



REVIEW

Open Access

Clinical significance of HIV-1 coreceptor usage

Hanneke Schuitemaker^{1,3*†}, Angélique B van 't Wout^{1,3†}, Paolo Lusso^{2*†}

Abstract

The identification of phenotypically distinct HIV-1 variants with different prevalence during the progression of the disease has been one of the earliest discoveries in HIV-1 biology, but its relevance to AIDS pathogenesis remains only partially understood. The physiological basis for the phenotypic variability of HIV-1 was elucidated with the discovery of distinct coreceptors employed by the virus to infect susceptible cells. The role of the viral phenotype in the variable clinical course and treatment outcome of HIV-1 infection has been extensively investigated over the past two decades. In this review, we summarize the major findings on the clinical significance of the HIV-1 coreceptor usage.

Introduction

The entry of human immunodeficiency virus (HIV) into cells is critically dependent on the sequential interaction of the viral envelope with two cell-surface receptors, the CD4 glycoprotein and a 7-transmembrane-domain chemokine receptor such as CCR5 or CXCR4. The evolutionary choice of HIV of exploiting chemokine receptors as entry gateways has established a tight biological bond between HIV and the chemokine system, turning the natural ligands of these receptors into specific viral inhibitors. The first encounter between the fields of HIV and chemokines occurred unexpectedly at the end of 1995 with the discovery that three chemokines of the CC family, RANTES (CC-chemokine ligand 5 or CCL5), MIP-1 α (CCL3) and MIP-1 β (CCL4), act as potent and specific natural inhibitors of HIV-1 infection [1]. A few months later, in the spring of 1996, a totally independent experimental approach led to the identification of a chemokine receptor, CXCR4, as a critical cell-surface coreceptor for HIV-1 entry [2]. These two complementary findings triggered an authentic chain reaction of further breakthroughs, most notably the discovery of the second major HIV-1 coreceptor (i.e., CCR5), the identification of a specific chemokine ligand for CXCR4 (i.e., SDF-1/CXCL12),

and the first definitive association of a genetic determinant (i.e., CCR5- Δ 32) with HIV-1 resistance (reviewed in [3]). Looking backward, the exploration of this uncharted area of investigation has greatly advanced our understanding of the biology and pathogenesis of HIV infection, opening new perspectives for the development of effective measures for the therapy and prevention of AIDS.

CCR5 and CXCR4: the two clinically relevant HIV-1 coreceptors

Although several chemokine receptors may function as HIV-1 coreceptors *in vitro*, multiple lines of clinical and experimental evidence indicate that only two of them, CCR5 and CXCR4, have *bona fide* clinical relevance (reviewed in [3]). Both CCR5 and CXCR4 are expressed, in combination with CD4, on all the relevant target cells for HIV-1, including primary CD4⁺ T cells, macrophages and dendritic cells. Individual viral isolates are presently classified based on their ability to use CCR5 (R5 variants), CXCR4 (X4 variants) or both coreceptors (R5X4 variants) [4]). The dual-tropic R5X4 viruses are further classified as Dual-R (R5X4 variants with more efficient use of CCR5 than of CXCR4) or Dual-X (R5X4 with more efficient use of CXCR4 than of CCR5) [5-7]. In the absence of a more accurate characterization, bulk viral isolates capable of using both coreceptors are designated dual/mixed (D/M) as their quasispecies may contain any mixture of the various phenotypic variants (Figure 1).

Ex vivo determination of HIV-1 coreceptor usage

Before the identification of the coreceptors, HIV-1 isolates were characterized based on their ability to infect

* Correspondence: H.Schuitemaker@amc.uva.nl; plusso@niaid.nih.gov

† Contributed equally

¹Department of Experimental Immunology, Sanquin Research, Landsteiner Laboratory, and Center for Infection and Immunity Amsterdam (CINIMA) at the Academic Medical Center of the University of Amsterdam, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands

²Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892, USA
Full list of author information is available at the end of the article


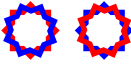

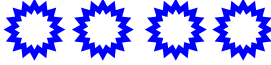
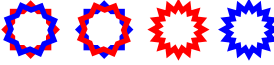

Coreceptor	CCR5	CCR5, CXCR4	CXCR4
MT-2	NSI	SI	SI
Trofile	R5	D/M	X4
Clone			
Isolate			
Tropism			
CD4⁺ T cell	memory	naïve and memory	naïve and memory
Thymocytes	-	++	+++
Precursors	-	++	+++
Macrophages	+++	+	+/-
Dendritic cells	+++	+	+/-
T cell lines	-	++	+++

Figure 1 Overview of coreceptor use and cell tropism of different HIV-1 variants. Individual viral isolates are classified based on their ability to use CCR5 (R5 variants), CXCR4 (X4 variants) or both coreceptors (R5X4 variants). Bulk viral isolates capable of using both coreceptors are designated dual/mixed (D/M) as their quasiespecies may contain any mixture of the various phenotypic variants. The cell tropism of each viral isolate is determined by the expression levels of CCR5 and CXCR4 on the various target cells.

and induce syncytia (multinucleated giant cells) in CD4⁺ T-cell lines that express CXCR4 but not CCR5. Using the MT-2 cell line as a prototype, viruses that did not infect MT-2 cells were designated non-syncytium inducing (NSI), while viruses that did infect MT-2 cells were designated syncytium inducing (SI). Today, we attribute this differential ability to the viral coreceptor usage, and MT-2-positive variants are defined as either X4 or R5X4 [4]. Of note, the MT-2 assay can only detect CXCR4-using variants, and the absence of viral growth in MT-2 can be due either to the exclusive presence of R5 variants or to the failure to isolate HIV. For a precise determination of the coreceptor use of HIV-1 isolates, cell lines such as U87 and GHOST transfected with CCR5, CXCR4, or other coreceptors, have been used [8,9]. More recently, several recombinant phenotypic assays to determine coreceptor usage have been developed, such as the Trofile assay (Monogram Biosciences) [10]. Patient plasma is used to generate pseudoviruses or infectious recombinant viruses that include full-length or partial viral envelopes derived from the patient's virus population. The recombinants are subsequently tested on indicator cell lines expressing CD4 and either CCR5 or CXCR4. While the sensitivity of the Trofile assay for detecting CXCR4-using HIV-1 is similar to that of the MT-2

test [11], recombinant phenotypic assays are able to distinguish pure R5, D/M and pure X4 populations.

HIV-1 coreceptor usage and genetic subtypes

The use of different coreceptors by HIV-1 is a general phenomenon that has been observed for viruses from all different genetic subtypes and circulating recombinant forms (CRFs) [12,13]. However, quantitative differences in the prevalence of CXCR4 usage among subtypes exist. Most of the initial studies on HIV-1 coreceptor usage evolution were performed on strains belonging to genetic subtype-B [14,15], which remains the best characterized subtype in terms of coreceptor usage. However, a more complex picture has been delineated with the extension of these studies to non-B HIV-1 subtypes.

One of the major discrepancies was the observation of a low frequency of CXCR4 usage among subtype-C strains [16-21]. Infection with subtype-C HIV-1 accounts for over half of the worldwide HIV-1 epidemics [22], and is as deadly as subtype-B infection. Although it has been suggested that the underrepresentation of CXCR4-using strains in these reports might be due to a sampling bias, later studies in treatment-experienced patients from Zimbabwe [23] and a recent study from South Africa have reported a higher prevalence of CXCR4-using variants than in the past [24],

suggesting an ongoing evolution of the subtype-C HIV-1 epidemic in Africa (see also below).

An inverse skewing in coreceptor usage, with an increased presence of CXCR4-using strains, has instead been reported for subtype-D HIV-1 [5,12,25]. This observation is consistent with the faster pace of disease progression reported for subtype-D infection both in Africa [25-27] and outside Africa [28]. An increased rate of CXCR4 usage has also been reported in a study on a limited number of subtype-AE isolates [29,30]. Even more complex is the situation with mixed genotypes with only a few isolates so far characterized [31,32]. In a recent study in Guinea Bissau, an increasing and generally high frequency of CXCR4 tropism (86%) was observed in CRF02_AG [33].

Molecular determinants of HIV-1 coreceptor usage

Studies conducted with subtype-B variants have shown that the HIV-1 coreceptor usage is determined by the viral envelope, primarily the second and third variable loops (V2 and V3) of the external HIV-1 envelope glycoprotein, gp120 [34-37]. Specifically, the presence of a positively-charged amino acid at either one or both of two specific positions in the V3 loop (positions 11 and 25) is strongly associated with a CXCR4-using phenotype in subtype-B primary isolates [38,39], suggesting that these amino acids play a crucial role in the interaction of gp120 with the coreceptors. Indeed, it has been shown that the V1V2 and V3 regions are involved in the interaction with CCR5 and CXCR4 [40-42]. This model is compatible with insights from structural studies in which V3 and a conserved coreceptor-binding site that includes the stem of the V1V2 loop are involved in coreceptor binding. In various trimeric models of the gp41-gp120 envelope glycoprotein complex, V2 directly interacts with V3 and both participate in coreceptor binding [43-46].

As shown for subtype-B HIV-1, the V3 loop also seems to be the principal genetic determinant of the coreceptor choice among subtype-D (10 variants

studied) and subtype-A (one variant studied) [12]. In later studies, subtype-C CXCR4-using strains did not show the same dependence as other subtypes on positively-charged residues in the V3 loop [47,48].

Predictive algorithms of HIV-1 coreceptor usage

The identification of viral genotypic changes associated with different coreceptor usage has led to the development of sequence-based algorithms to predict coreceptor usage. Starting with the 11/25 rule derived from subtype-B variants [39], efforts have concentrated mainly on identifying sequence patterns in the V3 loop. Most genotypic predictors of coreceptor usage incorporate information from across the V3 region (**Table 1**) [49-53], in some cases along with genotypic correlates outside the V3 [54]. Some methods also incorporate clinical data [55]. Progress is also being made on the inclusion of structural information to assist prediction [56], as well as on the ability to discriminate between X4 and R5X4 virus [57]. On cloned viruses belonging to genetic subtype B, the specificity and sensitivity of most predictive methods exceed 90% and 80%, respectively. However, the sensitivity drops below 50% when bulk uncloned sequences and non-subtype B viruses are assayed. Moreover, technical limitations to the generation of unambiguous DNA sequences from the HIV-1 envelope region that has insertions and deletions that prevent the generation of interpretable electropherograms, interfere with a predictive determination of tropism in a significant fraction of patient samples. This in combination with the limited predictive power obviously has implications for a clinical diagnostic application of bulk sequencing technology and current predictive algorithms for HIV-1 coreceptor usage. To date, studies of genotypic predictors have been retrospective with patient samples selected based on availability of phenotypic tropism determinations. Prospective studies will be needed to firmly establish the clinical usefulness of genotypic tropism determination.

As discussed below, the selective pressure that leads to the emergence of CXCR4-using strains is complex and

Table 1 Bioinformatic predictors of HIV-1 coreceptor use based on V3 loop sequence

Predictor	URL	Method	Ref
R5/X4 and NSI/SI network	http://cancer.med.unc.edu/swanstromlab/resources.html	Neural network	Resch et al, 2001
WebPSSM	http://indra.mullins.microbiol.washington.edu/webpssm/ http://fortinbras.us/cgi-bin/fssm/fssm.pl	PSSM	Jensen et al, 2003
WetCat	http://genomiac2.ucsd.edu:8080/wetcat/v3.html	SVM	Pillai et al, 2003
Geno2pheno	http://coreceptor.bioinf.mpi-inf.mpg.de/	SVM	Sing et al, 2007
V3SD	The source code for prediction and analysis is available upon request.	SVM	Sander et al, 2007
R5/X4-pred	http://yjxy.ujv.edu.cn/R5-X4_pred.rar	Random forests	Xu et al, 2007
ANN	Not available	Neural network	Lamers et al, 2008
hiv-dskernel	http://genome.ulaval.ca/hiv-dskernel	SVM	Boisvert et al, 2008

variable, and clinically relevant CXCR4-using minorities may coexist with a predominant R5 virus, even though they remain rare enough to go undetected by sequencing [58]. In addition, not all determinants of coreceptor usage lie within the V3 loop, the region employed by most current predictors [35,59]. Accurate prediction is also complicated by the fact that the V3-C4 region of the envelope gene, which has the greatest influence on tropism, also has a relatively high rate of diversity although the V3 region itself does contain conserved segments [60]. This complicates the interpretation of sequences, especially when so-called bulk sequencing is employed. This method captures only the most prevalent sequence (>25% of the total quasispecies), and the results become difficult to interpret when many genetic variants are present simultaneously. However, continuous progress is being made, especially with the application of next generation sequencing [61].

Clinical trials with CCR5 antagonists have indicated that patients with detectable CXCR4-using virus are unlikely to show a significant decrease in viral load in response to CCR5 antagonists [62,63]. Therefore, prior to initiating treatment with CCR5 inhibitors, patients are now screened to exclude those who harbor CXCR4-using variants. Both phenotypic and genotypic assays have been developed for this screening, but the Monogram Trofile assay is currently the only clinically validated test and has been used to screen the largest number of patients [10,64]. As more combined V3 genotype-phenotype data become available, genotypic predictors of coreceptor usage are likely to become a viable alternative to phenotypic assays. Indeed, recent data from the HOMER cohort and the MOTIVATE and MERIT trials show that genotype-based methods performed on populations-based samples, or "bulk" sequence data, are equivalent to the Trofile assay and that deep sequencing can actually improve the phenotypic results [65,66].

Coreceptor usage in primary HIV-1 infection

Coreceptor usage and HIV-1 transmission

Despite an extensive literature on the subject, the process of *in vivo* transmission of HIV-1 remains largely unknown and most of the current models are essentially conjectural. In fact, there are virtually insurmountable difficulties in studying the earliest events of HIV-1 infection in the human species, while nonhuman primate models, albeit useful, provide only a partial and, in all likelihood, non-physiological picture. In fact, most of the observations on the acute phase of HIV-1 infection in humans are made several weeks or even months after the initial transmission event, typically in subjects who manifest clinical signs of acute retroviral syndrome. With this caveat in mind, the bulk of evidence indicates

that R5 HIV-1 variants are largely prevalent during the acute phase of infection [67,68]. Even if both R5 and CXCR4-using variants are present in the donor, most often only the R5 variants are detected in the recipient [69-71]. Whether this early R5 predominance reflects a *bona fide* transmission bias or a superior *in vivo* fitness of R5 strains during the early phase of infection remains uncertain.

Several studies have attempted to correlate the predominance of R5 HIV-1 strains during the acute phase with a biological bottleneck inherent to the genital mucosa, variously related to trapping and inactivation of CXCR4-using virus by mucin and innate antiviral proteins, preferential transcytosis of R5 viruses in endothelial cells and/or preferential amplification of R5 viruses by resident macrophages, dendritic cells and/or Langerhans cells (reviewed in [72]). In reality, however, no conclusive evidence has been provided to indicate that CXCR4-using strains are less able or unable to sustain mucosal transmission. For example simian/human chimeric immunodeficiency viruses (SHIV) bearing an X4 HIV-1 envelope can be readily transmitted via the mucosal route in macaques, and have widely been used as a reference model [73].

Another important element that is rarely taken into consideration in the HIV-1 transmission equation is the transmitter bias, due to the fact that people with replicating CXCR4-using viruses may be in a more advanced stage of their disease and less prone to engage in risky sexual behavior [74]. If a majority of transmissions occur from asymptomatic individuals who at that time still only harbor R5 variants, then this would indeed contribute to the scarcity of transmission of CXCR4-using variants.

Post-transmission events

Another potential contributing factor in the marked R5-variant predominance during acute primary HIV-1 infection is a post-transmission amplification bias. In peripheral blood, the high proportion of CCR5⁺CD4⁺ T cells that are recruited during acute HIV-1 infection may favor R5 variants to replicate and thereby outcompete putative co-transmitted CXCR4-using variants. Moreover, the high proportion of memory/activated CCR5⁺CD4⁺ T cells present in the mucosal-associated lymphoid tissue, which is considered a major site of HIV-1 replication during primary infection [75], may provide the optimal environment for preferential R5 HIV-1 amplification. Of importance, these cells also express high levels of integrin $\alpha 4\beta 7$, an important facilitator of HIV-1 infection [76].

Although primary infection with CXCR4-using HIV-1 strains is believed to be a rare event, mixed R5/X4 primary infections have been clearly documented in a few

patients studied longitudinally from an early stage post-infection [77,78]; interestingly, however, the CXCR4-using component was selectively cleared from plasma with the transition to the chronic phase in which only R5 variants could be recovered, raising the possibility that mixed R5/X4 transmission may in fact be more frequent than it appears, albeit underestimated due to late sampling, subsequent to the disappearance of the CXCR4-using component from plasma. In a recent retrospective evaluation of a large number of patients (n = 390) enrolled in the PRIMO cohort in France between 1996 and 2007, a relatively high prevalence (15.9%) of predicted CXCR4-using viruses was documented during primary infection [79]. However, subsequent phenotypic analysis of the patients in this study infected with non-subtype B variants (n=131) showed that genotypic predictions overestimated the proportion of CXCR4-using variants in non-subtype B infected patients, resulting in a much lower prevalence (0.8%) of actual CXCR4-using variants [80]. In agreement, a low prevalence of CXCR4-using viruses (4-6%) was observed in three recent cohorts of seroconverters: one from the United States (n=150) during the period 1999-2003 [81], one from France (n=133) during the period 1995-2008 [82], and one from the Netherlands (n=46) during the period 2003-2008 (ABW et al., unpublished observations). Moreover, detailed investigations of individuals experiencing primary infection and sampled prior to seroconversion (Fiebig stage I-V) using single genome amplification have revealed a consistent pattern of CCR5 dependence at this stage [67,68,83-88] indicating that any putative post-transmission amplification bias would have to occur within the first few days of exposure.

Protective effect of congenital CCR5 deficiencies

The most convincing argument in favor of the *bona fide* predominance of R5 variants in the initial transmission events is the high degree of protection from HIV-1 infection conferred by genetic deficiency of CCR5. Subjects that are homozygotes for the *CCR5-Δ32* allele are highly protected from HIV-1 infection [89]. Indeed, this genotype is enriched among individuals who remain seronegative despite high-risk sexual behavior [90,91] as well among hemophiliacs who had remained HIV negative despite exposure to batches of clotting factor that were known to be the cause of HIV infection in other hemophiliacs [92]. *CCR5*wt/*Δ32* heterozygosity does not confer protection from HIV-1 infection, but this genotype has been associated with a significantly lower viral set point and delayed disease progression [93-96]. A second crippling polymorphism, m303, that introduces a premature stop codon in the *CCR5* gene has been identified in exposed-uninfected subjects [97].

Despite the strong protective effect conferred by congenital CCR5 deficiencies, a handful of infected *CCR5-Δ32* homozygotes have been reported, all invariably harboring CXCR4- dependent HIV-1 strains [98]. Interestingly, two *CCR5-Δ32*^{+/+} individuals were found to harbor an inherently (not mixed) dual-tropic virus (R5X4) that remained stable over time [99], suggesting that maintenance of the CCR5-using envelope conformation might provide a selective advantage *in vivo* despite the absence of a usable receptor. Although the viral load in infected *CCR5-Δ32*^{+/+} subjects tends to be low, a rapid depletion of circulating CD4⁺ T cells has been noted [98]. However, the very limited number of individuals characterized thus far makes it difficult to evaluate whether the clinical and virological course of an X4 HIV-1 infection *d'emblée* differs from that of conventional R5 HIV-1 infection in individuals with wild-type *CCR5* genes. In this respect, viral variants with X4/DM phenotype have been detected in individual case reports of acute HIV-1 infections with peculiarly severe clinical manifestations [100-103], but again their number is too limited to draw any reliable conclusions and it is difficult to exclude other confounding cofactors.

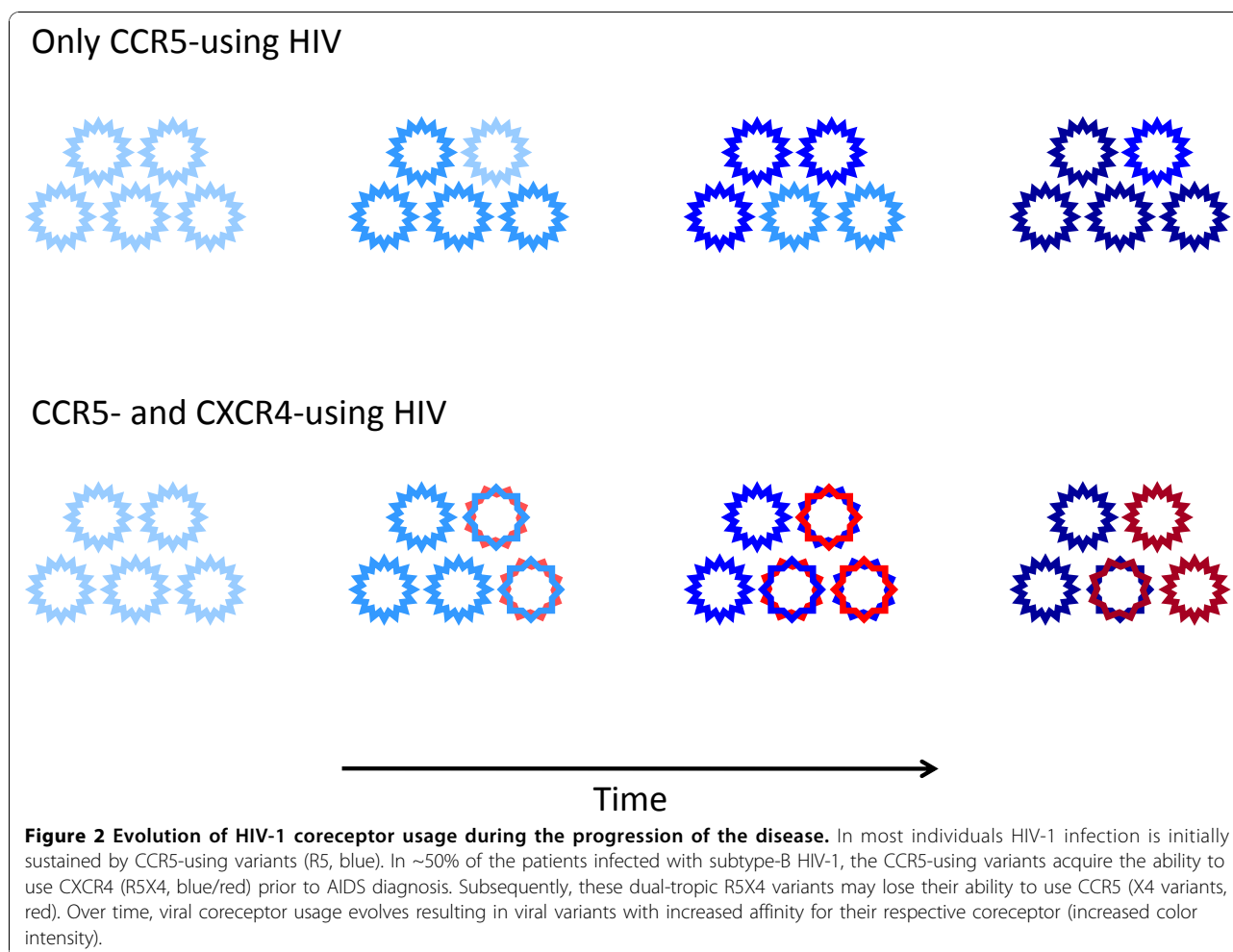
Role of minor coreceptors

Usage of the so-called "minor" coreceptors during primary HIV-1 infection has only marginally been investigated. Interestingly, a high frequency of CCR3 usage during primary infection has been detected by direct cloning and expression of viral sequences, while *in vitro* culture apparently selects against CCR3 usage leading to the expansion of variants with exclusive CCR5 usage [104]. By contrast, in a few cases of primary infection with subtype- C HIV-1 studied, all of R5 phenotype, there was a surprisingly high frequency of usage of GPR15, APJ and CXCR6, but not of CCR3 [105,106]. Further studies are warranted to address the relevance of minor coreceptors in HIV-1 transmission.

Coreceptor usage in chronic HIV-1 infection

***In vivo* evolution of HIV-1 coreceptor usage**

During the asymptomatic phase of HIV-1 infection a homogeneous R5 virus population is commonly present that generally has the ability to replicate efficiently in both T cells and macrophages [70,71]. In many patients, a typical pattern of viral evolution has been documented during the course of the infection with the emergence, usually in concomitance with the earliest signs of disease progression, of CXCR4-using variants. However, this pattern is not consistently observed in all patients progressing to AIDS (**Figure 2**). For example, in patients infected with subtype-B HIV-1, variants that use CXCR4 can be isolated from approximately half of the patients who have developed AIDS [15,24,33,106-109]. Of note,



such variants may first appear during the asymptomatic phase of infection, before AIDS is diagnosed [106], albeit after an initial decline of CD4⁺ T cells [110]. Patients progressing to AIDS often harbor viral populations that can use multiple coreceptors including CCR5, CXCR4 and one or more minor coreceptors [15]. Whether a promiscuous coreceptor usage provides a selective advantage for HIV remains uncertain since most of the minor coreceptors show a low and/or tissue-specific expression pattern.

Until recently, the general consensus was that CXCR4-using variants only emerge in a certain proportion of HIV-1 infected individuals, which varies according to the viral genetic subtype. However, recent evidence has challenged this view suggesting that the prevalence of CXCR4 usage is continuously evolving over time. Data from the Amsterdam Cohort Studies on HIV infection and AIDS (ACS) show a continuously ongoing X4 conversion rate at the population level, even after AIDS diagnosis (H.S. et al., unpublished results). Thus, it seems that the emergence of

CXCR4 usage is a matter of time and that some HIV-1 infected individuals die before they develop CXCR4-using variants. Molecular cloning of the viral quasispecies has documented the frequent presence of DNA sequences predictive of CXCR4 usage in blood cells even in the absence of detectable replication of such variants, as well as transient appearances of CXCR4-using strains on a background of sustained R5 persistence [50,111,112]. These observations further challenge the simplistic concept of a single and irreversible coreceptor-switching event, depicting a complex dynamic state where variant predominance is a continuously evolving process governed by multiple interactive factors. After the appearance of CXCR4-using variants, R5 variants remain present in the vast majority of patients [108,113]. Pure X4 virus populations are rather infrequently detected and often restricted to late-stage disease [114], although they have been documented earlier in rare cases of *ab initio* X4 transmission [70,115,116] often associated with the absence of CCR5 expression in the host [98].

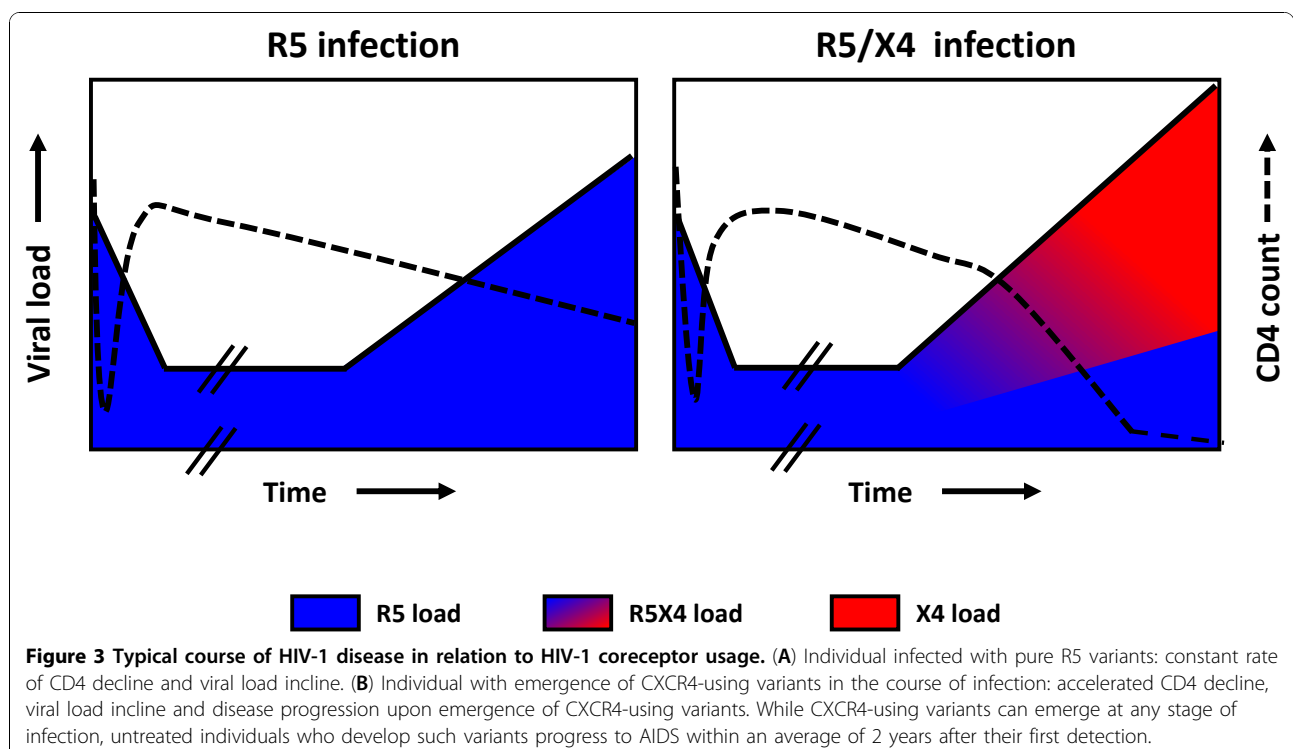
Origin of CXCR4-using HIV-1 variants

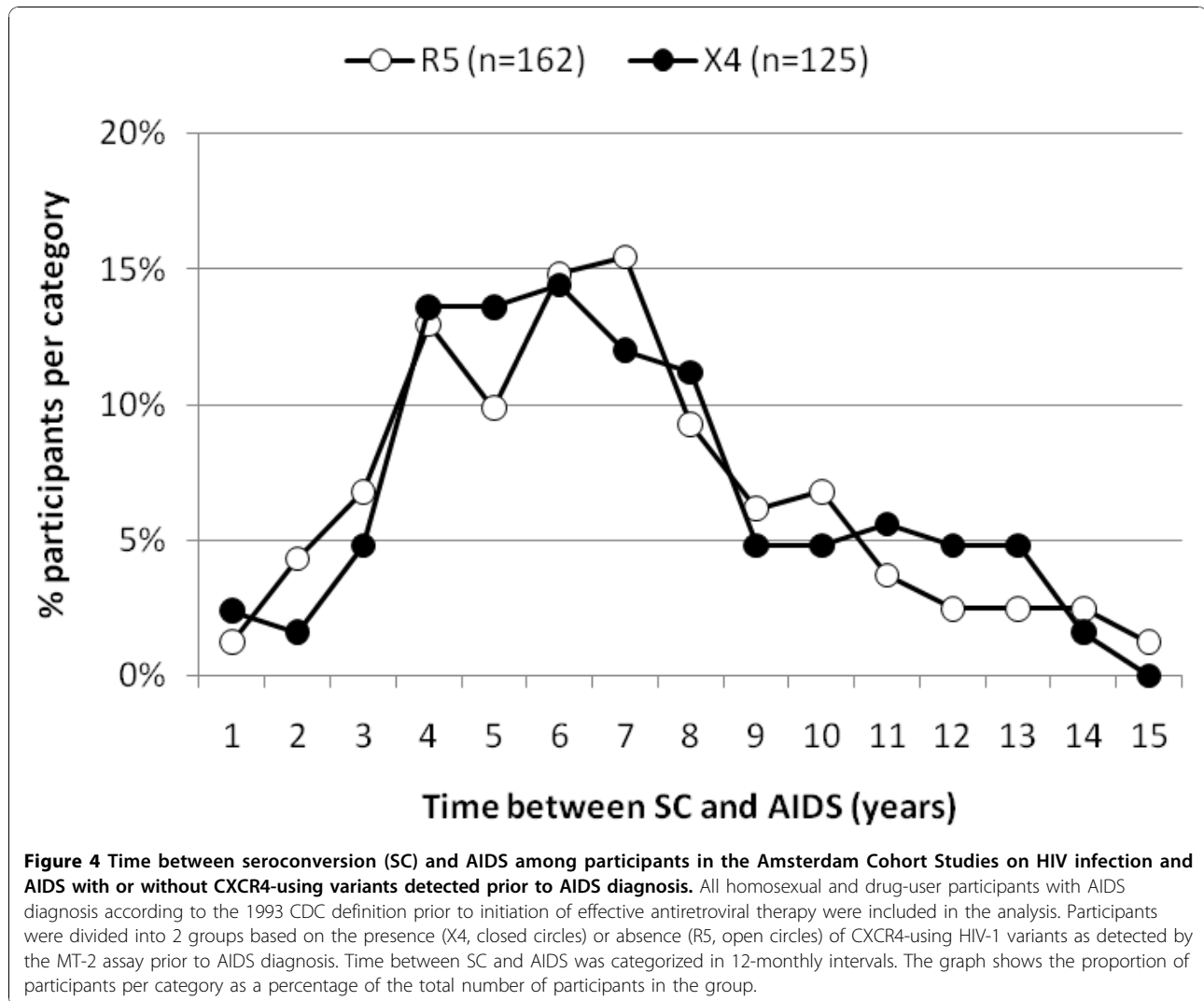
Several lines of evidence suggest that CXCR4-using variants evolve *in vivo* from pre-existing R5 variants. Phylogenetic analysis of longitudinally obtained HIV-1 variants from individual patients has shown that although R5 and X4 variants from a single patient form separate monophyletic clusters in an unrooted tree, they do cluster together when an appropriate out-group is used. Although this implies that R5 and CXCR4-using variants from a single individual are more related to each other than to HIV-1 variants from another person, it does not exclude that both variants may be initially transmitted together and then differentially expressed *in vivo*, with CXCR4-using variants maintained in a cryptic condition until immune deterioration occurs. However, the first detectable CXCR4-using variants are more closely related to the R5 variants obtained at the same time than co-existing R5 and CXCR4-using variants derived at later time points. Moreover, recently emerged CXCR4-using variants generally maintain the ability to use CCR5 and in phylogenetic trees these dual-tropic R5X4 populations cluster between pure R5 and later-stage CXCR4-using virus populations derived from the same individual. These observations are compatible with the hypothesis that the first CXCR4-using variants evolve from pre-existing R5 variants within the same individual, rather than remaining silent for many years after an initial independent transmission.

Clinical significance of CXCR4-using HIV-1 variants: cause or consequence of immune deterioration?

The emergence of CXCR4-using HIV-1 variants in a patient is almost invariably associated with a subsequent increase in the rate of decline of circulating CD4⁺ T cells, an accelerated disease progression, and a poor prognosis for survival [106] (**Figure 3**). Indeed, studies in the era preceding the introduction of combination antiretroviral therapy (cART) demonstrated a significant acceleration in the rate of CD4⁺ T-cell decline after the first detection of CXCR4-using variants [106,107]. However, the presence of CXCR4-using variants is not an obligatory prerequisite for disease progression and a significant proportion of individuals progress to AIDS and AIDS-related death while harboring exclusively R5 HIV-1 variants. Notably, the time from seroconversion to AIDS is not significantly different between individuals who harbor only R5 variants as compared to individuals with detectable CXCR4-using variants (**Figure 4**).

The accelerated CD4⁺ T-cell decline after the first appearance of CXCR4-using HIV-1 variants seems to be determined by the greater proportion of CD4⁺ T cells that express CXCR4, which provides a larger target-cell pool for the virus. Indeed, up to 90% of CD4⁺ T cells express CXCR4 whereas CCR5 expression is restricted to a smaller subset (15-35%) that express a memory/activated phenotype [117-119]. Specifically, CXCR4, but not CCR5, is expressed on naïve CD4⁺ T cells [118], a large population of non-antigen-primed T cells. Thus,





naïve CD4⁺ T cells are exclusively a target for CXCR4-using variants [120]. The pathological consequences of infection of naïve CD4⁺ T cells may be dramatic, resulting in the depletion of the largest pool of CD4⁺ T cells in the body. Considering the potential beneficial effect of an expanded target-cell population, it is puzzling that CXCR4-using variants emerge in only a proportion of infected individuals [106] even though a limited number of amino acid substitutions in V3 is sufficient to confer CXCR4-using capability to R5 variants [38,121].

It has been hypothesized that the absence of CXCR4-using variants early in HIV-1 infection may be due to their higher vulnerability to the host adaptive immune responses, in particular neutralizing antibodies. The fact that these variants appear more frequently after the initial decline of CD4⁺ T cells indeed suggests that they may represent a peculiar, congenic form of opportunistic infection. In a recent study, we found that the first

appearing CXCR4-using variants are more sensitive to neutralizing antibodies directed against the CD4 binding site than their co-existing R5 variants [122]. A greater sensitivity to both monoclonal antibodies and sera from infected patients was also documented in sequential isolates derived from a cohort of perinatally-infected children (P.L. et al., unpublished data). Further evidence for a greater sensitivity of CXCR4-using variants to antibody-mediated neutralization has come from the characterization of a conserved neutralization epitope within the V3 domain of gp120: such epitope, designated D19, is invariably cryptic in R5 variants of different genetic subtypes, but it is consistently exposed in CXCR4-using variants, rendering such variants sensitive to neutralization by a specific antibody [123]. A role of cell-mediated immune responses was instead suggested by the re-emergence of CXCR4-using strains in dually-infected (R5+X4 SHIV) macaques after *in vivo* depletion of CD8⁺

T cells [124]. Additional data pointing to a lower *in vivo* fitness of CXCR4-using variants have come from the results of clinical trials with the CCR5- antagonist maraviroc (MVC). Resistance to MVC was shown to develop by two mechanisms: a reduced drug susceptibility associated with changes in the V3 loop that allow the R5 virus to use CCR5 in its MVC-bound conformation [125] or the emergence of variants that use CXCR4 [58]. In the latter scenario, the CXCR4-using virus seems to originate from an unrecognized pretreatment reservoir, indicating that screening assay sensitivity remains to be improved. Interestingly, however, the circulating virus was shown to revert to the R5 phenotype following cessation of MVC, indicating that the selective pressure acting against CXCR4 usage was preserved.

Altogether, the above observations suggest that effective host immune responses may exert a selective pressure that hinders the emergence of CXCR4-using variants. However, when the host immune competence begins to fade during the progression of HIV-1 disease, such pressure would start to wane, paving the way for the emergence of CXCR4-using variants. Although the host immune surveillance may explain, at least in part, the absence of CXCR4- using variants during the asymptomatic phase of HIV-1 infection, it cannot justify the apparent scarcity of CXCR4-using variants in the very early phase of infection when neutralizing antibodies and cytotoxic T lymphocytes are still absent. As discussed above, this may result from a lack of transmission or to the inability of recently transmitted CXCR4-using virus to compete with co-existing R5 HIV-1 during the earliest phase of infection.

R5 HIV-1 evolution in AIDS_progressors without coreceptor switch

As stated above, a significant fraction of patients progresses to full-blown AIDS without experiencing an overt switch to CXCR4 usage. However, accumulating evidence indicates that in spite of their "monogamous" CCR5 use late isolates from these patients are inherently more pathogenic [126] and RANTES-resistant than early isolates [127,128]. In line with these observation is the ability of late-stage CCR5-restricted HIV-1 variants to use chimeric coreceptors in which parts of CCR5 have been replaced with segments of CXCR4 (R5 broad), whilst early CCR5-using HIV-1 variants are restricted to the use of wild-type CCR5 (R5 narrow) [127,129,130].

This *in vivo* evolution of CCR5-restricted HIV-1 in humans is similar to that observed in nonhuman primates infected with SIV, which never acquires CXCR4 usage even though its pathogenicity increases during the late disease stages [131]. Moreover, we have observed that the ability of R5 isolates to replicate in macrophages is progressively reduced during the course of

infection, resulting in a predominantly T-cell tropic R5 HIV-1 quasispecies even before the progression to AIDS [108]. In about half of subtype-B HIV-1 infected individuals, this shift towards full T-cell tropism precedes the emergence of CXCR4 using HIV-1 variants.

R5X4 HIV-1 variants as an intermediate evolutionary stage

Evidence suggests that the evolutionary changes in the V3 loop involved in the coreceptor-usage switch are gradual and accretive, and that dual coreceptor usage (R5X4) represents an intermediate transitional phase. R5X4 viruses can be more efficient in using either CCR5 (dual-R) or CXCR4 (dual-X), or can use both coreceptors with similar efficiency [5-7]. As mentioned above, there is a continued evolution in viral coreceptor usage *in vivo*, resulting in a broad range of coreceptor affinities within the HIV-1 quasispecies. The limited number of amino acid substitutions required to confer CXCR4-using capability to R5 variants *in vitro* [38,121], combined with the high mutation rate of HIV-1 and the larger population of target cells expressing CXCR4, would predict an early and nearly universal emergence of CXCR4-using variants during chronic infection. However, the fact that CXCR4-using variants seem to develop successfully only once during HIV-1 infection [112,132], the scarcity of R5 virus variants with intermediate genotypes, and the fact that the newly emerged CXCR4-using variants differ from coexisting R5 variants by more than the minimally required number of amino acid mutations [133] altogether suggest that the virus evolves to the CXCR4-using phenotype through less-fit intermediate stages. The late emergence of CXCR4- using variants might be explained by an inability of these intermediate variants to compete with the well-established R5 virus population in spite of their broader cellular host range. Once established, however, R5X4 viruses may have developed the optimal fitness to predominate during the transition phase [134], although they may eventually be outcompeted by HIV-1 variants with a pure X4 phenotype.

Evolution of co-existing R5 and CXCR4-using HIV-1 variants

The progressive divergence between co-existing R5 and CXCR4-using clones in phylogenetic trees reflects the continuous evolution of the variable loops of gp120 following the acquisition of CXCR4 usage. This implies that the structure of gp120 continues to evolve to optimize its interaction with the coreceptors. This evolution is accompanied by improved coreceptor-binding affinity, which in turn is reflected in a decreasing sensitivity of R5 variants to inhibition by CCR5-binding chemokines and small- molecule CCR5 antagonists [127,128,135,136], as well as of CXCR4-using variants to SDF-1 and the synthetic antagonist AMD3100 [137].

Once established, CXCR4-using variants are likely to be more replication competent than R5 variants, given the broader target-cell range and the generally higher replication kinetics of CXCR4-using HIV-1 *in vitro* [132,138,139]. In light of these considerations, it seems paradoxical that R5 variants persist and may even expand *in vivo* after the emergence of CXCR4-using HIV-1 [113]. The most likely explanation for the observed coexistence of R5 and CXCR4-using variants is their distinct target cell range within an infected patient [120,140,141]. R5 variants seem to reside mainly in activated/memory CD45RO⁺CD4⁺ T cells, which express CCR5, whereas CXCR4-using variants reside in both CD45RA⁺ and CD45RO⁺CD4⁺ T cells [120]. Interestingly, evidence for frequent recombination events between co-existing R5 and CXCR4-using variants has been reported [142,143]. While phylogenetic trees based on envelope sequences show a clear-cut separation of coexisting R5 and CXCR4-using virus variants, this separation is not observed when the phylogenetic tree is based on *gag* sequences. Apparently, R5 and CXCR4-using variants can co-infect cells that express both coreceptors, thus allowing for the recombination of their genetic material.

Interestingly, the distribution of R5 and CXCR4-using variants in different blood compartments may vary. Two recent studies reported a higher prevalence of predicted CXCR4-using envelopes in PBMC than in plasma [144,145], although a third study could not confirm this discrepancy [146]. Differences in the sensitivity of the coreceptor usage predictors used may be responsible for the different study outcomes. Thus, the relevance of the choice of patient material for the determination of HIV-1 coreceptor usage remains to be established.

Evolutionary dynamics of coreceptor usage in different HIV-1 subtype epidemics

The different prevalence of CXCR4-using variants among different HIV-1 genetic subtypes remains puzzling. As CXCR4-using variants emerge after an accumulation of mutations, the different prevalence observed among different subtypes and CRFs may reflect the same phenomenon at the population level [147], although a direct relationship between evolutionary rate and development of CXCR4 usage has not been specifically investigated. Based on a series of recent observations, however, it is tempting to speculate that the prevalence of CXCR4-using HIV-1 is increasing with the age of the subtype epidemics (**Figure 5**). Indeed, phylogenetic studies have revealed that the subtype-D epidemic, which has the highest prevalence of CXCR4-using variants, is one of the oldest, while the subtype-C epidemic, which has a much lower prevalence of CXCR4-using variants, is considered one of the most recently emerged [148,149]

(UNAIDS). The subtype-B HIV-1 epidemic has an intermediate pattern, both in terms of age and prevalence of CXCR4-using HIV-1 [150]. This assumption is highly speculative and not supported by all the data available at present. However, if confirmed, it would imply that all the subtype epidemics are evolving towards a higher prevalence of CXCR4-using HIV-1 variants although it is conceivable that each epidemic might reach a point of equilibrium beyond which such prevalence will not further increase.

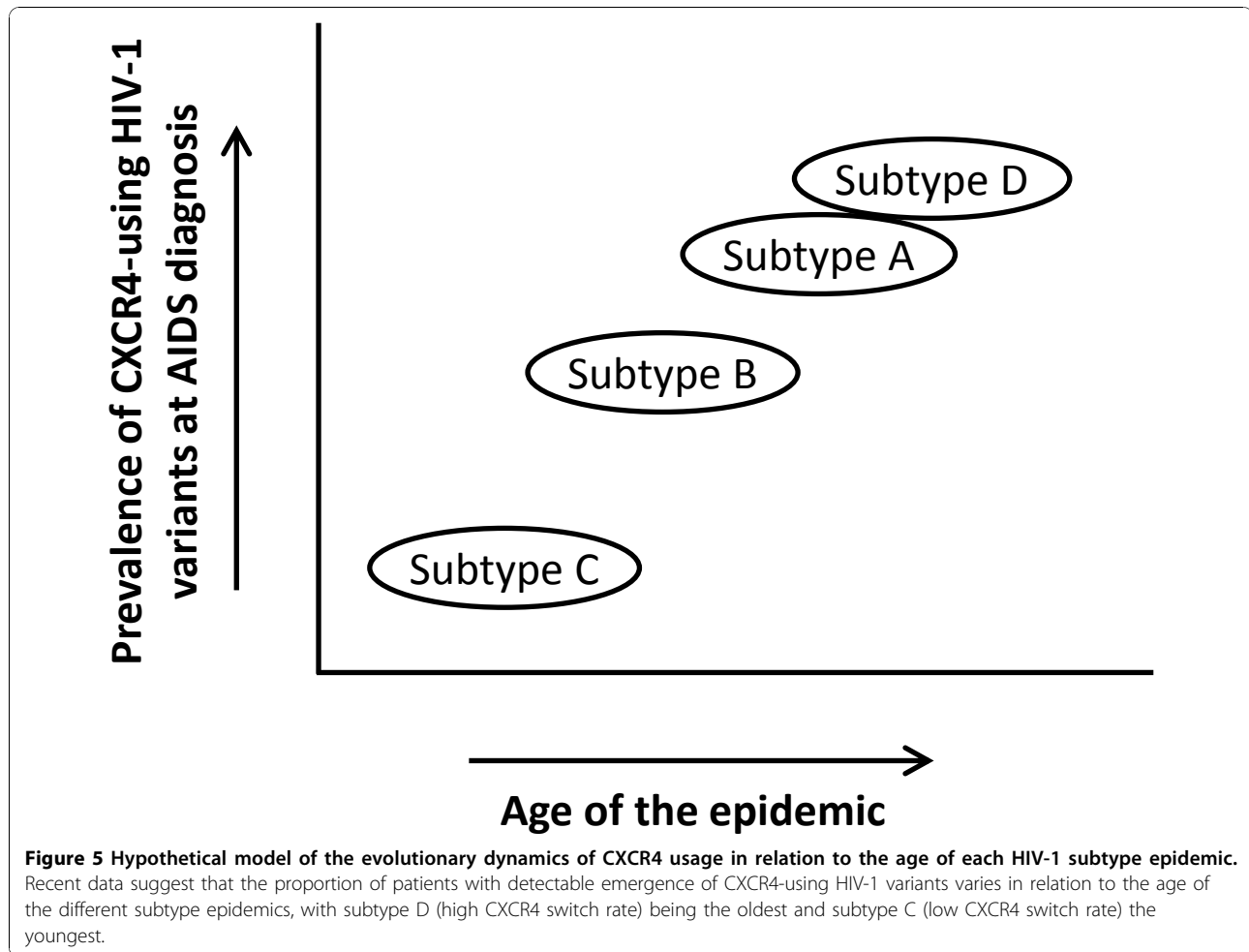
HIV-1 coreceptor usage and antiretroviral therapy

Early studies of zidovudine monotherapy showed that the beneficial effect was mainly limited to persons who do not develop CXCR4-using variants [151,152]. Moreover, a trend towards increased emergence of CXCR4-using variants under zidovudine therapy was observed [151]. Further studies confirmed a differential efficacy of certain HIV-1 reverse transcriptase inhibitors against R5 and CXCR4-using viruses, with zidovudine showing a higher efficacy against R5 viruses and didanosine against CXCR4-using viruses [153,154]. This could be explained by the differential efficiency of activation of zidovudine and didanosine in CCR5-expressing activated memory T cells and CXCR4-expressing naive and resting memory T cells. Drugs that are activated equally in both types of target cells, such as lamivudine, or that did not require activation, such as the protease inhibitor (PI) ritonavir, showed equal activity against both variants [154,155].

Although several studies have reported differential responses to single-agent ART according to coreceptor usage of the HIV-1 variant present in the patient, the association between clinical efficacy of currently used cART regimens and coreceptor usage has not been rigorously evaluated. In 80-90% of asymptomatic treatment-naïve patients, only R5 virus is found [156-159], whereas recent cross-sectional studies demonstrated CXCR4-using virus in 40-55% of patients with previous antiretroviral exposure [114,159-161], probably reflecting the generally lower CD4 counts in individuals who are eligible for initiating cART [162].

Once patients are started on cART, both increased and decreased frequencies of CXCR4-using viruses have been reported [23,163-167], but neither seems to predict treatment success [168]. In patients experiencing virological failure under ART, HIV-1 tropism shifts in either direction (R5>X4 or X4>R5) have been reported in 13.2%–28.6% of subjects [162,169]. For suppressive cART, an early study reported rates of tropism shifts as high as 37.5% [167], but most recent evidence suggests that HIV-1 tropism shifts under suppressive ART are rare (3- 11%) [146,170].

While initial data from the HOMER cohort showed that baseline sequences predictive of CXCR4 usage were



associated with increased risk of clinical progression during cART [171], the presence of CXCR4-using variants at baseline was not predictive of survival or treatment response after adjusting for other baseline parameters [172,173]. Instead, it was significantly associated with lower CD4 counts regardless of antiretroviral treatment exposure. Similar results were recently obtained both in a UK cohort [174] and in a Dutch cohort (ABW et al, unpublished results). It is still unclear whether the effectiveness of different regimens (e.g. PI containing or not) varies according to the coreceptor usage of the HIV-1 variants harbored by the patient at baseline.

Conclusion

The identification of two major HIV-1 coreceptors, CCR5 and CXCR4, the finding of their differential expression on various HIV-1 target cells, and the discovery of viral variants with differential ability to use them have significantly advanced our understanding of the clinical course of HIV-1 infection and the efficacy of

antiviral therapy. The establishment of a connection between HIV-1 and the chemokine system has resulted in the development of a new drug class that directly interferes with CCR5 usage by HIV-1. These drugs not only increase the range of therapeutic options for the systemic treatment of HIV-1-infected individuals, but can also be employed as topical microbicides to prevent HIV-1 acquisition at the mucosal level. Despite these extraordinary successes, many questions regarding the clinical significance of HIV-1 coreceptor usage remain unanswered. Finding an answer to these questions may pave the way toward a deeper understanding of AIDS pathogenesis and a more effective control of the HIV-1 epidemics worldwide.

Acknowledgements

This work was supported in part by the Intramural Research Program of the NIAID, NIH, Bethesda, Maryland. We wish to thank all the individuals who ever worked in the Department of Clinical Viro-Immunology at the Sanquin Blood Supply Foundation and/or at the Laboratory for Viral Immune Pathogenesis at the Academic Medical Center for their helpful discussion on the topic of HIV-1 variability in AIDS pathogenesis. We thank Dr Andrew

Leigh-Brown for his helpful suggestion on the different ages of the HIV-1 subtype epidemics.

This article has been published as part of *Journal of Translational Medicine* Volume 9 Supplement 1, 2011: Differential use of CCR5 vs. CSCR4 by HIV-1. Pathogenic, Translational and Clinical Open Questions. The full contents of the supplement are available online at <http://www.translational-medicine.com/supplements/9/S1>.

Author details

¹Department of Experimental Immunology, Sanquin Research, Landsteiner Laboratory, and Center for Infection and Immunity Amsterdam (CINIMA) at the Academic Medical Center of the University of Amsterdam, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands. ²Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892, USA. ³Present address: Crucell Holland BV, Leiden, The Netherlands.

Competing interests

The authors declare no competing interests.

Published: 27 January 2011

References

- Cocchi F, DeVico AL, Garzino-Demo A, Arya SK, Gallo RC, Lusso P: **Identification of RANTES, MIP-1 alpha, and MIP-1 beta as the major HIV-suppressive factors produced by CD8+ T cells.** *Science* 1995, **270**:1811-1815.
- Feng Y, Broder CC, Kennedy PE, Berger EA: **HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor.** *Science* 1996, **272**:872-877.
- Lusso P: **HIV and the chemokine system: 10 years later.** *Embo J* 2006, **25**:447-456.
- Berger EA, Doms RW, Fenyo EM, Korber BT, Littman DR, Moore JP, Sattentau QJ, Schuitemaker H, Sodroski J, Weiss RA: **A new classification for HIV-1.** *Nature* 1998, **391**:240.
- Huang W, Eshleman SH, Toma J, Fransen S, Stawiski E, Paxinos EE, Whitcomb JM, Young AM, Donnell D, Mmro F, et al: **Coreceptor tropism in human immunodeficiency virus type 1 subtype D: high prevalence of CXCR4 tropism and heterogeneous composition of viral populations.** *J Virol* 2007, **81**:7885-7893.
- Irlbeck DM, Amrine-Madsen H, Kitrinis KM, Labranche CC, Demarest JF: **Chemokine (C-C motif) receptor 5-using envelopes predominate in dual/mixed-tropic HIV from the plasma of drug-naive individuals.** *Aids* 2008, **22**:1425-1431.
- Yi Y, Isaacs SN, Williams DA, Frank I, Schols D, De Clercq E, Kolson DL, Collman RG: **Role of CXCR4 in cell-cell fusion and infection of monocyte-derived macrophages by primary human immunodeficiency virus type 1 (HIV-1) strains: two distinct mechanisms of HIV-1 dual tropism.** *J Virol* 1999, **73**:7117-7125.
- Deng H, Liu R, Ellmeier W, Choe S, Unutmaz D, Burkhardt M, Di Marzio P, Marmon S, Sutton RE, Hill CM, et al: **Identification of a major co-receptor for primary isolates of HIV-1.** *Nature* 1996, **381**:661-666.
- Cecilia D, KewalRamani VN, O'Leary J, Volsky B, Nyambi P, Burda S, Xu S, Littman DR, Zolla-Pazner S: **Neutralization profiles of primary human immunodeficiency virus type 1 isolates in the context of coreceptor usage.** *J Virol* 1998, **72**:6988-6996.
- Whitcomb JM, Huang W, Fransen S, Limoli K, Toma J, Wrin T, Chappay C, Kiss LD, Paxinos EE, Petropoulos CJ: **Development and characterization of a novel single-cycle recombinant-virus assay to determine human immunodeficiency virus type 1 coreceptor tropism.** *Antimicrob Agents Chemother* 2007, **51**:566-575.
- Coakley E, Reeves JD, Huang W, Mangas-Ruiz M, Maurer I, Harskamp AM, Gupta S, Lie Y, Petropoulos CJ, Schuitemaker H, van 't Wout AB: **Comparison of human immunodeficiency virus type 1 tropism profiles in clinical samples by the Trofile and MT-2 assays.** *Antimicrob Agents Chemother* 2009, **53**:4686-4693.
- De Wolf F, Hogervorst E, Goudsmit J, Fenyo EM, Rubsamen-Waigmann H, Holmes H, Galvao-Castro B, Karita E, Wasi C, Sempala SD, et al: **Syncytium-inducing and non-syncytium-inducing capacity of human immunodeficiency virus type 1 subtypes other than B: phenotypic and genotypic characteristics.** *WHO Network for HIV Isolation and Characterization. AIDS Res Hum Retroviruses* 1994, **10**:1387-1400.
- Zhang YJ, Dragic T, Cao Y, Kostrikis L, Kwon DS, Littman DR, KewalRamani VN, Moore JP: **Use of coreceptors other than CCR5 by non-syncytium-inducing adult and pediatric isolates of human immunodeficiency virus type 1 is rare in vitro.** *J Virol* 1998, **72**:9337-9344.
- 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**:621-628.
- Scarlatti G, Tresoldi E, Bjorndal A, Fredriksson R, Colognesi C, Deng HK, Malnati MS, Plebani A, Siccardi AG, Littman DR, et al: **In vivo evolution of HIV-1 co-receptor usage and sensitivity to chemokine-mediated suppression.** *Nat Med* 1997, **3**:1259-1265.
- Abebe A, Demissie D, Goudsmit J, Brouwer M, Kuiken CL, Pollakis G, Schuitemaker H, Fontanet AL, Rinke de Wit TF: **HIV-1 subtype C syncytium- and non-syncytium-inducing phenotypes and coreceptor usage among Ethiopian patients with AIDS.** *Aids* 1999, **13**:1305-1311.
- Batra M, Tien PC, Shafer RW, Contag CH, Katzenstein DA: **HIV type 1 envelope subtype C sequences from recent seroconverters in Zimbabwe.** *AIDS Res Hum Retroviruses* 2000, **16**:973-979.
- Bjorndal A, Sonnerborg A, Tscherning C, Albert J, Fenyo EM: **Phenotypic characteristics of human immunodeficiency virus type 1 subtype C isolates of Ethiopian AIDS patients.** *AIDS Res Hum Retroviruses* 1999, **15**:647-653.
- Peeters M, Vincent R, Perret JL, Lasky M, Patrel D, Liegeois F, Courgnaud V, Seng R, Matton T, Molinier S, Delaporte E: **Evidence for differences in MT2 cell tropism according to genetic subtypes of HIV-1: syncytium-inducing variants seem rare among subtype C HIV-1 viruses.** *J Acquir Immune Defic Syndr Hum Retrovirol* 1999, **20**:115-121.
- Ping LH, Nelson JA, Hoffman IF, Schock J, Lamers SL, Goodman M, Vernazza P, Kazembe P, Maida M, Zimba D, et al: **Characterization of V3 sequence heterogeneity in subtype C human immunodeficiency virus type 1 isolates from Malawi: underrepresentation of X4 variants.** *J Virol* 1999, **73**:6271-6281.
- Tscherning C, Alaeus A, Fredriksson R, Bjorndal A, Deng H, Littman DR, Fenyo EM, Albert J: **Differences in chemokine coreceptor usage between genetic subtypes of HIV-1.** *Virology* 1998, **241**:181-188.
- Hemelaar J, Gouws E, Ghys PD, Osmanov S: **Global and regional distribution of HIV-1 genetic subtypes and recombinants in 2004.** *Aids* 2006, **20**:W13-23.
- Johnston ER, Zijenah LS, Mutetwa S, Kantor R, Kittinunvorakoon C, Katzenstein DA: **High frequency of syncytium-inducing and CXCR4-tropic viruses among human immunodeficiency virus type 1 subtype C-infected patients receiving antiretroviral treatment.** *J Virol* 2003, **77**:7682-7688.
- Connell BJ, Michler K, Capovilla A, Venter WD, Stevens WS, Papathanasopoulos MA: **Emergence of X4 usage among HIV-1 subtype C: evidence for an evolving epidemic in South Africa.** *Aids* 2008, **22**:896-899.
- Kaleebu P, Nankya IL, Yirrell DL, Shafer LA, Kyosimire-Lugemwa J, Lule DB, Morgan D, Beddows S, Weber J, Whitworth JA: **Relation between chemokine receptor use, disease stage, and HIV-1 subtypes A and D: results from a rural Ugandan cohort.** *J Acquir Immune Defic Syndr* 2007, **45**:28-33.
- Baeten JM, Chohan B, Lavreys L, Chohan V, McClelland RS, Certain L, Mandaliya K, Jaoko W, Overbaugh J: **HIV-1 subtype D infection is associated with faster disease progression than subtype A in spite of similar plasma HIV-1 loads.** *J Infect Dis* 2007, **195**:1177-1180.
- Vasan A, Renjifo B, Hertzmark E, Chaplin B, Msamanga G, Essex M, Fawzi W, Hunter D: **Different rates of disease progression of HIV type 1 infection in Tanzania based on infecting subtype.** *Clin Infect Dis* 2006, **42**:843-852.
- Easterbrook PJ, Smith M, Mullen J, O'Shea S, Chrystie I, de Ruiter A, Tatt ID, Geretti AM, Zuckerman M: **Impact of HIV-1 viral subtype on disease progression and response to antiretroviral therapy.** *J Int AIDS Soc* 13:4.
- Yu XF, Wang Z, Beyrer C, Celentano DD, Khambonruang C, Allen E, Nelson K: **Phenotypic and genotypic characteristics of human immunodeficiency virus type 1 from patients with AIDS in northern Thailand.** *J Virol* 1995, **69**:4649-4655.
- Utaipat U, Duerr A, Rudolph DL, Yang C, Butera ST, Lupo D, Pisell T, Tangmunkongvorakul A, Kamtorn N, Nantachit N, et al: **Coreceptor utilization of HIV type 1 subtype E viral isolates from Thai men with HIV**

- type 1-infected and uninfected wives. *AIDS Res Hum Retroviruses* 2002, **18**:1-11.
31. Tebit DM, Zekeng L, Kaptue L, Salminen M, Krausslich HG, Herchenroder O: **Genotypic and phenotypic analysis of HIV type 1 primary isolates from western Cameroon.** *AIDS Res Hum Retroviruses* 2002, **18**:39-48.
 32. Zhong P, S BU, Konings F, Urbanski M, Ma L, Zekeng L, Ewane L, Agyingi L, Agwara M, Saa , *et al*: **Genetic and biological properties of HIV type 1 isolates prevalent in villagers of the Cameroon equatorial rain forests and grass fields: further evidence of broad HIV type 1 genetic diversity.** *AIDS Res Hum Retroviruses* 2003, **19**:1167-1178.
 33. Esbjornsson J, Mansson F, Martinez-Arias W, Vincic E, Biague AJ, da Silva ZJ, Fenyo EM, Norrgren H, Medstrand P: **Frequent CXCR4 tropism of HIV-1 subtype A and CRF02_AG during late-stage disease—indication of an evolving epidemic in West Africa.** *Retrovirology* 2010, **7**:23.
 34. Chesebro B, Nishio J, Peryman S, Cann A, O'Brien W, Chen IS, Wehrly K: **Identification of human immunodeficiency virus envelope gene sequences influencing viral entry into CD4-positive HeLa cells, T-leukemia cells, and macrophages.** *J Virol* 1991, **65**:5782-5789.
 35. Groenink M, Fouchier RA, Broersen S, Baker CH, Koot M, van't Wout AB, Huisman HG, Miedema F, Tersmette M, Schuitemaker H: **Relation of phenotype evolution of HIV-1 to envelope V2 configuration.** *Science* 1993, **260**:1513-1516.
 36. O'Brien WA, Koyanagi Y, Namazie A, Zhao JQ, Digne A, Idler K, Zack JA, Chen IS: **HIV-1 tropism for mononuclear phagocytes can be determined by regions of gp120 outside the CD4-binding domain.** *Nature* 1990, **348**:69-73.
 37. Shioda T, Levy JA, Cheng-Mayer C: **Small amino acid changes in the V3 hypervariable region of gp120 can affect the T-cell-line and macrophage tropism of human immunodeficiency virus type 1.** *Proc Natl Acad Sci U S A* 1992, **89**:9434-9438.
 38. De Jong JJ, De Ronde A, Keulen W, Tersmette M, Goudsmit J: **Minimal requirements for the human immunodeficiency virus type 1 V3 domain to support the syncytium- inducing phenotype: analysis by single amino acid substitution.** *J Virol* 1992, **66**:6777-6780.
 39. Fouchier RA, Groenink M, Kootstra NA, Tersmette M, Huisman HG, Miedema F, Schuitemaker H: **Phenotype-associated sequence variation in the third variable domain of the human immunodeficiency virus type 1 gp120 molecule.** *J Virol* 1992, **66**:3183-3187.
 40. Cho MW, Lee MK, Carney MC, Berson JF, Doms RW, Martin MA: **Identification of determinants on a dualtropic human immunodeficiency virus type 1 envelope glycoprotein that confer usage of CXCR4.** *J Virol* 1998, **72**:2509-2515.
 41. Hoffman TL, Stephens EB, Narayan O, Doms RW: **HIV type I envelope determinants for use of the CCR2b, CCR3, STRL33, and APJ coreceptors.** *Proc Natl Acad Sci U S A* 1998, **95**:11360-11365.
 42. Ross TM, Cullen BR: **The ability of HIV type 1 to use CCR-3 as a coreceptor is controlled by envelope V1/V2 sequences acting in conjunction with a CCR-5 tropic V3 loop.** *Proc Natl Acad Sci U S A* 1998, **95**:7682-7686.
 43. Kwong PD, Wyatt R, Robinson J, Sweet RW, Sodroski J, Hendrickson WA: **Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody.** *Nature* 1998, **393**:648-659.
 44. Kwong PD, Wyatt R, Sattentau QJ, Sodroski J, Hendrickson WA: **Oligomeric modeling and electrostatic analysis of the gp120 envelope glycoprotein of human immunodeficiency virus.** *J Virol* 2000, **74**:1961-1972.
 45. Moulard M, Lortat-Jacob H, Mondor I, Roca G, Wyatt R, Sodroski J, Zhao L, Olson W, Kwong PD, Sattentau QJ: **Selective interactions of polyanions with basic surfaces on human immunodeficiency virus type 1 gp120.** *J Virol* 2000, **74**:1948-1960.
 46. Rizzuto CD, Wyatt R, Hernandez-Ramos N, Sun Y, Kwong PD, Hendrickson WA, Sodroski J: **A conserved HIV gp120 glycoprotein structure involved in chemokine receptor binding.** *Science* 1998, **280**:1949-1953.
 47. Coetzer M, Cilliers T, Ping LH, Swanstrom R, Morris L: **Genetic characteristics of the V3 region associated with CXCR4 usage in HIV-1 subtype C isolates.** *Virology* 2006, **356**:95-105.
 48. Jensen MA, Coetzer M, van 't Wout AB, Morris L, Mullins JL: **A reliable phenotype predictor for human immunodeficiency virus type 1 subtype C based on envelope V3 sequences.** *J Virol* 2006, **80**:4698-4704.
 49. Boisvert S, Marchand M, Lavolette F, Corbeil J: **HIV-1 coreceptor usage prediction without multiple alignments: an application of string kernels.** *Retrovirology* 2008, **5**:110.
 50. Jensen MA, Li FS, van 't Wout AB, Nickle DC, Shriner D, He HX, McLaughlin S, Shankarappa R, Margolick JB, Mullins JL: **Improved coreceptor usage prediction and genotypic monitoring of R5-to-X4 transition by motif analysis of human immunodeficiency virus type 1 env V3 loop sequences.** *J Virol* 2003, **77**:13376-13388.
 51. Pillai S, Good B, Richman D, Corbeil J: **A new perspective on V3 phenotype prediction.** *AIDS Res Hum Retroviruses* 2003, **19**:145-149.
 52. Resch W, Hoffman N, Swanstrom R: **Improved success of phenotype prediction of the human immunodeficiency virus type 1 from envelope variable loop 3 sequence using neural networks.** *Virology* 2001, **288**:51-62.
 53. Xu S, Huang X, Xu H, Zhang C: **Improved prediction of coreceptor usage and phenotype of HIV-1 based on combined features of V3 loop sequence using random forest.** *J Microbiol* 2007, **45**:441-446.
 54. Hoffman NG, Seillier-Moiseiwitsch F, Ahn J, Walker JM, Swanstrom R: **Variability in the human immunodeficiency virus type 1 gp120 Env protein linked to phenotype- associated changes in the V3 loop.** *J Virol* 2002, **76**:3852-3864.
 55. Sing T, Low AJ, Beerenwinkel N, Sander O, Cheung PK, Domingues FS, Buch J, Daumer M, Kaiser R, Lengauer T, Harrigan PR: **Predicting HIV coreceptor usage on the basis of genetic and clinical covariates.** *Antivir Ther* 2007, **12**:1097-1106.
 56. Sander O, Sing T, Sommer I, Low AJ, Cheung PK, Harrigan PR, Lengauer T, Domingues FS: **Structural descriptors of gp120 V3 loop for the prediction of HIV-1 coreceptor usage.** *PLoS Comput Biol* 2007, **3**:e58.
 57. Lamers SL, Salemi M, McGrath MS, Fogel GB: **Prediction of R5, X4, and RSX4 HIV-1 coreceptor usage with evolved neural networks.** *IEEE/ACM Trans Comput Biol Bioinform* 2008, **5**:291-300.
 58. Westby M, Lewis M, Whitcomb J, Youle M, Pozniak AL, James IT, Jenkins TM, Perros M, van der Ryst E: **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**:4909-4920.
 59. Huang W, Toma J, Fransen S, Stawiski E, Reeves JD, Whitcomb JM, Parkin N, Petropoulos CJ: **Coreceptor tropism can be influenced by amino acid substitutions in the gp41 transmembrane subunit of human immunodeficiency virus type 1 envelope protein.** *J Virol* 2008, **82**:5584-5593.
 60. Gaschen B, Taylor J, Yusim K, Foley B, Gao F, Lang D, Novitsky V, Haynes B, Hahn BH, Bhattacharya T, Korber B: **Diversity considerations in HIV-1 vaccine selection.** *Science* 2002, **296**:2354-2360.
 61. Swenson LC, Moores A, Low AJ, Thielen A, Dong W, Woods C, Jensen MA, Wynhoven B, Chan D, Glascock C, Harrigan PR: **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.
 62. Fatkenheuer G, Nelson M, Lazzarin A, Konourina I, Hoepelman AI, Lampiris H, Hirschel B, Tebas P, Raffi F, Trottier B, *et al*: **Subgroup analyses of maraviroc in previously treated R5 HIV-1 infection.** *N Engl J Med* 2008, **359**:1442-1455.
 63. Gulick RM, Lalezari J, Goodrich J, Clumeck N, DeJesus E, Horban A, Nadler J, Clotet B, Karlsson A, Wohlfeiler M, *et al*: **Maraviroc for previously treated patients with R5 HIV-1 infection.** *N Engl J Med* 2008, **359**:1429-1441.
 64. Su Z, Gulick RM, Krambrink A, Coakley E, Hughes MD, Han D, Flexner C, Wilkin TJ, Skolnik PR, Greaves WL, *et al*: **Response to vicriviroc in treatment-experienced subjects, as determined by an enhanced-sensitivity coreceptor tropism assay: reanalysis of AIDS clinical trials group A5211.** *J Infect Dis* 2009, **200**:1724-1728.
 65. McGovern RA, Dong W, Zhong X, Mo T, Knapp D, Thielen A, Chapman D, Lewis M, James IT, Heera J, *et al*: **Population-based sequencing of the V3-loop is comparable to the enhanced sensitivity Trofile assay in predicting virologic response to maraviroc of treatment-naïve patients in the MERIT trial.** *In 17th Conference on Retroviruses and Opportunistic Infections; San Francisco, CA* 2010.
 66. Swenson LC, Dong W, Mo T, Thielen A, Jensen MA, Chapman D, James IT, Heera J, Valdez H, Harrigan PR: **Large-scale application of deep sequencing using 454 technology to HIV tropism screening.** *In 17th*

- Conference on Retroviruses and Opportunistic Infections; San Francisco, CA* 2010.
67. Keele BF, Giorgi EE, Salazar-Gonzalez JF, Decker JM, Pham KT, Salazar MG, Sun C, Grayson T, Wang S, Li H, *et al*: **Identification and characterization of transmitted and early founder virus envelopes in primary HIV-1 infection.** *Proc Natl Acad Sci U S A* 2008, **105**:7552-7557.
 68. Salazar-Gonzalez JF, Bailes E, Pham KT, Salazar MG, Guffey MB, Keele BF, Derdeyn CA, Farmer P, Hunter E, Allen S, *et al*: **Deciphering human immunodeficiency virus type 1 transmission and early envelope diversification by single-genome amplification and sequencing.** *J Virol* 2008, **82**:3952-3970.
 69. Long EM, Rainwater SM, Lavreys L, Mandaliya K, Overbaugh J: **HIV type 1 variants transmitted to women in Kenya require the CCR5 coreceptor for entry, regardless of the genetic complexity of the infecting virus.** *AIDS Res Hum Retroviruses* 2002, **18**:567-576.
 70. van 't Wout AB, Kootstra NA, Mulder-Kampinga GA, Albrecht-van Lent N, Scherpbier HJ, Veenstra J, Boer K, Coutinho RA, Miedema F, Schuitemaker H: **Macrophage-tropic variants initiate human immunodeficiency virus type 1 infection after sexual, parenteral, and vertical transmission.** *J Clin Invest* 1994, **94**:2060-2067.
 71. Zhu T, Mo H, Wang N, Nam DS, Cao Y, Koup RA, Ho DD: **Genotypic and phenotypic characterization of HIV-1 patients with primary infection.** *Science* 1993, **261**:1179-1181.
 72. Margolis J, Shattock R: **Selective transmission of CCR5-utilizing HIV-1: the 'gatekeeper' problem resolved?** *Nat Rev Microbiol* 2006, **4**:312-317.
 73. Harouse JM, Tan RC, Gettie A, Dailey P, Marx PA, Luciw PA, Cheng-Mayer C: **Mucosal transmission of pathogenic CXCR4-utilizing SHIVSF33A variants in rhesus macaques.** *Virology* 1998, **248**:95-107.
 74. Dukers NH, Goudsmit J, de Wit JB, Prins M, Weverling GJ, Coutinho RA: **Sexual risk behaviour relates to the virological and immunological improvements during highly active antiretroviral therapy in HIV-1 infection.** *Aids* 2001, **15**:369-378.
 75. Veazey RS, DeMaria M, Chalifoux LV, Shvetz DE, Pauley DR, Knight HL, Rosenzweig M, Johnson RP, Desrosiers RC, Lackner AA: **Gastrointestinal tract as a major site of CD4+ T cell depletion and viral replication in SIV infection.** *Science* 1998, **280**:427-431.
 76. Arthos J, Cicala C, Martinelli E, Macleod K, Van Ryk D, Wei D, Xiao Z, Veenstra TD, Conrad TP, Lempicki RA, *et al*: **HIV-1 envelope protein binds to and signals through integrin alpha4beta7, the gut mucosal homing receptor for peripheral T cells.** *Nat Immunol* 2008, **9**:301-309.
 77. Cornelissen M, Mulder-Kampinga G, Veenstra J, Zorgdrager F, Kuiken C, Hartman S, Dekker J, van der Hoek L, Sol C, Coutinho R, *et al*: **Syncytium-inducing (SI) phenotype suppression at seroconversion after intramuscular inoculation of a non-syncytium-inducing/SI phenotypically mixed human immunodeficiency virus population.** *J Virol* 1995, **69**:1810-1818.
 78. Lathey JL, Pratt RD, Spector SA: **Appearance of autologous neutralizing antibody correlates with reduction in virus load and phenotype switch during primary infection with human immunodeficiency virus type 1.** *J Infect Dis* 1997, **175**:231-232.
 79. Frange P, Galimand J, Goujard C, Deveau C, Ghosn J, Rouzioux C, Meyer L, Chaix ML: **High frequency of X4/DM-tropic viruses in PBMC samples from patients with primary HIV-1 subtype-B infection in 1996-2007: the French ANRS CO06 PRIMO Cohort Study.** *J Antimicrob Chemother* 2009, **64**:135-141.
 80. Frange P, Chaix ML, Raymond S, Galimand J, Deveau C, Meyer L, Goujard C, Rouzioux C, Izopet J: **Low frequency of CXCR4-using viruses in patients at the time of primary HIV-1 non-B infection.** *J Clin Microbiol* 2010.
 81. Huang W, Toma J, Stawiski E, Fransen S, Wrin T, Parkin N, Whitcomb JM, Coakley E, Hecht FM, Deeks SG, *et al*: **Characterization of human immunodeficiency virus type 1 populations containing CXCR4-using variants from recently infected individuals.** *AIDS Res Hum Retroviruses* 2009, **25**:795-802.
 82. Raymond S, Delobel P, Mavigner M, Cazabat M, Encinas S, Souyris C, Bruel P, Sandres-Saune K, Marchou B, Massip P, Izopet J: **CXCR4-using viruses in plasma and peripheral blood mononuclear cells during primary HIV-1 infection and impact on disease progression.** *Aids* 2009, **23**:2305-2312.
 83. Abrahams MR, Anderson JA, Giorgi EE, Seoighe C, Mlisana K, Ping LH, Athreya GS, Treurnicht FK, Keele BF, Wood N, *et al*: **Quantitating the multiplicity of infection with human immunodeficiency virus type 1 subtype C reveals a non-poisson distribution of transmitted variants.** *J Virol* 2009, **83**:3556-3567.
 84. Bar KJ, Li H, Chamberland A, Tremblay C, Routy JP, Grayson T, Sun C, Wang S, Learn GH, Morgan CJ, *et al*: **Wide variation in the multiplicity of HIV-1 infection among injection drug users.** *J Virol* 2010, **84**:6241-6247.
 85. Haaland RE, Hawkins PA, Salazar-Gonzalez J, Johnson A, Tichacek A, Karita E, Manigart O, Mulenga J, Keele BF, Shaw GM, *et al*: **Inflammatory genital infections mitigate a severe genetic bottleneck in heterosexual transmission of subtype A and C HIV-1.** *PLoS Pathog* 2009, **5**:e1000274.
 86. Li H, Bar KJ, Wang S, Decker JM, Chen Y, Sun C, Salazar-Gonzalez JF, Salazar MG, Learn GH, Morgan CJ, *et al*: **High Multiplicity Infection by HIV-1 in Men Who Have Sex with Men.** *PLoS Pathog* 2010, **6**:e1000890.
 87. Masharsky AE, Dukhovlinova EN, Verevchkin SV, Tousseva OV, Skochilov RV, Anderson JA, Hoffman I, Cohen MS, Swanson R, Kozlov AP: **A substantial transmission bottleneck among newly and recently HIV-1-infected injection drug users in St Petersburg, Russia.** *J Infect Dis* 2010, **201**:1697-1702.
 88. Salazar-Gonzalez JF, Salazar MG, Keele BF, Learn GH, Giorgi EE, Li H, Decker JM, Wang S, Baalwa J, Kraus MH, *et al*: **Genetic identity, biological phenotype, and evolutionary pathways of transmitted/founder viruses in acute and early HIV-1 infection.** *J Exp Med* 2009, **206**:1273-1289.
 89. Liu R, Paxton WA, Choe S, Ceradini D, Martin SR, Horuk R, MacDonald ME, Stuhlmann H, Koup RA, Landau NR: **Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection.** *Cell* 1996, **86**:367-377.
 90. Dean M, Carrington M, Winkler C, Huttley GA, Smith MW, Allikmets R, Goedert JJ, Buchbinder SP, Vittinghoff E, Gomperts E, *et al*: **Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CCR5 structural gene. Hemophilia Growth and Development Study, Multicenter AIDS Cohort Study, Multicenter Hemophilia Cohort Study, San Francisco City Cohort, ALIVE Study.** *Science* 1996, **273**:1856-1862.
 91. Samson M, Libert F, Doranz BJ, Rucker J, Liesnard C, Farber CM, Saragosti S, Lapoumeroulie C, Cognaux J, Forceille C, *et al*: **Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene.** *Nature* 1996, **382**:722-725.
 92. Kupfer B, Kaiser R, Brackmann HH, Effenberger W, Rockstroh JK, Matz B, Schneeweis KE: **Protection against parenteral HIV-1 infection by homozygous deletion in the C-C chemokine receptor 5 gene.** *Aids* 1999, **13**:1025-1028.
 93. de Roda Husman AM, Koot M, Cornelissen M, Keet IP, Brouwer M, Broersen SM, Bakker M, Roos MT, Prins M, de Wolf F, *et al*: **Association between CCR5 genotype and the clinical course of HIV-1 infection.** *Ann Intern Med* 1997, **127**:882-890.
 94. Ioannidis JP, Rosenberg PS, Goedert JJ, Ashton LJ, Benfield TL, Buchbinder SP, Coutinho RA, Eugen-Olsen J, Gallart T, Katzenstein TL, *et al*: **Effects of CCR5-Delta32, CCR2-64I, and SDF-1 3'A alleles on HIV-1 disease progression: An international meta-analysis of individual-patient data.** *Ann Intern Med* 2001, **135**:782-795.
 95. Michael NL, Chang G, Louie LG, Mascola JR, Dondero D, Birx DL, Sheppard HW: **The role of viral phenotype and CCR-5 gene defects in HIV-1 transmission and disease progression.** *Nat Med* 1997, **3**:338-340.
 96. Michael NL, Louie LG, Rohrbaugh AL, Schultz KA, Dayhoff DE, Wang CE, Sheppard HW: **The role of CCR5 and CCR2 polymorphisms in HIV-1 transmission and disease progression.** *Nat Med* 1997, **3**:1160-1162.
 97. Quillent C, Oberlin E, Braun J, Rousset D, Gonzalez-Canali G, Metais P, Montagnier L, Virelizier JL, Arenzana-Seisdedos F, Beretta A: **HIV-1-resistance phenotype conferred by combination of two separate inherited mutations of CCR5 gene.** *Lancet* 1998, **351**:14-18.
 98. Sheppard HW, Celum C, Michael NL, O'Brien S, Dean M, Carrington M, Dondero D, Buchbinder SP: **HIV-1 infection in individuals with the CCR5-Delta32/Delta32 genotype: acquisition of syncytium-inducing virus at seroconversion.** *J Acquir Immune Defic Syndr* 2002, **29**:307-313.
 99. Gray L, Churchill MJ, Keane N, Sterjovski J, Ellett AM, Purcell DF, Pombourios P, Kol C, Wang B, Saksena NK, *et al*: **Genetic and functional analysis of R5X4 human immunodeficiency virus type 1 envelope glycoproteins derived from two individuals homozygous for the CCR5delta32 allele.** *J Virol* 2006, **80**:3684-3691.
 100. Dalmau J, Puertas MC, Azuara M, Marino A, Frahm N, Mothe B, Izquierdo-Useros N, Buzon MJ, Paredes R, Matas L, *et al*: **Contribution of**

- immunological and virological factors to extremely severe primary HIV type 1 infection. *Clin Infect Dis* 2009, **48**:229-238.
101. Markowitz M, Mohri H, Mehndru S, Shet A, Berry L, Kalyanaraman R, Kim A, Chung C, Jean-Pierre P, Horowitz A, et al: **Infection with multidrug resistant, dual-tropic HIV-1 and rapid progression to AIDS: a case report.** *Lancet* 2005, **365**:1031-1038.
102. Mohri H, Markowitz M: **In vitro characterization of multidrug-resistant HIV-1 isolates from a recently infected patient associated with dual tropism and rapid disease progression.** *J Acquir Immune Defic Syndr* 2008, **48**:511-521.
103. Nielsen C, Pedersen C, Lundgren JD, Gerstoft J: **Biological properties of HIV isolates in primary HIV infection: consequences for the subsequent course of infection.** *Aids* 1993, **7**:1035-1040.
104. Aasa-Chapman MM, Aubin K, Williams I, McKnight A: **Primary CCR5 only using HIV-1 isolates does not accurately represent the in vivo replicating quasi-species.** *Virology* 2006, **351**:489-496.
105. Isaacman-Beck J, Hermann EA, Yi Y, Ratcliffe SJ, Mulenga J, Allen S, Hunter E, Derdeyn CA, Collman RG: **Heterosexual transmission of human immunodeficiency virus type 1 subtype C: Macrophage tropism, alternative coreceptor use, and the molecular anatomy of CCR5 utilization.** *J Virol* 2009, **83**:8208-8220.
106. Koot M, Keet IP, Vos AH, de Goede RE, Roos MT, Coutinho RA, Miedema F, Schellekens PT, Tersmette M: **Prognostic value of HIV-1 syncytium-inducing phenotype for rate of CD4+ cell depletion and progression to AIDS.** *Ann Intern Med* 1993, **118**:681-688.
107. Richman DD, Bozzette SA: **The impact of the syncytium-inducing phenotype of human immunodeficiency virus on disease progression.** *J Infect Dis* 1994, **169**:968-974.
108. Schuitemaker H, Koot M, Kootstra NA, Dercksen MW, de Goede RE, van Steenwijk RP, Lange JM, Schattenkerk JK, Miedema F, Tersmette M: **Biological phenotype of human immunodeficiency virus type 1 clones at different stages of infection: progression of disease is associated with a shift from monocytotropic to T-cell-tropic virus population.** *J Virol* 1992, **66**:1354-1360.
109. Koot M, Vos AH, Keet RP, de Goede RE, Dercksen MW, Terpstra FG, Coutinho RA, Miedema F, Tersmette M: **HIV-1 biological phenotype in long-term infected individuals evaluated with an MT-2 cocultivation assay.** *Aids* 1992, **6**:49-54.
110. Koot M, van Leeuwen R, de Goede RE, Keet IP, Danner S, Eeftinck Schattenkerk JK, Reiss P, Tersmette M, Lange JM, Schuitemaker H: **Conversion rate towards a syncytium-inducing (SI) phenotype during different stages of human immunodeficiency virus type 1 infection and prognostic value of SI phenotype for survival after AIDS diagnosis.** *J Infect Dis* 1999, **179**:254-258.
111. Ida S, Gatanaga H, Shioda T, Nagai Y, Kobayashi N, Shimada K, Kimura S, Iwamoto A, Oka S: **HIV type 1 V3 variation dynamics in vivo: long-term persistence of non-syncytium-inducing genotypes and transient presence of syncytium-inducing genotypes during the course of progressive AIDS.** *AIDS Res Hum Retroviruses* 1997, **13**:1597-1609.
112. Shankarappa R, Margolick JB, Gange SJ, Rodrigo AG, Upchurch D, Farzadegan H, Gupta P, Rinaldo CR, Learn GH, He X, et al: **Consistent viral evolutionary changes associated with the progression of human immunodeficiency virus type 1 infection.** *J Virol* 1999, **73**:10489-10502.
113. Koot M, van 't Wout AB, Kootstra NA, de Goede RE, Tersmette M, Schuitemaker H: **Relation between changes in cellular load, evolution of viral phenotype, and the clonal composition of virus populations in the course of human immunodeficiency virus type 1 infection.** *J Infect Dis* 1996, **173**:349-354.
114. Melby T, Despirito M, Demasi R, Heilek-Snyder G, Greenberg ML, Graham N: **HIV-1 coreceptor use in triple-class treatment-experienced patients: baseline prevalence, correlates, and relationship to enfuvirtide response.** *J Infect Dis* 2006, **194**:238-246.
115. Huang W, Eshleman SH, Toma J, Stawiski E, Whitcomb JM, Jackson JB, Guay L, Musoke P, Parkin N, Petropoulos CJ: **Vertical transmission of X4-tropic and dual-tropic HIV-1 in five Ugandan mother-infant pairs.** *Aids* 2009, **23**:1903-1908.
116. Weiss SH, Goedert JJ, Gartner S, Popovic M, Waters D, Markham P, di Marzo Veronese F, Gail MH, Barkley WE, Gibbons J, et al: **Risk of human immunodeficiency virus (HIV-1) infection among laboratory workers.** *Science* 1988, **239**:68-71.
117. Berkowitz RD, Beckerman KP, Schall TJ, McCune JM: **CXCR4 and CCR5 expression delineates targets for HIV-1 disruption of T cell differentiation.** *J Immunol* 1998, **161**:3702-3710.
118. Bleul CC, Wu L, Hoxie JA, Springer TA, Mackay CR: **The HIV coreceptors CXCR4 and CCR5 are differentially expressed and regulated on human T lymphocytes.** *Proc Natl Acad Sci U S A* 1997, **94**:1925-1930.
119. de Roda Husman AM, Blaak H, Brouwer M, Schuitemaker H: **CC chemokine receptor 5 cell-surface expression in relation to CC chemokine receptor 5 genotype and the clinical course of HIV-1 infection.** *J Immunol* 1999, **163**:4597-4603.
120. Blaak H, van 't Wout AB, Brouwer M, Hooibrink B, Hovenkamp E, Schuitemaker H: **In vivo HIV-1 infection of CD45RA(+)/CD4(+) T cells is established primarily by syncytium-inducing variants and correlates with the rate of CD4(+) T cell decline.** *Proc Natl Acad Sci USA* 2000, **97**:1269-1274.
121. Pastore C, Nedellec R, Ramos A, Pontow S, Ratner L, Mosier DE: **Human immunodeficiency virus type 1 coreceptor switching: V1/V2 gain-of-fitness mutations compensate for V3 loss-of-fitness mutations.** *J Virol* 2006, **80**:750-758.
122. Bunnik EM, Quakkelaar ED, van Nuenen AC, Boeser-Nunnink B, Schuitemaker H: **Increased neutralization sensitivity of recently emerged CXCR4-using human immunodeficiency virus type 1 strains compared to coexisting CCR5-using variants from the same patient.** *J Virol* 2007, **81**:525-531.
123. Lusso P, Earl PL, Sironi F, Santoro F, Ripamonti C, Scarlatti G, Longhi R, Berger EA, Burastero SE: **Cryptic nature of a conserved, CD4-inducible V3 loop neutralization epitope in the native envelope glycoprotein oligomer of CCR5-restricted, but not CXCR4-using, primary human immunodeficiency virus type 1 strains.** *J Virol* 2005, **79**:6957-6968.
124. Harouse JM, Buckner C, Gettie A, Fuller R, Bohm R, Blanchard J, Cheng-Mayer C: **CD8+ T cell-mediated CXC chemokine receptor 4-simian/human immunodeficiency virus suppression in dually infected rhesus macaques.** *Proc Natl Acad Sci U S A* 2003, **100**:10977-10982.
125. Westby M, Smith-Burchnell C, Mori J, Lewis M, Mosley M, Stockdale M, Dorr P, Ciarabella G, Perros M: **Reduced maximal inhibition in phenotypic susceptibility assays indicates that viral strains resistant to the CCR5 antagonist maraviroc utilize inhibitor-bound receptor for entry.** *J Virol* 2007, **81**:2359-2371.
126. Scoggins RM, Taylor JR Jr., Patrie J, van't Wout AB, Schuitemaker H, Camerini D: **Pathogenesis of primary R5 human immunodeficiency virus type 1 clones in SCID-hu mice.** *J Virol* 2000, **74**:3205-3216.
127. Karlsson I, Antonsson L, Shi Y, Oberg M, Karlsson A, Albert J, Olde B, Owman C, Jansson M, Fenyo EM: **Coevolution of RANTES sensitivity and mode of CCR5 receptor use by human immunodeficiency virus type 1 of the R5 phenotype.** *J Virol* 2004, **78**:11807-11815.
128. Koning FA, Kwa D, Boeser-Nunnink B, Dekker J, Vingerhoed J, Hiemstra H, Schuitemaker H: **Decreasing sensitivity to RANTES (regulated on activation, normally T cell-expressed and -secreted) neutralization of CC chemokine receptor 5-using, non-syncytium-inducing virus variants in the course of human immunodeficiency virus type 1 infection.** *J Infect Dis* 2003, **188**:864-872.
129. Cavarelli M, Karlsson I, Zanchetta M, Antonsson L, Plebani A, Giaquinto C, Fenyo EM, De Rossi A, Scarlatti G: **HIV-1 with multiple CCR5/CXCR4 chimeric receptor use is predictive of immunological failure in infected children.** *PLoS One* 2008, **3**:e3292.
130. Karlsson I, Antonsson L, Shi Y, Karlsson A, Albert J, Leitner T, Olde B, Owman C, Fenyo EM: **HIV biological variability unveiled: frequent isolations and chimeric receptors reveal unprecedented variation of coreceptor use.** *Aids* 2003, **17**:2561-2569.
131. Kimata JT, Kuller L, Anderson DB, Dailey P, Overbaugh J: **Emerging cytopathic and antigenic simian immunodeficiency virus variants influence AIDS progression.** *Nat Med* 1999, **5**:535-541.
132. van 't Wout AB, Blaak H, Ran LJ, Brouwer M, Kuiken C, Schuitemaker H: **Evolution of syncytium-inducing and non-syncytium-inducing biological virus clones in relation to replication kinetics during the course of human immunodeficiency virus type 1 infection.** *J Virol* 1998, **72**:5099-5107.
133. Kuiken CL, de Jong JJ, Baan E, Keulen W, Tersmette M, Goudsmit J: **Evolution of the V3 envelope domain in proviral sequences and isolates of human immunodeficiency virus type 1 during transition of the viral biological phenotype.** *J Virol* 1992, **66**:5704.

134. Pastore C, Ramos A, Mosier DE: **Intrinsic obstacles to human immunodeficiency virus type 1 coreceptor switching.** *J Virol* 2004, **78**:7565-7574.
135. Koning FA, Koevoets C, van der Vorst TJ, Schuitmaker H: **Sensitivity of primary R5 HTV-1 to inhibition by RANTES correlates with sensitivity to small-molecule R5 inhibitors.** *Antivir Ther* 2005, **10**:231-237.
136. Repits J, Oberg M, Esbjornsson J, Medstrand P, Karlsson A, Albert J, Fenyo EM, Jansson M: **Selection of human immunodeficiency virus type 1 R5 variants with augmented replicative capacity and reduced sensitivity to entry inhibitors during severe immunodeficiency.** *J Gen Virol* 2005, **86**:2859-2869.
137. Stalmeijer EH, Van Rij RP, Boeser-Nunnink B, Visser JA, Naarding MA, Schols D, Schuitmaker H: **In vivo evolution of X4 human immunodeficiency virus type 1 variants in the natural course of infection coincides with decreasing sensitivity to CXCR4 antagonists.** *J Virol* 2004, **78**:2722-2728.
138. Asjo B, Morfeldt-Manson L, Albert J, Biberfeld G, Karlsson A, Lidman K, Fenyo EM: **Replicative capacity of human immunodeficiency virus from patients with varying severity of HIV infection.** *Lancet* 1986, **2**:660-662.
139. Connor RI, Mohri H, Cao Y, Ho DD: **Increased viral burden and cytopathicity correlate temporally with CD4+ T-lymphocyte decline and clinical progression in human immunodeficiency virus type 1-infected individuals.** *J Virol* 1993, **67**:1772-1777.
140. Ostrowski MA, Chun TW, Justement SJ, Motola I, Spinelli MA, Adelsberger J, Ehler LA, Mizell SB, Hallahan CW, Fauci AS: **Both memory and CD45RA +CD62L+ naive CD4(+) T cells are infected in human immunodeficiency virus type 1-infected individuals.** *J Virol* 1999, **73**:6430-6435.
141. van Rij RP, Blaak H, Visser JA, Brouwer M, Rientsma R, Broersen S, de Roda Husman AM, Schuitmaker H: **Differential coreceptor expression allows for independent evolution of non-syncytium-inducing and syncytium-inducing HIV-1.** *J Clin Invest* 2000, **106**:1039-1052.
142. Mild M, Esbjornsson J, Fenyo EM, Medstrand P: **Frequent intrapatient recombination between human immunodeficiency virus type 1 R5 and X4 envelopes: implications for coreceptor switch.** *J Virol* 2007, **81**:3369-3376.
143. van Rij RP, Worobey M, Visser JA, Schuitmaker H: **Evolution of R5 and X4 human immunodeficiency virus type 1 gag sequences in vivo: evidence for recombination.** *Virology* 2003, **314**:451-459.
144. Verhofstede C, Vandekerckhove L, Eygen W, Demecheleer E, Vandembroucke I, Winters B, Plum J, Vogelaers D, Stuyver L: **CXCR4-using HIV type 1 variants are more commonly found in peripheral blood mononuclear cell DNA than in plasma RNA.** *J Acquir Immune Defic Syndr* 2009, **50**:126-136.
145. Edo-Matas D, van Gils MJ, Bowles EJ, Navis M, Rachinger A, Boeser-Nunnink B, Stewart-Jones GB, Kootstra NA, van 't Wout AB, Schuitmaker H: **Genetic composition of replication competent clonal HIV-1 variants isolated from peripheral blood mononuclear cells (PBMC), HIV-1 proviral DNA from PBMC and HIV-1 RNA in serum in the course of HIV-1 infection.** *Virology* 2010, **405**:492-504.
146. Seclen E, Del Mar Gonzalez M, De Mendoza C, Soriano V, Poveda E: **Dynamics of HIV tropism under suppressive antiretroviral therapy: implications for tropism testing in subjects with undetectable viraemia.** *J Antimicrob Chemother* 2010, **65**:1493-1496.
147. Abecasis AB, Vandamme AM, Lemey P: **Quantifying differences in the tempo of human immunodeficiency virus type 1 subtype evolution.** *J Virol* 2009, **83**:12917-12924.
148. Gray RR, Tatem AJ, Lamers S, Hou W, Laeyendecker O, Serwadda D, Sewankambo N, Gray RH, Wawer M, Quinn TC, *et al*: **Spatial phylogenetics of HIV-1 epidemic emergence in east Africa.** *Aids* 2009, **23**:F9-F17.
149. Worobey M, Gemmel M, Teuwen DE, Haselkorn T, Kunstman K, Bunce M, Muyembe JJ, Kabongo JM, Kalengayi RM, Van Marck E, *et al*: **Direct evidence of extensive diversity of HIV-1 in Kinshasa by 1960.** *Nature* 2008, **455**:661-664.
150. Gilbert MT, Rambaut A, Wlasiuk G, Spira TJ, Pitchenik AE, Worobey M: **The emergence of HIV/AIDS in the Americas and beyond.** *Proc Natl Acad Sci U S A* 2007, **104**:18566-18570.
151. Koot M, Schellekens PT, Mulder JW, Lange JM, Roos MT, Coutinho RA, Tersmette M, Miedema F: **Viral phenotype and T cell reactivity in human immunodeficiency virus type 1-infected asymptomatic men treated with zidovudine.** *J Infect Dis* 1993, **168**:733-736.
152. Karlsson A, Parsmyr K, Aperia K, Sandstrom E, Fenyo EM, Albert J: **MT-2 cell tropism of human immunodeficiency virus type 1 isolates as a marker for response to treatment and development of drug resistance.** *J Infect Dis* 1994, **170**:1367-1375.
153. van 't Wout AB, de Jong MD, Kootstra NA, Veenstra J, Lange JM, Boucher CA, Schuitmaker H: **Changes in cellular virus load and zidovudine resistance of syncytium-inducing and non-syncytium-inducing human immunodeficiency virus populations under zidovudine pressure: a clonal analysis.** *J Infect Dis* 1996, **174**:845-849.
154. van 't Wout AB, Ran LJ, de Jong MD, Bakker M, van Leeuwen R, Notermans DW, Loeliger AE, de Wolf F, Danner SA, Reiss P, *et al*: **Selective inhibition of syncytium-inducing and nonsyncytium-inducing HIV-1 variants in individuals receiving didanosine or zidovudine, respectively.** *J Clin Invest* 1997, **100**:2325-2332.
155. van 't Wout AB, Ran LJ, Nijhuis M, Tijnagel JM, de Groot T, van Leeuwen R, Boucher CA, Schuitmaker H, Schuurman R: **Efficient inhibition of both syncytium-inducing and non-syncytium-inducing wild-type HIV-1 by lamivudine in vivo.** *Aids* 1998, **12**:1169-1176.
156. Brumme ZL, Goodrich J, Mayer HB, Brumme CJ, Henrick BM, Wynhoven B, Asselin JJ, Cheung PK, Hogg RS, Montaner JS, Harrigan PR: **Molecular and clinical epidemiology of CXCR4-using HIV-1 in a large population of antiretroviral-naive individuals.** *J Infect Dis* 2005, **192**:466-474.
157. Moyle GJ, Wildfire A, Mandalia S, Mayer H, Goodrich J, Whitcomb J, Gazzard BG: **Epidemiology and predictive factors for chemokine receptor use in HIV-1 infection.** *J Infect Dis* 2005, **191**:866-872.
158. Poveda E, Briz V, de Mendoza C, Benito JM, Corral A, Zahonero N, Lozano S, Gonzalez-Lahoz J, Soriano V: **Prevalence of X4 tropic HIV-1 variants in patients with differences in disease stage and exposure to antiretroviral therapy.** *J Med Virol* 2007, **79**:1040-1046.
159. Coakley E, Petropoulos CJ, Whitcomb JM: **Assessing chemokine co-receptor usage in HIV.** *Curr Opin Infect Dis* 2005, **18**:9-15.
160. Hunt PW, Harrigan PR, Huang W, Bates M, Williamson DW, McCune JM, Price RW, Spudich SS, Lampiris H, Hoh R, *et al*: **Prevalence of CXCR4 tropism among antiretroviral-treated HIV-1-infected patients with detectable viremia.** *J Infect Dis* 2006, **194**:926-930.
161. Wilkin TJ, Su Z, Kuritzkes DR, Hughes M, Flexner C, Gross R, Coakley E, Greaves W, Godfrey C, Skolnik PR, *et al*: **HIV type 1 chemokine coreceptor use among antiretroviral-experienced patients screened for a clinical trial of a CCR5 inhibitor: AIDS Clinical Trial Group A5211.** *Clin Infect Dis* 2007, **44**:591-595.
162. Briz V, Poveda E, del Mar Gonzalez M, Martin-Carbonero L, Gonzalez-Gonzalez R, Soriano V: **Impact of antiretroviral therapy on viral tropism in HIV-infected patients followed longitudinally for over 5 years.** *J Antimicrob Chemother* 2008, **61**:405-410.
163. Equils O, Garratty E, Wei LS, Plaeger S, Tapia M, Deville J, Krogstad P, Sim MS, Nielsen K, Bryson YJ: **Recovery of replication-competent virus from CD4 T cell reservoirs and change in coreceptor use in human immunodeficiency virus type 1-infected children responding to highly active antiretroviral therapy.** *J Infect Dis* 2000, **182**:751-757.
164. Philpott S, Weiser B, Anastos K, Kitchen CM, Robison E, Meyer WA 3rd, Sacks HS, Mathur-Wagh U, Brunner C, Burger H: **Preferential suppression of CXCR4-specific strains of HIV-1 by antiviral therapy.** *J Clin Invest* 2001, **107**:431-438.
165. Skrabal K, Trouplin V, Labrosse B, Obry V, Diamond F, Hance AJ, Clavel F, Mammano F: **Impact of antiretroviral treatment on the tropism of HIV-1 plasma virus populations.** *Aids* 2003, **17**:809-814.
166. Galan I, Jimenez JL, Gonzalez-Rivera M, De Jose MI, Navarro ML, Ramos JT, Mellado MJ, Gurbindo MD, Bellon JM, Resino S, *et al*: **Virological phenotype switches under salvage therapy with lopinavir-ritonavir in heavily pretreated HIV-1 vertically infected children.** *Aids* 2004, **18**:247-255.
167. Delobel P, Sandres-Saune K, Cazabat M, Pasquier C, Marchou B, Massip P, Izopet J: **R5 to X4 switch of the predominant HIV-1 population in cellular reservoirs during effective highly active antiretroviral therapy.** *J Acquir Immune Defic Syndr* 2005, **38**:382-392.
168. Saracino A, Monno L, Cibelli DC, Punzi G, Brindicci G, Ladisa N, Tartaglia A, Lagioia A, Angarano G: **Co-receptor switch during HAART is independent of virological success.** *J Med Virol* 2009, **81**:2036-2044.
169. Lehmann C, Daumer M, Boussaad I, Sing T, Beerwinkler N, Lengauer T, Schmeisser N, Wyen C, Fatkenheuer G, Kaiser R: **Stable coreceptor usage of**

HIV in patients with ongoing treatment failure on HAART. *J Clin Virol* 2006, **37**:300-304.

170. Soulie C, Marcelin AG, Ghosn J, Amellal B, Assoumou L, Lambert S, Duvivier C, Costagliola D, Katlama C, Calvez V: **HIV-1 X4/R5 co-receptor in viral reservoir during suppressive HAART.** *Aids* 2007, **21**:2243-2245.
171. Brumme ZL, Dong WW, Yip B, Wynhoven B, Hoffman NG, Swanstrom R, Jensen MA, Mullins JI, Hogg RS, Montaner JS, Harrigan PR: **Clinical and immunological impact of HIV envelope V3 sequence variation after starting initial triple antiretroviral therapy.** *Aids* 2004, **18**:F1-9.
172. Low AJ, Dong W, Chan D, Sing T, Swanstrom R, Jensen M, Pillai S, Good B, Harrigan PR: **Current V3 genotyping algorithms are inadequate for predicting X4 co-receptor usage in clinical isolates.** *Aids* 2007, **21**:F17-24.
173. Low AJ, Marchant D, Brumme CJ, Brumme ZL, Dong W, Sing T, Hogg RS, Montaner JS, Gill V, Cheung PK, Harrigan PR: **CD4-dependent characteristics of coreceptor use and HIV type 1 V3 sequence in a large population of therapy-naive individuals.** *AIDS Res Hum Retroviruses* 2008, **24**:219-228.
174. 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**:1617-1623.

doi:10.1186/1479-5876-9-S1-S5

Cite this article as: Schuitemaker *et al.*: Clinical significance of HIV-1 coreceptor usage. *Journal of Translational Medicine* 2010 **9**(Suppl 1):S5.

**Submit your next manuscript to BioMed Central
and take full advantage of:**

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at
www.biomedcentral.com/submit

