Characterization of human immunodeficiency virus type 1 (HIV-1) diversity and tropism in 145 patients with primary HIV-1 infection

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Abstract: In the context of sexual transmission of human immunodeficiency virus type 1 (HIV-1), current findings suggest that the mucosal barrier is the major site of viral selection, transforming the complex inoculum to a small, homogeneous founder virus population. We analyzed HIV-1 transmission in relation to viral and host characteristics within the Zurich primary HIV-1 infection study. METHODS: Clonal HIV-1 envelope sequences (on average 16 clones/patient) were isolated from the first available plasma samples during the early phase of infection from 145 patients with primary HIV-1 infection. Phylogenetic and tropism analyses were performed. Differences of viral diversities were investigated in association with several parameters potentially influencing HIV-1 transmission, e.g., concomitant sexually transmitted infections (STIs) and mode of transmission. RESULTS: Median viral diversity within env C2-V3-C3 region was 0.39% (range 0.04%-3.23%). Viral diversity did not correlate with viral load, but it was slightly correlated with the duration of infection. Neither transmission mode, gender, nor STI predicted transmission of more heterogeneous founder virus populations that were found in 16 of 145 patients (11%; diversity >1%). Only 2 patients (1.4%) were assuredly infected with CXCR4-tropic HIV-1 within a R5/X4-tropic–mixed population, as revealed and confirmed using several genotypic prediction algorithms and phenotypic assays. CONCLUSIONS: Our findings suggest that transmission of multiple HIV-1 variants might be a complex process that is not dependent on mucosal factors alone. CXCR4-tropic viruses can be sexually transmitted in rare instances, but their clinical relevance remains to be determined.

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Characterization of Human Immunodeficiency Virus Type 1 (HIV-1) Diversity and Tropism in 145 Patients With Primary HIV-1 Infection

Philip Rieder, Beda Joos, Alexandra U. Scherrer, Herbert Kuster, Dominique Braun, Christina Grube, Barbara Niederöst, Christine Leemann, Sara Gianella, a Karin J. Metzner, Jürg Böni, Rainer Weber, and Huldrych F. Günthard

1 Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich; and 2 Swiss National Center for Retroviruses, Institute of Medical Virology, University of Zurich, Switzerland

(See the Editorial Commentary by Loes, on pages 1280–2.)

Background. In the context of sexual transmission of human immunodeficiency virus type 1 (HIV-1), current findings suggest that the mucosal barrier is the major site of viral selection, transforming the complex inoculum to a small, homogeneous founder virus population. We analyzed HIV-1 transmission in relation to viral and host characteristics within the Zurich primary HIV-1 infection study.

Methods. Clonal HIV-1 envelope sequences (on average 16 clones/patient) were isolated from the first available plasma samples during the early phase of infection from 145 patients with primary HIV-1 infection. Phylogenetic and tropism analyses were performed. Differences of viral diversities were investigated in association with several parameters potentially influencing HIV-1 transmission, eg, concomitant sexually transmitted infections (STIs) and mode of transmission.

Results. Median viral diversity within env C2-V3-C3 region was 0.39% (range 0.04%–3.23%). Viral diversity did not correlate with viral load, but it was slightly correlated with the duration of infection. Neither transmission mode, gender, nor STI predicted transmission of more heterogeneous founder virus populations that were found in 16 of 145 patients (11%; diversity >1%). Only 2 patients (1.4%) were assuredly infected with CXCR4-tropic HIV-1 within a R5/X4-tropic–mixed population, as revealed and confirmed using several genotypic prediction algorithms and phenotypic assays.

Conclusions. Our findings suggest that transmission of multiple HIV-1 variants might be a complex process that is not dependent on mucosal factors alone. CXCR4-tropic viruses can be sexually transmitted in rare instances, but their clinical relevance remains to be determined.

The extensive genetic diversity of human immunodeficiency virus type 1 (HIV-1) is a tremendous challenge regarding the development of broadly effective vaccines and also for antiretroviral treatment. The genetic bottleneck during HIV-1 transmission may be an Achilles heel of HIV-1 [1]. The mucosal barrier could be a key factor in driving the genetically complex viral inoculum to a homogeneous founder population. The impact of the mucosal barrier may depend on the anatomy, physiology, concurrent sexual transmitted infections (STIs), and sexual practice [2–4]. However, a genetic bottleneck has also been observed in intravenous drug users (IVDU), indicating mechanisms independent of the mucosa [3, 5].

During transmission, there is also strong selection for viral variants using CCR5 as a coreceptor [6, 7]. In-depth characterization of HIV-1 tropism in larger groups of patients during primary HIV-1 infection
Research and Reference Reagent Program (Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health). Syncytium-inducing phenotypes were determined in MT-2 cell culture assays [24]. If available, primary isolates, derived from both plasma and peripheral blood mononuclear cells (PBMCs), were tested. In addition, GHOST cell lines expressing CCR5 or CXCR4 and CD4 or CD4 alone were used to determine coreceptor usage. Cells were infected with primary virus isolates of each patient at a multiplicity of infection of 0.06. Expression of green fluorescent protein under control of the HIV-2 long terminal repeat promoter was detected by fluorescence microscopy [25].

**MATERIALS AND METHODS**

**Patient Characteristics**

Patients were enrolled in the ZPHI study (http://clinicaltrials.gov, ID = NCT00537966) [13–17]. Acute/recent PHI was confirmed in all patients according to previously published definitions [15]. We estimated a date of infection for each patient by integrating all available clinical and laboratory data [15, 18]. During the first visit, each patient was checked for symptoms and physical signs of concurrent STIs and serology for syphilis. When dysuria, genital ulcers, groin, and/or rectal pain was present, urine and/or rectal swabs were subjected to polymerase chain reaction (PCR) analysis for *Neisseria gonorrhoeae*, *Chlamydia trachomatis* (when positive, specific PCR for lym- phogranuloma venereum associated L-serovars was performed), and Herpes simplex.

**Sequencing**

RNA extraction, amplification, cloning, and sequencing of HIV-1 *env* C2-V3-C3 fragments were performed according to Rieder et al [15] by modification of previously described methods [19, 20].

**Genotypic Prediction of HIV-1 Coreceptor Usage**

V3-loop sequences were interpreted by three different genotypic prediction tools: 1) Web-PSSM http://indra.mullins.microbiol.washington.edu/webpssm/, 2) Wetcat http://genomiac2.ucsd.edu:8080/wetcat/v3.html, and 3) geno2phen[coreceptor] [7] (http://coreceptor.bioinf.mpi-inf.mpg.de/index.php). We used geno2phen[coreceptor] with a false-positive rate of 5.75% [21]. Wetcat analysis is based on support vector machine (SVM) classifier. Position-specific scoring matrices (PSSM) predictions were performed by HIV-1 subtype B and C SINSI matrices for all non-C subtypes and clade C, respectively.

**Phenotyping Assays**

The cell lines MT-2 (D Richman, University of California, San Diego [22]), GHOST Parental Cell Line, and GHOST Cell Transfectants GHOST CXCR4 and Hi-5 (VN KewalRamani and DR Littman, New York, NY [23]) were obtained from the AIDS Research and Reference Reagent Program (Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health). Syncytium-inducing phenotypes were determined in MT-2 cell culture assays [24]. If available, primary isolates, derived from both plasma and peripheral blood mononuclear cells (PBMCs), were tested. In addition, GHOST cell lines expressing CCR5 or CXCR4 and CD4 or CD4 alone were used to determine coreceptor usage. Cells were infected with primary virus isolates of each patient at a multiplicity of infection of 0.06. Expression of green fluorescent protein under control of the HIV-2 long terminal repeat promoter was detected by fluorescence microscopy [25].

**Phylogenetic Analyses**

Sequences were edited with SeqMan-5.08 software (DNASTAR Inc., Madison, WI), aligned with MAFFT-6.240 [26, 27], manually corrected, and tested for hypermutations by Hypermut 2.0 (www.hiv.lanl.gov). Molecular evolutionary analyses were conducted using MEGA-4 [28]. Neighbor-joining phylogenetic (NJ) trees were constructed by MEGA-4 as well as Seqboot, Dndist, and “neighbor and consensus” (PHYLIP-3.68, distributed by J Felsenstein, University of Washington, Seattle). The reference strain HIV-1_HXB2 (GenBank accession no. K03455) and other B and non-B strains were used as outgroup references and bootstrapping (1000 or 100 replications with MEGA-4 and PHYLIP-3.68, respectively). Pol sequences were obtained from the Swiss HIV Cohort study drug resistance database [29]. Pairwise distances were computed by using MEGA-4 [28]. Nucleotide diversities were obtained using the Tamura-Nei model. All reported sequences have been deposited in GenBank under accession numbers GU471280 to GU471390, GU471407 to GU471578, and JF958169 to JF960135.

**Statistical Analyses**

Statistical analyses were performed using GraphPad Prism version 5 (GraphPad Software, San Diego, CA) and STATA 11 SE (StataCorp, College Station, TX). Nonparametric tests were used for group comparison (Mann-Whitney U test). Multiple linear regression was used to analyze the association of viral diversity with viral and host baseline characteristics. The following variables were considered in the model: Age, sex, transmission category, STI, acute retroviral syndrome (ARS), viral load, CD4-cell count, viral subtype, and estimated date of infection (EDI).

**RESULTS**

**Patient Characteristics**

We analyzed 145 patients comprising 131 males and 14 females who were enrolled in the ongoing ZPHI study (Table 1). The modes of transmission according to patients’ statements included homosexual (73%), heterosexual (22%), bisexual (2%), IVDU (1.4%), and others (1.4%). Concomitant STIs have been
diagnosed in 20 patients (14%). HIV-1 subtype B was most prevalent (80%) followed by CRF01_AE (9%), C (3%), and A (3%). The remaining 5% of patients were infected with HIV-1 subtypes F1, G, CRF02_AG, and CRF12_BF.

One hundred twenty-five patients were diagnosed during documented acute HIV-1 infection. Of those, 120 patients (96%) had an ARS [13] and 87 (70%) had a negative or indeterminate Western blot (WB). Recent infection was diagnosed in 20 patients. Baseline blood samples were available within a median of 6 weeks (range 2–18) after EDI in acute and within 12 (range 7–24) weeks in recently infected patients. Fiebig staging [30] was possible for 117 patients: 2 patients were assigned to stage II, 14 patients to stage III, 52 patients to stage IV, 20 patients to stage V, 19 patients to stage V/VI, and 10 patients to stage VI.

**Phylogenetic Reconstruction**

NJ trees containing clonal C2-V3-C3 sequences from 113 (78%) patients formed individual clusters with bootstrap values of 100%. Clusters with bootstrap values <100% were found in 32 patients, most of whom also harbored closely related pol sequences (median genetic distance 0.0062; range 0–0.0124) except for 4 patients forming 2 clusters. Possible contamination was ruled out by analyzing an independent sample from each of these 4 patients. Sequences from all patients showed no clustering with reference clones used in our laboratory.

**Validity of Viral Diversity at Baseline**

In total, 2268 clones (median 16 clones/patient; range 10–16) spanning C2-V3-C3 region of the env gene were derived from plasma HIV-1 RNA of 145 PHI patients early after transmission (median 6 weeks, range 2–24). The baseline viral diversities ranged from 0.04% to 3.23% (median 0.39%). To obtain representative samples of quasispecies, reverse transcription-PCR was performed in duplicate using HIV-1 RNA extracts from 1 ml of plasma. The viral load of the plasma samples ranged from 2.4 to 7.6 (median 5.3) log_{10} copies/ml. Despite this broad distribution, viral loads and nucleotide diversities were not correlated (Figure 1). The viral loads of 10 patients were <5000 copies/ml; however, viral diversities (median 0.44%; range 0.11–1.25%) were similar to patients with high viral loads (P = .662). Thus, the sequenced clones were representative of the actual plasma virus populations in vivo as previously shown in chronically infected patients [20]. In addition, single genome amplification (SGA) median 12 sequences/sample) was performed in a subset of 25 patients, and the resulting viral diversities correlated well with clonal sequencing (slope = 1.05, intercept = −0.28%, r² = 0.681; data not shown). The viral

<p>| Table 1. Baseline Characteristics of 145 Patients With Primary HIV-1 Infection |
|--------------------------------------------------|---|---|---|</p>
<table>
<thead>
<tr>
<th>Number of patients</th>
<th>Total patients</th>
<th>Female</th>
<th>Acute infection</th>
<th>Recent infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>145</td>
<td>100</td>
<td>125</td>
<td>20</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td></td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Male</td>
<td>131</td>
<td>90</td>
<td>112</td>
<td>95</td>
</tr>
<tr>
<td>Female</td>
<td>14</td>
<td>10</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>HIV-1 subtype B</td>
<td>116</td>
<td>80</td>
<td>99</td>
<td>79</td>
</tr>
<tr>
<td>Transmission mode</td>
<td>116</td>
<td>80</td>
<td>99</td>
<td>79</td>
</tr>
<tr>
<td>Homosexual</td>
<td>106</td>
<td>73</td>
<td>91</td>
<td>73</td>
</tr>
<tr>
<td>Heterosexual</td>
<td>32</td>
<td>22</td>
<td>28</td>
<td>22</td>
</tr>
<tr>
<td>Bisexual</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>IVDU</td>
<td>2</td>
<td>1.4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Others</td>
<td>2</td>
<td>1.4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>STIsc</td>
<td>20</td>
<td>14</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>HIV-1 drug resistance transmittedd</td>
<td>6</td>
<td>4</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Acute retroviral syndrome</td>
<td>136</td>
<td>94</td>
<td>120</td>
<td>96</td>
</tr>
<tr>
<td>Negative or indeterminate Western Blot</td>
<td>87</td>
<td>60</td>
<td>87</td>
<td>70</td>
</tr>
<tr>
<td>Estimated duration of infection (weeks)</td>
<td>median (min-max)</td>
<td>median (min-max)</td>
<td>median (min-max)</td>
<td>median (min-max)</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Viral load (log_{10} copies of HIV-1 RNA/ml plasma)</td>
<td>5.3 (2.4–7.6)</td>
<td>4.8 (4.2–6.7)</td>
<td>5.5 (2.4–7.6)</td>
<td>5.0 (3.2–6.5)</td>
</tr>
<tr>
<td>CD4⁺ T cells (cells/µl blood)</td>
<td>414 (87–1295)</td>
<td>454 (87–1295)</td>
<td>374 (127–1295)</td>
<td>503.5 (87–965)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>36 (19–70)</td>
<td>32 (19–55)</td>
<td>37 (19–70)</td>
<td>34 (20–58)</td>
</tr>
</tbody>
</table>

Abbreviations: IVDU, intravenous drug users; STIs, sexually transmitted infections; HIV-1, human immunodeficiency virus type 1.
a Other subtypes: CRF01_AE, C, A, F1, G, CRF02_AG, CRF12_BF.
b One case needle stick; 1 case either IVDU or heterosexual.
c Concomitant STIs: syphilis and/or chlamydia and/or gonorrhea.
d International AIDS Society-USA mutation.
diversities increased slightly with time since EDI (EDI was described in previously published methods [15, 18]) (Figure 2), supporting the reliability of the EDI calculation used. As also shown in Figure 2, negative WBs have been detected up to 10 weeks and positive WBs between 4 and 24 weeks after EDI, demonstrating the need to include clinical data into the diagnostic staging process.

Viral Diversity in Relation to Host and Viral Characteristics

In our study population, the median viral diversity shortly after transmission did not deviate among different sexual transmission categories (Figure 3A). Twenty patients, including 1 female, 5 men having sex with females, and 14 men having sex with men (MSM), were diagnosed with a concomitant STI, but their median viral diversity did not deviate from those without concomitant STI (P = .703; Figure 3B). Next, we analyzed in more detail the relation between diversity and transmission mode among MSM depending on the encountered mucosa. Analyzed sexual practices were insertive anal intercourse, receptive anal intercourse, and unsafe oral intercourse. Patients practicing various sexual activities or with unknown transmission route were excluded from this analysis. No significant differences among distinct transmission modes were observed (Figure 3C).

We also derived the viral subtypes from individual pol sequences to examine their possible effect on diversity (Figure 4). Four patients had been infected with HIV-1 subtype C. They showed a median viral diversity of 1.07% (range 0.54–1.89%), which significantly deviated from those observed in HIV-1 subtype B (P = .011). In addition, no association between viral diversity and viral tropism was found (for tropism see below). Moreover, multiple linear regression testing the association of viral diversity with EDI, age, CD4-cell count, viral load, viral subtype, ARS, STI, and transmission mode showed no relationship except for EDI (slope = 0.031 [95% confidence interval = 0.001–0.061], P = .046, r^2 = 0.151).

Characteristics of Patients With High Viral Diversity

We identified 16 subjects (11%) with a viral diversity higher than 1% despite a short estimated duration of infection (median 7 weeks; range 4–14). Of the 14 males 3 had a concomitant STI. One of the heterosexual men was infected either by sexual contact or by sharing needles. For detailed patient characteristics see Table 2. Overall, no patient’s or transmission’s characteristics were overrepresented in the group of patients with viral diversity higher than 1%. These patients were infected with HIV-1 subtype A (1 of 4, 25%), B (12 of 116, 10%), and C (3 of 4; 75%).

Phylogenetic analyses of the C2-V3-C3 region in the 16 patients with viral diversities >1% showed 3 different tree patterns. In 4 patients, a starlike diversification was seen, whereas in 8 patients the viral clones appeared to be subdivided into 2 to 4 subclusters, each with diversity below 1% and average genetic distances between these intrapatient subgroups of 2.14% (range 1.11–4.37%). In the remaining 4 patients, a mixture of these 2
described patterns was observed including subgroups showing viral diversity of more than 1%.

**Viral Tropism**

Using PSSM, SVMwetcat, and geno2pheno[coreceptor] to predict viral tropism in clonal C2-V3-C3 sequences, X4-tropic viruses or R5/X4 mixed populations were predicted for 10%, 19%, and 14% of the patients, respectively (Table 3). In 102 patients (70%), all three prediction algorithms were consistent in predicting R5-tropic viral variants. The three bioinformatic tools yielded conflicting coreceptor usage predictions in 27% of the patients. In 4 patients (3%), all three tools predicted X4-tropic strains, either as a mixed population in 3 cases or as a pure X4-tropic population in 1 case. Full-length \( env \) sequences obtained by SGA showed the same quasispecies composition and mutation patterns as clonal C2-V3-C3 sequences in these 4 patients (data not shown). Next, HIV-1 coreceptor usage was determined by phenotypic assays using 175 available primary isolates (from 117 patients) derived from plasma and PBMCs (Table 4). In the first step, all isolates were screened by the MT-2
cell assay and if X4-tropism was detected, this was confirmed by the GHOST cell assay, allowing also differentiation of mixtures of R5/X4 double users, respectively. In 1 of the 4 patients with concordant genotypic X4-tropism prediction and in 1 patient with concordant CCR5-tropism prediction, a mixture of R5/X4-tropic viruses was detected in the PBMC isolate but not in the isolate obtained from plasma.

**DISCUSSION**

To investigate the complexity of transmitted HIV-1 populations, we determined viral diversity by HIV-1 envelope C2-V3-C3 clonal sequencing and assessed potential factors associated with transmission of heterogeneous viral populations in 145 patients enrolled in a single center primary HIV-1 infection cohort (ZPHI study). This analysis revealed three major findings: 1) heterogeneous virus populations (diversity >1%) were found in 11% of all patients; 2) neither concomitant STI, gender, nor differences in sexual practices could be identified as factors associated with transmission of heterogeneous virus populations; and 3) genotypic prediction of coreceptor tropism by 3 computational tools unambiguously characterized transmitted viruses of 73% of all patients. Seventy percent of patients harbored R5-tropic viruses, and 3% (4 patients) were predicted as harboring X4-tropic and R5/X4-tropic viruses, respectively. However, in only 1 of those, CXCR4-usage could be confirmed phenotypically. In addition, 1 patient consistently predicted as harboring R5-tropic viruses, showed phenotypically mixed R5/X4-tropic viruses. Furthermore, results remained genotypically ambiguous in 27%.

Despite various efforts [2–5, 31, 32], factors associated with increased viral heterogeneity, which is found in ~10%–20% of PHI patients, have not been fully elucidated. However, this
result is not surprising given the difficulties to identify large populations of well-characterized patients with PHI and the multifactorial nature affecting diversity upon transmission. In particular, interpretation of results may be complicated by, 1) differences in patient populations with regard to time of sampling after infection; 2) disparities in ethnicity and living environments such as access to health care, sanitation conditions, and nutritional status; 3) potential selection bias of patients enrolled in PHI studies; 4) uncertainties concerning sexual practices adopted; 5) the lack of knowledge of potential host genetic factors; and 6) different methodologies used to investigate early transmitted virus.

Our finding of complex HIV-1 populations in 11% of patients is in line with previous findings [2], although at a lower range. A likely explanation for these differences might be different frequencies of index patients transmitting a homogeneous virus population while they are in the acute phase of their HIV-1 infection. Another potential explanation is our rather conservative approach, compared with others [1], to search for complex viral populations using a cutoff of 1% diversity to differentiate homogeneous from heterogeneous transmitted viruses.

In contrast to previous studies [3, 31–33], no elevated complexity of transmitted viruses was found in the 20 patients with a concomitant STI during PHI. STIs clearly increase the risk of HIV-1 transmission by enhancing infectiousness and susceptibility [34]. The lower viral diversity in our patients might be due to the time of the transmission of the STI. Ongoing and already established STIs prior to HIV-1 infection may be of higher importance to render the mucosal barrier more susceptible for HIV-1 infection than those transmitted concurrently with HIV-1 or shortly thereafter. In our setting, the vast majority of patients reported first occurrence of STI symptoms very closely to ARS symptoms, suggesting that STIs were cotransmitted with HIV-1. In contrast, Haaland et al [31] reported that their Zambian and Rwandan seroconverter patients had symptoms and signs of STIs that were already present at the time of the last seronegative visit, suggesting that those STIs were present for comparatively longer durations, and therefore, mucosa may have been damaged more severely at the time of HIV-1 infection. Another difference between cohorts may be the proportion of female sex workers included [4]. In this group, the prevalence of STIs and the occurrence of mucosal microtrauma are higher than those in males or in females in general [3, 4, 35]. Female sex workers did not participate in our study.

HIV-1 subtype C may be associated with greater propensity for transmission possibility than other HIV-1 group M subtypes [34]. In our study, the 4 patients infected with HIV-1 subtype C showed a higher median viral diversity compared with HIV-1 subtype B-infected patients (P = .011). Viral diversity of CRF01_AE and other circulating recombinant forms were not significantly lower compared with HIV-1 subtype B, but none of those patients was infected with a more complex population (>1%). Whether higher diversity seen in our HIV-1 subtype C cases is based on HIV-1 subtype-specific viral properties or cannot be proven at this time. Similar diversities found in HIV-1 subtypes B and C in other PHI cohorts question the biological relevance of our finding [33]. However, it has to be noted that comparisons between those 2 HIV-1 subtypes were based on different study settings in different countries and at different centers [33]. Thus, ultimately, only studies of larger patient cohorts enrolled under similar conditions will yield an answer to this question.

By combining 3 different genotypic prediction algorithms, we found that 4 PHI patients (3%) possibly harbored CXCR4-tropic viruses during acute HIV-1 infection. However, in only 1 of those patients, X4-tropic strains could be confirmed phenotypically. In addition, from 1 patient with consistent R5-coreceptor prediction, X4-tropic strains could be isolated within a R5/X4 mixture. Thus, in our study, transmission of relevant replication competent X4 variants seemed to be a rare event. These findings are in line with Raymond et al [8] who reported 6.4% of plasma viruses as X4-tropic and R5/X4-tropic mixtures, respectively, in patients with acute HIV-1 infection. In contrast, a recent study using ultra-deep sequencing and genotypic prediction reported X4-tropic variants in ~50% of a small number of patients with acute HIV-1 infection [9]. Discrepant results are not surprising given the methodological difficulties regarding assays, prediction algorithms used, studied populations, and sample sizes. At present, the clinical relevance of transmitted X4-tropic minority variants is not known, because thresholds for frequencies of X4-tropic minority variants to predict maraviroc response during PHI are not available [36]. Furthermore, the problem with genotypic prediction is that it cannot predict replication competence of these minority species. On the other hand, phenotypic assays using primary viral isolates overcome this limitation but may lack sensitivity due

### Table 4. Phenotypic Prediction of HIV-1 Tropism of Primary HIV-1 Isolates Obtained From Plasma and PBMCs of Primary HIV-1–Infected Patients

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of patients predicted by 3 algorithms</td>
</tr>
<tr>
<td>X4</td>
<td>4</td>
</tr>
<tr>
<td>R5</td>
<td>102</td>
</tr>
<tr>
<td>inconsistent</td>
<td>39</td>
</tr>
</tbody>
</table>

a Predicted by 3 bioinformatic tools: Geno2pheno, PSSM, and SVMwetcat.
b Detected in peripheral blood mononuclear cells (PBMCs) with MT-2 and GHOST cell assay.
c Detected in PBMCs with MT-2 but not with GHOST cell assay.
to initial selection process during their propagation in cell cultures [37]. Recombinant phenotypic assays using PCR-amplified HIV-1 envelope may have an improved sensitivity profile, but they give only information on tropism of single envelopes amplified and not on replication competence of these viruses. Taken together, despite a considerable amount of previous work [8–10, 38, 39], frequency of clinically relevant transmitted CXCR4-using viruses is still not known, but in our study transmission of such viruses seemed to occur only rarely.

In summary, our findings suggest that transmission of complex virus founder populations may not depend solely on mucosal factors. In addition, transmission of clinically relevant CXCR4-using virus strains seems to remain a rare event, and caution is warranted when predicting coreceptor usage by genotypic algorithms alone, because concordance of those tools is still limited.

**Notes**

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**Potential conflicts of interest.** H. F. G. has been an adviser and/or consultant for GlaxoSmithKline, Abbott, Novartis, Boehringer Ingelheim, Gilead Sciences, Roche, Merck Sharp & Dohme, Tibotec, and Bristol-Myers Squibb. In addition, H. F. G. has received unrestricted research and educational grants from Roche, Abbott, Bristol-Myers Squibb, Gilead Sciences, GlaxoSmithKline, ViIV Healthcare, Tibotec and Merck Sharp & Dohme as well as travel grants from Roche, Abbott, Bristol-Myers Squibb, Gilead Sciences, GlaxoSmithKline, ViIV Healthcare, Tibotec, and Merck Sharp & Dohme (all money went to the institution). K. J. M. has received travel grants and honoraria from Gilead, Roche Diagnostics, GlaxoSmithKline, Bristol-Myers Squibb, Tibotec, and Abbott, and he has received a research grant from Gilead. P. R. has received travel grants from Gilead Sciences. R. W. has received travel grants from Abbott, Boehringer Ingelheim, Bristol-Myers Squibb, Gilead Sciences, GlaxoSmithKline, Merck Sharp & Dohme, Pfizer, Roche, TRB Chemidica, and Tibotec. B. J., A. U. S., H. K., D. B., C. G., B. N., C. L., J. B., and S. G. have no conflicts of interest.

**Author contributions.** Study concept, design, supervision: Günthard Acquisition of laboratory data: Rieder, Joos, Böni, Leemann, Niederöst, Kuster, and Metzner

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