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Switch of predicted HIV-1 tropism in treated subjects and its association with disease progression

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Abstract

Dynamics of human immunodeficiency virus type 1 (HIV-1) tropism after antiretroviral therapy (ART) initiation and their association with disease progression are poorly investigated.

This was a cohort study on subjects from the ICONA cohort receiving ART with persistently detectable (PD) or persistently undetectable (PU) viral load (VL) and with stored plasma or peripheral blood mononuclear cell (PBMC) samples at 2 time-points (T1, T2) after ART initiation. HIV-1 co-receptor tropism was determined by V3-loop sequencing and the geno2pheno algorithm. A switch in viral tropism was defined if the tropism classification at T2 differed from that observed at T1. Time to disease progression, defined as the occurrence of a new acquired immune deficiency syndrome (AIDS)-defining event/death from T2, was also evaluated.

One hundred ninety-five patients were analyzed (124 PD, 71 PU). Over a median follow-up of 22.6 (19.8–28.1) months, PD and PU patients showed similar rates (95% confidence interval) of switch to a non-R5 virus [PD: 6.9 (3.7–11.2)/100-person-years of follow-up (PYFU); PU: 8.0 (3.4–14.5)/100-PYFU; $P=0.63$] and of switch to a R5 virus [PD: 15.4 (7.3–26.4)/100-PYFU; PU: 8.1 (2.5–16.7)/100-PYFU; $P=0.38$]. Switch to non-R5 virus was predicted by nadir CD4+ before T1.

Twenty-two (18%) PD and 4 (6%) PU subjects experienced disease progression ($P=0.02$). The risk of disease progression was independently associated with a switch in co-receptor tropism (adjusted hazard ratio = 4.06, 95% CI: 1.20–13.80, $P=0.03$) as well as age, AIDS diagnosis, nadir CD4+ before T2, current CD4+, and VL.

Switch of HIV-1 tropism under ART occurs in both directions, with similar rates in subjects with PD or PU VL and it might be predictive of future unfavorable clinical outcome.

Abbreviations: 95% CI = 95% confidence interval, AIDS = acquired immune deficiency syndrome, ART = antiretroviral therapy, FPR = false positive rate, HCV = hepatitis C virus, HIV-1 = human immunodeficiency virus type 1, HR = hazard ratio, IQR = interquartile range, NNRTI = non-nucleoside reverse transcriptase inhibitor, NRTI = nucleoside reverse transcriptase inhibitor, PBMC = peripheral blood mononuclear cell, PD = persistently detectable (viral load), PU = persistently undetectable (viral load), PYFU = person-years of follow-up, RR = relative risk, T1 = time-point 1, T2 = time-point 2, VIF = variance inflation factor, VL = viral load.

Keywords: CCR5, disease progression, FPR, HIV, tropism switch

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1. Introduction

Cross-sectional evaluation of human immunodeficiency virus type 1 (HIV-1) co-receptor usage by means of phenotypic assays and/or V3 population genotyping[1] has been intensively studied in untreated primary[2] and chronic HIV-1 infection[3,4] and in patients on virological failure.[5,6] The impact of baseline tropism on virological response and CD4 gains after antiretroviral therapy (ART) initiation has also been investigated in several studies including or not CCR5 inhibitors.[7–9]

The frequency and implications of co-receptor switch in HIV-1 treated subjects unexposed to CCR5 inhibitors are instead poorly understood. Longitudinal analyses have been performed on co-receptor usage before and after ART initiation in subjects on suppressive therapy,[10–15] virological failure,[15–19] or after therapy interruption,[20,21] yielding controversial results. HIV-1 co-receptor usage has been determined longitudinally under ART in paired plasma[16,17] or peripheral blood mononuclear cell (PBMC) samples[10,12–15] in a very limited subset of patients.

There are several studies that have evaluated the relationship between the co-receptor tropism and the risk of clinical disease progression in ART-naïve subjects or after treatment initiation,[9,22–24] on the other hand, whether HIV-1 tropism switch under ART pressure might be associated with the risk of clinical progression has never been previously investigated.

The aim of this study was to determine the rate of HIV-1 tropism switch in subjects under ART both in presence of persistently detectable (PD) or undetectable (PU) viral load (VL). The association between tropism switch and disease progression was also evaluated.

2. Methods

This is a longitudinal cohort study on adult HIV-1 treated subjects enrolled in the ICONA Foundation Study Cohort, with available paired samples of plasma or cells stored at 2 time-points after ART initiation. Briefly, the ICONA Foundation Cohort is a cohort of HIV-infected patients which superseded the original I. CO.N.A. (Italian Cohort of Antiretroviral-Naïve Patients) study, recruiting HIV-positive patients when still ART-naïve. At their enrollment, subjects provide written informed consent to include their clinical and laboratory data in the ICONA database for scientific purposes.

The ICONA database collects the method of VL quantification and the corresponding detection limit; this information was available for the analyses and used to correctly classify as detectable or undetectable the recorded VL values. VL values $>$ 50 copies/mL were classified as detectable; VL values $<$ 50 copies/mL or below the detection limit in use in each center were classified as undetectable.

Two groups of treated HIV-1 subjects were retrospectively identified based on the recorded VL determinations: subjects with PD VL at and between the 2 considered time-points; subjects with PU VL at and between the 2 considered time-points. Patients treated with maraviroc were excluded from this analysis.

In both groups, the first sample was the nearest to the ART initiation (and approximately at least 6 months after starting), while the second sample was taken approximately 2 years after the date of first sample, with a median interval between the 2 samples of 22.9 (20–28.5) months.

Co-receptor tropism was determined on HIV-RNA from plasma samples in PD subjects and on proviral DNA extracted from PBMC samples in PU subjects.

A single polymerase chain reaction (PCR) product per sample was subjected to standard population sequencing. Sequences were analyzed with Seqscape software v2.5 (Applied Biosystems, Foster City, CA) as previously described.[25–27] Nucleotide mixtures were considered if the second highest peak in the electropherogram was >25%. V3 sequences with more than 8 nucleotide mixtures were discarded. The V3 sequences are available on request.

Co-receptor tropism was inferred with the geno2pheno algorithm setting the false positive rate (FPR) at 10% for plasma samples and 20% for PBMC samples,[1] based on previous studies[28,29] showing concordant results in the determination of co-receptor tropism for the same individual whether plasma RNA or proviral DNA was used. Co-receptor tropism for all PD patients was determined only on plasma RNA at either time-point; similarly in PU patients, co-receptor tropism was assessed only on proviral DNA at either time-point. Clonal prediction was employed for classifying sequences. Clinical isolates were classified as R5 if FPR >10% (RNA) or >20% (DNA) and non-R5 if FPR <10% (RNA) or <20% (DNA). Non-R5 tropism included either R5/X4-coreceptors (dual or mixed tropism) and X4-coreceptor.

Baseline for the statistical analysis used to estimate the rate of tropism switch and factors associated with switch was defined as the date of the first sample (T1).

A co-receptor switch (from R5 to non-R5 or from non-R5 to R5) defined an unstable tropism and was considered to occur if the HIV tropism classification at the second time-point (T2) differed from that observed at T1, otherwise tropism was considered as stable (R5 or non-R5 at both time-points).

Disease progression was defined as the occurrence of a new clinical acquired immune deficiency syndrome (AIDS)-defining event or death; time to disease progression was calculated as the time elapsed since T2 up to the first new AIDS-defining event or death or last available clinical follow-up visit (whichever occurred first).

2.1. Statistical analysis

Patients' main characteristics were described as median (interquartile range, IQR) for continuous variables or proportions for categorical variables. Continuous variables were compared using the Wilcoxon rank sum test or the Kruskal–Wallis test, as appropriate. Differences between proportions were tested by the χ^2 or Fisher exact test.

The incidence rate of co-receptor tropism switch was calculated as the number of switches per 100 person-years of follow-up (PYFU); separate estimates were calculated for switch from non-R5 to R5-tropic viruses and from R5 to non-R5-tropic viruses. Rates were reported with the corresponding 95% confidence interval (95% CI).

Univariate and multivariate Poisson regression models were performed to estimate the risk of tropism switch in relation to the subjects' virological group (PD vs PU) and other baseline characteristics. Separate models were used to identify predictors of the risk of switch to R5-tropic virus and of the risk of switch to non-R5-tropic virus, and estimates were presented as relative risk (RR) with 95% CI; covariates with a P-value $\leq$0.20 in the univariate analysis were considered for entry into the multivariate models in addition to age, sex, baseline CD4+, and subjects' virological group (PU vs PD). Collinearity between variables was assessed by means of the variance inflation factors (VIF); the largest VIF for a single covariate was 1.6, indicating that all variables could be included in the model together.

Mixed-effect linear models, with an unstructured covariance matrix, were used to examine CD4+ trend during the T1–T2 interval or from baseline (T1) until disease progression or last available visit, whichever occurred first; estimates of CD4+ trajectories over time (slope ± standard error) were calculated using random intercept and random slope of months of follow-up to model the within-patient errors.

Univariate and multivariate Cox proportional hazard models were calculated to evaluate the influence of co-receptor tropism switch and other covariates on the risk of disease progression (occurrence of a new AIDS-defining event or death since T2); the contribution of each covariate was expressed by the hazard ratio (HR) with the corresponding 95% CI. The multivariate model included demographic variables, hepatitis C virus (HCV) co-infection (a factor with a potential impact on disease progression) and those confounders with a $P \leq 0.10$ in the univariate analysis: age, sex, HCV co-infection, AIDS diagnosis before T2, CD4 nadir before T2, years of ART treatment, co-receptor tropism switch, current CD4+, current HIV-RNA.

In all statistical analyses, a $P < 0.05$ was considered statistically significant. The analyses were performed using SAS Software, release 9.2 (SAS Institute, Cary, NC).

3. Results

3.1. Patients and their characteristics

HIV-1 V3 loop sequences were successfully obtained from 195 subjects (124 PD, 71 PU) satisfying the study inclusion criteria at baseline (T1) and at T2; baseline was 10.0 (5.2–16.3) months after the date of ART initiation.

The main baseline characteristics of PD and PU subjects are shown in Table 1; PD subjects were younger, more frequently intravenous drug users, more likely to be co-infected with HCV (41.1% vs 23.9%, $P = 0.01$), with an earlier calendar year of ART initiation, more frequently treated with a nucleoside reverse transcriptase inhibitor (NRTI)-based regimen (37.9% vs 7.0%, $P < .001$), as compared with PU patients. The median number of

Table 1

Baseline demographic, clinical, and laboratory characteristics of 195 HIV-1 treated subjects included in the analysis.

Characteristic	Total (n = 195)	PD patients (n = 124)	PU patients (n = 71)	P
Age, y	37 (33–42)	36 (32–40)	40 (34–45)	0.01*
Males	134 (69.0%)	85 (68.6%)	49 (69.0%)	0.95†
Race				>0.99†
White	184 (94.4%)	117 (94.4%)	67 (94.4%)	
Other	11 (5.6%)	7 (5.6%)	4 (5.6%)	
Mode of HIV transmission				0.01†
IDU	70 (35.9%)	55 (44.4%)	15 (21.1%)	
MSM	42 (21.5%)	21 (16.9%)	21 (29.6%)	
Heterosexual	73 (37.4%)	43 (34.7%)	30 (42.3%)	
Other	10 (5.1%)	5 (4%)	5 (7%)	
HCV-Ab				0.01†
Positive	68 (34.9%)	51 (41.1%)	17 (23.9%)	
Negative	112 (57.4%)	62 (50.0%)	50 (70.4%)	
Unknown	15 (7.7%)	11 (8.9%)	4 (5.6%)	
HbsAg				0.54†
Positive	12 (6.1%)	9 (7.2%)	3 (4.2%)	
Negative	169 (86.7%)	104 (83.9%)	65 (91.5%)	
Unknown	14 (7.2%)	11 (8.9%)	3 (4.2%)	
Previous diagnosis of AIDS				0.16†
Yes	31 (15.9%)	16 (12.9%)	15 (21.1%)	
No	164 (84.1%)	108 (87.1%)	56 (78.9%)	
Nadir CD4+ before baseline (cells/μL)	259 (105–403)	308 (148–428)	209 (73–332)	0.01*
≤200	74 (38.0%)	39 (31.5%)	35 (49.3%)	0.02†
>200	121 (62.0%)	85 (68.6%)	36 (50.7%)	
Type of ART				<0.001†
NRTI-based	52 (26.7%)	47 (37.9%)	5 (7.0%)	
NNRTI-based	37 (19.0%)	15 (12.1%)	22 (31.0%)	
PI-based	106 (54.3%)	62 (50.0%)	44 (62.0%)	
Years since first HIV positive test	3.7 (1.3–9.9)	5.1 (1.4–10.9)	2.8 (1.2–7.9)	0.14*
Calendar year of ART initiation	1998 (1997–2001)	1998 (1997–1998)	2001 (1998–2004)	<0.001*
Months since ART initiation	10.0 (5.2–16.3)	6.7 (4.7–12.9)	11.8 (8.6–23.6)	<0.001*
Baseline calendar year	1999 (1998–2002)	1998 (1998–1999)	2001 (1999–2005)	<0.001*
HIV-RNA (copies/mL)	2233 (60–14,800)	9900 (2400–36,950)	50 (50–50)	<0.001*
CD4+ (cells/μL)	448 (257–653)	443 (232–625)	468 (297–658)	0.26*
CD8+ (cells/μL)	965 (706–1323)	949 (757–1335)	1054 (691–1271)	0.87*
CD4+/CD8+ ratio	0.43 (0.27–0.63)	0.42 (0.26–0.57)	0.49 (0.31–0.68)	0.07*
Hemoglobin at baseline (g/dL)	14.1 (13.0–14.8)	14.0 (12.6–14.6)	14.4 (13.8–15.3)	0.01*

Results are median (IQR) or frequency (%), as appropriate.

ART = antiretroviral therapy, IDU = intravenous drug users, MSM = men who have sex with men, NNRTI = non-nucleoside reverse transcriptase inhibitor, NRTI = nucleoside reverse transcriptase inhibitor, PD = persistently detectable viral load between T1 and T2, PI = protease inhibitor, PU = persistently undetectable viral load between T1 and T2.

* By Wilcoxon rank sum test.

† By χ^2 or Fisher exact test, as appropriate.

VL determinations between the 2 time-points was 6 (5–8) and 8 (6–10) in PD and PU subjects, respectively ($P<.001$). In PD subjects, HIV-RNA area-under-the-curve between the 2 time-points was 3.82 (3.31–4.26) $\log_{10}$copies/mL/d. Overall, subtype B HIV-1 was assigned to 183 patients [117/124 (94%) in PD and 66/71 (93%) in PU, $P=0.76$].

3.2. HIV-1 tropism switch

At baseline (T1), 93 (75%) PD patients were predicted to harbor an R5 virus in comparison to 46 PU (65%) subjects, with no significant difference ($P=0.14$). At T2, there were 90 (73%) PD subjects with an R5 virus after a median follow-up of 22.1 (19.2–24.7) months and 43 (61%) PU subjects had an R5 strain after a median follow-up of 24.4 (21.0–31.6) months (difference not statistically significant: $P=0.11$).

There were 101 (81%) PD and 58 (82%) PU subjects with stable co-receptor tropism and 23 (19%) PD and 13 (18%) PU subjects experienced a co-receptor tropism switch between the 2 time-points (Fig. 1). The rate of co-receptor switch was similar between PD and PU subjects (Fig. 1). Among PD subjects, the switch was mainly from a non-R5 to an R5 strain ($P=0.04$) while no difference was found among PU subjects ($P=0.81$).

No association was found between all the considered baseline demographic, clinical, and laboratory characteristics or the subjects' group (i.e., being PD or PU) with the risk of tropism switch to R5, while nadir CD4+ before T1 was the only factor associated with the risk of tropism switch to non-R5 [adjusted RR (per 100-cells/μL higher)=0.55, 95% CI: 0.35–0.86, $P=0.01$] (Table 2).

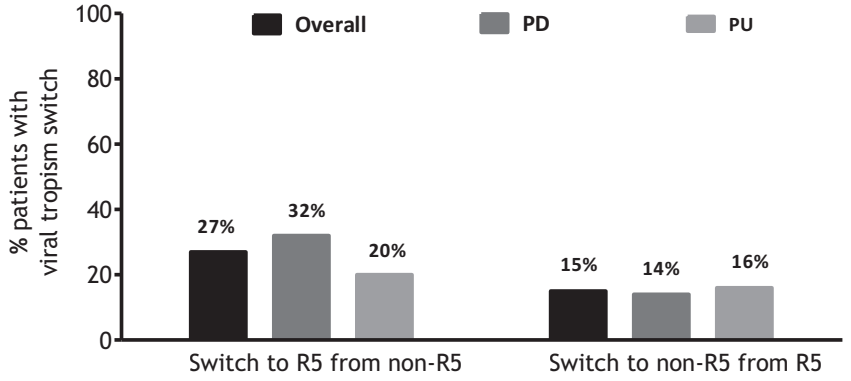
When PD and PU patients were considered separately, no association was observed between all the considered factors and the risk of tropism switch to R5-tropic virus (data not shown) or the risk of switch to non R5-tropic virus (Table 3).

We explored the association between co-receptor switch and changes in CD4+ and VL during the T1–T2 time interval (Table 4): a less favorable CD4+ trend was observed in subjects with tropism switch in comparison to patients with stable tropism. Results were similar when restricting to the switch from a non-R5 to an R5 strain or vice versa.

3.3. Association between co-receptor switch and HIV-1 disease progression

After a median time of 5.4 (2.5–9.3) years from T2, 26 (13%) patients developed at least one AIDS event or died: 22/124 (18%) among the PD group and 4/71 (6%) among PU subjects, respectively ($P=0.02$). Among the 22 PD subjects, 11 patients had at least one AIDS event and 17 patients died (11 without AIDS events); among PU subjects, 2 patients had new AIDS events, 2 patients died. Clinical details of PD and PU subjects with disease progression are reported in Table 5.

PD subjects showed a faster disease progression and were associated with a higher risk of disease progression as compared with PU subjects (unadjusted HR=3.09, 95% CI: 1.06–9.00, $P=0.04$].



	Non-R5 tropic virus at baseline (T1)				R5 tropic virus at baseline (T1)			
	Overall (n=56)	PD (n=31)	PU (n=25)	p-value	Overall (n=139)	PD (n=93)	PU (n=46)	p-value
False positive rate at baseline (T1), median (IQR)	1.7 (0-6-5.8)	1.8 (0.5-5.3)	1.7 (0.6-7.1)	0.691[a]	48.7 (28.8-74.4)	44.4 (23.9-68.6)	59.1 (36.2-77.6)	0.026[a]
False positive rate at T2, median (IQR)	3.7 (1.7-19.5)	4.1 (1.7-31.1)	2.2 (1.7-7.1)	0.189[a]	35.2 (19.1-65.4)	31.6 (17.1-51.8)	43.3 (24.7-73.6)	0.035[a]
Time interval between the 2 time points (years), median (IQR)	2.1 (1.9-2.6)	1.9 (1.7-2.5)	2.1 (2.0-2.7)	0.010[a]	1.8 (1.6-2.2)	1.8 (1.6-2.0)	1.9 (1.6-2.5)	0.069[a]
Number of patients with co-receptor switch	15	10	5		21	13	8	
PYFU	127	65	62	-	288	188	100	-
Rate (95% CI) of co-receptor switch (per 100-PYFU)	11.8 (6.6-18.6)	15.4 (7.3-26.4)	8.1 (2.5-16.7)	0.383[c]	7.3 (4.2-10.4)	6.9 (3.7-11.2)	8.0 (3.4-14.5)	0.627[c]

Abbreviations: PD, persistently detectable patients; PU, persistently undetectable patients; IQR, interquartile range; 95% CI, 95% confidence interval; PYFU, person years of follow-up.
a by Wilcoxon rank sum test;
b by Poisson regression. Rate comparisons (switch to non-R5-tropic virus vs switch to R5-tropic virus): among failing patients: p=0.047; among suppressed patients: p=0.806.

Figure 1. Co-receptor tropism switch in subjects receiving antiretroviral therapy (ART) with persistently detectable (PD) or persistently undetectable (PU) viral load.

Table 2

Poisson regression: unadjusted and adjusted relative risk of co-receptor switch in the 195 HIV-1 treated subjects considered in the analysis.

	Risk of switch to R5-tropic virus (n = 56)				Risk of switch to non-R5-tropic virus (n = 139)			
Characteristic	Unadjusted RR (95% CI)	*P*	Adjusted RR (95% CI)	*P*	Unadjusted RR (95% CI)	*P*	Adjusted RR (95% CI)	*P*
Age (per 10-years older)	0.87 (0.42–1.80)	0.71	0.88 (0.42–1.85)	0.73	1.06 (0.77–1.44)	0.74	1.50 (0.85–2.64)	0.17
Sex								
Males vs females	0.94 (0.32–2.75)	0.91	0.98 (0.30–3.22)	0.97	0.98 (0.57–1.66)	0.92	0.86 (0.32–2.31)	0.76
Race								
White vs other	NA*	NA*	–	–	0.51 (0.23–1.13)	0.10	0.28 (0.07–1.13)	0.10
Mode of HIV transmission		0.66	–	–		0.74	–	–
IDU	0.96 (0.26–3.56)	0.95			0.80 (0.45–1.43)	0.45		
MSM	1.60 (0.49–5.26)	0.44			0.85 (0.43–1.68)	0.64		
Heterosexual	Ref	–			Ref	–		
Other	NA†	NA†			1.06 (0.37–3.03)	0.92		
HCV-Ab		0.39	–	–		0.93	–	–
Positive	0.41 (0.09–1.86)	0.25			0.96 (0.56–1.62)	0.87		
Negative	Ref	–			Ref	–		
Unknown	1.26 (0.28–5.67)	0.77			0.83 (0.30–2.33)	0.72		
HbsAg		0.18		0.23		0.54	–	–
Positive	2.63 (0.71–9.72)	0.15	2.42 (0.62–9.47)	0.20	1.24 (0.45–3.43)	0.68		
Negative	Ref	–	Ref	–	Ref	–		
Unknown	2.95 (0.80–10.9)	0.11	2.84 (0.74–11.0)	0.13	0.52 (0.17–2.13)	0.36		
Previous diagnosis of AIDS							–	–
Yes vs no	1.42 (0.45–4.47)	0.56	–	–	1.58 (0.86–2.92)	0.14		
Nadir CD4+ prior to baseline (per 100-cells/μL higher)	1.03 (0.82–1.28)	0.81	–	–	0.84 (0.73–0.97)	0.02	0.55 (0.35–0.86)	0.01
>200 vs ≤200 cells/μL	0.88 (0.31–2.50)	0.81			0.60 (0.37–0.99)	0.04		
Type of ART		0.96	–	–		0.81	–	–
NRTI-based	1.00 (0.29–3.43)	>0.99			0.83 (0.46–1.50)	0.54		
NNRTI-based	1.19 (0.35–4.08)	0.78			0.87 (0.47–1.64)	0.68		
PI-based	Ref	–			Ref	–		
Years since first HIV positive test (per year longer)	0.98 (0.88–1.08)	0.67	–	–	1.02 (0.97–1.07)	0.48	–	–
Calendar year of ART initiation (per year longer)	1.05 (0.89–1.24)	0.58	–	–	1.03 (0.95–1.12)	0.41	–	–
Months since ART initiation (per 12-months longer)	1.08 (0.59–1.97)	0.81	–		0.97 (0.76–1.24)	0.83	–	–
Baseline calendar year (per year increase)	1.05 (0.90–1.23)	0.56	–	–	1.03 (0.96–1.12)	0.42	–	–
Subjects' virological group (PD vs PU)	1.73 (0.59–5.06)	0.32	1.49 (0.49–4.51)	0.49	0.74 (0.45–1.23)	0.24	1.67 (0.52–5.33)	0.38
Baseline HIV-RNA (per $\log_{10}$ copies/mL higher)	1.29 (0.87–1.92)	0.20	–	–	NE	–		
Baseline CD4+ (per 100-cells/μL higher)	1.01 (0.87–1.18)	0.88	0.99 (0.82–1.18)	0.87	0.93 (0.85–1.03)	0.15	1.34 (0.98–1.84)	0.07
Baseline CD8+ (per 100-cells/μL higher)	0.94 (0.83–1.07)	0.35	–	–	0.97 (0.92–1.02)	0.27		
Baseline CD4+/CD8+ ratio (per 0.2-unit higher)	1.09 (0.80–1.48)	0.59	–	–	0.76 (0.52–1.11)	0.16	0.76 (0.42–1.39)	0.35
Baseline hemoglobin (per g/dL higher)	0.76 (0.45–1.28)	0.30	–	–	1.07 (0.88–1.30)	0.52		

ART = antiretroviral therapy, IDU = intravenous drug users, MSM = men who have sex with men, NA = not applicable, NE = not evaluated (because all suppressed patients had undetectable HIV-RNA at baseline), NNRTI = non-nucleoside reverse transcriptase inhibitor, NRTI = nucleoside reverse transcriptase inhibitor, PD = persistently detectable patients, PI = protease inhibitor, PU = persistently undetectable patients, Ref = reference, RR = relative risk.

* No switch to R5-tropic virus occurred among subjects with non-white race.

† No switch to R5-tropic virus occurred among subjects with other mode of HIV transmission.

Table 3

Poisson regression: unadjusted and adjusted relative risk of switch to non-R5-tropic virus in the 139 HIV-1 treated subjects with an R5-tropic virus at baseline.

	PD patients (n = 93)				PU patients (n = 46)			
Characteristic	**Unadjusted RR (95% CI)**	***P***	**Adjusted RR (95% CI)**	***P***	**Unadjusted RR (95% CI)**	***P***	**Adjusted RR (95% CI)**	***P***
Age (per 10-years older)	0.93 (0.44–1.95)	0.85	0.72 (0.32–1.63)	0.43	1.98 (1.02–3.82)	0.04	1.42 (0.63–3.21)	0.40
Sex								
Males vs females	1.11 (0.34–3.60)	0.86	1.35 (0.38–4.75)	0.63	0.70 (0.17–2.95)	0.63	0.78 (0.16–3.75)	0.76
Previous diagnosis of AIDS								
Yes vs no	4.13 (1.35–12.64)	0.01	1.55 (0.36–6.61)	0.55	0.88 (0.11–7.18)	0.91	–	–
Nadir CD4+ before baseline (per 100-cells/μL higher)	0.60 (0.41–0.88)	0.01	0.63 (0.30–1.36)	0.24	0.76 (0.48–1.20)	0.20	0.54 (0.26–1.13)	0.09
Baseline calendar year (per year increase)	1.05 (0.81–1.36)	0.71	–	–	1.23 (0.99–1.53)	0.07	1.13 (0.87–1.46)	0.36
Baseline HIV-RNA (per $\log_{10}$ copies/mL higher)	1.68 (0.93–3.03)	0.09	1.23 (0.63–2.40)	0.55	NE	–	NE	–
Baseline CD4+ (per 100-cells/μL higher)	0.73 (0.56–0.95)	0.02	1.27 (0.71–2.25)	0.43	1.02 (0.85–1.22)	0.83	1.24 (0.91–1.70)	0.19
Baseline CD4+/CD8+ ratio (per 0.2-unit higher)	0.42 (0.22–0.80)	0.01	0.54 (0.18–1.61)	0.24	1.11 (0.76–1.60)	0.62	–	–

Race, mode of HIV transmission, HCV-Ab, HbsAg, years since HIV positive test, calendar year of ART initiation, months since ART initiation, baseline CD8+, and baseline hemoglobin were also tested and did not influence the risk of switch to non-R5-tropic virus either in persistently detectable (PD) or undetectable patients (PU).

95% CI = 95% confidence interval, NE = not evaluated (as all suppressed patients had undetectable HIV-RNA at baseline), PD = persistently detectable patients, PU = persistently undetectable patients, RR = relative risk.

Patients with tropism switch appeared to have faster disease progression rather than those with stable R5 or non-R5 co-receptor tropism and the risk of disease progression was higher among subjects with tropism switch (to R5 or non-R5) in comparison to those with stable tropism (R5 or non-R5) (HR = 2.39, 95% CI: 1.03–5.54, $P = 0.04$].

At multivariate analysis, the risk of disease progression was more likely in older subjects, in subjects with an AIDS diagnosis before T2, with a lower CD4+ count nadir before T2, with lower current CD4+, with higher current VL, and in subjects with co-receptor switch rather than those with stable tropism (Table 6).

4. Discussion

The dynamics of co-receptor tropism switch in HIV-1 infected subjects under ART pressure remain poorly explored. Our findings show that in approximately 2 years of follow-up under ART, co-receptor usage is stable in 80% of cases, either in case of

Table 4

Immuno-virological trend between the 2 time points according to co-receptor switch and patients' virological group.

	Crude CD4+ slope (cells/μL per month)		HIV-RNA AUC ($\log_{10}$ copies/mL per day)	
Characteristic	**Estimate (standard error)***	***P****	**Median (IQR)**	***P**†*
Subjects' virological group				
PD patients	0.13 (± 0.74)	<0.001	3.82 (3.31–4.26)	<0.001
PU patients	4.78 (± 0.89)		1.69 (1.69–1.69)	
Co-receptor switch				
Stable non-R5 virus	3.09 (± 1.50)	0.07	2.38 (1.69–3.80)	0.35
Switch to R5 virus	2.75 (± 2.55)		3.76 (1.69–4.24)	
Stable R5 virus	1.78 (± 0.69)	0.04	3.26 (1.69–3.95)	0.85
Switch to non-R5 virus	0.36 (± 1.61)		3.53 (1.69–4.26)	
Stable virus (non-R5 or R5)	2.18 (± 0.66)	0.01	3.25 (1.69–3.89)	0.51
Switch to R5 or to non-R5 virus	1.51 (± 1.37)		3.57 (1.69–4.25)	
Co-receptor switch among PD patients				
Stable virus (non-R5 or R5)	0.23 (± 0.82)	>0.99	3.67 (3.27–4.25)	0.02
Switch to R5 or to non-R5 virus	−0.26 (± 1.72)		4.13 (3.61–4.89)	
Co-receptor switch among PU patients				
Stable virus (non-R5 or R5)	5.00 (± 0.99)	<0.001	1.69 (1.69–1.69)	NE
Switch to R5 or to non-R5 virus	3.90 (± 2.06)		1.69 (1.69–1.69)	

AUC = area under the curve, IQR = interquartile range, NE = not evaluated (because all suppressed patients with or without co-receptor shift had always undetectable HIV-RNA between the two time points), PD = persistently detectable patients, PU = persistently undetectable patients.

* By univariate mixed linear model.

† By Wilcoxon rank sum test.

Table 5

Characteristics of 26 patients with disease progression from T2.

Subjects' virological group	Patient ID	Viral tropism evolution	Nadir CD4+	CD4+ at T1	CD4+ at T2	FPR at T1	FPR at T2	T1 date	T2 date	AIDS event date	Type of AIDS event	Death date
PU patients	1	Stable non-R5	26	74	246	0.6	0.4	Nov 17, 1998	Jul 13, 2001	Jun 24, 2008	Cervical cancer	
	2	Stable R5	48	909	1177	91.4	88.5	Dec 11, 1999	Oct 1, 2001			Dec 17, 2009
	3	Stable R5	62	630	620	55.1	78.8	Apr 3, 2000	Nov 30, 2001			Sep 14, 2006
	4	Shift to non-R5	93	286	238	77.6	15.6	May 2, 2001	Jun 25, 2003	Apr 7, 2004	Wasting syndrome	
										Apr 7, 2004	AIDS dementia complex	
PD patients	5	Stable non-R5	227	438	691	5.3	1.7	Jun 29, 1998	May 16, 2000			Jan 13, 2002
	6	Stable non-R5	3	257	214	7.4	4.0	Feb 16, 2000	Mar 7, 2002	Nov 15, 2007	Wasting syndrome	Apr 4, 2008
										Mar 15, 2008	*Pneumocystis jiroveci* pneumonia	
	7	Stable non-R5	201	566	495	0.2	0	Jun 17, 1998	Feb 5, 2001			Sep 13, 2007
	8	Stable R5	26	724	640	29.2	31.4	Jun 15, 2000	Mar 15, 2002	Feb 13, 2009	Esophageal candidiasis	
	9	Stable R5	209	209	230	15.7	17.0	Dec 14, 1998	Mar 13, 2001			Sep 9, 2003
	10	Stable R5	13	820	520	89.1	87.4	Mar 23,1999	Jan 24, 2002	Sep 11, 2006	Kaposi sarcoma	Apr 30, 2013
										Jun 28, 2007	Cerebral toxoplasmosis	
										Jun 28, 2007	CMV infection	
										Jun 28, 2007	Wasting syndrome	
										Aug 7, 2012	CMV infection	
										Mar 9, 2013	*P. jiroveci* pneumonia	
	11	Stable R5	35	247	120	24.7	39.6	Jul 21, 1998	Feb 10, 2000			Mar 11, 2000
	12	Stable R5	224	270	445	77.2	43.0	Aug 10, 1998	Apr 06, 2000	Oct 15, 2001	Non-Hodgkin lymphoma	
	13	Stable R5	203	338	308	95.7	86.2	Mar 16, 1999	Nov 7, 2000			Nov 22, 2009
	14	Stable R5	21	68	622	95.8	70.5	Apr 23, 1998	Jan 17, 2000	Feb 18, 2004	Extrapulmonary tuberculosis	
	15	Stable R5	232	452	287	56.9	65.4	Oct 20, 1998	Apr 19, 2000	Aug 1, 2001	Herpes simplex; esophagitis/pneumonia	
	16	Stable R5	484	1264	484	64.0	67.2	Mar 12, 1998	Feb 22, 2000			Jun 10, 2000
	17	Stable R5	10	135	187	42.3	38.1	Feb 10, 1998	Jan 15, 2000			Dec 30, 2005
	18	Stable R5	169	169	345	90.7	90.7	Feb 9, 1999	Oct 16, 2001			Jan 26, 2006
	19	Stable R5	622	769	1026	85.9	33.0	Jun 10, 1998	Apr 11, 2000			Oct 30, 2005
	20	Shift to R5	32	374	75	5.3	33.9	Sep 28, 1998	Apr 23, 2001	May 29, 2001	AIDS dementia complex	Jun 9, 2001
	21	Shift to R5	3	33	34	0.2	20.8	Sep 1, 1999	Feb 18, 2002	Oct 9, 2002	Esophageal candidiasis	Feb 18, 2006
										Oct 9, 2002	Wasting syndrome	
										Jan 24, 2003	Extrapulmonary cryptococcosis	
	22	Shift to R5	8	977	292	0.7	50.2	Mar 31, 1998	Feb 17, 2000	Jan 9, 2001	Esophageal candidiasis	
	23	Shift to R5	86	133	263	0.5	80.5	Feb 24, 1998	Oct 21, 1999			Aug 16, 2012
	24	Shift to R5	61	215	61	1.7	48.9	Apr 30, 2002	Apr 6, 2005			Sep 24, 2005
	25	Shift to non-R5	2	104	47	13.1	1.9	Mar 15, 1999	Oct 15, 2000	Nov 6, 2004	Cerebral toxoplasmosis	Jan 4, 2008
	26	Shift to non-R5	6	228	258	23.6	9.2	Feb 2, 1999	Sep 20, 2000	Aug 7, 2001	Esophageal candidiasis	Oct 15, 2003
										Jan 25, 2002	Cerebral toxoplasmosis	
										Jan 25, 2002	Esophageal candidiasis	
										Mar 10, 2003	Wasting syndrome	

FPR = false positive rate, PD = persistently detectable patients, PU = persistently undetectable patients, T1 = time-point 1 (baseline), T2 = time-point 2.

Table 6

Cox proportional hazard models: unadjusted and adjusted relative risk of HIV disease progression in the 195 HIV-1 treated subjects.

	Univariate analysis		Multivariate analysis	
Characteristic	**Unadjusted HR (95% CI)**	***P***	**Adjusted HR (95% CI)**	***P***
Age (per 10-years older)	1.10 (0.88–1.38)	0.40	1.74 (1.24–2.44)	0.01
Sex				
Males vs females	1.08 (0.48–2.45)	0.86	0.67 (0.20–2.29)	0.53
Years of HIV infection (per 5-years longer)	0.90 (0.62–1.32)	0.60	–	–
Years of ART (per 5-years longer)	0.50 (0.24–1.00)	0.05	0.61 (0.22–1.64)	0.32
HCV co-infection				
Yes vs no	0.80 (0.35–1.85)	0.60	0.58 (0.17–1.95)	0.38
AIDS diagnosis prior to T2				
Yes vs no	2.69 (1.18–6.12)	0.02	5.86 (1.51–22.75)	0.01
Nadir CD4+ before T2 (per 100-cells/μL higher)	0.83 (0.66–1.04)	0.10	0.75 (0.59–0.94)	0.01
Calendar year of T2 (per year increase)	0.88 (0.73–1.06)	0.17	–	–
Current HIV-RNA (per $\log_{10}$ copies/mL higher)	1.99 (1.48–2.66)	<0.001	1.59 (1.03–2.47)	0.04
HIV-RNA between T1 and T2				
PD vs PU	3.09 (1.06–9.00)	0.04	–	–
CD4+ at T2 (per 100-cells/μL higher)	0.77 (0.65–0.92)	0.01	–	–
Current CD4+ (per 100-cells/μL higher)	0.49 (0.38–0.64)	<0.001	0.55 (0.40–0.75)	<0.001
Viral tropism				
Switch vs stable	2.39 (1.03–5.54)	0.04	4.06 (1.20–13.80)	0.03

95% CI = 95% confidence interval, ART = antiretroviral therapy, HCV = hepatitis C virus, HR = hazard ratio, PD = persistently detectable patients, PU = persistently undetectable patients, T1 = time-point 1, T2 = time-point 2.

successful control of viral replication or in case of persistent detectable HIV-1 viremia. Switch of HIV-1 tropism under ART occurs in both directions, with similar rates in subjects with PD or PU VL. Although comparisons with previous studies are difficult because of differences in study design, length of follow-up, methodologies, and cutoffs used for co-receptor determination, our data confirmed on a large-scale findings reported in a small case series. Switch from non-R5 to R5 virus has been described in detail in a previous study evaluating the co-receptor usage in 40 treated failing patients with a median interval between the 2 time-points of about 20 months.[16] Co-receptor usage was stable in 87% of patients, conversion from X4 to R5 or from R5 to X4 was observed in 2 patients, respectively. In another study[19] on 76 subjects with drug-resistant viremia >1000 copies/mL on a stable ART regimen for >4 months and with a median of 3 tropism determinations (by means of a phenotypic recombinant virus assay) over 9 months, the rate of tropism switch was about 10% and occurred in both directions; 12% of patients infected with R5-tropic virus at baseline switched to D/M-tropic virus by 1 year and 11% infected with D/M-tropic virus at baseline switched to R5 or X4-tropic virus over the same period. In this study, the apparent tropism switches were considered as oscillations in the amount of X4-tropic virus around the limit of assay detection. Nevertheless, a possible alternative interpretation might be that the switch of the co-receptor usage from X4 to R5 under ART pressure may indeed truly occur, independently from CD4 level and undetectability of VL. In fact, a tropism switch from non-R5 to R5 virus has also been observed in a very small series of patients on suppressive ART[10–15] or interrupting successful ART.[20,21]

Both R5 and non-R5 variants have been shown to persist in viral reservoirs after prolonged suppression of plasma VL below the detection limit.[10–15] Preliminary studies documented that the stability of CCR5 co-receptor usage is correlated with the maintenance of HIV-RNA <2.5 copies/mL.[12] Correlation analyses between stability of the co-receptor usage and residual viremia, changes in proviral DNA and viral replicative capacity[30] will provide new insights into the mechanisms of co-receptor tropism switching in subjects with virological suppression.

We were not able to identify predictors of tropism switch with the only exception of CD4 nadir that was predictive of the switch from R5 to non-R5 which might be along the lines of previous studies showing that the presence of non-R5 virus is associated with low nadir CD4 values.[31,32]

Results of the impact of tropism switch on long-term risk of disease progression in subjects receiving ART are lacking. Previous studies evaluated the risk of disease progression in relation to co-receptor tropism by geno2pheno algorithm determined at a single time-point yielding conflicting results.[9,33]

In our analysis, the presence of unstable co-receptor tropism, irrespectively of tropism switch direction, was associated with less favorable CD4 changes and with an increased risk of disease progression, especially in subjects with PD VL. Our results might contribute to explain why a substantial number of patients (62%) diagnosed at a late stage of HIV-1 infection had only R5-tropic virus strains.[4]

Main limitations of our study included the small number of individuals experiencing the study outcomes and, consequently, the power to detect predictive factors of co-receptor tropism switch or the disease progression risk and the possibility to evaluate if the direction of tropism switch may differently affect the risk of disease progression. In addition, we cannot exclude that some individuals with stable tropism might also have experienced tropism switch before ART initiation, between ART initiation and T1, or between T2 and the date of disease progression.

In conclusion, HIV-1 tropism switch under ART pressure was detected in both directions and it appears to occur with similar rates in subjects with PD or PU VL. An unstable tropism might be associated with a less favorable CD4 change and an increased risk of disease progression, independently of established risk factors

for HIV progression. Larger studies are needed to confirm our hypothesis.

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