

Title:

UCLA1 Aptamer Inhibition of Human Immunodeficiency Virus Type 1 Subtype C Primary Isolates in Macrophages and Selection of Resistance

Hazel Tumelo Mufhandu^{1*}, Kabamba Bankoledi Alexandre¹, Elin Solomonovna Gray^{2#}, Lynn Morris^{2, 3} and Makobetsa Khati^{1, 4}

¹Emerging Health Technologies Platform, Biosciences Unit, CSIR, Pretoria, South Africa, ²Center for HIV and STI's, National Institute for Communicable Diseases, Johannesburg, South Africa, ³University of the Witwatersrand, Johannesburg, South Africa, ⁴Department of Medicine, Groote Schuur Hospital and University of Cape Town, Cape Town, South Africa,

[#]Current address: Edith Cowan University, School of Medical Sciences, Joondalup, Western Australia, Australia

H. T Mufhandu: hmufhandu@csir.co.za *Corresponding author

K. B Kabamba: aalexandre@csir.co.za

E. S Gray: e.gray@ecu.edu.au

L. Morris: lynnm@nicd.ac.za

M. Khati: mkhati@csir.co.za

ABSTRACT

We have previously shown that the aptamer, UCLA1, is able to inhibit HIV-1 replication in peripheral blood mononuclear cells (PBMCs) by binding to residues in gp120. In this study we examined whether UCLA1 was effective against HIV-1 subtype C isolates in monocyte-derived macrophages (MDMs). Of 4 macrophage-tropic isolates tested, 3 were inhibited by UCLA1 in the low nanomolar range ($IC_{80} < 29$ nM). One isolate that showed reduced susceptibility (< 50 nM) to UCLA1 contained mutations in the $\alpha 5$ helix next to the CD4 and co-receptor (CoR) binding complex. To further evaluate aptamer resistance, two primary viruses were subjected to increasing concentrations of UCLA1 over a period of 84 days in PBMCs. One isolate showed a 7-fold increase in IC_{80} (351 nM) associated with genetic changes, some of which were previously implicated in resistance. This included F223Y in the C2 region and P369L within the CD4 and CoR binding complex. A second isolate showed a 3-fold increase in IC_{80} (118 nM) but failed to show any genetic changes. Collectively, these data show that UCLA1 can efficiently block HIV-1 infection in MDMs and PBMCs with escape mutations arising in some isolates after prolonged exposure to the aptamer. This supports the further development of the UCLA1 aptamer as a HIV-1 entry inhibitor.

Keywords

HIV-1

Subtype C

Aptamer

UCLA1

gp120 mutations

Monocyte-derived macrophages

INTRODUCTION

The AIDS epidemic is a major global health crisis with over 36 million people living with HIV and high rates of new infections in many countries (www.who.int/hiv/en). HIV-1 subtype C is the most dominant viral subtype found mostly in sub-Saharan Africa and India with over 70% of new global HIV infections occurring in these regions. While the development of a vaccine is considered essential to control the HIV epidemic, this has proven to be a major challenge largely due to the high genetic variability and evolutionary rates of HIV [1]. The widespread use of anti-retroviral therapy has had a significant impact on the epidemic with over 15 million people currently being treated. However, anti-retroviral drugs trigger viral evolution and select for resistance [2, 3] and as such newer drugs and agents need to be developed.

UCLA1 is a shortened and modified RNA aptamer derived from the parental B40 aptamer [4, 5]. This molecule has been shown to bind to HIV-1 gp120 with high specificity and affinity and to potently inhibit HIV-1 subtype C entry. Our previous study revealed UCLA1 binding sites within the CoR binding site, at the base of the V3 loop, and in the bridging sheet within the conserved V1/V2 stem-loop of gp120 [6]. As a promising anti-HIV-1 candidate, UCLA1 may complement existing ARV regimens or be used in HIV prevention strategies, such as a microbicide. The study of resistance against any inhibitor of HIV-1 is necessary to identify the nature and number of mutations associated with viral escape. It also enables studies to assess

the prevalence of naturally-occurring resistant strains and hence the likelihood of efficacy.

In addition to CD4⁺ T lymphocytes, HIV-1 infects blood monocytes and tissue macrophages which are thought to play an important role in the maintenance of viral reservoirs [7, 8]. Furthermore, these cells may play a role in HIV-1 transmission at mucosal sites. Thus potential entry inhibitors of macrophage infection could not only block the transmission of HIV at mucosal surfaces but also prevent seeding of reservoirs. Thus, the current study examined UCLA1 inhibition efficacy against HIV-1 subtype C clinical isolates in MDMs and evaluated the generation of UCLA1 escape mutations in PBMCs.

MATERIALS AND METHODS

Isolation of monocyte-derived macrophages

Macrophages were derived from blood monocytes [9]. Briefly, PBMCs were freshly isolated from HIV-seronegative blood donors using the Ficoll-Hypaque method [10] and re-suspended in Lonza X-VIVO-10 culture media (Whitehead Scientific, S.A), supplemented with 10% fetal bovine serum (FBS, Gibco Invitrogen, South Africa). Cells were incubated for 90 min at 37°C in 5% CO₂ in T150 flasks and washed to remove non-adherent cells. The adherent monocytes were incubated overnight in the supplemented X-VIVO-10 media and the monolayer disrupted by adding chilled PBS at 4°C for 1 hr [11]. Re-suspended monocytes were cultured in Rosewell Park Memorial Institute (RPMI) medium supplemented with 10% FBS (10% RPMI growth media) and 5 ng/ml human granulocyte macrophage colony stimulating factor (hGM-

CSF) (Roche Diagnostics, Mannheim, Germany) for 5-7 days to enable differentiation into macrophages.

Phenotypic characterization of monocyte-derived macrophages

Phenotype confirmation of the differentiated macrophages was performed by fluorescence activated cell sorting (FACS) analysis [9, 11]. Briefly, the macrophages were fixed and indirect cell surface staining performed with CD14/AF514 MAbs (Whitehead Scientific, S.A, Invitrogen Life Technologies, S.A) which stained undifferentiated monocytes, CD68/AF488 or MHCII/AF488 MAbs stained differentiated monocytes (macrophages) and CD3/PE-Cy5.5 (Caltag Laboratories, Thailand) stained T lymphocytes. The cells were acquired on the BD FACSCalibur flow cytometer (Immunocytometry Systems, CA, USA) and analyzed with the BD CellQuest Pro software, version 5.1 (BD Biosciences, NJ, USA).

Screening of macrophage-tropic viruses

A panel of 25 HIV-1 subtype C replication competent primary isolates were screened for macrophage tropism. These included four viruses from the CAPRISA 002 Acute Infection study cohort [12], five from the Durban female sex workers cohort [13], eight paediatric isolates [14], five from HIV/TB co-infected patients and three AIDS patients co-infected with *Cryptococcus meningitis* [15]. Twenty of the viruses were R5, four were R5X4 [14, 15] and one X4 virus [15]. An R5-tropic subtype B primary virus, ADA, known to infect macrophages was used as a reference strain. Briefly, viral stocks were grown in 2×10^6 PBMCs which were CD8 T cells depleted using the RosetteSep human CD8 depletion cocktail method (Separation Scientific, South Africa). The cells were re-suspended in RPMI medium plus 20% FBS, 50 $\mu\text{g/ml}$

gentamicin (Sigma-Aldrich, South Africa) and 5% IL-2 (Roche, South Africa) (complete medium). Phytohemagglutinin (PHA) (Sigma-Aldrich, South Africa) was added to the media for activation of the lymphocytes. Culture supernatants were tested weekly for p24 antigen using the Vironostika HIV-1 Antigen Microelisa System (Biomerieux, South Africa) and expanded when high levels of p24 antigen (100-200 ng/ml) were present. Cultures were fed twice weekly with complete medium and weekly with fresh PHA-activated donor PBMCs for 3 weeks. Virus-containing supernatants were clarified by centrifugation and stored at -70°C until use.

HIV-1 infection of MDMs

MDMs ($0.5 - 1.0 \times 10^6$ cells/ml) were infected with 500 - 2500 TCID₅₀ of the primary viruses in 24-well tissue culture plates and incubated overnight at 37°C, 5% CO₂ [9]. Control wells with only cells and media were included. Virus inoculum was aspirated and the cells washed with 10% RPMI growth media. The cells were then re-suspended in 10% RPMI growth media and incubated for a further 6 days at 37°C, 5% CO₂. The cultures were harvested every 7th day and maintained for a maximum of 42 days.

Neutralization of HIV-1 in MDMs

Neutralization of HIV-1 subtype C infection in MDMs by UCLA1 anti-gp120 RNA aptamer was done as previously described for PBMCs [9]. Briefly, UCLA1 was used at a starting concentration of 100 nM with 3-fold serial dilutions in RPMI supplemented with 5% FBS (growth media). Virus supernatants at 500 - 2500 TCID₅₀ were added to the serially diluted aptamer and incubated for 1 hr at 37°C, 5% CO₂. Differentiated macrophages were seeded at 1.0×10^6 cells/ml in 96-well culture

plates. Half of the volume of the cell supernatants was aspirated and the aptamer/virus mix added to the cells and incubated overnight at 37°C, 5% CO₂. The assay was performed in triplicate and included control wells without aptamer. The cells were washed 3 times by aspirating the virus inoculum from each well and adding 250 µl of RPMI growth media. The cells were then incubated for a further 6 days in RPMI growth media at 37°C, 5% CO₂. On day 7, 50 µl of virus supernatants were harvested from each well and the cultures replenished with an equal volume of RPMI growth media and incubated at 37°C, 5% CO₂ for a further 7 days. The supernatants were harvested every 7th day for a maximum of 21 days. The harvested viral supernatants were inactivated with 1.25% Empigen BB detergent and stored at 4°C in zip-lock plastic bags until further use.

HIV-1 p24-antigen immunoassay

Macrophages were evaluated for infection and neutralization using the HIV-1 chemiluminescent p24 antigen ELISA as previously described [16]. Briefly, p24 antigen was captured from a detergent lysate of virions by a polyclonal antibody adsorbed to 96-well plate. Bound p24 was detected with an alkaline phosphatase-conjugated anti-p24 MAb and a luminescent detection system. Luminescence was measured with the Wallac 1420 Victor Multilabel luminometer (Separation Scientific, S.A). The neutralization titers were determined at 70% level of reduction in p24 antigen production of the test cultures compared with the negative control cultures (without inhibitor).

Evaluation of resistance to UCLA1

Two primary isolates that showed different co-receptor usage, RP1 (R5X4-tropic) and Du422 (R5-tropic) were used to evaluate resistance to the aptamer. Both viruses were shown to be sensitive to UCLA1 in PBMCs in the previous study [6]. For this, 1000 TCID₅₀ of each virus was cultured in the presence of increasing concentrations of the aptamer in 1×10^6 CD8-depleted PBMCs. The starting concentration of the aptamer was the IC₈₀ of each virus tested. Virus cultures without UCLA1 were used as negative controls. All cultures were maintained in 5% RPMI growth media supplemented with 0.05 µg/ml of IL-2. The cultures were passaged every 7th day and new cultures set up with 10% of the harvest from the previous culture in freshly isolated CD8-depleted PBMCs. UCLA1 concentration was increased whenever the viral growth, measured by p24 antigen ELISA, was similar or higher than the negative control cultures.

Isolation, amplification and sequencing of HIV-1 RNA

Isolation of viral RNA from cultures was performed with the QIAamp mini spin viral isolation kit (QIAGEN, S.A). The extracted RNA was reverse transcribed into cDNA using OFM19 primer: 5'-GCA CTC AAG GCA AGC TTT ATT GAG GCT TA-3' and Superscript III reverse transcriptase (Invitrogen). The envelope gene was amplified by nested PCR using the High Fidelity Platinum Taq DNA polymerase (Invitrogen) as described previously [17]. The amplified DNA was purified with the QIAquick PCR purification Kit (QIAGEN), quantified and sequenced using an ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, S.A). The sequences were resolved on an ABI 3100 automated genetic analyzer and were

assembled and edited using Sequencher (version 4.0), (Gene Codes, Ann Arbor, MI), Clustal X (v 1.83) and Bioedit (v 5.0.9) software.

RESULTS

Screening for macrophage-tropic viruses

Purified populations of macrophages were derived from blood monocytes after culture in hGM-CSF. Surface staining with either MHC Class II or CD68 mAbs confirmed that 99.9% of the monocytes had differentiated into macrophages (Supplementary Figures 1A and 1B). Twenty-five HIV-1 subtype C primary viruses were screened for macrophage tropism with >10 ng p24 indicative of viral replication (Supplementary Table 1). Five isolates showed an increase in p24 antigen levels over the 42 days of culture (Figure 1A), and were considered to be macrophage-tropic viruses. Peak p24 titers were in the range of 20.7-107.8 ng at 21 days of infection for four of the isolates. Du156 showed the lowest levels of infection with a peak p24 concentration of 3.7 ng/ml on day 35. Consequently, 4 subtype C isolates (CM1, COT9, Du422 and SW14) were used to evaluate UCLA1 neutralization in MDMs. The subtype B isolate, ADA, was included as a macrophage-tropic reference strain. This virus showed the highest levels of replication with a p24 value peaking at 407.9 ng a week earlier on day 14 (Figure 1B).

Neutralization of HIV-1 primary isolates in MDMs

The UCLA1 aptamer was tested at a starting concentration of 100 nM against 4 HIV-1 subtype C R5-tropic viruses. This was informed by our previous data where the same concentration was tested against HIV-1 subtype C primary viruses in PBMCs [6]. Three viruses tested in MDMs were neutralized in a dose-dependent manner

with IC_{80} values in the range of 6-29 nM (Figure 2). A fourth isolate, CM1 was neutralized but the inhibition was not high enough to obtain an IC_{80} value and was considered to have reduced susceptibility. Examination of gp160 sequence of this isolate revealed R476K, V491I, N492K, V496I and K500S mutations in the $\alpha 5$ helix next to the CD4 and CoR binding complex which may have contributed to the reduced susceptibility (Supplementary Figure 2). The subtype B isolate (ADA) was neutralized with an IC_{80} value of 30 nM in MDMs.

Generation of UCLA1 resistant isolates

We next assessed whether UCLA1 was able to select for viral resistance *in vitro*. Two primary isolates, RP1 (R5X4-tropic) and Du422 (R5-tropic), that were inhibited by UCLA1 in PBMCs in the previous study [6], were cultured in PBMCs with escalating concentrations of the aptamer for 84 and 77 days, respectively (Figure 3). Control cultures without aptamer were also assessed. Culture supernatants were collected once a week and tested for p24 antigen levels. The viral growth pattern varied during resistance selection and in some instances was markedly reduced when compared with the control cultures thus confirming UCLA1 anti-HIV activity in the test cultures (Figure 3 and Supplementary Tables 2A and 2B). For example, this was observed for RP1 at day 35 where the p24 for the test culture (with UCLA1) was 6-fold (3.8 ng/ml) less than the control culture (25.2 ng/ml) (Figure 3 and Supplementary Table 2A). The IC_{80} values for RP1 and Du422 at the end of selection period was 351 nM and 118 nM, that is, 7-fold and 3-fold respectively compared to p24 values before selection (Table 1).

Sequence analysis of UCLA1-selected isolates

Viral RNA from the culture supernatants were used to sequence the gp160 gene to assess for mutations within the *env* gene that might have occurred due to the selective pressure from UCLA1. No mutations were generated in the Du422 isolate, including the control culture, despite its ability to tolerate slightly increased UCLA1 concentrations. Six mutations were detected in RP1, at the base of the V3 loop (R322Q), within the V4 loop (N410S), in β 3 of the bridging sheet next to the V1/V2 loop (R202T), in the C2 region between β 4 and β 5 sheets (F223Y), within the CD4 and CoR binding complex in the α 3 helix (P369L) and in the α 5 helix next to the CD4 and CoR binding complex within the β 24 sheet (K476R) (Figures 4A and B). The P369L mutation was also present in the RP1 control virus cultured in the absence of aptamer.

DISCUSSION

The study examined the ability of the UCLA1 aptamer to inhibit HIV-1 subtype C infection of primary isolates in MDMs and evaluated selection of viral resistance against the aptamer. UCLA1 exhibited higher neutralization potency in MDMs compared to our previous data with PBMCs. Viral resistance against UCLA1 neutralization was evaluated by propagation under escalating concentrations of the aptamer. The selective pressure of UCLA1 resulted in resistance of one of the two viruses tested although this took time and was associated with multiple genetic changes in the HIV-1 envelope gene.

Primary viruses were screened for macrophage tropism before testing for UCLA1 inhibition. As expected, only R5-tropic primary viruses were competent to infect MDMs as their co-receptor, CCR5, serves as the major receptor on macrophages for

HIV [18]. When tested for antiviral activity in MDM cells, UCLA1 was able to inhibit entry of all four subtype C macrophage-tropic primary isolates tested. Its efficacy was demonstrated to be more potent in MDMs ($IC_{80} < 29$ nM) than in PBMCs ($IC_{80} < 94$ nM) [6]. The higher efficiency of UCLA1 against macrophages may be due to viral entry determinants, such as CD4 binding which is lower in MDMs [19]. A previous study identified V3 loop determinants of R5 macrophage-tropism suggesting that a conformational change in the V3 loop increased exposure of the CD4bs [19-21]. Given that we observed binding of UCLA1 in the V3 loop [4, 6] a similar exposure of the CD4bs to UCLA1 might have occurred which may explain the higher aptamer potency in MDMs. Blood monocytes and tissue macrophages are known to function as virus reservoirs *in vivo* and thus serve as obstacles to HIV clearance due to their contribution to persistent HIV infection [11]. Therefore, UCLA1 an efficient entry inhibitor of macrophage infection, may be useful to eliminate the burden of infection as well as limit the size of the virus reservoirs although there are obstacles such as latently infected, resting CD4⁺ T cells and persistence of HIV in infected individuals receiving antiretroviral therapy [22] to address before the aptamer can be considered as a viral reservoir inhibitor.

Selection of RP1 resistance by UCLA1 in PBMCs resulted in a 7-fold increase in IC_{80} values and generation of genetic changes in the envelope gene. Based on our previous data the F223Y mutation within the C2 region has been shown to decrease binding of the UCLA1 parental aptamer (B40t77) to HIV-1BaL gp120 [23] and might also be an escape mutation. The P369L mutation in the C3 region was observed to confer resistance to UCLA1 inhibition by site-directed mutagenesis in our earlier study [6]. However, P369L was also present in the control virus that had no aptamer

selection pressure suggesting that it is unstable and occurs spontaneously. An Arginine at residue 476 in the α 5-helix selected in RP1 has been identified as one of the essential sites of antibody gp120-core binding and for optimal viral infectivity [24]. The site is highly conserved across different subtypes of HIV-1, predominantly occurring as R476 and mutating the epitope results in loss of infectivity [24]. Therefore, the RP1 isolate may have generated the K476R mutation in order to gain fitness and survive the increasing concentrations of UCLA1. R476 was also found to occur naturally in CM1 and may have contributed to the reduced sensitivity seen in this isolate. The R202T in the bridging sheet, R322Q in the V3 loop and N410S in the V4 loop were observed for the first time here but their role requires further study. Thus, five mutations (R202T, F223Y, R322Q, N410S and K476R) and the previously discovered P369L mutation were identified in this study using *in vitro* selection with UCLA1 of a primary isolate. It will be important however to confirm these as UCLA1 escape mutations by site-directed mutagenesis of Env-pseudotyped viruses as was done previously for P369L [6] and also assess if escape in MDMs follows the same pathways.

Overall, our results indicate that selection of mutations associated with UCLA1 resistance required extended exposure to the drug (3 months) probably due to the very low nanomolar concentration levels that were utilized. Since the current study, together with our previous study [6] has shown that the aptamer is robust even at low nanomolar quantities, it may serve as a good anti-HIV-1 entry inhibitor that requires lower concentrations of the aptamer and thus cost effective as a product. This is the first study to report the *in vitro* generation of escape mutations, over a period of 12 weeks, against anti-gp120 aptamers using primary viruses. While only two isolates

were studied here, they provide important proof-of-principle data and identify key mutations for validation. Thus, further studies with more primary isolates will contribute to a better understanding of HIV-1 escape mechanism from neutralizing aptamers.

Competing interests

The authors declare that they have no competing interests.

Author's contributions

HTM designed the study, performed all experiments, analysed data and wrote the manuscript.

KBA contributed with study design, analysed data and co-authored manuscript.

ESG provided guidance with some experiments and co-authored manuscript.

LM provided the clinical samples and guidance with all experiments and co-authored manuscript.

MK conceived and designed the study, provided all the required reagents and co-authored manuscript.

Acknowledgements

We thank Johanna Ledwaba, Mary Phoswa and Sarah Cohen from the NICD for technical assistance. The authors acknowledge the donation of UCLA1 aptamer by Ian McGowan, William James and Brian Sproat under the NIH Aptamer Microbicide Program, grant 5U01AI066734-03. This work was funded by The Young Researcher Establishment Fund (YREF 2007 029), the Poliomyelitis Research Foundation

(08/37) and the Wits Medical Faculty Research Endowment Fund (001 410 MUFH011 TS).

References

- [1] D.M. Tebit, I. Nankya, E.J. Arts, Y. Gao, HIV diversity, recombination and disease progression: how does fitness "fit" into the puzzle?, *AIDS Rev*, 9 (2007) 75-87.
- [2] E. Seclen, M. Gonzalez Mdel, M. Lapaz, C. Rodriguez, J. del Romero, A. Aguilera, C. de Mendoza, V. Soriano, E. Poveda, Primary resistance to maraviroc in a large set of R5-V3 viral sequences from HIV-1-infected patients, *J Antimicrob Chemother*, 65 (2010) 2502-2504.
- [3] J.B. Presloid, I.S. Novella, RNA Viruses and RNAi: Quasispecies Implications for Viral Escape, *Viruses*, 7 (2015) 3226-3240.
- [4] C. Cohen, M. Forzan, B. Sproat, R. Pantophlet, I. McGowan, D. Burton, W. James, An aptamer that neutralizes R5 strains of HIV-1 binds to core residues of gp120 in the CCR5 binding site, *Virology*, 381 (2008) 46-54.
- [5] M. Khati, M. Schuman, J. Ibrahim, Q. Sattentau, S. Gordon, W. James, Neutralization of infectivity of diverse R5 clinical isolates of human immunodeficiency virus type 1 by gp120-binding 2'F-RNA aptamers, *J Virol*, 77 (2003) 12692-12698.
- [6] H.T. Mufhandu, E.S. Gray, M.C. Madiga, N. Tumba, K.B. Alexandre, T. Khoza, C.K. Wibmer, P.L. Moore, L. Morris, M. Khati, UCLA1, a synthetic derivative of a gp120 RNA aptamer, inhibits entry of human immunodeficiency virus type 1 subtype C, *J Virol*, 86 (2012) 4989-4999.
- [7] A. Kumar, G. Herbein, The macrophage: a therapeutic target in HIV-1 infection, *Molecular and cellular therapies*, 2 (2014) 10.
- [8] H. Koppensteiner, R. Brack-Werner, M. Schindler, Macrophages and their relevance in Human Immunodeficiency Virus Type I infection, *Retrovirology*, 9 (2012) 82.
- [9] S. Crowe, Maslin, C., Ellery, P., and Kedzierska, K., *Current Protocols in Immunology*. Eds: Coligen., J.E., Bierer, B.E., Margulies, D.H., et al., National Institute of Health. John Wiley & Sons, Inc., *Current Protocols in Immunology*, volume 3 (2004) pp 12.14.14-12.14.13.
- [10] R. Bures, A. Gaitan, T. Zhu, C. Graziosi, K.M. McGrath, J. Tartaglia, P. Caudrelier, R. El Habib, M. Klein, A. Lazzarin, D.M. Stablein, M. Deers, L. Corey, M.L. Greenberg, D.H. Schwartz, D.C. Montefiori, Immunization with recombinant canarypox vectors expressing membrane-anchored glycoprotein 120 followed by glycoprotein 160 boosting fails to generate antibodies that neutralize R5 primary

isolates of human immunodeficiency virus type 1, *AIDS Res Hum Retroviruses*, 16 (2000) 2019-2035.

[11] M. Collin, W. James, S. Gordon, Development of techniques to analyse the formation of HIV provirus in primary human macrophages, *Res Virol*, 142 (1991) 105-112.

[12] F. van Loggerenberg, K. Mlisana, C. Williamson, S.C. Auld, L. Morris, C.M. Gray, Q. Abdool Karim, A. Grobler, N. Barnabas, I. Iriogbe, S.S. Abdool Karim, Establishing a cohort at high risk of HIV infection in South Africa: challenges and experiences of the CAPRISA 002 acute infection study, *PLoS One*, 3 (2008) e1954.

[13] R. Bures, L. Morris, C. Williamson, G. Ramjee, M. Deers, S.A. Fiscus, S. Abdool-Karim, D.C. Montefiori, Regional clustering of shared neutralization determinants on primary isolates of clade C human immunodeficiency virus type 1 from South Africa, *J Virol*, 76 (2002) 2233-2244.

[14] I. Choge, T. Cilliers, P. Walker, N. Taylor, M. Phoswa, T. Meyers, J. Viljoen, A. Violari, G. Gray, P.L. Moore, M. Papathanosopoulos, L. Morris, Genotypic and phenotypic characterization of viral isolates from HIV-1 subtype C-infected children with slow and rapid disease progression, *AIDS Res Hum Retroviruses*, 22 (2006) 458-465.

[15] T. Cilliers, J. Nhlapo, M. Coetzer, D. Orlovic, T. Ketas, W.C. Olson, J.P. Moore, A. Trkola, L. Morris, The CCR5 and CXCR4 coreceptors are both used by human immunodeficiency virus type 1 primary isolates from subtype C, *J Virol*, 77 (2003) 4449-4456.

[16] J.A. McKeating, A. McKnight, J.P. Moore, Differential loss of envelope glycoprotein gp120 from virions of human immunodeficiency virus type 1 isolates: effects on infectivity and neutralization, *J Virol*, 65 (1991) 852-860.

[17] F. Gao, S.G. Morrison, D.L. Robertson, C.L. Thornton, S. Craig, G. Karlsson, J. Sodroski, M. Morgado, B. Galvao-Castro, H. von Briesen, S. Beddows, J. Weber, P.M. Sharp, G.M. Shaw, B.H. Hahn, Molecular cloning and analysis of functional envelope genes from human immunodeficiency virus type 1 sequence subtypes A through G. The WHO and NIAID Networks for HIV Isolation and Characterization, *J Virol*, 70 (1996) 1651-1667.

[18] B. Lee, M. Sharron, L.J. Montaner, D. Weissman, R.W. Doms, Quantification of CD4, CCR5, and CXCR4 levels on lymphocyte subsets, dendritic cells, and differentially conditioned monocyte-derived macrophages, *Proc Natl Acad Sci U S A*, 96 (1999) 5215-5220.

[19] T. Musich, P.J. Peters, M.J. Duenas-Decamp, M.P. Gonzalez-Perez, J. Robinson, S. Zolla-Pazner, J.K. Ball, K. Luzuriaga, P.R. Clapham, A conserved determinant in the V1 loop of HIV-1 modulates the V3 loop to prime low CD4 use and macrophage infection, *J Virol*, 85 (2011) 2397-2405.

[20] M.J. Duenas-Decamp, P.J. Peters, D. Burton, P.R. Clapham, Determinants flanking the CD4 binding loop modulate macrophage tropism of human immunodeficiency virus type 1 R5 envelopes, *J Virol*, 83 (2009) 2575-2583.

[21] C.J. Duncan, Q.J. Sattentau, Viral determinants of HIV-1 macrophage tropism, *Viruses*, 3 (2011) 2255-2279.

[22] T.W. Chun, A.S. Fauci, HIV reservoirs: pathogenesis and obstacles to viral eradication and cure, *AIDS*, 26 (2012) 1261-1268.

[23] M.K. Joubert, N. Kinsley, A. Capovilla, B.T. Sewell, M.A. Jaffer, M. Khati, A modeled structure of an aptamer-gp120 complex provides insight into the mechanism of HIV-1 neutralization, *Biochemistry*, 49 (2010) 5880-5890.

[24] J. Pietzsch, J.F. Scheid, H. Mouquet, F. Klein, M.S. Seaman, M. Jankovic, D. Corti, A. Lanzavecchia, M.C. Nussenzweig, Human anti-HIV-neutralizing antibodies frequently target a conserved epitope essential for viral fitness, *J Exp Med*, 207 (2010) 1995-2002.

FIGURE LEGENDS

Figure 1: Infectivity of HIV-1 primary viruses in monocyte-derived macrophages (MDMs). **(A):** Screening of HIV-1 subtype C primary isolates for macrophage infectivity. **(B):** Infectivity of macrophage tropic HIV-1 subtype B ADA primary virus. Viruses were cultured for a maximum of 42 days to evaluate their infectivity in MDMs. Infectivity was measured as HIV-1 Gag p24 production using supernatants that were harvested weekly.

Figure 2: Representative dose-dependent neutralization graphs of HIV-1 subtype C primary isolates in MDM. UCLA1 aptamer was used at a starting concentration of 100 nM. Neutralization was measured as a reduction in p24 production compared to the control without aptamer. The IC₈₀ values were calculated as p24 antigen titers causing 80% reduction of p24 antigen production compared with the virus control (without UCLA1). The assay was performed at least three times, in triplicate, for each primary isolate tested. Statistical significant difference of UCLA1 inhibition

compared to the virus control was determined by t-test and indicated by asterisks where * and *** indicate p values of less than 0.05 and 0.001, respectively. NS indicates no significance.

Figure 3: In vitro selection of UCLA1 resistant viruses. HIV-1 subtype C primary isolates (RP1 and Du422) were cultured in PBMCs with increasing concentrations of UCLA1 for a maximum period of 84 days. The concentration of the aptamer was increased only when p24 production in the UCLA1 culture was the similar or higher than the control culture. RP1 and Du422 were isolated and sequenced at day 77 and 84, respectively.

Figure 4: Selection of envelope mutations in RP1 after culture with UCLA1. **(A):** Amino acid sequence alignment of RP1 propagated under increasing concentrations of UCLA1 (RP1 UCLA1) compared with the wild-type RP1 sequence (RP1) and RP1 propagated without aptamer (RP1 Control). The amino acid changes that were observed after propagation, at 84 days, are highlighted in red for RP1 UCLA1 sequence and in green for the RP1 Control sequence. The numbering is according to the HxB2 sequence. **(B):** Structural representation of UCLA1 escape mutations generated under increasing concentrations of the aptamer. Residues implicated in UCLA1 escape are in red spheres and their locations within gp120 are mentioned in parenthesis. The numbering is according to the HxB2 sequence. Coordinates were taken from the structure of gp120JRFL core with V3 ligated with CD4 and X5 (Protein Data Bank accession no. 2B4C). The X5 antibody is removed from the depicted structure. Gp120 is represented in green and CD4 in magenta. The

structure was generated with PyMOL (DeLano Scientific LLC, South San Francisco, CA [<http://www.pymol.org>]).

TABLE LEGEND

Table 1: Pre- and post- selection IC_{80} values of UCLA1 against HIV-1 primary isolates in PBMCs.