## Supplemental Digital Content

### Swiss HIV Cohort Study (SHCS)

Clinical and genotypic data was obtained from the Swiss HIV Cohort Study (SHCS). The SHCS is a swisswide, clinic-based cohort study with continuous enrolment and at least semiannual study visits [1]. The SHCS has been approved by ethical committees of all participating institutions, and written informed consent was obtained from participants.

The SHCS resistance database contains all genotypic HIV resistance tests performed by the 4 authorized laboratories in Switzerland, stored in SmartGene's (Zug, Switzerland) Integrated Database Network System (IDNS, version 3.4.0) [2]. All available/w/ sequences (6500) until May 2008 were used for the first phylogenetic analysis to select candidates for the confirmatory *env* sequencing. A second analysis (12'303 *pol* sequences) was performed in May 2009 to assess the relation to the overall HIV-1 infected population in the SHCS.

The SHCS is representative of at least 50% of the HIV infected population of Switzerland, 70% of AIDS cases and 75% of patients receiving antiretroviral treatment [3-5].

### Patients and samples

Patients were enrolled in the Zurich Primary HIV Infection Study (ZPHI, http://clinicaltrials.gov, ID NCT00537966) [6-9]. All presented with documented acute or recent primary HIV-1 infection. Acute HIV-1 infection was defined as acute retroviral syndrome (ARS) and negative or indeterminate Western blot in the presence of a positive p24 Ag and/or detectable plasma HIV-1 RNA; or a documented seroconversion with or without symptoms during the last 90 days. Patients with recent HIV-1 infection had a possible ARS, positive Western blot and detectable HIV-RNA, in addition to a negative HIV-gpl20 avidity [8] or detuned assay [10] or a documented acute HIV-1 infection, however, referral to our center occurred more than 90 days after the estimated date of infection. Early ART was offered to patients with acute infection followed by termination of early ART as an option after having achieved one year of viral suppression below 50 copies/ml (details according to study protocol, see clinicaltrials.gov). The ZPHI-study has been approved by the ethical committee and written informed consent was obtained from all participants. All patients received mandatory counselling with regards to safe sex practises at enrolment into the study and again before early ART was stopped. Moreover, self reported sexual behaviour was assessed by structured interviews every six months. Blood samples were collected from all persons at the time of enrolment before early ART and sequentially in at least three month intervals. Detailed patient characteristics are presented in Table S1. Information was treated strictly anonymously. No contact tracing was performed based on sequence information.

### Estimated timepoint of infection

We estimated a date of infection for each patient as accurate as possible. For this all available information was integrated (first positive, last negative test, negative, indeterminate and positive Western blot results, avidity assay, unambiguous risk contacts, onset of symptoms of the acute retroviral syndrome). The following scenarios were addressed: Fully negative Western blot. If the patient did have a single risk contact that he was aware of within the last three weeks of the test this date was taken. If he did

**Table S1. Patient characteristics at baseline.**

| | Total PHI | | Acute | | Recent | |
|---|---|---|---|---|---|---|
| number of patients | 111 | (100%) | 97 | (100%) | 14 | (100%) |
| male | 98 | (88%) | 85 | (88%) | 13 | (93%) |
| female | 13 | (12%) | 12 | (12%) | 1 | (7%) |
| viral subtype B[a] | 87 | (78%) | 74 | (76%) | 13 | (93%) |
| transmission group | | | | | | |
| homosexual | 78 | (70%) | 68 | (70%) | 10 | (71%) |
| heterosexual | 27 | (24%) | 24 | (25%) | 3 | (21%) |
| i.v drug use | 2 | (2%) | 2 | (2%) | | |
| bisexual | 2 | (2%) | 1 | (1%) | 1 | (7%) |
| others[b] | 2 | (2%) | 2 | (2%) | | |
| STDs[c] | 16 | (14%) | 12 | (12%) | 4 | (29%) |
| resistance transmitted | 7 | (6%) | 6 | (6%) | 1 | (7%) |
| negative or indeterminate Western Blot | 75 | (68%) | 74 | (76%) | 1 | (7%) |
| time interval (weeks)[d] from infection to first sample | 6 | (2-24) | 5 | (2-13) | 13.5 | (7-24) |
| viral load[d] (log 10 copies of viral RNA / ml) | 5.3 | (2.4-7.6) | 5.4 | (2.4-7.6) | 5.0 | (3.2-6.1) |
| CD4+ (cells / ul)[d] | 398 | (87-1295) | 385 | (127-1120) | 429 | (87-965) |
| age[d] | 37 | (19-70) | 38 | (19-70) | 34 | (29-58) |

[a]other subtypes: CRF01AE, C, A, F, G, CRF02AE, CFR12BF
[b]needle stick (n=l); i.v. drug use or heterosexual (n=l)
[c]sexually transmitted diseases: syphilis and/or chlamydia and/or gonorrhea
[d]median (range)

not know for sure in this case we assumed the infection to have taken place 14 days before the Western blot date. If a Western blot was indeterminate and the patient had an unambiguous risk contact the date of this risk contact was taken, if it was within 2-6 weeks before the date of the Western blot. If several risk contacts were present, then a higher and lower range was estimated and the mean was calculated. In general, if an ARS was present then onset of symptoms was assumed to have occurred 10 to 30 days after the infection took place. If no ARS was present but seroconversion within 6 months or less was clearly documented, a positive risk date was taken as the estimated infection time point if a single unambiguous contact was available (e.g. one single unprotected sexual contact with a known HIV-1 infected partner). If such one was not identifiable the mean between the two tests was taken. Of note, the estimated infection timepoints seemed to be quite accurate because there is a significant correlation between the estimated time after infection and increasing C2-V3-C3 diversity (r2=0.07, p=0.05).

## Estimation of Infection Rates

Based on the estimated dates of HIV transmission, we calculated cluster-specific infection rates. Exposure time for each recipient patient was defined as the cumulative time with HIV RNA > 1000 copies/mL for all previously infected members of the same cluster. To reflect that not all individuals were equally likely to transmit the virus to a recipient patient, the contribution of time to the denominator of rates was weighted according to the maximum likelihood genetic viral similarity between the recipient patient and a previously infected member of a cluster.

## Sequencing Methods

***RNA extraction.*** One ml blood plasma was centrifuged for 45 min at 50'000 x g and 16°C. RNA was resuspended and extracted by using RNeasy Mini Kit as described [11].

**Amplification ofHIV-1** *env.* Reverse transcription-PCR was performed in a one-tube reaction in order to minimize the risk of contamination using QIAGEN OneStep RT-PCR Enzyme Kit (Qiagen, Chatsworth, CA). For cDNA synthesis the primer V3Bout (5'-ATTACAGTAGAAAAATTCCCCT-3') was used and the primers V3Fin (5'-GAACAGGACCATGTA-CAAATGTCAGCACAGTACAAT-3') and V3Bin (5'-GCGTTAAAGCTTCTGGGTCCCCTCCTGAG-3') were used for PCR. When the Reverse Transcription PCR failed to amplify the alternative primers mfl66 (5'-TACTTCCTGCCACATGTTTATAAATTGTTT-TAT-3') and mfl70 (5'-AAATGTCAGCACAGTA-CAATGTACACATGG-3') were used. For each sample duplicate 30|il reactions using 3|il template RNA were performed to ensure representative sampling of HIV-1 genomes. The thermocycling conditions were 30 min at 50°C and 15 min at 95°C, followed by 50 cycles of 10 seconds, 10 seconds, and 60 seconds at 95°C, 60°C, and 72°C, respectively, and 1 cycle of 10 min at 72°C.

***Cloning and sequencing.*** Two |il of the pooled and purified PCR products was ligated into the plasmid vector pDrive using the QIAGEN PCR Cloning kit (Qiagen, Chatsworth, CA). 16 individual clones were picked after bacterial transformation and cultured in a microwell plate at 37°C for 3 hours in LB medium, then diluted 1:100 in water and incubated at 95°C for 5 min. One |il of this mix was used in a PCR with 30-nl reaction volume using the primers M13 (5' GTAAAAC-GACGGCCAGT 3') and T7 (5' GTAATACGACT-CACTATAG 3'). In a previous study we showed that no artefacts are introduced by the additional PCR step [12]. One \A of each clonal PCR product containing approximately 20 to 30 ng DNA was sequenced in both directions with the primers Ml3 and T7 using BigDye chain terminator chemistry and the automated sequencer ABI 3100 (Applied Biosystems, Rotkreuz, Switzerland).

**Table S2. Forward transmission events.**

| cluster | transmission | VL at timepoint of transmission[a] | Genetic distance between *pol* sequences[b] | Genetic distance between C-2-V3-C3 sequences[b] | probability of transmission chain |
|---|---|---|---|---|---|
| A | ZPHIA1->ZPHIA2 | 33478 | 0.39 % | 0.62 % | very high[c] |
| B | ZPHIB1–>ZPHIB2 | 930 | 0.79 % | 1.63 % | high |
| C | ZPHIC1->ZPHIC2 | 2237 | 0.12% | 0.31 % | very high[c] |
| E | ZPHIE1->ZPHIE2 | 1690000 | 0.08 % | 0.00 % | high |
| E | ZPHIE1->ZPHIE3 | 314 | 0.08 % | 0.60 % | possible |
| E | ZPHI E2 -> ZPHI E3 | 1039 | 0.00 % | 0.31% | possible |
| E | ZPHI E2 -> ZPHI E4 | 27681 | 0.85 % | 2.88 % | possible |
| E | ZPHI E3 -> ZPHI E4 | 7679 | 0.85 % | 3.41 % | possible |
| E | ZPHI E2 -> ZPHI E5 | 27800 | 1.09% | 5.49% | possible |
| E | ZPHI E3 -> ZPHI E5 | 6650 | 1.09% | 5.96% | possible |
| D | ZPHI Dl-> ZPHI D2 | <50 | 1.26% | 0.92 % | unlikely |

[a]The viral load of the source at the time point of recipient infection is integrated from the viral load from the last sample before and the earliest sample after the time point of onward transmission and indicated in copies/ml.
[b]Pairwise distance calculation with Tamura-Nei model
[c]Transmission within a stable monogamous partnership

## References


1. Ledergerber B, Egger M, Opravil M, Telenti A, Hirschel B, Battegay M, *et al.* **Clinical progression and virological failure on highly active antiretroviral therapy in HIV-1 patients: a prospective cohort study. Swiss HIV Cohort Study** *Lancet* 1999,353:863-868
2. von Wyl V, Yerly S, Boni J, Burgisser P, Klimkait T, Battegay M, *et al.* **Emergence of HIV-1 drug resistance in previously untreated patients initiating combination antiretroviral treatment: a comparison of different regimen types.** *Arch Intern Med* 2007,167:1782-1790.
3. Schoeni-Affolter F, Rickenbach M, Furrer H, Rudin C, Giinthardt HF, Ledergerber B, *et al.* **COHORT PROFILE: The Swiss HIV Cohort Study** *in press* 2009
4. Yerly S, Junier T, Gayet-Ageron A, Amari EB, von Wyl V, Gunthard HF, *et al.* **The impact of transmission clusters on primary drug resistance in newly diagnosed HIV-1 infection.** *AIDS* 2009,23:1415-1423.
5. Metzner KJ, Rauch P, von Wyl V, Leemann C, Grube C, Kuster H, *et al.* **Efficient suppression of minority quasispecies of drug-resistant viruses present at primary HIV-1 infection by RTV- boosted protease inhibitor containing ART.** *in press* 2009.
6. Aceto L, Karrer U, Grube C, Oberholzer R, Hasse B, Presterl E, *et al.* **[Primary HIV-1 infection in Zurich: 2002-2004].** *Praxis (Bern 1994)* 2005,94: 1199-1205.
7. Huber M, Fischer M, Misselwitz B, Manrique A, Kuster H, NiederA^jst B, *et al.* **Complement Lysis Activity in Autologous Plasma Is Associated with Lower Viral Loads during the Acute Phase of HIV-1 Infection.** *PLoS Med* 2006,3:e441.
8. Rusert P, Kuster H, Joos B, Misselwitz B, Gujer C, Leemann C, *et al.* **Virus Isolates during Acute and Chronic Human Immunodeficiency Virus Type 1 Infection Show Distinct Patterns of Sensitivity to Entry Inhibitors** *J. Virol.* 2005,79:8454-8469.
9. Huber M, von Wyl V, Ammann CG, Kuster H, Stiegler G, Katinger H, *et al.* **Potent human immunodeficiency virus-neutralizing and complement lysis activities of antibodies are not obligatorily linked.** *J Virol* 2008,82:3834-3842.
10. Janssen RS, Satten GA, Stramer SL, Rawal BD, O'Brien TR, Weiblen BJ, *et al.* **New Testing Strategy to Detect Early HIV-1 Infection for Use in Incidence Estimates and for Clinical and Prevention Purposes.** *JAMA* 1998,280:42-48.
11. Fischer M, Huber W, Kallivroussis A, Ott P, Opravil M, Luthy R, *et al.* **Highly sensitive methods for quantitation of human immunodeficiency virus type 1 RNA from plasma, cells, and tissues.** *JClinMicrobiol* 1999,37:1260-1264.
12. Joos B, Trkola A, Fischer M, Kuster H, Rusert P, Leemann C, *et al.* **Low human immunodeficiency virus envelope diversity correlates with low in vitro replication capacity and predicts spontaneous control of plasma viremia after treatment interruptions.** *J Virol* 2005,79:9026-9037.