirals for the evaluation of antiviral compounds against HIV-1 (13). WE Human fetal thymus and liver were obtained through services provided by a nonprofit organization (Advanced Bioscience Resources, Alameda, CA) in accordance with federal, state, and local regulations. Coimplantation of small thymus and liver fragments under the kidney capsule to create SCID-hu Thy/Liv mice and inoculation of the Thy/Liv implants with HIV-1 was carried out as described (12, 13) in a cohort of 40 mice each was implanted with tissues from a single donor.

C34 peptide synthesis, generation of the drug affinity complex moiety (maleimide propionic acid and amino ethyl ethoxy acetic acid linker), and the conjugation of maleimido-C34 to cysteine-34 of human serum albumin (HSA) to generate PC-1505 were performed as reported previously (6-9, 16, 17). Mass
spectrometry of each purified sample confirmed the most abundant protein product corresponded to a 1:1 covalent complex of HSA with the maleimido derivative, and reverse-phase HPLC analysis of the purified sample confirmed the removal of essentially all unbound (free) maleimido derivative. PC-1505 was prepared in 8 mM sodium octanoate in 1.5% polysorbate. Drugs were administered to the mice (6 or 7 mice per group) by subcutaneous injection or oral gavage at the indicated dosage levels (peptide alone excluding albumin for PC-1505) beginning 24 h before direct injection of 1,000 TCID$_{50}$ HIV-1 NL4-3 or RPMI 1640 (mock infection) into each Thy/Liv implant. We chose NL4-3 as the inoculum because we have validated the SCID-hu model with licensed antiretroviral drugs (including T-20) against this molecular clone (13) and have previously demonstrated the potent activity of PC-1505 against NL4-3 challenge in the mice (16). Implants were collected 21 days after inoculation and dispersed into single-cell suspensions for quantification of p24 by ELISA and viral RNA by branched DNA assay and were stained with antibodies to CD3, CD4, CD8, p24, and MHC class I (W6/32) for analysis of T-cell subsets by multiparameter flow cytometry as described previously (12, 13, 15). The limit of detection of HIV-1 RNA by the bDNA assay was 1.5 log$_{10}$ copies per $10^6$ implant cells. Animal protocols were approved by the UCSF Institutional Animal Care and Use Committee.

One single dose of 200 mg/kg PC-1505 (fusion inhibitor dosage excluding the albumin) reduced implant viral RNA by 3.3 log$_{10}$ and p24 by >95% compared to untreated infected mice (Fig. 1). Four of six treated mice had no detectable p24, and one of six mice had no detectable p24 or HIV-1 RNA. One single lower dose of 60 mg/kg PC-1505 reduced viral RNA by 2.0 log$_{10}$ and p24 by 95%. Although 200 mg/kg of PC-1505 had potent antiviral activity, reducing HIV RNA in the implants by
>1,000 fold compared to untreated mice (Fig. 1), the Thy/Liv implants were not completed protected from infection. This is the result of performing the inoculation by injection of the viral inoculum directly into a highly susceptible target organ – a significant challenge to complete protection with a drug that is giving a single time before virus exposure.

Both dosage levels of PC-1505 also reduced the percentage of Gag-p24+ thymocytes and interferon-α-induced MHC class I expression on DP thymocytes (Fig. 1). A single dose of PC-1505 in these mice also completely protected immature and mature T-cells in the Thy/Liv implants from virus-mediated cytopathicity and depletion in terms of total implant cellularity, thymocyte viability, percentage of immature cortical CD4+CD8+ (double-positive, DP) thymocytes and reduction in the CD4/CD8 ratio (Fig. 2). Although the differences in implant cellularity between PC-1505- and Truvada-treated groups appeared modest, this reflects the 21-day time point we chose for implant collection, a point before thymocyte depletion becomes severe after inoculation with the X4 HIV NL4-3. For instance, the mean percentage of DP thymocytes (of live thymocytes) was 80% for the mock-infected group and 60% for the untreated infected group (Fig. 2); based on a previous study (14), the DP percentage would have been reduced to ~10% by 28 days. For each of the cellular parameters in Figure 2, the PC 1505 treated groups have means as high (or higher) than the mock-infected groups, indicating complete protection from thymocyte depletion as assessed by these specific endpoints.

In stark contrast to our findings with PC-1505, a single oral preexposure dose of Truvada (200 mg/kg tenofovir DF and 130 mg/kg emtricitabine) 24 h before virus inoculation reduced viral RNA by only 0.8 log10 and p24 by 59% and was substantially less effective in preventing thymocyte depletion. One single lower dose
of Truvada (60 mg/kg tenofovir DF and 40 mg/kg emtricitabine) had no detectable antiviral or cytoprotective activity. (For comparison, this lower dose of Truvada is approximately 12 times lower than the daily human dosage of 300 mg tenofovir DF and 200 mg emtricitabine). In studies performed by Denton et al. in the humanized Bone marrow/Liver/Thymus (BLT) mouse model, Truvada PrEP prevented HIV-1 infection after intravaginal (1) and intrarectal and intravenous HIV-1 exposure (2), although these studies differed from ours (single oral administration) in that once-daily intraperitoneal injections of the drugs (200 mg/kg tenofovir DF and 130 mg/kg emtricitabine) were performed for 7 consecutive days starting 2 days before inoculation. For comparison purposes, the human dosage level of Truvada is

This study confirms the highly potent in vivo anti-HIV-1 activity of PC-1505 and establishes a proof-of-principle for this new class of albumin-peptide conjugates. Agents with this kind of sustained activity hold promise for targeting early events in the transmission of HIV as is the case for gel-based microbicides. Furthermore, unlike most other classes of antivirals, albumin-peptide conjugates offer the unique opportunity to optimize, and perhaps personalize, the peptide sequence of the fusion inhibitor (or combination of peptide sequences) for effective activity against many multidrug-resistant strains of HIV. The potent activity we observed for a single preexposure dose supports further preclinical and clinical development of this promising long-acting fusion inhibitor and confirms the usefulness of the SCID-hu Thy/Liv mouse model for evaluation of in vivo antiretroviral efficacy and preexposure prophylaxis.
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FIG. 1. PC-1505 has more sustained antiviral activity than Truvada against HIV-1 NL4-3 with a single preexposure dose. SCID-hu Thy/Liv mice were treated with one subcutaneous injection of PC-1505 (60 or 200 mg/kg of peptide alone excluding albumin) or one oral dose of Truvada (60 mg/kg tenofovir DF plus 40 mg/kg emtricitabine or 200 mg/kg tenofovir DF plus 130 mg/kg emtricitabine) 24 h before virus inoculation. Antiviral efficacy was assessed by determining HIV-1 RNA, p24, percentage of Gag-p24+ thymocytes, and MHC-I expression on DP thymocytes for treated versus untreated mice. The columns represent the means, and the open circles represent individual mice from the same cohort 21 days after virus (or mock) inoculation. *, P < 0.05; **, P < 0.01 compared to untreated HIV-1-infected mice by the Mann-Whitney U test.

FIG. 2. PC-1505 has more sustained cytoprotective activity than Truvada against HIV-1 NL4-3 with a single preexposure dose. SCID-hu Thy/Liv mice were treated with one subcutaneous injection of PC-1505 (60 or 200 mg/kg of peptide alone excluding albumin) or one oral dose of Truvada (60 mg/kg tenofovir DF plus 40 mg/kg emtricitabine or 200 mg/kg tenofovir DF plus 130 mg/kg emtricitabine) 24 h before virus inoculation. Thymocyte protection was assessed by total implant cellularity, thymocyte viability, percentage of DP thymocytes, and CD4/CD8 ratio for treated versus untreated mice. The columns represent the means, and the open circles represent individual mice from the same cohort 21 days after virus (or mock) inoculation. *, P < 0.05; **, P < 0.01 compared to untreated HIV-1-infected mice by the Mann-Whitney U test.


Figure 2

[Bar charts showing changes in total cells, live thymocytes, DP thymocytes, and CD4:CD8 ratio with different treatments.]