European Mitochondrial Haplogroups Are Associated With CD4+ T Cell Recovery in HIV-infected Patients on Combination Antiretroviral Therapy

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ABSTRACT

Background: There is substantial interindividual variability in the rate and extent of CD4+ T cell (CD4+) recovery after starting combination antiretroviral therapy (cART). The aim of our study was to determine whether mitochondrial DNA (mtDNA) haplogroups are associated with recovery of CD4+ in HIV-infected patients on cART.

Methods: We carried out a retrospective study on 275 naïve cART patients with CD4+ counts <350 cells/mm^3, who were followed-up during at least 24 months after initiating cART. mtDNA genotyping was performed by Sequenom's MassARRAY platform.

Results: Patients within cluster JT and haplogroup J had a lower chance of achieving a CD4+ count ≥500 cells/mm^3 than patients within cluster HV and haplogroup H (hazard ratio (HR)=0.68 (p=0.058) and HR=0.48 (p=0.010), respectively). The time of follow-up during which the CD4+ count was ≥500 cells/mm^3 was longer in haplogroups HV and H than in haplogroups JT and J(20 months versus 6.2 months (p=0.049) and 20 months versus 0 months (p=0.047), respectively). Additionally, haplogroups HV and H had higher chances of achieving CD4+ count ≥500 cells/mm^3 during at least 12, 36, 48, and 60 months post-cART initiation compared to patients within haplogroups JT and J. Patients within haplogroup T only had a lower chance of achieving CD4+ count ≥500 cells/mm^3 during at least 48 months and 60 months post-cART initiation.

Conclusion: European mitochondrial haplogroups might influence CD4+ recovery in HIV-infected patients following initiation with cART. Haplogroups J and T appear to be associated with a worse profile of CD4+ recovery, whereas haplogroup H was associated with a better CD4+ reconstitution.

Key-words: mitochondria; polymorphisms; AIDS; antiretroviral therapy; immune system reconstitution

INTRODUCTION

Mitochondria are essential organelles that provide energy to eukaryotic cells via oxidative phosphorylation and regulate cellular survival via control of apoptosis while playing a key role in the innate immune response against viral infections.[1] Systemic mitochondrial dysfunction is evident during acquired immune deficiency syndrome (AIDS) progression, such as in the form of mitochondrial genome (mtDNA) depletion, increased reactive oxygen species (ROS) formation, antioxidant enzyme deficiency, and increased oxidative damage in patients with accelerated disease.[2] In addition, mitochondrial toxicity contributes to serious side effects observed in human immunodeficiency virus (HIV)-infected individuals treated with nucleoside reverse transcriptase inhibitors (NRTIs).[3] These drugs, such as didanosine, zidovudine, and stavudine, are potent inhibitors of mitochondria DNA polymerase gamma; and zidovudine may inhibit thymidine kinase 2 and cause reduced levels of endogenous nucleotides thereby decreasing synthesis of mtDNA.[3]

Mutations in mtDNA have been acquired throughout human history, and thus the human population has been subdivided into a number of discrete mitochondrial clades or haplogroups, which are defined on the basis of specific mtDNA polymorphisms.[4] In European Caucasians, 4 major haplogroups or clusters (HV, U, JT, and IWX) and several minor haplogroups have been identified (H, V, pre-V, J, T, Uk, W, X, I, etc.).[4] Variations in mtDNA have been directly associated with susceptibility to disorders such as cancer, sepsis, diabetes, and degenerative diseases.[5] In HIV infection, mtDNA haplogroup H has been associated with a low likelihood of AIDS progression and/or severe immunodeficiency.[6] Associations with metabolic disturbances and liver fibrosis progression have also been detected in patients coinfected with hepatitis C virus.[7, 8] Moreover, mtDNA haplogroups J and T that are infected with HIV have increased likelihoods of AIDS progression and/or CD4+ count <200 cells/mm^3,[6] metabolic disturbances,[7] and peripheral neuropathy;[9] but lower chances of lipoatrophy [10] and neuroretinal disorder. [11]

The CD4+ T cell is the primary cellular target of HIV, and a continuous loss of CD4+ T cells leads to immunodeficiency, opportunistic diseases, and death.[12, 13] Hence, the CD4+ T cell count in peripheral blood represents the principal surrogate marker for clinical symptoms and AIDS-defining illnesses;[14] and it is also a major factor in the decision to initiate combination antiretroviral therapy (cART) in HIV-infected individuals.[15, 16]The primary goal of cART is to reduce the plasma HIV-RNA to below detectable levels. In this setting, sustained HIV suppression may restore and preserve immunologic function, decreasing both AIDS-defining and non-AIDS-defining complications, and prolonging life.[15, 16] However, there is substantial interindividual variability in the rate and extent of CD4+ T cell recovery after starting cART.[17] Up to 30-40% of cART-treated patients fail to achieve substantial increases in CD4+ count and may continue to develop the disease.[17-21]

85 The aim of our study was to determine whether mtDNA haplogroups are associated with CD4+ T cell
86 recovery in cART-naïve HIV-infected patients after the initiation of cART.
87

PATIENTS AND METHODS

Patients

We carried out a retrospective study on HIV-infected patients, who started cART between 1996 and 2010 in Hospital Gregorio Marañón (Madrid, Spain). This work was conducted in accordance with the Declaration of Helsinki. All patients gave their written informed consent to be included in the study and the Institutional Ethics Committee approved the study.

This population belonged to a cohort that has been followed according to recommendations from the GESIDA/Spanish AIDS Plan regarding antiretroviral treatment in adults with HIV infection.[15] The criteria for inclusion were naïve cART patients with CD4+ count values <350 cells/mm^3 during the chronic phase of infection, DNA samples for genotyping mtDNA polymorphisms, data of CD4 and CV at least every six months, and a follow-up of at least 24 months after the initiation of cART.

We conducted blood sample collections from HIV-infected patients who were in the hospital between January 2010 and June 2010 (six months). From a total of 1500 patients who started cART between 1996 and 2010, we only obtained blood samples from 960 of them. Of those, only 392 patients had baseline CD4+ values <350 cells/mm^3 and CD4 and CV data for at least every six months. Next, only 325 patients had a follow-up of at least 24 months after the initiation of cART.

Of these 325 patients, we performed the assay for DNA genotyping but 27 patients were excluded because we were unable to genotype the mtDNA polymorphisms to determine their mitochondrial haplogroup. Additionally, to make this study more uniform, we excluded 33 patients who were not of the European ‘‘N’’ mitochondrial macro-haplogroup, which is ancestral to almost all European and many Eurasian haplogroups.[4] In the end, we only analyzed 275 HIV-infected patients who started cART. In addition, 162 healthy blood donors (negative for HIV, HCV, and HBV infection) from the “Centro de Transfusión de la Comunidad de Madrid” participated as a control group. Their age and gender characteristics were similar to the HIV-infected patients.

Clinical and laboratory marker data

Data were collected by chart and database review with a standard questionnaire in order to obtain baseline data such as age, sex, HIV risk group, Centers for Disease Control and Prevention (CDC) clinical category, baseline CD4+ T cells and plasma HIV-RNA, hepatitis C virus (HCV) serology, and cART regimen.

Following initiation of cART, patients were monitored every 3 - 6 months with measurements of CD4+ T cells and plasma HIV-RNA. Plasma HIV-RNA was measured using the third-generation branched DNA assay (Quantiplex version 3.0; Siemens, Barcelona, Spain), which displays a low

detection limit of 50 copies/mL. T-cell subsets in peripheral blood were quantified by flow cytometry (FACScan, Becton-Dickinson Immunocytometry Systems, San Jose, CA, USA).

mtDNA Genotyping

Total DNA was extracted from peripheral blood with Qiagen columns (QIAamp DNA Blood Midi/Maxi; Qiagen, Hilden, Germany). DNA samples were sent to the Spanish National Genotyping Center (CeGen; http://www.cegen.org/). Genotyping was performed by using Sequenom's MassARRAY platform (San Diego, CA, USA) using the iPLEX® Gold assay design system.

Individuals within the European N macro cluster were further separated into the most common European major haplogroups or clusters (HV, IWX, U, and JT) and haplogroups (H, V, pre-V, J, T, I, W and X) according to 14 polymorphisms in the mtDNA (see **supplementary figure (SF) 1**). All patients were of European ancestry because individuals not within the N macro cluster were excluded from the study.

Outcome variables

The main outcome variables were: i) the temporal trend in CD4+ T cell counts after starting cART; ii) the ability to achieve a value of CD4+ count ≥500 cells/mm^3 during follow-up; iii) the total time with CD4+ count ≥500 cells/mm^3 during follow-up; and iv) the ability to achieve and maintain CD4+ count ≥500 cells/mm^3 over an extended period of time (at least 12, 24, 36, 48, and 60 months).

The total time with CD4+ count ≥500 cells/mm^3 was calculated taking into account the time elapsed between consecutive visits with the specific event (CD4+ count ≥500 cells/mm^3). The counting was only interrupted when there were at least two consecutive visits without these two outcome variables. Thus, no patient may have 100% of follow-up time with CD4+ count ≥500 cells/mm^3.

Statistical Analysis

Due to the distribution of patients within haplogroups, we analyzed the data according to 4 major haplogroups or clusters (HV, IWX, U, and JT) and 3 haplogroups (H, J, and T) separately. Statistical analysis was performed by SPSS 19.0 software (SPSS INC, Chicago, IL, USA). All tests were two-tailed with p-values <0.05 considered significant.

Categorical data and proportions were analyzed by using the chi-squared test or Fisher's exact test. Mann-Whitney U tests were used to compare data between independent groups. Kaplan-Meier and Cox regression analyses were used to analyze the time to achieve the first value of CD4+ count ≥500 cells/mm^3 as an outcome. Cox regression test was adjusted by baseline characteristics such as gender, age, HCV infection, AIDS, CD4+ T cells/mm^3, HIV-RNA, and NRTIs with mitochondrial toxicity (Zidovudine, Didanosine, Stavudine) in the initial cART regimen.

A Generalized Linear Models (GLM) with a log-link was used to compare the time with CD4+ count ≥500 cells/mm^3 among different mtDNA clusters and haplogroups. Logistic regression analyses were also performed to calculate the likelihood of achieving CD4+ count values ≥500 cells/mm^3 for a long period of time according to mtDNA haplogroups. All regression tests were adjusted by gender, age, HCV infection, AIDS, CD4+ T cells/mm^3 and HIV-RNA at baseline, percentage of the whole follow-up period when HIV-RNA <50 copies/mL, and NRTIs with mitochondrial toxicity (Zidovudine, Didanosine, Stavudine) in the initial cART.

RESULTS

Characteristics of the study population

Table 1 shows the characteristics of 275 HIV-infected patients who self-identified as "white" and had a western European, or N, mitochondrial macro cluster. We did not find any significant differences among haplogroups in epidemiological, clinical, or antiretroviral therapy characteristics. In addition, no patient received TDF and ddI in combination. Additionally, the median of follow-up time among patients from the different European haplogroups was higher than 100 months, and we did not see significant differences among groups.

SF 2 shows the frequencies of mtDNA haplogroups in patients and healthy controls. We did not find any significant differences between groups in the frequencies of mtDNA haplogroups, and the distribution of mtDNA haplogroups across our HIV-infected patients was similar to data found by other authors concerning HIV infection within a Caucasian population.[6, 22, 23] The haplogroups Pre-V, V, I, X, and W had frequencies of less than 5% (**SF 2**) and were therefore included in broader clusters to minimize type I errors in statistical analyses. Thus, the genetic association tests were performed on the major haplogroups or clusters HV, U, JT, and IXW and on the haplogroups H, J, and T.

European haplogroups and temporal trends in CD4+ T cell counts after starting cART

Patients within cluster HV had higher median CD4+ count than patients within cluster JT at the 60th month (p= 0.026) and the 72nd month (p= 0.035) of follow-up (**Figure 1A**). In addition, patients within haplogroup H had higher median CD4+ count than patients within haplogroup J at the 48th month (p= 0.026), the 60th month (p= 0.014), and the 72nd month (p= 0.037) of follow-up (**Figure 1B**).

European haplogroups and time to achieve CD4+ count ≥500 cells/mm^3

In the Kaplan-Meier analysis, more than half of the patients reached CD4+ count values ≥500 cells/mm^3 in all analyzed haplogroups but patients within cluster JT and haplogroup J had slower CD4+ recovery, because they took longer to reach the initial CD4+ count value ≥500 cells/mm^3 (**SF3**). The adjusted Cox regression showed that patients within cluster JT and haplogroup J had a lower likelihood of achieving a CD4+ count value ≥500 cells/mm^3 than patients within cluster HV and haplogroup H (hazard ratio (HR)= 0.68 (**Figure 2A;** p= 0.058) and HR= 0.48 (**Figure 2B;** p= 0.010), respectively).

European haplogroups and time with CD4+ count ≥500 cells/mm^3

The time of follow-up during which CD4+ count was ≥500 cells/mm^3 was longer in patients within cluster HV than in patients within cluster JT (median of 20 months versus 6.2 months; p= 0.029)

(**Figure 3A**). Furthermore, patients within haplogroup H had a longer time with CD4+ count ≥500 cells/mm^3 than patients within haplogroup J (median of 20 months versus 0 months; p=0.024) (**Figure 3B**). When a GLM adjusted by the most relevant clinical and epidemiological variables was performed, patients within cluster HV and haplogroup H had higher values of time with CD4+ count ≥500 cells/mm^3 than patients within cluster JT and haplogroup J (p= 0.004 and p= 0.027, respectively).

European haplogroups and long-term CD4+ T cells recovery

Additionally, cluster JT and haplogroups J and T had the lowest percentage of patients who achieved CD4+ count values ≥500 cells/mm^3 for a long period of time, while cluster HV and haplogroup H had the highest values (see **SF4**). After adjusting for the most relevant clinical and epidemiological variables, we found significant values for achieving CD4+ count values ≥500 cells/mm^3 during at least 12, 36, 48, and 60 months within cluster HV and haplogroup H versus patients within cluster JT, and haplogroups J and T (**Figure 4**). Thus, cluster HV patients had higher chances than cluster JT patients of achieving CD4+ count ≥500 cells/mm^3 during at least 12 months (OR= 2.17; p= 0.039), 36 months (OR= 3.11; p= 0.008), 48 months (OR= 4.42; p= 0.003), and 60 months (OR= 5.20; p= 0.003) (**Figure 4A**). Patients within haplogroup H had higher chances than patients within haplogroup J of achieving CD4+ count ≥500 cells/mm^3 during at least 12 months (OR= 3.14; p= 0.019), 36 months (OR= 3.53; p= 0.026), 48 months (OR= 3.53; p= 0.042), and 60 months (OR= 4.34; p= 0.044) (**Figure 4B**). Finally, patients within haplogroup H had higher chances than patients within haplogroup T of achieving CD4+ count ≥500 cells/mm^3 during at least 48 months (OR= 6.31; p= 0.021) and 60 months (OR= 6.24; p= 0.040) (**Figure 4C**).

DISCUSSION

Our study showed that there was a relationship between mtDNA haplogroups and CD4+ T cell recovery. Cluster HV and haplogroup H were associated with successful CD4+ reconstitution while cluster JT and haplogroup J were associated with worse CD4+ T cell recovery. The association of haplogroup T with a worse CD4+ T cell reconstitution was only evident when we analyzed long-term CD4+ recovery (CD4+ count ≥500 cells/mm^3 during at least 48 and 60 months). Moreover, we did not find any significant differences among patients within clusters U and IXW, possibly because the number of patients in these haplogroups was low and/or CD4+ recovery appeared to be in between haplogroups HV/H and JT/J/T.

The molecular mechanism underlying the functional differences between these mitochondrial haplogroups could be the reason for the different CD4+ T cell recovery characteristics during cART. Mitochondrial haplogroup H has demonstrated higher activity in the electron transport chain, producing higher quantities of ATP and ROS than other haplogroups, such as J, which exhibits lower energy efficiency.[24, 25] Hendrickson *et al*. reported associations of haplogroups J and U (lower ATP and ROS production) with increased prevalences of progression to AIDS and/or values of CD4+ <200 cells/mm^3 in an analysis using longitudinal data from several U.S. cohorts, whereas the more tightly coupled haplogroup H (higher ATP and ROS production) was associated with a decreased prevalence of AIDS progression and death among therapy-naïve Caucasian patients.[6] On the other hand, a cross-sectional study did not find associations between mtDNA haplogroups and current CD4+ count or plasma HIV-RNA among a population of predominantly ART-treated patients.[26] In two recent studies among ART-naïve Africans patients, the mtDNA haplogroup L2 was associated with changes in T-cell activation from baseline to 48-weeks as well as with poorer CD4+ recovery.[27, 28] To our knowledge, our study shows for the first time an association between European mtDNA haplogroups and CD4+ recovery in Caucasian HIV-infected patients on cART during a long-term follow-up period.

Contradictory results have been shown, however, since haplogroup H may lead to higher production of ROS,[25] which would increase the oxidative damage in the immune system during HIV infection. [29] However, haplogroup H has been associated with a delay in AIDS progression because ROS production may enhance innate immunity.[6] Furthermore, higher rates of ROS production may lead to an up-regulation of antioxidant defenses without causing severe immune damage,[30] which may contribute to maintaining good immune function, ensuring good control of HIV replication and, in turn, decreasing oxidative stress and apoptosis.[31, 32] Besides, it is possible that the degree of energy efficiency could have a larger impact on the pathophysiology of HIV infection than the generation of ROS. This would be a reason for patients within cluster HV and haplogroups H to have more successful CD4+ T cell reconstitution than patients within cluster JT, and haplogroups J and T.

There is great controversy about when to start cART. The decision of when to initiate cART requires weighing the benefits treatment has in terms of morbidity and mortality against the risks.[15, 16] Although randomized clinical trials clearly demonstrate the benefits of starting cART in HIV-infected patients with CD4+ count <350 cells/mm^3,[33] there is also an evidentiary basis to recommend starting cART in persons with CD4+ count >350 cells/mm^3. HIV-infected persons with CD4+ count in the range of 350-500 cells/mm^3 have increased rates of some comorbidities such as non-AIDS-defining malignancies and vascular, kidney, and liver disease,[34-39] probably due to uncontrolled HIV replication and increased T-cell activation and inflammation.[40-45] The amount of data supporting early initiation of therapy is less when the CD4+ count increases to >500 cells/mm^3, and concerns remain over the unknown overall benefit, long-term risks, and cumulative additional costs associated with earlier treatment.[15, 16] In addressing this doubt, our data could provide information to make a decision on when to start cART. Patients within haplogroups J and T (worse CD4+ reconstitution) would not wait until CD4+ count was <350 cells/mm^3 before initiating cART. On the contrary, they might be treated when CD4+ count was in the range 350-500 cells/mm^3 or even better when CD4+ count was still >500 cells/mm^3. Conversely, patients within haplogroup H (better CD4+ reconstitution) might wait until CD4+ count falls into the range of 350-500 cells/mm^3. However, we must emphasize that the decision over when to initiate cART in therapy-naïve HIV-infected patients is multifactorial, and a recommendation for delaying the initiation of cART simply because a given patient has a favorable haplogroup should be taken with caution; however, the information provided by the genotyping of mitochondrial haplogroups should not be disregarded.

There are factors that may influence the extent of CD4 recovery in patients on cART, however they have not been studied in detail in this work. Among them is the cART modality. [46, 47] However, with the study design and patients available for this work, it is difficult to analyze the effect of a drug or combination of specific drugs because HIV therapies were prescribed in function of the availability of these drugs from 1996 to 2010. In addition, these initial HIV treatments were modified during the follow-up at the discretion of individual physician according to the needs of each patient. Moreover, our cohort may be relatively outdated taking into account that more than 30% of patients initiated cART with zidovudine, and our results might not be able to be extrapolated to the current antiretroviral regimens. However, after a given cART achieves an undetectable HIV viral load, we think that the influence of the drug used might be secondary except for the side effects that may result in poorer adherence, or that the drug has direct effects on mitochondrial function. Thus, we have included in the regression model the adjustment of two key variables such as the percentage of the whole follow-up period with HIV-RNA <50 copies/mL (indirect measure of cART adherence) and the inclusion of NRTIs with mitochondrial toxicity (Zidovudine, Didanosine, Stavudine) in the cART.

Another important factor is HCV infection, which may influence the extent of CD4+ recovery in patients on cART.[48, 49] Therefore, in our study, we have included test results for HCV antibodies for adjusting the logistic regression analysis; but we have not included the value of the PCR test for HCV (active hepatitis C). Initially, the large majority of patients with HCV antibodies had chronic hepatitis C. Afterwards, during the follow-up, a high percentage of HIV/HCV coinfected patients were treated with interferon alpha and ribavirin, clearing HCV infection in about 50% of these patients. However, such as with cART, it is difficult to analyze the effect of HCV clearance with the study design and patients available for this work. Moreover, the regression analysis was not adjusted by hypersplenism, a condition particularly important in patients with cirrhosis due to chronic hepatitis C.[50] In our cohort, around 40% of patients had HCV infection at baseline. However, according to our estimates, we think that only about 7% of patients had cirrhosis in 2002 and 12% of patients in 2010; and of these, only 25% had a Child-Pugh score of B or C (unpublished data). This means that around 1% of our patients may have had hypersplenism, a very low number that would have had little effect on our results.

This study has other limitations that must be taken into account to ensure correct interpretation of the data. First, this is a retrospective study and, therefore, the case record is selected a priori from patients surviving long enough to yield sufficient follow-up (at least 24 months for this analysis). Thus, nonresponders or advanced patients showing early mortality on therapy are excluded. Second, the sample size is limited, which may have impaired the ability to detect less robust associations and may affect the adjustment of the regression models when using a large number of covariates. Additionally, we did not do detailed analyses on some of the haplogroups when sample size was low. Third, we did not have reliable data about adherence to cART, but we used the percentage of time with HIV-RNA <50 copies/mL during the entire follow-up time as representative of adherence, and this was used in the multivariate models to adjust the OR values. Although adherence is a critical determinant of the efficacy of cART, ideal measures remain elusive and are subject to errors. However, almost always, a good adherence is accompanied by a control of HIV viral load (HIV-RNA <50 copies/mL).[51] In addition, HIV viral load data is an objective assessment of adherence. Fourth, although these results suggest that variations in mtDNA may influence CD4+ reconstitution, we do not have any direct functional measurements of mitochondrial oxidative phosphorylation or apoptosis in these subjects to provide additional data on the potential mechanism. Future studies will need to include such measurements. Fifth, this study was carried out on Caucasian patients who started cART with a CD4+ count <350 cells/mm^3 thus the results of our study are only truly applicable to this setting.

In summary, European mitochondrial haplogroups might influence CD4+ T cell recovery in HIV-infected patients after the initation of cART. Haplogroups J and T appear to be associated with a worse profile of CD4+ T cell recovery, whereas haplogroup H was associated with a better CD4+ reconstitution. Due to the small sample size, our results should be considered more as preliminary data than as definitive results. Replication of these results with independent larger studies may allow for a more specific assessment of mtDNA subhaplogroups associated with even more pronounced differences in CD4 recovery.

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Transparency declarations

The authors do not have a commercial or other association that might pose a conflict of interest.

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AUTHORS CONTRIBUTIONS

JB and SR participated in the study concept and design. JB, DM, JC, JCL, PC and TAE participated in patient selection, collection of samples and acquisition of data. MGF, AFR, MAJS, MGA, and YC participated in sample preparation, DNA isolation and genotyping pre-procedure, and contributed with critical revision of the manuscript. JMB and SR performed all statistical analysis. SR supervised the study. All authors revised the manuscript from a draft by SR.

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Figure 1. Temporal trends of median CD4+ (cells/mm^3) according to European haplogroups in HIV-infected patients on cART. (*), significant differences ($p<0.05$) between haplogroups HV and JT, and between haplogroups H and J were extracted from a Mann-Whitney test.

Figure 2. Likelihood of achieving the first value of CD4+ count ≥500 cells/ mm^3. Data were extracted from Cox proportional hazards test adjusted by baseline characteristics such as gender, age, HCV infection, AIDS, CD4+/mm^3, HIV-RNA, and the inclusion of NRTIs with mitochondrial toxicity (Zidovudine, Didanosine, Stavudine) in the initial cART.

Figure 3. Summary of the median time with CD4+ count ≥500 cells/mm^3 in HIV-infected patients on cART according to European haplogroups. P-values were extracted from a Mann-Whitney test.

Figure 4. Likelihood of achieving CD4+ count ≥500 cells/mm^3 over an extended period of time according to European haplogroups JT, J and T among HIV-infected patients on cART. Data were extracted from logistic regression test adjusted by gender, age, HCV infection, AIDS, CD4+/mm^3 and HIV-RNA at baseline, percentage of time with HIV-RNA <50 copies/mL in respect to the whole follow-up period, and NRTIs with mitochondrial toxicity (Zidovudine, Didanosine, Stavudine) in the initial cART.

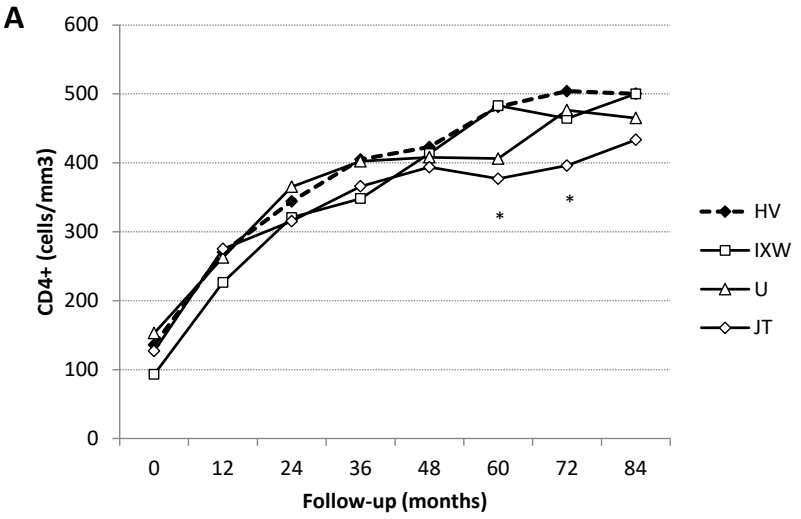
Table 1. Clinical, immunologic, and virologic characteristics of the HIV-1-infected patients at baseline.

	Clusters or major-Haplogroups				
	HV	**IXW**	**U**	**JT**	**All**
No.	133	23	68	51	275
Male	99 (74.4%)	17 (73.9%)	52 (76.5%)	40 (78.4%)	208 (75.6%)
Age (years)	38.1 (32 - 45.1)	38.6 (30.7 - 41.3)	37.6 (32.6 - 50.1)	36.8 (32 - 46)	37.8 (32 - 45.1)
Clinical category C (CDC)	62 (46.6%)	12 (52.2%)	28 (41.2%)	29 (56.9%)	131 (47.6%)
Intravenous drug use (IDU)	47 (35.3%)	8 (34.8%)	31 (45.6%)	19 (37.3%)	105 (38.2%)
HCV infection	51 (38.3%)	9 (39.1%)	33 (48.5%)	19 (37.3%)	112 (40.7%)
HIV markers					
CD4+ T cells/mm^3	136 (48 - 225)	93 (51 - 252)	153 (74 - 241)	127 (50 - 230)	136 (50 - 231)
Log_{10} plasma HIV-RNA (copies/mL)	4.56 (3.89 - 5.28)	4.95 (4.27 - 5.51)	4.55 (4.09 - 5.19)	4.93 (4.24 - 5.42)	4.71 (4.06 - 5.30)
First cART regimen					
Nucleoside analogue (NRTI)					
Zidovudine	49 (36.8%)	4 (17.4%)	23 (33.8%)	21 (41.2%)	97 (35.3%)
Azlcitabine	4 (3%)	0 (0%)	0 (0%)	1 (2%)	5 (1.8%)
Didanosine	19 (14.3%)	8 (34.8%)	9 (13.2%)	12 (23.5%)	48 (17.5%)
Stavudine	41 (30.8%)	9 (39.1%)	26 (38.2%)	16 (31.4%)	92 (33.5%)
Lamivudine	96 (72.2%)	18 (78.3%)	49 (72.1%)	39 (76.5%)	202 (73.5%)
Abacavir	13 (9.8%)	1 (4.3%)	11 (16.2%)	3 (5.9%)	28 (10.2%)
Tenofovir	30 (22.6%)	4 (17.4%)	13 (19.1%)	8 (15.7%)	55 (20%)
Emtricitabine	24 (18%)	2 (8.7%)	13 (19.1%)	5 (9.8%)	44 (16%)
Protease inhibitor (PI)					
Ritonavir	43 (32.3%)	9 (39.1%)	19 (27.9%)	13 (25.5%)	84 (30.5%)
Nelfinavir	7 (5.3%)	1 (4.3%)	1 (1.5%)	4 (7.8%)	13 (4.7%)
Lopinavir	23 (17.3%)	8 (34.8%)	9 (13.2%)	8 (15.7%)	48 (17.5%)
Saquinavir	7 (5.3%)	0 (0%)	4 (5.9%)	2 (3.9%)	13 (4.7%)
Indinavir	42 (31.6%)	6 (26.1%)	21 (30.9%)	16 (31.4%)	85 (30.9%)
Amprenavir	4 (3%)	0 (0%)	1 (1.5%)	1 (2%)	6 (2.2%)
Atazanavir	5 (3.8%)	0 (0%)	4 (5.9%)	0 (0%)	9 (3.3%)

	Clusters or major-Haplogroups				
	HV	**IXW**	**U**	**JT**	**All**
Non-Nucleoside analogue (NNRTI)					
Nevirapine	12 (9%)	3 (13%)	10 (14.7%)	9 (17.6%)	34 (12.4%)
Efavirenz	26 (19.5%)	4 (17.4%)	15 (22.1%)	9 (17.6%)	54 (19.6%)
Others					
Enfuvirtide	1 (0.8%)	0 (0%)	0 (0%)	0 (0%)	1 (0.4%)
Raltegravir	1 (0.8%)	1 (4.3%)	1 (1.5%)	0 (0%)	3 (1.1%)
cART protocols					
3 NRTI	4 (3%)	1 (4.3%)	2 (2.9%)	2 (3.9%)	9 (3.3%)
2 NRTI + 1 PI	53 (39.8%)	8 (34.8%)	20 (29.4%)	19 (37.3%)	100 (36.4%)
2 NRTI + 1 NNRTI	32 (24.1%)	6 (26.1%)	21 (30.9%)	16 (31.4%)	75 (27.3%)
2 NRTI + 2 PI	35 (26.3%)	6 (26.1%)	18 (26.5%)	11 (21.6%)	70 (25.5%)
3 NRTI + 1 NNRTI	5 (3.8%)	0 (0%)	4 (5.9%)	0 (0%)	9 (3.3%)
Others	4 (3%)	2 (8.7%)	3 (4.4%)	3 (5.9%)	12 (4.4%)
2 years on cART initiation					
Same regimen as the cART initiation (%)	55/125 (44%)	12/22 (54.5%)	34/65 (52.3%)	23/47 (48.9%)	124/259 (47.9%)
No. of line therapy	2 (1 - 2)	1 (1 - 2)	1 (1 - 2)	1 (1 - 2)	1 (1 - 2)
5 years on cART initiation					
Same regimen as the cART initiation (%)	10/92 (10.9%)	6/19 (31.6%)	8/48 (16.7%)	8/37 (21.6%)	32/196 (16.3%)
No. of line therapy	2 (2 - 3)	2 (1 - 3)	2 (2 - 3)	2 (2 - 3.5)	2 (2 - 3)
8 years on cART initiation					
Same regimen as the cART initiation (%)	6/62 (9.7%)	1/13 (7.7%)	4/36 (11.1%)	1/22 (4.5%)	12/133 (9%)
No. of line therapy	3 (2 - 4)	3 (2 - 4)	3 (2 - 4)	3 (2 - 4)	3 (2 - 4)

Values are expressed as median (percentile 25 - percentile 75) and absolute count (percentage). HCV: Hepatitis C virus. HIV-1: Human immunodeficiency virus type 1. HIV-RNA: plasma HIV load. cART: combination antiretroviral therapy. NRTI: nucleoside analogue HIV reverse transcriptase inhibitor. NNRTI: non-nucleoside analogue HIV reverse transcriptase inhibitor. PI: protease inhibitor. CDC: Center for Disease Control and Prevention.

Figure 1.


A
600
500
400
300
200
100
0
CD4+ (cells/mm3)
0
12
24
36
48
60
72
84
Follow-up (months)
*
*
HV
IXW
U
JT

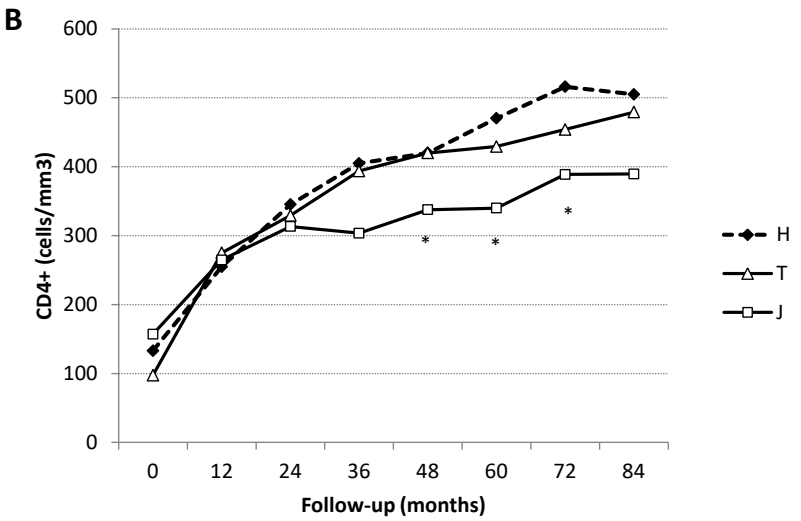

B
600
500
400
300
200
100
0
CD4+ (cells/mm3)
0
12
24
36
48
60
72
84
Follow-up (months)
*
*
*
H
T
J

Figure 2.

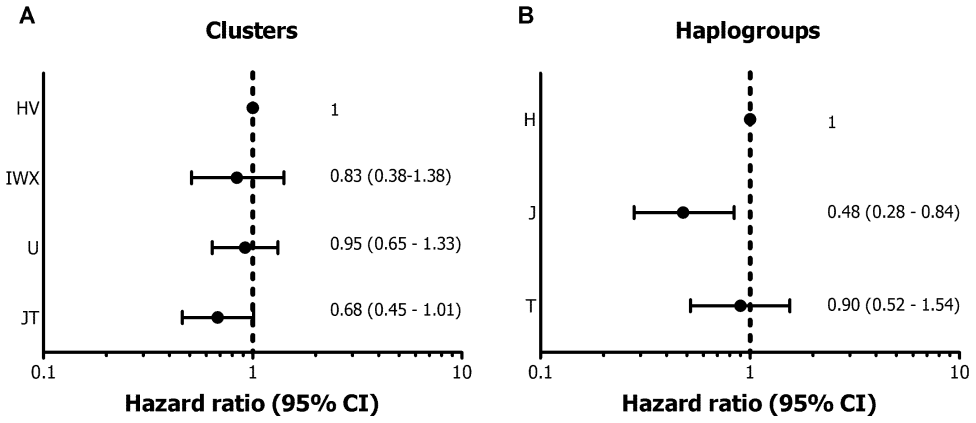

A
Clusters
HV
IWX
U
JT
1
0.83 (0.38-1.38)
0.95 (0.65 - 1.33)
0.68 (0.45 - 1.01)
0.1
1
10
Hazard ratio (95% CI)
B
Haplogroups
H
J
T
1
0.48 (0.28 - 0.84)
0.90 (0.52 - 1.54)
0.1
1
10
Hazard ratio (95% CI)

Figure 3.

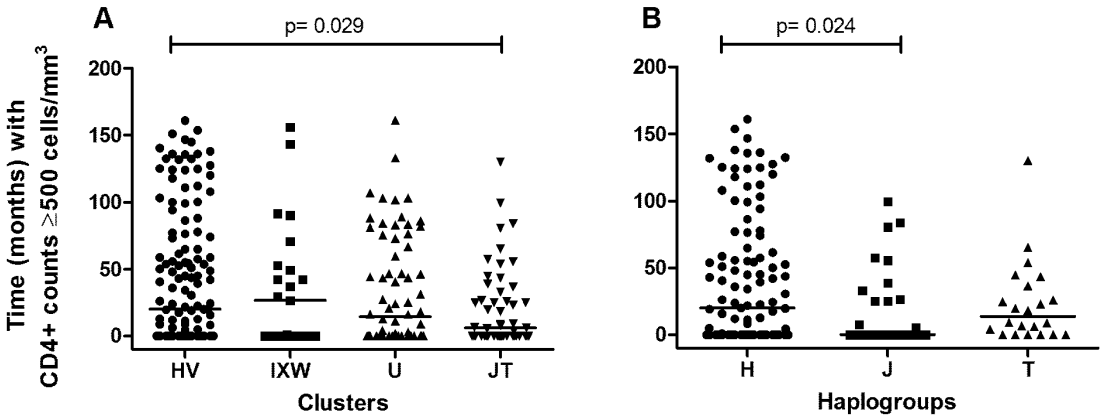
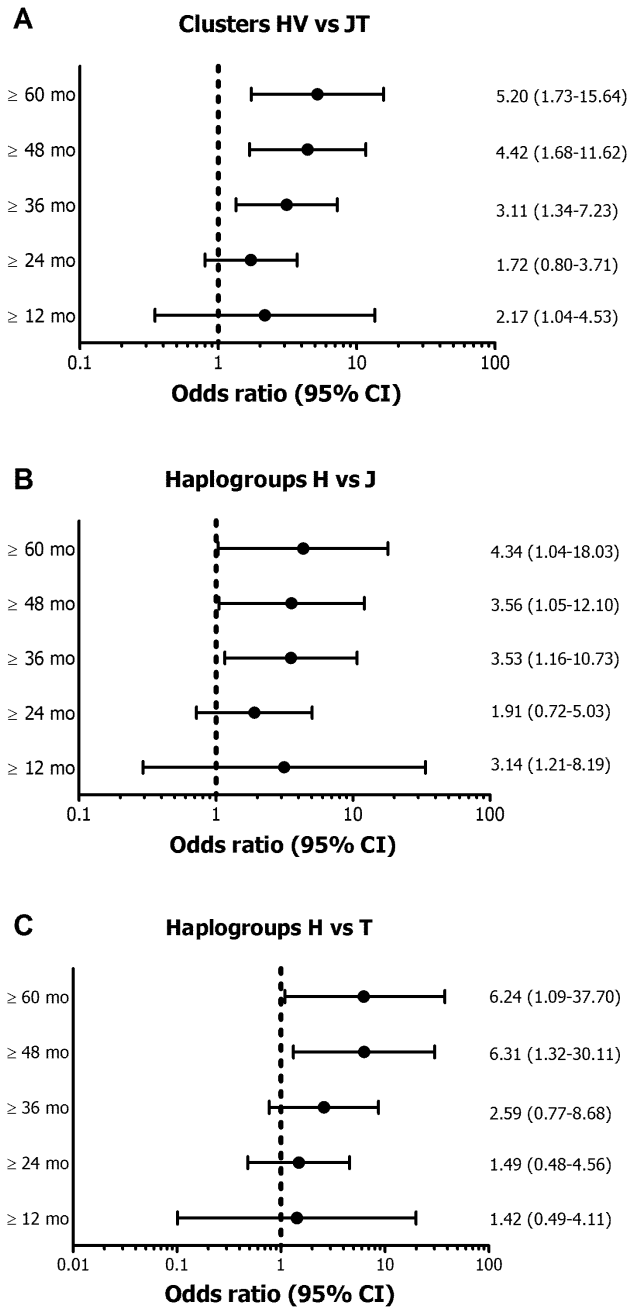

A
p= 0.029
Time (months) with CD4+ counts ≥500 cells/mm3
200
150
100
50
0
HV
IXW
U
JT
Clusters
B
p= 0.024
200
150
100
50
0
H
J
T
Haplogroups

Figure 4.


A
Clusters HV vs JT
≥ 60 mo
≥ 48 mo
≥ 36 mo
≥ 24 mo
≥ 12 mo
5.20 (1.73-15.64)
4.42 (1.68-11.62)
3.11 (1.34-7.23)
1.72 (0.80-3.71)
2.17 (1.04-4.53)
0.1
1
10
100
Odds ratio (95% CI)
B
Haplogroups H vs J
≥ 60 mo
≥ 48 mo
≥ 36 mo
≥ 24 mo
≥ 12 mo
4.34 (1.04-18.03)
3.56 (1.05-12.10)
3.53 (1.16-10.73)
1.91 (0.72-5.03)
3.14 (1.21-8.19)
0.1
1
10
100
Odds ratio (95% CI)
C
Haplogroups H vs T
≥ 60 mo
≥ 48 mo
≥ 36 mo
≥ 24 mo
≥ 12 mo
6.24 (1.09-37.70)
6.31 (1.32-30.11)
2.59 (0.77-8.68)
1.49 (0.48-4.56)
1.42 (0.49-4.11)
0.01
0.1
1
10
100
Odds ratio (95% CI)