

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

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31

32 **Running title: CD4 decline following PHI according to tropism**

33

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44 **Abstract:**

45

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

50

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

57

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

65

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

70 **Introduction**

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

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

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

97 **Patients and Methods**

98

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

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

114

115 The HIV envelope gene was amplified and sequenced from frozen plasma samples collected
116 at enrolment in the cohort and HIV tropism was determined using Geno2Pheno algorithm
117 with a false-positive rate (FPR) of 10%. We used specific validated algorithms to predict
118 tropism of CRF02_AG,¹⁴ D¹⁵ and CRF01_AE¹⁶ subtype viruses. Genotypic prediction of
119 tropism for other non-B subtype viruses was done similarly to B subtype viruses, according to
120 the French ANRS algorithm (www.hivfrenchresistance.org). All tropism determinations were
121 performed in the same Virology Laboratory of Saint-Louis Hospital in Paris, France.

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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.

141 **Results**

142 *Characteristics at enrolment*

143 A total of 1387 patients were eligible for inclusion in the study. Their characteristics are
144 shown in Table 1, with the key finding being that median time between estimated date of
145 seroconversion and enrolment into a CASCADE cohort was one month (IQR 0-3) and median
146 time between cohort enrolment and cART initiation was 21 months. At enrolment, 202 of
147 1387 (14.6% (95% CI: 12.7-16.5%)) harboured an X4/DM-tropic virus and their baseline
148 characteristics did not differ from the 1185 harbouring a R5-tropic virus as regards to age,
149 gender, year of enrolment, transmission group, CD4 count and HIV viral load. The only
150 difference was HIV subtype; the prevalence of X4/DM-tropic viruses was higher in subtype B
151 (16.4%) than in non-B subtypes viruses (6.3%, $p < 0.001$) (Table 1).

152

153 *CD4 T cell count decline according to HIV-1 tropism*

154 The CD4 dynamics were modelled according to tropism (Figure 1). CD4 decrease slopes were
155 not significantly different according to HIV-1 tropism during the first 30 months following
156 seroconversion: the slope of CD4 T cell decrease was $-0.13 \sqrt{\text{CD4}}/\text{month}$ and -0.16
157 $\sqrt{\text{CD4}}/\text{month}$ in patients harbouring a R5 or X4/DM virus, respectively. This difference did
158 not reach statistical significance ($p=0.08$, Table 2). For example, starting from 500 CD4 T
159 cells/ mm^3 , the model predicted that a patient harbouring a R5-tropic virus would reach a CD4
160 T cell count of 476/ mm^3 after 12 months of follow-up without cART, while a patient
161 harbouring a X4/DM tropic would reach a mean of 449 CD4 T cells/ mm^3 at the same time
162 point of follow-up. No marked change in these results was found after adjusting for age, year
163 of seroconversion (<2002 , [2002-2005], [2005-2007], and ≥ 2007), and baseline HIV viral load.

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165

166 *Time to cART initiation according to HIV-1 tropism*

167 A total of 225 patients did not initiate cART during follow-up: 17% with a R5-tropic virus
168 and 13% with a X4/DM tropic virus ($p=0.23$). The Kaplan-Meier estimates of the median
169 delay between enrolment and cART initiation was 20.76 months in patients harbouring an R5-
170 tropic virus (IQR 0.72 – 51) and 22.86 months in patients with a X4/DM tropic virus (IQR
171 0.49 – 47) , with no statistically significant difference (logrank test $p=0.32$; Figure 2).

172

173 *Sensitivity analysis*

174

175 Although no statistically significant interaction was found between viral subtype and tropism
176 in the model, we also ran separately the analysis in patients harbouring a B subtype virus and
177 non-B virus, and found similar results. Only after excluding patients from the ANRS –
178 PRIMO cohort, we found a statistically significant difference, albeit modest, in CD4 T cell
179 count slope according to HIV-1 tropism, with a steeper slope for X4/DM than for R5 tropic
180 viruses ($p=0.02$). For example, starting from 500 CD4 T cells/mm³, the model predicted that a
181 patient harbouring a R5-tropic virus would reach a CD4 T cell count of 376/mm³ after 24
182 months of follow-up without cART, while a patient harbouring a X4/DM tropic would reach a
183 mean of 333 CD4 T cells/mm³ at the same time point of follow-up. At 30 months of follow-
184 up, the CD4 T cell count would be 348/mm³ for a patient harbouring a R5-tropic virus and
185 297/mm³ for a patient harbouring a X4/DM-tropic virus. This difference remained statistically
186 significant after adjusting for age, year of seroconversion (<2002, [2002-2005[, [2005-2007[,
187 and ≥ 2007), and HIV viral load ($p=0.01$). Again, no statistically significant interaction was
188 found between viral subtype and tropism.

189 **Discussion**

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

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

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

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

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

217

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

226 Interestingly, we did find a statistically significant difference in CD4 T cell count slopes
227 according to HIV-1 tropism when restricting the analysis to all but the ANRS- PRIMO cohort.

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

235 The value of determining HIV-1 tropism at the time of PHI is questionable now that all
236 national and international guidelines recommend rapid initiation of cART in patients
237 diagnosed at the time of PHI. Maraviroc, a CCR5-antagonist, is also not listed among the
238 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.
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- 333 1. Balandya E, Sheth S, Sanders K, Wieland-Alter W, Lahey T. Semen protects CD4+ target
334 cells from HIV infection but promotes the preferential transmission of R5 tropic HIV. *J Immunol*
335 2010; **185**: 7596-604.
- 336 2. van't Wout AB, Kootstra NA, Mulder-Kampinga GA, et al. Macrophage-tropic variants
337 initiate human immunodeficiency virus type 1 infection after sexual, parenteral, and vertical
338 transmission. *J Clin Invest* 1994; **94**: 2060-7.
- 339 3. Alkhatib G, Combadiere C, Broder CC, et al. CC CKR5: a RANTES, MIP-1alpha, MIP-
340 1beta receptor as a fusion cofactor for macrophage-tropic HIV-1. *Science* 1996; **272**: 1955-8.
- 341 4. Blanpain C, Libert F, Vassart G, Parmentier M. CCR5 and HIV infection. *Receptors*
342 *Channels* 2002; **8**: 19-31.
- 343 5. Brumme ZL, Goodrich J, Mayer HB, et al. Molecular and clinical epidemiology of
344 CXCR4-using HIV-1 in a large population of antiretroviral-naive individuals. *J Infect Dis* 2005;
345 **192**: 466-74.
- 346 6. Schuitemaker H, Koot M, Kootstra NA, et al. Biological phenotype of human
347 immunodeficiency virus type 1 clones at different stages of infection: progression of disease is
348 associated with a shift from monocyctotropic to T-cell-tropic virus population. *J Virol* 1992; **66**:
349 1354-60.
- 350 7. Daar ES, Kesler KL, Petropoulos CJ, et al. Baseline HIV type 1 coreceptor tropism
351 predicts disease progression. *Clin Infect Dis* 2007; **45**: 643-9.
- 352 8. de Mendoza C, Rodriguez C, Garcia F, et al. Prevalence of X4 tropic viruses in patients
353 recently infected with HIV-1 and lack of association with transmission of drug resistance. *J*
354 *Antimicrob Chemother* 2007; **59**: 698-704.
- 355 9. Frange P, Galimand J, Goujard C, et al. High frequency of X4/DM-tropic viruses in
356 PBMC samples from patients with primary HIV-1 subtype-B infection in 1996-2007: the French
357 ANRS CO06 PRIMO Cohort Study. *J Antimicrob Chemother* 2009; **64**: 135-41.
- 358 10. Raymond S, Delobel P, Mavigner M, et al. Development and performance of a new
359 recombinant virus phenotypic entry assay to determine HIV-1 coreceptor usage. *J Clin Virol*
360 2010; **47**: 126-30.
- 361 11. Markowitz M, Mohri H, Mehandru S, et al. Infection with multidrug resistant, dual-tropic
362 HIV-1 and rapid progression to AIDS: a case report. *Lancet* 2005; **365**: 1031-8.
- 363 12. Raymond S, Delobel P, Mavigner M, et al. CXCR4-using viruses in plasma and peripheral
364 blood mononuclear cells during primary HIV-1 infection and impact on disease progression.
365 *AIDS* 2010; **24**: 2305-12.
- 366 13. Pantazis N, Porter K, Costagliola D, et al. Temporal trends in prognostic markers of
367 HIV-1 virulence and transmissibility: an observational cohort study. *Lancet HIV* 2014; **1**: e119-26.
- 368 14. Raymond S, Delobel P, Mavigner M, et al. Genotypic prediction of human
369 immunodeficiency virus type 1 CRF02-AG tropism. *J Clin Microbiol* 2009; **47**: 2292-4.
- 370 15. Raymond S, Delobel P, Chaix ML, et al. Genotypic prediction of HIV-1 subtype D
371 tropism. *Retrovirology* 2011; **8**: 56.
- 372 16. Raymond S, Delobel P, Rogez S, et al. Genotypic prediction of HIV-1 CRF01-AE
373 tropism. *J Clin Microbiol* 2013; **51**: 564-70.
- 374 17. Krastinova E, Seng R, Yeni P, et al. Is clinical practice concordant with the changes in
375 guidelines for antiretroviral therapy initiation during primary and chronic HIV-1 infection? The
376 ANRS PRIMO and COPANA cohorts. *PLoS One* 2013; **8**: e71473.
- 377 18. Ghosn J, Galimand J, Raymond S, et al. X4 tropic multi-drug resistant quasi-species
378 detected at the time of primary HIV-1 infection remain exclusive or at least dominant far from
379 PHI. *PLoS One* 2011; **6**: e23301.

- 380 19. Waters L, Mandalia S, Randell P, Wildfire A, Gazzard B, Moyle G. The impact of HIV
381 tropism on decreases in CD4 cell count, clinical progression, and subsequent response to a first
382 antiretroviral therapy regimen. *Clin Infect Dis* 2008; **46**: 1617-23.
- 383 20. Soulie C, Charpentier C, Flandre P, et al. Natural evolution of CD4+ cell count in
384 patients with CD4 >350 or >500 cells/mm³ at the time of diagnosis according to HIV-1
385 coreceptor tropism. *J Med Virol* 2012; **84**: 1853-6.
- 386 21. Touloumi G, Pantazis N, Pillay D, et al. Impact of HIV-1 subtype on CD4 count at HIV
387 seroconversion, rate of decline, and viral load set point in European seroconverter cohorts. *Clin*
388 *Infect Dis* 2013; **56**: 888-97.
- 389 22. Mlisana K, Werner L, Garrett NJ, et al. Rapid disease progression in HIV-1 subtype C-
390 infected South African women. *Clin Infect Dis* 2014; **59**: 1322-31.
- 391 23. Raymond S, Delobel P, Mavigner M, et al. Correlation between genotypic predictions
392 based on V3 sequences and phenotypic determination of HIV-1 tropism. *AIDS* 2008; **22**: F11-6.
- 393 24. Recordon-Pinson P, Soulie C, Flandre P, et al. Evaluation of the genotypic prediction of
394 HIV-1 coreceptor use versus a phenotypic assay and correlation with the virological response to
395 maraviroc: the ANRS GenoTropism study. *Antimicrob Agents Chemother* 2010; **54**: 3335-40.
- 396 25. Mortier V, Dauwe K, Vancoillie L, et al. Frequency and predictors of HIV-1 co-receptor
397 switch in treatment naive patients. *PLoS One* 2013; **8**: e80259.
- 398 26. Raymond S, Nicot F, Carcenac R, et al. No selection of CXCR4-using variants in cell
399 reservoirs of dual-mixed HIV-infected patients on suppressive maraviroc therapy. *AIDS* 2016;
400 **30**: 965-8.
- 401 27. Laanani M, Ghosn J, Essat A, et al. Impact of the Timing of Initiation of Antiretroviral
402 Therapy During Primary HIV-1 Infection on the Decay of Cell-Associated HIV-DNA. *Clin Infect*
403 *Dis* 2015; **60**: 1715-21.
- 404 28. Ghosn J, Delaugerre C. Can we avoid treatment interruption studies in the search for an
405 HIV cure? *AIDS* 2015; **29**: 1575-7.
- 406 29. Saez-Cirion A, Bacchus C, Hocqueloux L, et al. Post-treatment HIV-1 controllers with a
407 long-term virological remission after the interruption of early initiated antiretroviral therapy
408 ANRS VISCONTI Study. *PLoS Pathog* 2013; **9**: e1003211.
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Table 1: Characteristics of eligible patients at the time of enrolment in CASCADE according to HIV-1 tropism (R5 versus X4/DM)

	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)
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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)
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414 ***1 missing value**

415 **** 116 missing values for viral load**

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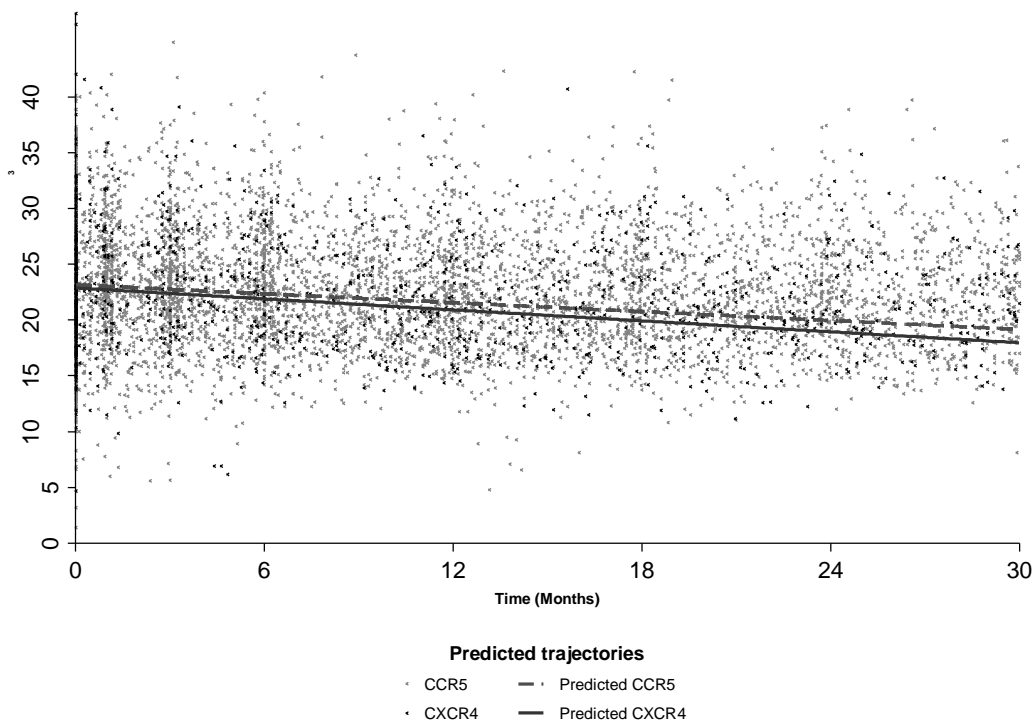
417 **Table 2: Spontaneous evolution of CD4 cell count in patients with R5-tropic virus versus**
 418 **X4/DM-tropic virus, from linear mixed-effects models**
 419

PARAMETER	ESTIMATE	SE*	P VALUE	ADJUSTED ESTIMATE**	SE*	P VALUE
FIRST $\sqrt{\text{CD4}}$ 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 $\sqrt{\text{CD4}}$/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

420 *Standard Error,**Adjusted for: age, year of seroconversion (in 4 categories according to
 421 percentiles <2002 ; \geq 2002 et <2005 ; \geq 2005 et <2007 ; \geq 2007), HIV viral load at PHI
 422

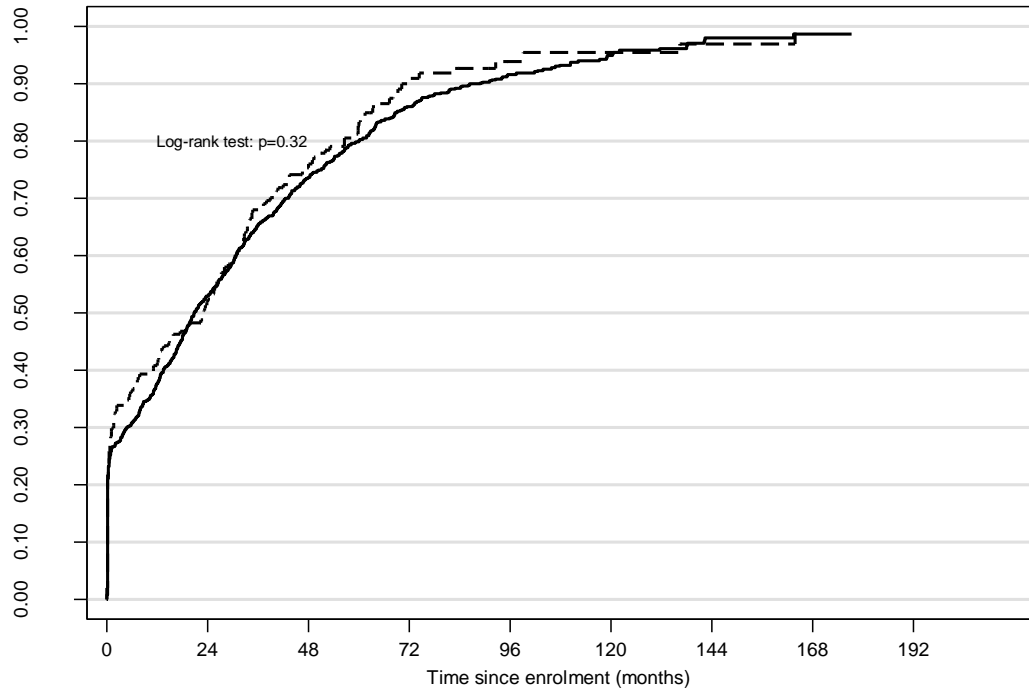
423 **Figure 1:** Estimated CD4 cell count decline from the piecewise linear mixed-effects model
424 according to tropism (dashed line represents the predicted estimated CD4 cell count decline
425 with CCR5 viruses and solid line represents the predicted estimated CD4 cell count decline
426 with CXCR4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

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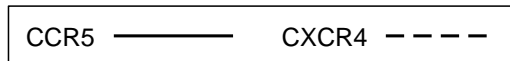
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433 **Figure 2: time to cART initiation according to HIV-1 tropism (Kaplan- Meier survival**
 434 **curves, log rank)** (dashed line represents cumulative probability of initiating cART in
 435 patients harbouring CCR5 viruses and solid line represents cumulative probability of initiating
 436 cART in patients harbouring CXCR4 viruses)
 437
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Number at risk

CCR5	1185	541	231	92	41	17	4	2	0	0
CXCR4	201	94	39	11	4	3	1	0	0	0



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