Clinical Infectious Diseases

MAJOR ARTICLE

Therapeutic Immune Recovery and Reduction of CXCR4-Tropic HIV-1

Joëlle Bader,[1] Martin Däumer,[6] Franziska Schöni-Affolter,[2,a] Jürg Böni,[3] Meri Gorgievski-Hrisoho,[4] Gladys Martinetti,[5] Alexander Thielen,[7] Thomas Klimkait,[1] for the Swiss HIV Cohort Study[b]

[1]Molecular Virology, Department of Biomedicine–Petersplatz, University of Basel, [2]Swiss HIV Cohort Study Data Center, University Hospital Lausanne, [3]Institute of Medical Virology, National Center for Retroviruses, University of Zürich, [4]Institute for Infectious Diseases, University of Berne, [5]Department of Microbiology, Ente Ospedaliero Cantonale, Bellinzona, Switzerland, [6]Institute for Immunogenetics, Kaiserslautern, and [7]Max Planck Institute for Informatics, Saarbrücken, Germany

Background. In the absence of therapy, CXCR4 (X4)–tropic human immunodeficiency virus type 1 (HIV-1) increases over time, associated with accelerated disease progression. In contrast, the majority of patients receiving long-term combination antiretroviral therapy (cART) present with CCR5 (R5)-tropic HIV-1 variants. It is unclear whether cART itself mediates the reduction of X4-tropic HIV-1. The current study aimed at assessing the tropism of viral integrates in patients' blood during fully suppressive cART.

Methods. The relative frequencies of X4-tropic proviral HIV-1 variants were determined by means of next-generation sequencing (False Positive Rate (FPR), 3.5%; R5- or X4 tropic variants occurring at less than 2% of the total virus population) for 35 treated patients in the Swiss HIV Cohort Study and followed longitudinally over time. Full viral suppression and a continuous CD4 T-cell recovery during cART were documented for all patients. Viral phylogenetic changes and sequence evolution were analyzed.

Results. The majority of patients (80%) experienced no frequency increase in X4-tropic proviruses during therapy. Although some proviral sequence evolution was demonstrable in >50% of these patients during therapy, this growing viral diversity was in no case paralleled by the emergence or expansion of X4-tropic provirus variants. In the remaining 20% of patients, the documented expansion of X4-tropic provirus was based on the outgrowth of single viral variants from minority populations already present before therapy initiation.

Conclusion. Our study demonstrates that X4-tropic HIV sharply declines in most patients during successful therapy, which indicates a preferential tropism-dependent provirus elimination in the immunocompetent host. The recently implemented World Health Organization strategies of immediate therapy initiation are fully in line with this gradual loss of X4 tropism during therapy. Moreover, the early use of coreceptor antagonists against the remaining CCR5-tropic viruses may be indicated.

Keywords. HIV; tropism; CXCR4-tropic; immune system.

For a successful cellular human immunodeficiency virus (HIV) infection it is essential that the viral envelope protein binds the CD4 receptor as well as an additional chemokine receptor. The choice of which receptor to use is centrally mediated by the interaction of variable loop 3 within the HIV-1 envelope to one of the chemokine receptors, CCR5 (R5) or CXCR4 (X4) [1].

It is long established that during early phases of infection >80% of all patients harbor HIV-1 strains with a tropism for the chemokine receptor R5. Hence, X4-tropic variants are rather rare in the beginning of infection and mostly appear later during the course of the disease, along with a deterioration of the immune system [2–5]. It is still not well understood how this switch from R5 to X4 tropism is triggered, but numerous reports have established a link between X4 tropism and an accelerated disease progression along with a faster decline in CD4 T cells [6, 7]. It has also been suggested that, in association with advanced disease, the weakened immune situation might play a key role by being less able to neutralize X4-tropic virus variants. This correlates with different glycosylation patterns of X4 viruses compared with R5-tropic virus variants [8–11]. Interestingly, a predominance of X4-tropic HIV-1 has also been clinically associated with an elevated expression of inflammatory markers [12]. Nevertheless, whether X4-tropic viruses are the cause or the consequence of disease progression is still not known.

Despite this general connection, it is important to note that with antiretroviral therapy (ART), even in advanced stages of disease, most patients carry mainly R5-tropic viruses in the blood. It remained somewhat puzzling that in the MOTIVATE-1 and MOTIVATE-2 studies, enrolling patients in salvage therapy situations after extended treatment periods, only 1.8% of the heavily pretreated patients presented at baseline with solely X4-tropic viruses in the circulating blood [13]. In addition,

Received 20 July 2016; editorial decision 21 October 2016; accepted 9 November 2016; published online November 12, 2016.

[a]Present affiliation: Public Office of Public Health, Berne, Switzerland.

[b]Study group members are listed in the Notes.

Correspondence: T. Klimkait, University of Basel, Department of Biomedicine, Molecular Virology, Petersplatz 10, 4009 Basel, Switzerland (thomas.klimkait@unibas.ch).

Clinical Infectious Diseases® 2017;64(3):295–300

DOI: 10.1093/cid/ciw737

the same studies showed that in most cases HIV-1, escaping under drug pressure of therapy with the R5-antagonist maraviroc, presented as X4-tropic virus. This obviously indicated a significant viral flexibility or archival availability of the respective HIV-1 variants. However, as soon as maraviroc therapy was suspended, and the virus was no longer forced to use the X4 coreceptor, the major HIV variant in the circulation returned to a solely CCR5-using form [14]. The latter observation supports a second crucial clinical fact: In patients receiving suppressive therapy, irrespective of the involvement of coreceptor antagonists, X4-tropic viruses rarely emerge, suggesting that these viruses might have a principal selective disadvantage compared with R5-tropic variants. Because no selective X4 receptor antagonists are available for clinical use, current modalities do not allow us to study the impact of a specific attack of X4-tropic virus in vivo.

The current study therefore aimed at following and characterizing in vivo the evolution of X4-tropic variants during periods of successful therapy. By applying next-generation sequencing, we investigated the dynamics of X4-tropic HIV before treatment and during suppressive combination ART (cART).

METHODS

Sample Selection and Tropism Testing

We included 70 peripheral blood mononuclear cell (PBMC) samples from 35 patients in the Swiss HIV Cohort Study (SHCS) for retrospective analysis. The study was conducted as a research project approved by the Scientific Board of the SHCS with approval of the ethical committees of all 7 participating study sites. Written consent had been obtained from all participants.

Aliquots of 2–5 × 10^6 Ficoll-isolated PBMCs, maintained frozen in liquid nitrogen in the biobank of the SHCS, were used to isolate the cell-associated proviral DNA; by means of a nested polymerase chain reaction protocol, the variable loop 3 region of HIV was amplified and sequenced on an ABI3130 gene analyzer. Specimens had been collected within 3 months before and 4 years after cART initiation. All patients were in the chronic infection phase with low CD4 T-cell counts at initiation (median CD4 T-cell count, 180/mm^3) and needed to present with undetectable virus load during therapy (monitored every 3 months) and a good CD4 T-cell response (increase of >200 cells/mm^3 during 4 years of therapy). Tropism analysis was performed on PBMC samples using the Geno2Pheno[454] software (Max Planck Institute for Informatics) to analyze sequences obtained with a MiSeq benchtop sequencer (Illumina).

Sequence and Phylogenetic Analysis

For sequence analysis, all calculations were performed with MEGA 6.0 software (Open source "Molecular Evolutionary Genetics Analysis"). The distance relatedness was calculated between the most prevalent variant and all remaining variants in a given sample. iTOL 2.0 software ("Interactive Tree of Life", EMBL, Germany) was used for phylogenetic tree visualization [15, 16].

Statistical Methods

Categorical data were compared using χ^2 tests, and continuous data using Mann-Whitney-Wilcoxon tests.

RESULTS

Patient Characteristics

Thirty-five patients were included in the study, comprising a total of 70 proviral samples. The patient population had a mean (SD) age of 49.6 (8.7) years, 68.6% were male, and 91.4% were white; 45.7% were men who have sex with men. Thus, the study population is reasonably balanced for sex. The median baseline CD4 T-cell count was 180/μL, and the median increase in CD4 T-cell during therapy was 459/μL. This reflects an overall very good immunological response among study participants and treatment initiation at advanced disease stages, with CD4 T-cell counts often <200/μL. The median viral load at baseline was 5.11 $\log_{10}$ copies/mL, also indicating a relatively high viral load at initiation, typically associated with a less favorable clinical course. Four (11.4%) of the 35 patients had a Δ32 heterozygous genotype; no patients were homozygous for the Δ32 mutation. Baseline characteristics are summarized in Table 1. First-line regimens included a nucleoside reverse-transcriptase inhibitor backbone that included 1 protease inhibitor (n = 19; 54.3%), 2 protease inhibitors (n = 8; 22.9%), or 1 nonnucleoside reverse-transcriptase inhibitor (n = 7; 20%) or was exclusively nucleoside reverse transcriptase inhibitor-based (n = 1; 2.9%). This reflects therapy regimens typical for the time period.

Sample Analysis

Sample Characteristics

The choice of the 70 specimens was strictly based on samples used in a study by Kaufmann et al [17] on CD4 T-cell recovery over time in ART-treated, HIV-1–infected individuals. The median read size was 45 833 reads per sample, with a median virus variant count per sample of 354.

With a false positive rate (FPR) setting of 3.5%, individual proviral X4-tropic HIV-1 variants could be identified in all samples except 1. With an FPR adjustment to 5%, the entire population of provirus variants in this single sample was called X4-tropic. (For bulk sequencing, the 5% cutoff is generally considered appropriate for identifying X4-tropic viruses) [18]. Applying a cutoff of 2%, as suggested by Swenson et al [19], categorizes all samples, in which X4-tropic viruses in a population remain below an abundance of 2%, as R5 tropic. With this adjusted rule, 40 samples (57.1%) were solely R5 tropic, and 30 (42.9%) contained X4-tropic viruses. Among the 40 samples with dominant R5 tropism, the mean percentage of X4-tropic minorities was 0.13% (range, 0%–0.91%). This low percentage is in good

Table 1. Baseline Characteristics in the 35 HIV-1–Infected, Treatment-Naive Patients

Characteristic	Patients, No. (%)[a]			*P* Value
	All Patients (n = 35)	Decrease in % of X4 Variants (n = 28)	Increase in % of X4 Variants (n = 7)	
Sex				
Male	24 (68.6)	19 (67.9)	5 (71.4)	.86
Female	11 (31.4)	9 (32.1)	2 (28.6)	
Age, mean (SD), y	49.6 (8.7)	49.5 (8.8)	50 (8.8)	.74
Ethnicity				.21
White	32 (91.4)	26 (92.8)	6 (85.7)	
Black	1 (2.9)		1 (14.3)	
Hispanic	1 (2.9)	1 (3.6)		
Asian	1 (2.9)	1(3.6)		
HIV transmission				.54
MSM	16 (45.7)	12 (42.9)	4 (57.1)	
Heterosexual	15 (42.9)	12 (42.9)	3 (42.9)	
IDU	4 (11.4)	4 (14.2)		
Baseline HIV RNA load, $\log_{10}$ copies/mL	5.14 (3.75–6.41)	4.99 (4.23–6.09)	5.53 (5.28 -6.41)	.02
Baseline proviral load, $\log_{10}$ copies/10^6 PBMCs	3.87 (2.42–5.47)	3.83 (2.42–5.05)	4.09 (3.67–5.47)	.14
T-cell count, median (range), cells/μL				
CD4 increase	459 (222–853)	434 (222–853)	463 (243–659)	.55
Baseline CD4	180 (7–511)	187 (9–511)	91 (7–242)	.17
Baseline CD8	756 (165–1595)	843 (165–1595)	652 (266–1288)	.40
Δ32 genotype				
Heterozygous	4 (11.4)	4 (14.3)	0	.29
Wild type	31 (88.6)	24 (85.7)	7 (100)	

Abbreviations: HIV 1, human immunodeficiency virus type 1; IDU, injection drug use; MSM, men who have sex with men; PBMCs, peripheral blood mononuclear cells; SD, standard deviation; X4, CXCR4.

[a]Data represent No. (%) of patients, unless otherwise specified.

accordance with observations by Swenson et al [19] suggesting that traces of X4-tropic viruses can be found in virtually every patient with ultradeep sequencing. In contrast, the mean percentage of R5-tropic variants in the 30 mainly X4-tropic samples was 47.25% (range, 0%–97.89%; $P \leq .001$). The maximum value of 97.89% indicates that in this case the 2% cutoff rule led to an X4 tropism assignment although the vast majority of all variants in this sample were R5-tropic according to the FPR.

Changes in X4-Tropic HIV Frequency During Therapy

Based on the literature, we had expected to observe an increase in X4 tropism over the time of infection. In contrast, the relative frequencies of proviral X4-tropic HIV-1 variants decreased or stayed stable over time in the vast majority of patients (28 of 35 patients: 80%), and it increased only in 7 patients (20%; $P < .001$) in our unselected study population. For each of the latter 7 patients with a gain in the percentage of X4 variants over time, we observed that 1 distinct provirus variant became prominent during suppressive therapy and eventually became solely responsible for the increase in the percentage of X4 variants. Moreover, in 6 of the 7 patients, the finally emerging variant had already been present as a minority before therapy initiation, with a mean abundance of 1.95% (range, 0.02%–6.99%) of the total proviral population. The emerging X4 variant was not detected in the pretreatment sample in only 1 patient.

Even when more stringent FPR thresholds (higher FPR value) were applied, the great majority of the patients experienced declining or steady frequencies of proviral X4-tropic HIV-1 over time (74.3% for an FPR of 5%, and 71.4% for an FPR of 10%). Note that in patients with diminishing viral X4 populations this reduction occurred in a slow gradual process (mean change, 28.33%), whereas in all patients with X4 persistence the increase in X4-tropic viruses progressed more rapidly toward an exclusive X4 representation in the viral population (mean change, 62.09%; $P < .001$). Univariate analysis of the baseline characteristics, which could have an influence on the increase in the percentage of X4-tropic variants, revealed that the only associated parameter was a higher viral load ($P = .02$) at therapy initiation for patients with an increase in that percentage (Table 1).

Possible Association With Proviral DNA Load

The dynamics of proviral DNA loads observed in this study confirm the previously reported findings that overall proviral loads decline during ART: In 80% of the patients in this study decreasing proviral loads were noted during successful treatment (Figure 1). In rarer cases, in which overall proviral loads increased during therapy, we did not observe a concomitant increase of the percentage of X4 tropic strains in the entire provirus population ($P = .34$). Taken together, this indicates that the proviral dynamics are not likely to be driven by properties of viral tropism.

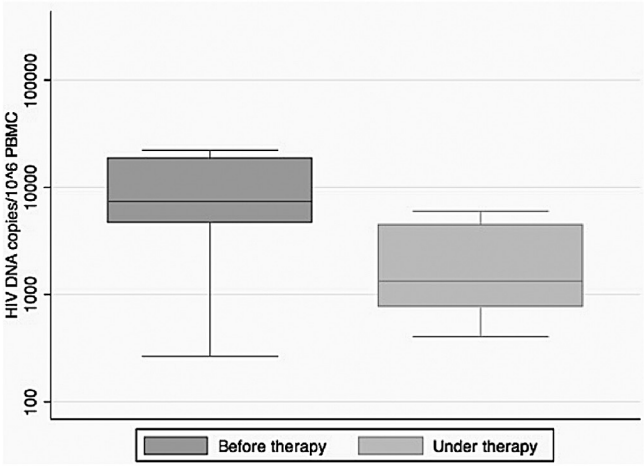

Figure 1. Block diagram of human immunodeficiency virus (HIV) proviral load copies per 1 million peripheral blood mononuclear cell (PBMCs) in patient populations at time points before or during treatment. Copy numbers are displayed on a logarithmic scale.

Molecular Characteristics: Viral Evolution and Diversity Over Time

During therapy, the majority of patients (60%) developed overall viruses with a markedly increasing diversity over time and therapy duration. In the same population an increase in the percentage of X4 variants was in no case associated with such diversification (P = .49). Figure 2A shows a phylogenetic tree representative of the patient group with decreasing diversity and declining percentage of X4 variants, and Figure 2B depicts a virus profile representative of patients with an increase in the percentage of X4 variants, accompanied by the emergence of a single virus variant. In particular, the apparent close sequence relatedness or clonality in the latter group seems to indicate a strong evolutionary advantage for the respective proviruses.

The limited availability of samples from the same individuals over time may pose a limitation and renders a judgment about the kinetics of the viral evolution difficult. However, as recently reported by our group, significant tropism stability over time is reflected by the fact that 85% of patients still presented R5-tropic viruses at 5-year follow-up [20]. Moreover, for each patient we were also able to analyze the next proviral sample available from the earliest time point after therapy initiation. This confirmed good proviral stability and stability of the proviral load over time (data not shown.)

Role of the Δ32 Deletion in the R5 Gene

A naturally occurring deletion in the CCR5 gene of the host (known as Δ32) negatively affects its function as HIV coreceptor. As a consequence, this mutation has the potential to favor the emergence of X4-tropic virus variants, and we therefore determined the Δ32 genotype in the CCR5 gene for all patients in the study. Thirty-one patients (88.6%) carried as homozygotes the wild-type CCR5 gene, 4 (11.4%) carried a heterozygous Δ32 genotype, and none

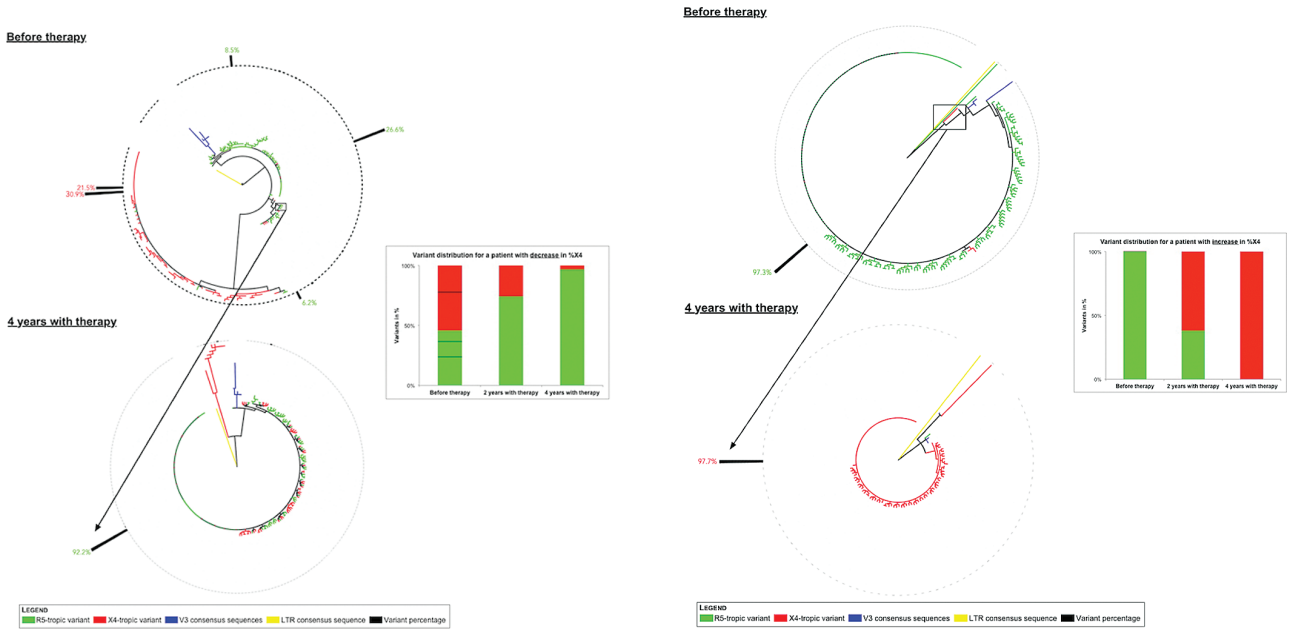

Figure 2. Phylogenetic trees and frequencies of viral variants before and during suppressive therapy. All present variable loop 3 (V3) variants are included for a representative patient with decreasing viral diversity and decreasing percentage of CXCR4 (X4) variants (*A*) or decreasing diversity and increasing percentage of X4 variants (*B*). CCR5 (R5)–tropic human immunodeficiency virus (HIV) variants are shown as green tree leaves; red leaves represent X4-tropic variants; blue leaves, consensus V3 sequences of the relevant subtypes A, B, and C; isolated yellow leaf represents a consensus HIV-1 subtype B Long terminal repeat sequence and was used for rooting. The length of the black bars indicates the frequency (percentage) of a given variant in the entire HIV sequence pool for that patient, indicated for the main provirus variants as a number. The inset with a bar chart represents the change in tropism frequencies considering all variants for the indicated time points. The single variant that led to the outgrowth during therapy is marked with a black arrow.

were homozygous for Δ32. The virologic outcome did not differ between these groups. Although the mutation should potentially provide a disadvantage to CCR5-using viruses and yield more X4 tropism, all patients with an increasing percentage of X4 variants were homozygous for the wild-type CCR5 gene ($P = .29$).

DISCUSSION

The association of X4-tropic HIV-1 with an accelerated CD4 T-cell loss has frequently been observed in the pre-ART era. Nevertheless, the mechanism of how this contributes to disease progression is still not understood, and in the context of ART the validity of this link has not yet been demonstrated. In contrast to the seemingly well-established trend that X4 viruses can prevail in late phases of HIV infection, our study somewhat unexpectedly revealed that for patients with effectively suppressed viral loads X4-tropic HIV variants follow a persistent downward trend. On the molecular level, for the great majority of patients, the percentage of X4-tropic variants in the population of HIV-infected cells declined over time during therapy, accompanied by a steady CD4 T-cell increase.

Even the use of most stringent FPR cutoffs (up 10%) did not change this primary study outcome. We would like to emphasize that the use of a higher FPR is not intended as a suggestion for clinical practice but solely for demonstrating the solidity of our data. Although our studies did not use a phenotypic test for verifying the genotype-based analysis, we do not anticipate major differences because earlier work had shown a good agreement of genotypic and phenotypic tropism determinations for subtype B viruses [21].

These deep-sequencing data convincingly support earlier findings that in the majority of treated patients the pretherapy tropism of free virus shifted from an X4 predominance to a mainly R5-tropic virus archive [20]. Of note, several mathematical modeling studies had already predicted that cART initiation will lead to a preferential suppression of X4-tropic variants [22–24]. The sharp contrast to the old observation with increasing X4 tropism may be due to the effects of therapy delaying pathogenesis and disease development.

Because we limited this study to patients with suppressed HIV viral loads (<20 copies/mL), free circulating virus was unlikely to be responsible for the observed cellular phenotype. Rather, our data suggest that the selective reduction of X4-tropic variants could be driven by the recovering immune system itself. This hypothesis receives further support from earlier reports, which have suggested that the recognition of the envelope glycoprotein by neutralizing antibodies could be centrally involved in the elimination of a viral variant. Moreover, because X4-tropic viruses tend to possess less glycosylated envelope proteins than R5-tropic viruses, the former might be more readily recognized and eliminated by the host defense [8–11]. Furthermore, it has been suggested that R5-tropic viruses might have a significant selection advantage during chronic infection, based on a higher escape from mechanisms of immune surveillance [25].

Another remarkable observation is a correlation with the natural hosts of the nonpathogenic simian immunodeficiency virus, the sooty mangabey and the African green monkey. These monkeys harbor solely R5-tropic virus strains and do not experience immune destruction during the course of an simian immunodeficiency virus infection. The infection does not seem to be associated with gut inflammation or a loss of the gut-associated lymphatic tissue. Both changes are quite characteristic for an advancing human HIV infection [26, 27].

The tropism dynamics observed in our study might therefore resemble the balanced situation in the natural simian host. Moreover, we speculate that during successful treatment the recovering immune system could preferentially target X4-tropic HIV variants and X4-infected cells. This would suffice to explain the "asymmetric" reduction of X4-tropic provirus in our study. Aside from the overwhelming trend toward declining X4-tropic provirus, our study also identified several patients with an increasing X4-tropic provirus population. Interestingly, in each case this was accompanied by the outgrowth of a single virus variant.

The high diversity and genetic instability of retroviruses renders it unlikely that a single variant of free, infectious HIV has given rise to such high sequence relatedness. It may rather be speculated that the respective provirus expansion originated from individual HIV-infected cells, originally as a small minority. Such a population may have already existed before therapy initiation, and a later triggering event may have resulted in a significant proliferation of the infected cells, along with that of a clonal proviral variant.

Of note, 2 earlier studies had reported similar findings. Westby et al [14] had shown that, after treatment with the CCR5 receptor antagonist maraviroc, the emerging X4-tropic variants stemmed from a pretreatment reservoir with an ancestral background different from that of the main R5-tropic variants in the circulation. A case study by Kordelas et al [28] and Verheyen et al [29] showed that a newly appearing X4 variant had emerged after stem cell transplantation from a pretreated minority [28, 29]. The observation of all 3 studies that the emerging variant originated from a preexisting virus minority along with the finding that this increase was not associated with a growing overall virus diversity over time are very compatible with the mechanism of a (pseudo)clonal expansion of initially rare, provirus-carrying CD4-positive cells. Indeed, the mechanism of such clonal virus expansion has recently been suggested for emerging HIV variants in the latent HIV pool [30].

In the search for predictors of the increase in the percentage of X4 variants, we observed an association with a higher baseline viral load but were unable to associate it with a Δ32 genotype. In contrast, even in patients with a heterozygous Δ32 genotype, the percentage of X4 variants declined, which indicates that also the assumed lower surface expression of CCR5 receptors did not favor X4 use by the virus in these patients. The second finding that in the immunocompetent host X4 tropism does not seem to be a favorable phenotype of HIV-1 is in full agreement with

clinical findings of the MOTIVATE studies, where a maraviroc-driven emergence of X4-tropic variants (maraviroc as CCR5 antagonist favored the escape of X4-tropic HIV-1) immediately reverted after suspending the CCR5 antagonist [14].

The findings of our study are well in line with the newly implemented World Health Organization concept of early treatment initiation as described in the START trial [31], both favoring the active involvement of the patient's immune function for the most successful therapy. In addition, our findings also support the early use of CCR5 receptor antagonists, which would exert a maximal effect on the dominantly present R5-tropic viruses. Because X4-tropic variants are known to be rare after infection and only accumulate later in disease, the preservation of a potent immune function itself may help suppress or eliminate X4-tropic virus variants. Further clinical studies on the role of the HIV tropism during infection are needed to verify our hypothesis, but the presented data lend support to the "treatment as prevention" strategy to help improving patient quality of life and to limit the further spread of disease.

Notes

Author contributions. J. Bader and T. K. conceived the project; J. Böni, M.G.H., G. M., and T. K. characterized and provided clinical specimens; J. Bader, M.D., and A.T. conducted the experimental work; J. Bader and F. S. A. analyzed data; J. Bader and T. K. wrote the manuscript; and all authors critically reviewed, discussed, and approved the content of the final manuscript and agreed to its submission.

Financial support. This study has been financed within the framework of the Swiss HIV Cohort Study (SHCS), supported by the Swiss National Science Foundation (grant 148522), by SHCS projects 521 and 739, and by the SHCS research foundation.

Potential conflicts of interest. All authors certify no potential conflicts of interest. The authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

SHCS membership. SHCS members include the following: V. Aubert, M. Battegay, E. Bernasconi, J. Böni, D. L. Braun, H. C. Bucher, C. Burton-Jeangros, A. Calm, M. Cavassini, G. Dollenmaier, M. Egger, L. Elzi, J. Fehr, J. Fellay, H. Furrer (chairman, Clinical and Laboratory Committee), C. A. Fux, M. G. H., H. Günthard (president, SHCS), D. Haerry (deputy, "Positive Council"), B. Hasse, H. H. Hirsch, M. Hoffmann, I. Hösli, C. Kahlert, L. Kaiser, O. Keiser, T. K., R. Kouyos, H. Kovari, B. Ledergerber, G. M., B. Martinez de Tejada, C. Marzolini, K. Metzner, N. Müller, D. Nadal, D. Nicca, G. Pantaleo, A. Rauch (chairman, Scientific Board), S. Regenass, C. Rudin (Chairman, Mother & Child Substudy), F. S. A. (head, Data Centre), P. Schmid, R. Speck, M. Stöckle, P. Tarr, A. Trkola, P. Vernazza, R. Weber, S. Yerly. SHCS data are gathered by the 5 Swiss university hospitals, 2 cantonal hospitals, 15 affiliated hospitals, and 36 private physicians (listed in http://www.shcs.ch/180-health-care-providers).

References

1. Berger EA, Murphy PM, Farber JM. Chemokine receptors as HIV-1 coreceptors: roles in viral entry, tropism, and disease. Annu Rev Immunol **1999**; 17:657–700.
2. Brumme ZL, Goodrich J, Mayer HB, et al. Molecular and clinical epidemiology of CXCR4-using HIV-1 in a large population of antiretroviral-naive individuals. J Infect Dis **2005**; 192:466–74.
3. Connor RI, Sheridan KE, Ceradini D, Choe S, Landau NR. Change in coreceptor use correlates with disease progression in HIV-1–infected individuals. J Exp Med **1997**; 185:621–8.
4. Moore JP, Kitchen SG, Pugach P, Zack JA. The CCR5 and CXCR4 coreceptors–central to understanding the transmission and pathogenesis of human immunodeficiency virus type 1 infection. AIDS Res Hum Retroviruses **2004**; 20:111–26.
5. Weinberger AD, Perelson AS. Persistence and emergence of X4 virus in HIV infection. Math Biosci Eng **2011**; 8:605–26.
6. Waters L, Mandalia S, Randell P, Wildfire A, Gazzard B, Moyle G. The impact of HIV tropism on decreases in CD4 cell count, clinical progression, and subsequent response to a first antiretroviral therapy regimen. Clin Infect Dis **2008**; 46:1617–23.
7. Weiser B, Philpott S, Klimkait T, et al; Swiss HIV Cohort Study. HIV-1 coreceptor usage and CXCR4-specific viral load predict clinical disease progression during combination antiretroviral therapy. AIDS **2008**; 22:469–79.
8. Clevestig P, Pramanik L, Leitner T, Ehrnst A. CCR5 use by human immunodeficiency virus type 1 is associated closely with the gp120 V3 loop N-linked glycosylation site. J Gen Virol **2006**; 87:607–12.
9. Pollakis G, Kang S, Kliphuis A, Chalaby MI, Goudsmit J, Paxton WA. N-linked glycosylation of the HIV type-1 gp120 envelope glycoprotein as a major determinant of CCR5 and CXCR4 coreceptor utilization. J Biol Chem **2001**; 276:13433–41.
10. Polzer S, Dittmar MT, Schmitz H, et al. Loss of N-linked glycans in the V3-loop region of gp120 is correlated to an enhanced infectivity of HIV-1. Glycobiology **2001**; 11:11–9.
11. Wei X, Decker JM, Wang S, et al. Antibody neutralization and escape by HIV-1. Nature **2003**; 422:307–12.
12. Hamlyn E, Hickling S, Porter K, et al; SPARTAC Investigators. Increased levels of CD4 T-cell activation in individuals with CXCR4 using viruses in primary HIV-1 infection. AIDS **2012**; 26:887–90.
13. Gulick RM, Lalezari J, Goodrich J, et al; MOTIVATE Study Teams. Maraviroc for previously treated patients with R5 HIV-1 infection. N Engl J Med **2008**; 359:1429–41.
14. Westby M, Lewis M, Whitcomb J, et al. Emergence of CXCR4-using human immunodeficiency virus type 1 (HIV-1) variants in a minority of HIV-1-infected patients following treatment with the CCR5 antagonist maraviroc is from a pretreatment CXCR4-using virus reservoir. J Virol **2006**; 80:4909–20.
15. Letunic I, Bork P. Interactive Tree Of Life (iTOL): an online tool for phylogenetic tree display and annotation. Bioinformatics **2007**; 23:127–8.
16. Letunic I, Bork P. Interactive Tree Of Life v2: online annotation and display of phylogenetic trees made easy. Nucleic Acids Res **2011**; 39(Web Server issue):W475–8.
17. Kaufmann GR, Perrin L, Pantaleo G, et al; Swiss HIV Cohort Study Group. CD4 T-lymphocyte recovery in individuals with advanced HIV-1 infection receiving potent antiretroviral therapy for 4 years: the Swiss HIV Cohort Study. Arch Intern Med **2003**; 163:2187–95.
18. Sierra S, Dybowski JN, Pironti A, et al. Parameters influencing baseline HIV-1 genotypic tropism testing related to clinical outcome in patients on maraviroc. PLoS One **2015**; 10:e0125502.
19. Swenson LC, Moores A, Low AJ, et al. Improved detection of CXCR4-using HIV by V3 genotyping: application of population-based and "deep" sequencing to plasma RNA and proviral DNA. J Acquir Immune Defic Syndr **2010**; 54:506–10.
20. Bader J, Schöni-Affolter F, Böni J, et al; Swiss HIV Cohort Study. Correlating HIV tropism with immunological response under combination antiretroviral therapy. HIV Med **2016**; 17:615–22.
21. Klimkait T. The XTrack system: application and advantage. Intervirology **2012**; 55:118–22.
22. Jiao Y, Wang P, Zhang H, et al. HIV-1 co-receptor usage based on V3 loop sequence analysis: preferential suppression of CXCR4 virus post HAART? Immunol Invest **2011**; 40:597–613.
23. Philpott S, Weiser B, Anastos K, et al. Preferential suppression of CXCR4-specific strains of HIV-1 by antiviral therapy. J Clin Invest **2001**; 107:431–8.
24. Ribeiro RM, Hazenberg MD, Perelson AS, Davenport MP. Naïve and memory cell turnover as drivers of CCR5-to-CXCR4 tropism switch in human immunodeficiency virus type 1: implications for therapy. J Virol **2006**; 80:802–9.
25. Gnanakaran S, Bhattacharya T, Daniels M, et al. Recurrent signature patterns in HIV-1 B clade envelope glycoproteins associated with either early or chronic infections. PLoS Pathog **2011**; 7:e1002209.
26. Broussard SR, Staprans SI, White R, Whitehead EM, Feinberg MB, Allan JS. Simian immunodeficiency virus replicates to high levels in naturally infected African green monkeys without inducing immunologic or neurologic disease. J Virol **2001**; 75:2262–75.
27. Chen Z, Zhou P, Ho DD, Landau NR, Marx PA. Genetically divergent strains of simian immunodeficiency virus use CCR5 as a coreceptor for entry. J Virol **1997**; 71:2705–14.
28. Kordelas L, Verheyen J, Beelen DW, et al; Essen HIV AlloSCT Group. Shift of HIV tropism in stem-cell transplantation with CCR5 delta32 mutation. N Engl J Med **2014**; 371:880–2.
29. Verheyen J, Thielen A, Sichtig N, et al. Breakthrough of preexisting X4-capable HIV after allogeneic stem-cell transplantation. Presented at: Conference on Retroviruses and Opportunistic Infections; February 23–26, **2015;** Seattle, WA.
30. Maldarelli F, Wu X, Su L, et al. HIV latency. Specific HIV integration sites are linked to clonal expansion and persistence of infected cells. Science **2014**; 345:179–83.
31. Lundgren JD, Babiker AG, Gordin F, et al; INSIGHT START Study Group. Initiation of antiretroviral therapy in early asymptomatic HIV infection. N Engl J Med **2015**; 373:795–807.