CD4 T cell decline following HIV seroconversion in individuals with and without CXCR4-tropic virus

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Running title: CD4 decline following PHI according to tropism

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Abstract:

Background: Data on natural clinical and immunological courses following HIV seroconversion with CXCR4-tropic or dual mixed (X4/DM) viruses are controversial. We compared spontaneous immunological outcome in patients harbouring a X4/DM virus at the time of seroconversion to those harbouring a CCR5-tropic (R5) virus.

Methods: Data from patients participating in CASCADE, a large cohort collaboration of HIV seroconverters, and with ≥2 years of follow-up since seroconversion were included. The HIV envelope gene was sequenced from frozen plasma samples collected at enrolment, and HIV tropism was determined using Geno2Pheno algorithm (FPR 10%). The spontaneous CD4 T cell evolution was compared by modeling CD4 kinetics using linear mixed models with random intercept and random slope.

Results: 1387 patients were eligible. Median time between seroconversion and enrolment was one month (range 0-3). At enrolment, 202 of 1387 (15%) harboured a X4/DM-tropic virus. CD4 decrease slopes were not significantly different according to HIV-1 tropism during the first 30 months following seroconversion. No marked change in these results was found after adjusting for age, year of seroconversion, and baseline HIV viral load. Time to antiretroviral treatment initiation was not statistically different between patients harbouring a R5 (20.76 months) and those harbouring a X4/DM-tropic virus (22.86 months, logrank test p=0.32).

Conclusion: In this large cohort collaboration, 15% of the patients harboured a X4/DM virus close to HIV seroconversion. Patients harbouring X4/DM tropic viruses close to seroconversion did not have an increased risk of disease progression, estimated by the decline in CD4 T cell count or time to cART initiation.

Introduction

HIV-1 enters into its target cells through a stepwise process including attachment to CD4 receptor on the cell surface, interaction with cell surface chemokine receptors, and fusion of the viral envelope and host cell membranes. Viral strains are classified as R5 when they only use the cysteine-cysteine receptor 5 (CCR5 or R5), X4 when they only use cysteine-X-cysteine receptor 4 (CXCR4 or X4) or X4/DM (dual/mixed) when both R5 and X4 viruses coexist in blood plasma. HIV transmitted through sexual activity is predominantly R5 tropic, as semen partly promotes transmission of R5 tropic viruses,[1] and because transmission of X4 tropic strains appears to be constrained whatever the route of transmission.[2-4] For this reason HIV variants isolated early in the course of infection use CCR5, along with CD4, to gain entry into cells,[5] while X4-tropic variants emerge late, and have also been associated with an accelerated decline of CD4 T cell count and progression to AIDS.[6,7] R5-tropic viruses are predominant during primary HIV-1 infection (PHI), although recent findings suggest that the prevalence of X4-tropic variants can reach up to 16% during PHI.[8-10] A rapid progression to AIDS has been reported in one patient shortly after primary infection with a dual-mixed X4/DM variant.[11] Cross-sectional studies performed at the time of PHI have not reported any difference in CD4 T cell count in those harbouring a X4 tropic virus compared to those harbouring a R5 tropic virus.[8-10] Longitudinal studies examining differences between R5 and X4 or dual mixed (X4/DM) viruses with regards to the natural clinical and immunological courses following HIV seroconversion are scarce and findings are conflicting. While some suggested that X4-tropic viruses present at PHI increase the risk of immunological progression,[12] others did not.[8] The major limitation of these longitudinal studies is their small sample size.

Here we assessed the impact of the presence of X4/DM variants (determined by genotypic assay) at the time of seroconversion on the subsequent natural evolution of CD4 T cell count

and on the time to combined antiretroviral treatment (cART) initiation in the large CASCADE collaboration cohort.

Patients and Methods

CASCADE is a collaboration of 28 cohorts of individuals with well estimated dates of HIV seroconversion (seroconverters). We used data pooled in September 2014, within EuroCoord. All collaborating cohorts received approval from their regulatory or national ethics review boards. Seroconversion dates were estimated as the midpoint between the last documented negative and first positive HIV antibody test dates for most participants (84.6%) with the interval between tests being 3 years or less. For the remaining individuals, seroconversion date was estimated through laboratory methods (PCR positivity in the absence of HIV antibodies or antigen positivity with four or fewer bands on western blot), or as the date of seroconversion illness with both an earlier negative and a later positive HIV test done within a time interval of 3 years or less.[13]

Data from patients participating in CASCADE were included in the present study if they had an interval of less than 2 years between a negative/positive ELISA or laboratory evidence of seroconversion, were enrolled after 1995, and had ≥2 years of follow-up since seroconversion, were ART-naive at enrolment, and had an available frozen sample within 12 months following seroconversion while ART-naive.

The HIV envelope gene was amplified and sequenced from frozen plasma samples collected at enrolment in the cohort and HIV tropism was determined using Geno2Pheno algorithm with a false-positive rate (FPR) of 10%. We used specific validated algorithms to predict tropism of CRF02_AG,[14] D[15] and CRF01_AE[16] subtype viruses. Genotypic prediction of tropism for other non-B subtype viruses was done similarly to B subtype viruses, according to the French ANRS algorithm (www.hivfrenchresistance.org). All tropism determinations were performed in the same Virology Laboratory of Saint-Louis Hospital in Paris, France.

Patient characteristics at the time of enrolment in the respective cohorts within CASCADE were compared using the Chi2 test and the Wilcoxon rank-sum test for categorical and continuous variables according to tropism R5 versus X4/DM, respectively. CD4 T cell count kinetics were analyzed on a square-root scale in order to obtain a normal distribution and stabilize the variance. We estimated the CD4 T cell dynamics over time, accounting for the correlation among repeated measurements within each individual, through linear mixed models with random intercept and random slope**.** Slopes of CD4 T cell counts were compared between the two groups. The mean CD4 count evolution was depicted by plotting the mixed model predictions. We examined evidence of an interaction between HIV-1 subtype and tropism. Time to cART initiation according to tropism was estimated by using Kaplan–Meier survival analysis and compared by log-rank test.

We performed several sensitivity analyses. First, because specific interpretation rules were used to predict tropism for non-B HIV-1 subtypes, we examined impact of HIV-1 tropism on CD4 T cell count evolution separately in B and in non-B HIV-1 subtypes. Second, because the French ANRS-PRIMO cohort accounted for half of the patients included in the study, and because French guidelines include specific therapeutic recommendations for PHI management,[17] we also performed the analysis without data from the ANRS – PRIMO cohort.

Results

Characteristics at enrolment

A total of 1387 patients were eligible for inclusion in the study. Their characteristics are shown in Table 1, with the key finding being that median time between estimated date of seroconversion and enrolment into a CASCADE cohort was one month (IQR 0-3) and median time between cohort enrolment and cART initiation was 21 months. At enrolment, 202 of 1387 (14.6% (95% CI: 12.7-16.5%)) harboured an X4/DM-tropic virus and their baseline characteristics did not differ from the 1185 harbouring a R5-tropic virus as regards to age, gender, year of enrolment, transmission group, CD4 count and HIV viral load. The only difference was HIV subtype; the prevalence of X4/DM-tropic viruses was higher in subtype B (16.4%) than in non-B subtypes viruses (6.3%, $p<0.001$) (Table 1).

CD4 T cell count decline according to HIV-1 tropism

The CD4 dynamics were modelled according to tropism (Figure 1). CD4 decrease slopes were not significantly different according to HIV-1 tropism during the first 30 months following seroconversion: the slope of CD4 T cell decrease was -0.13 √CD4/month and -0.16 √CD4/month in patients harbouring a R5 or X4/DM virus, respectively. This difference did not reach statistical significance ($p=0.08$, Table 2). For example, starting from 500 CD4 T cells/mm^3, the model predicted that a patient harbouring a R5-tropic virus would reach a CD4 T cell count of 476/mm^3 after 12 months of follow-up without cART, while a patient harbouring a X4/DM tropic would reach a mean of 449 CD4 T cells/mm^3 at the same time point of follow-up. No marked change in these results was found after adjusting for age, year of seroconversion (<2002, [2002-2005[, [2005-2007[, and ≥2007), and baseline HIV viral load.

Time to cART initiation according to HIV-1 tropism

A total of 225 patients did not initiate cART during follow-up: 17% with a R5-tropic virus and 13% with a X4/DM tropic virus (p=0.23). The Kaplan-Meier estimates of the median delay between enrolment and cART initiation was 20.76 months in patients harbouring an R5-tropic virus (IQR 0.72 – 51) and 22.86 months in patients with a X4/DM tropic virus (IQR 0.49 – 47) , with no statistically significant difference (logrank test p=0.32; Figure 2).

Sensitivity analysis

Although no statistically significant interaction was found between viral subtype and tropism in the model, we also ran separately the analysis in patients harbouring a B subtype virus and non-B virus, and found similar results. Only after excluding patients from the ANRS – PRIMO cohort, we found a statistically significant difference, albeit modest, in CD4 T cell count slope according to HIV-1 tropism, with a steeper slope for X4/DM than for R5 tropic viruses (p=0.02). For example, starting from 500 CD4 T cells/mm^3, the model predicted that a patient harbouring a R5-tropic virus would reach a CD4 T cell count of 376/mm^3 after 24 months of follow-up without cART, while a patient harbouring a X4/DM tropic would reach a mean of 333 CD4 T cells/mm^3 at the same time point of follow-up. At 30 months of follow-up, the CD4 T cell count would be 348/mm^3 for a patient harbouring a R5-tropic virus and 297/mm^3 for a patient harbouring a X4/DM-tropic virus. This difference remained statistically significant after adjusting for age, year of seroconversion (<2002, [2002-2005[, [2005-2007[, and ≥2007), and HIV viral load (p=0.01). Again, no statistically significant interaction was found between viral subtype and tropism.

Discussion

Here we show, in the largest sample size to date, that HIV-1 X4/DM tropic viruses can be identified in a significant proportion of patients enrolled close to seroconversion, and that X4/DM tropic viruses are not significantly associated with a faster decline in CD4 T cell count.

Despite the fact that semen promotes the transmission of R5-tropic viruses, we showed here that, in a large sample size with more than 95% of patients having acquired HIV through sexual transmission, almost 15% of these patients harboured X4/DM tropic viruses close to seroconversion. Such a proportion of X4/DM tropic viruses at the time of seroconversion is in keeping with other smaller earlier studies performed in France and in Spain.[8,9] These X4/DM viruses, when detected at the time of seroconversion, are dominant and quasi-exclusive and persist for lengthy periods of time.[16,18]

To the best of our knowledge, our study, by using the CASCADE collaboration cohort, has included the largest number of patients enrolled close to seroconversion. Unlike previous reports in chronically infected naïve patients or in patients with advanced HIV disease,[7,19,20] we show that, in recent infection, patients harbouring X4/DM tropic viruses did not have an increased risk of disease progression, estimated by the decline in CD4 T cell count or time to cART initiation.

We were also able to address the issue of HIV-1 subtype as 18% (n=254) of participants were infected with non-B subtypes. Some HIV-1 subtypes may have an impact on CD4 count at HIV seroconversion and CD4 rate of decline, but such subtypes are rare in CASCADE.[21] Mlisana et al showed that HIV-1 C subtype was associated with a rapid disease progression and a faster decline in CD4 T cell count.[22] Only one X4/DM tropic virus belonged to C subtype in our study. Of note, the Geno2Pheno test used to predict viral tropism has been validated for B subtype viruses.[23,24] Thus, specific rules have been generated for the

prediction of HIV-1 CRF02_AG, CRF01_AE and D subtype viruses,[14-16] but such specific rules are not available for other non-B subtype viruses. We did not find an impact of HIV-1 tropism on CD4 T cell count slopes according to HIV-1 subtype (B versus non-B).

A potential limitation might be that data on genotypic resistance to nucleoside and non-nucleoside reverse transcriptase inhibitors, protease and integrase inhibitors were not available for the current study, but we have shown previously that the frequency of R5X4 viruses among patients infected with resistant viruses was similar to that in those harbouring wild-type viruses.[9] Another limitation might be the lack of tropism assessment during follow-up. Indeed, some patients harbouring a R5-tropic virus at the time of seroconversion might have experienced a switch to X4-tropic virus during follow-up. However, such a coreceptor switch in the early course of the disease and without drug-selective pressure is very rare.[25]

Interestingly, we did find a statistically significant difference in CD4 T cell count slopes according to HIV-1 tropism when restricting the analysis to all but the ANRS- PRIMO cohort. We performed this sensitivity analysis because (i) the ANRS – PRIMO cohort accounted for half of the patients enrolled in the present study and (ii) French antiretroviral treatment guidelines during PHI might have differed from other countries in the past, with a more systematic and rapid antiretroviral treatment initiation during PHI.[17] Indeed, rapid treatment initiation at the time of PHI may have offset the potential role of HIV-1 tropism on the subsequent CD4 T cell count natural slope. Although statistically significant, the difference in the CD4 T cell count reached after 24 months of follow-up may not be clinically relevant.

The value of determining HIV-1 tropism at the time of PHI is questionable now that all national and international guidelines recommend rapid initiation of cART in patients diagnosed at the time of PHI. Maraviroc, a CCR5-antagonist, is also not listed among the preferred antiretrovirals to be used for first line cART. Recent data, however, suggest that the

239 presence of CXCR4-using viruses at the time of PHI was associated with the virological
240 failure of cART initiated during PHI.[26] In addition, there is a growing interest in such patients,
241 diagnosed and started on cART at the time of PHI, because they might be the best candidates
242 for future studies addressing functional cure.[27-29] Such studies require structured treatment
243 interruptions, thus, HIV-1 tropism might also prove helpful in selecting the best candidates.
244

Acknowledgments:

CASCADE Steering Committee: Julia Del Amo (Chair), Laurence Meyer (Vice Chair), Heiner C. Bucher, Geneviève Chêne, Osamah Hamouda, Deenan Pillay, Maria Prins, Magda Rosinska, Caroline Sabin, Giota Touloumi.

CASCADE Co-ordinating Centre: Kholoud Porter (Project Leader), Ashley Olson, Andrea Cartier, Lorraine Fradette, Sarah Walker, Abdel Babiker.

CASCADE Clinical Advisory Board: Heiner C. Bucher, Andrea De Luca, Martin Fisher, Roberto Muga

CASCADE Collaborators: Australia PHAEDRA cohort (Tony Kelleher, David Cooper, Pat Grey, Robert Finlayson, Mark Bloch) Sydney AIDS Prospective Study and Sydney Primary HIV Infection cohort (Tony Kelleher, Tim Ramacciotti, Linda Gelgor, David Cooper, Don Smith); Austria Austrian HIV Cohort Study (Robert Zangerle); Canada South Alberta clinic (John Gill); Estonia Tartu Ülikool (Irja Lutsar); France ANRS CO3 Aquitaine cohort (Geneviève Chêne, Francois Dabis, Rodolphe Thiebaut), ANRS CO4 French Hospital Database (Dominique Costagliola, Marguerite Guiguet), Lyon Primary Infection cohort (Philippe Vanhems), French ANRS CO6 PRIMO cohort (Marie-Laure Chaix, Jade Ghosn), ANRS CO2 SEROCO cohort (Laurence Meyer, Faroudy Boufassa); Germany German HIV-1 seroconverter cohort (Osamah Hamouda, Karolin Meixenberger, Norbert Bannert, Barbara Bartmeyer); Greece AMACS (Anastasia Antoniadou, Georgios Chrysos, Georgios L. Daikos); Greek Haemophilia cohort (Giota Touloumi, Nikos Pantazis, Olga Katsarou); Italy Italian Seroconversion Study (Giovanni Rezza, Maria Dorrucci), ICONA cohort (Antonella d'Arminio Monforte, Andrea De Luca.) Netherlands Amsterdam Cohort Studies among homosexual men and drug users (Maria Prins, Ronald Geskus, Jannie van der Helm, Hanneke Schuitemaker); Norway Oslo and Ulleval Hospital cohorts (Mette Sannes, Oddbjorn Brubakk, Anne-Marte Bakken Kran); Poland National Institute of Hygiene (Magdalena Rosinska); Spain Badalona IDU hospital cohort (Roberto Muga, Jordi Tor), Barcelona IDU Cohort (Patricia Garcia de Olalla, Joan Cayla), CoRIS-scv (Julia del Amo, Santiago Moreno, Susana Monge); Madrid cohort (Julia Del Amo, Jorge del Romero), Valencia IDU cohort (Santiago Pérez-Hoyos); Sweden Swedish InfCare HIV Cohort, Sweden (Anders Sönnerborg); Switzerland Swiss HIV Cohort Study (Heiner C. Bucher, Huldrych Günthard, Alexandra Scherrer); Ukraine Perinatal Prevention of AIDS Initiative (Ruslan Malyuta); United Kingdom Public Health England (Gary Murphy), UK Register of HIV Seroconverters (Kholoud Porter, Anne Johnson, Andrew Phillips, Abdel Babiker), University College London (Deenan Pillay); African cohorts: Genital Shedding Study (US: Charles Morrison; Family Health International, Robert Salata, Case Western Reserve University, Uganda: Roy Mugerwa, Makerere University, Zimbabwe: Tsungai Chipato, University of Zimbabwe); International AIDS Vaccine Initiative (IAVI) Early Infections Cohort (Kenya, Rwanda, South Africa, Uganda, Zambia: Matt A. Price, IAVI, USA; Jill Gilmour, IAVI, UK; Anatoli Kamali, IAVI, Kenya; Etienne Karita, Projet San Francisco, Rwanda).

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Phillips, University College London, UK, Kholoud Porter, University College London, UK; Claire Thorne, University College London, UK.

EuroCoord Council of Partners: Jean-Pierre Aboulker, Institut National de la Santé et de la Recherche Médicale, France; Jan Albert, Karolinska Institute, Sweden; Silvia Asandi, Romanian Angel Appeal Foundation, Romania; Geneviève Chêne, University of Bordeaux, France; Dominique Costagliola (chair), INSERM, France; Antonella d'Arminio Monforte, ICoNA Foundation, Italy; Stéphane De Wit, St. Pierre University Hospital, Belgium; Peter Reiss, Stichting HIV Monitoring, Netherlands; Julia Del Amo, Instituto de Salud Carlos III, Spain; José Gatell, Fundació Privada Clínic per a la Recerca Bíomèdica, Spain; Carlo Giaquinto, Fondazione PENTA, Italy; Osamah Hamouda, Robert Koch Institut, Germany; Igor Karpov, University of Minsk, Belarus; Bruno Ledergerber, University of Zurich, Switzerland; Jens Lundgren, Region Hovedstaden, Denmark; Ruslan Malyuta, Perinatal Prevention of AIDS Initiative, Ukraine; Claus Møller, Cadpeople A/S, Denmark; Kholoud Porter, University College London, United Kingdom; Maria Prins, Academic Medical Centre, Netherlands; Aza Rakhmanova, St. Petersburg City AIDS Centre, Russian Federation; Jürgen Rockstroh, University of Bonn, Germany; Magda Rosinska, National Institute of Public Health, National Institute of Hygiene, Poland; Manjinder Sandhu, Genome Research Limited; Claire Thorne, University College London, UK; Giota Touloumi, National and Kapodistrian University of Athens, Greece; Alain Volny Anne, European AIDS Treatment Group, France.

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Funding:

This study was funded by CASCADE collaboration in EuroCOORD.

Transparency declaration:

Authors have no conflict of interest to declare with regards to the present study.

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410 **Table 1: Characteristics of eligible patients at the time of enrolment in CASCADE**
411 **according to HIV-1 tropism (R5 versus X4/DM)**
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	ALL	R5 N=1185	X4/DM N=202
Sex, % (n)			
Male	87 (1213)	88 (1040)	86 (173)
Age at enrolment Median (IQR), years	35 (29-41)	35 (29-41)	35 (29-40)
Time of follow-up before cART Median (IQR), months	21 (0.7-50)	20.76 (0.72 – 51)	22.86 (0.49-47)
Year of enrolment Median (IQR)	2005 (2002-2007)	2005 (2002-2007)	2005 (2001-2007)
Time between seroconversion and enrolment Median (IQR), months	0.9 (0.3-2.7)	0.9 (0.3-2.7)	0.8 (0.3-3.2)
Transmission group, % (n)			
Homosexual / bisexual	73 (1016)	73 (864)	75 (152)
Heterosexual	21 (284)	21 (247)	18 (37)
Other, IV, haemophilia	3 (46)	3 (35)	6 (11)
Missing	3 (41)	3 (39)	1 (2)
Ethnic origin, % (n)			
White	69 (956)	69 (818)	68 (138)
African & other (6 Asians)	8 (110)	9 (101)	5(9)
Missing values	23 (321)	22 (266)	27 (55)
Subtype			
Subtype B	70.7 (980)	69 (819)	80 (161)
CRF02_AG	0.8 (11)	1 (11)	0 (0)
Other	17.5 (243)	19 (227)	8 (16)
missing	11 (153)	11 (128)	12 (25)
Clinical AIDS, % (n) during follow-up	5 (74)	5 (63)	5 (11)
ART treatment initiated, % (n) during follow up in the cohort (at anytime)	84 (1162)	83 (987)	87 (175)
Number of CD4 measurements Median	6 (1-11)	6 (1-11)	6 (1-11)

(IQR)			
CD4 cell count at PHI diagnosis* (Median (IQR) cells/mm^3)	508 (377-673)	510 (378-672)	498 (366-678)
HIV viral load at PHI diagnosis**			
Median (IQR) log_{10}c/mL	4.9 (4.2-5.5)	5.0 (4.3-5.5)	4.9 (4.2-5.4)

***1 missing value**

**** 116 missing values for viral load**

Table 2: Spontaneous evolution of CD4 cell count in patients with R5-tropic virus versus X4/DM-tropic virus, from linear mixed-effects models

PARAMETER	ESTIMATE	SE*	P VALUE	ADJUSTED ESTIMATE **	SE*	P VALUE
FIRST √CD4 FOLLOWING SEROCONVERSION (IN R5)	23.42	0.47	**<.0001**			
X4 VS R5	-0.27	0.39	0.50	-0.24	0.39	0.53
SLOPE √CD4/MONTH						
R5	-0.13	0.01		-0.14	0.01	
X4	-0.16	0.02		-0.17	0.02	
X4 VS R5	-0.03	0.02	0.08	-0.04	0.02	0.06

*Standard Error,**Adjusted for: age, year of seroconversion (in 4 categories according to percentiles **<2002** ; ≥2002 et <2005 ; ≥2005 et <2007 ; ≥2007), HIV viral load at PHI

Figure 1: Estimated CD4 cell count decline from the piecewise linear mixed-effects model according to tropism (dashed line represents the predicted estimated CD4 cell count decline with CCR5 viruses and solid line represents the predicted estimated CD4 cell count decline with CXCR4 viruses).

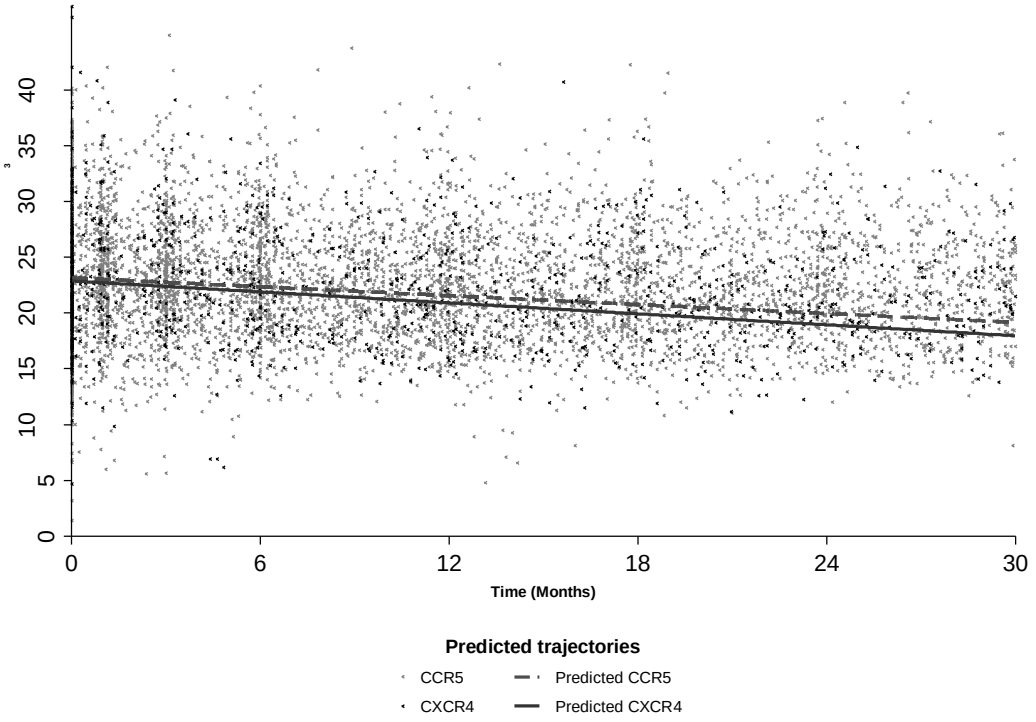

Figure 2: time to cART initiation according to HIV-1 tropism (Kaplan- Meier survival curves, log rank) (dashed line represents cumulative probability of initiating cART in patients harbouring CCR5 viruses and solid line represents cumulative probability of initiating cART in patients harbouring CXCR4 viruses)