Original Article

Title: *IL7RA* polymorphisms predict the CD4+ recovery in HIV patients on cART
Short title: *IL7RA* polymorphisms and CD4+ recovery

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Character count of Title: 69
Character count of Running Head: 37
Word count of Abstract: 241
Word count of Keywords: 5

Word count of Text: 2435
Count of References: 25
Count of Tables: 2
Count of Figures: 1

ABSTRACT

Background: The *IL7RA* polymorphisms have recently been associated associated with CD4+ T-cell decline in untreated HIV-infected subjects, and CD4+ T-cell recovery in patients on combination antiretroviral therapy (cART). The aim of this study was to evaluate whether *IL7RA* polymorphisms are associated with CD4+ T cell recovery in HIV-infected patients on long-term cART.

Study design: We performed a retrospective study in 151 naïve cART patients with severe immunodeficiency (CD4+ counts ≤200 cells/mm^3). *IL7RA* polymorphisms genotyping was performed by using Sequenom's MassARRAY platform. The outcome variable was the time to achieve the first value of CD4+ count ≥500 cells/mm^3 during the follow-up.

Results: Two different trends of CD4+ T cell recovery were found in Kaplan-Meier analysis. During the first 48 months, 60/151 (39.7%) of the patients reached CD4+ T cell values ≥500 cells/mm^3, and no differences were observed between *IL7RA* genotypes. After the first 48 months of follow-up, 27/151 (17.8%) of the patients reached CD4+ T cell values ≥500 cells/mm^3**,** with a different pattern of CD4+ recovery depending on *IL7RA* genotype. Patients with rs10491434 TT genotype and rs6897932 TT genotype were more likely of achieving CD4+ value ≥500 cells/mm^3 than patients with rs10491434 CT/CC genotype (adjusted hazard ratio (aHR)=3.59; p=0.005) and patients with rs6897932 CC/CT genotype (aHR=11.7; p<0.001).

Conclusions: The *IL7RA* polymorphisms seem to be associated with CD4+ T cell recovery in HIV-infected patients who started cART with severe immunodeficiency, in the second phase of CD4+ T cell recovery after long-term cART.

Key-words: IL7RA; polymorphisms; AIDS; antiretroviral therapy; immune system reconstitution

BACKGROUND

The CD4+ T cell is the primary cellular target of human immunodeficiency virus (HIV), and a continuous loss of CD4+ T cells leads to acquired immune deficiency syndrome (AIDS) progression and death [1, 2]. Hence, CD4+ T cell count in peripheral blood represents the principal surrogate marker for AIDS-defining illnesses [3]; and it is also a major factor in the decision to initiate combination antiretroviral therapy (cART) in HIV-infected individuals [4, 5].

The cART in HIV-infected individuals usually reduces the plasma HIV-RNA to undetectable levels, restore CD4+ T cell levels, and preserves immunologic function [4, 5]. However, there is substantial variability in the rate and extent of CD4+ T cell recovery after starting cART [6]. A significant percentage of cART-treated patients fail to achieve substantial increases in CD4+ T cell count and it may contribute to increase the risk of both acquired immune deficiency syndrome (AIDS) and non-AIDS morbidity and mortality [7-9]. To date, the best baseline predictors of poor immune recovery are several clinical features (advanced age, hepatitis C coinfection, high pretreatment HIV viral load, and low CD4+ T cell nadir), genetic factors (CCR5 polymorphisms, mitochondrial haplogroups), immune activation (CD38+, HLA-DR+), among others [10, 11]. However, the immunological mechanisms causing immune recovery are not completely understood and an unexplained variability in treatment outcome still remains unknown, suggesting that other host factors may play an important role in CD4+ T cell recovery.

The CD4+ T cell recovery after cART is biphasic, with an initial steep increase by proliferation and redistribution of CD4+ T cells from lymphoid tissue to the blood stream (first phase), followed by a slower increase due to de-novo production of T cells in the thymus (second phase) [12]. Interleukin-7 (IL-7) is a critical factor for T cell homeostasis by promoting thymic output of T cell, and development, survival, and proliferation of T cells [13]. The responsiveness of IL-7 is dependent on the expression of the IL-7 receptor, which is a heterodimer consisting of the common cytokine receptor gamma chain (CD132) and α-chain of the IL-7 receptor (IL7Rα or CD127) [13]. The IL7Rα is encoded by *IL7RA* gene and *IL7RA* polymorphisms (rs10491434 and rs6897932) have recently been associated with CD4+ T cell decline in untreated whites HIV-infected subjects [14], and CD4+ T cell recovery in HIV-infected patients on cART [15, 16].

OBJECTIVES

The aim of this study was to evaluate whether *IL7RA* polymorphisms (rs10491434 and rs6897932) are associated with CD4+ T cell recovery in HIV-infected patients on long-term cART.

STUDY DESIGN

Patients

We carried out a retrospective study in a cohort of HIV-infected patients who started cART with severe immunodeficiency between 1996 and 2010, which has been described in detail in a recent article [10]. 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 (Grupo de Estudio del SIDA-SEIMC, Spanish AIDS Plan) regarding antiretroviral treatment in adults with HIV infection [4]. The criteria for inclusion were naïve cART patients with CD4+ T cell count values ≤200 cells/mm^3, DNA samples for genotyping *IL7RA* polymorphisms and data of CD4+ T cell and plasma HIV-RNA at least every six months. We only included patients with a follow-up of at least 24 months after the initiation of cART to be sure that all patients had a minimum follow-up time to reach values above 500 CD4+/mm^3. Besides, all patients who did not achieve undetectable viral load after the first 6 months of follow-up were excluded. Afterwards, the follow-up of individuals was stopped when two consecutive measurements of viral load above 1000 copies/ml with more than 3 months apart in the study period were found (censored data).

We conducted blood sample collections from HIV-infected patients who were in 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, 216 patients had baseline CD4+ T cell values ≤200 cells/mm^3 and data of CD4+ T cell and plasma HIV-RNA for at least every six months, but only 184 of the patients had a follow-up of at least 24 months after the initiation of cART. Afterwards, we included 172 patients because 12 patients were excluded due to the fact that we were unable to genotype the *IL7RA* polymorphisms. A further 21 patients were excluded based on not achieving undetectable viral load in the first 6 months (low adhesion, resistance to HIV treatment, etc.), leaving 151 patients for analysis.

Clinical and laboratory data

Clinical and epidemiological data were obtained from medical records. 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).

IL7RA polymorphisms genotyping

We selected two *IL7RA* polymorphisms located in two putative regulatory regions [rs10491434 (3′UTR) and rs6897932 (exon 6)], which have been recently related to AIDS progression in naïve patients and CD4+ T cell recovery in patients on cART [14, 15, 17]. Genomic DNA was extracted from peripheral blood with Qiagen kit (QIAamp DNA Blood Midi/Maxi; Qiagen, Hilden, Germany). DNA samples were genotyped at the Spanish National Genotyping Center (CeGen; http://www.cegen.org/) for genotyping *IL7RA* polymorphisms by using Sequenom's MassARRAY platform (San Diego, CA, USA) and the iPLEX® Gold assay design system. Note that two patients did not have genotyping data for rs10491434.

Outcome variable

The main outcome variable was to achieve a value of CD4+ T cell count ≥500 cells/mm^3 at a defined time during the follow-up.

Statistical Analysis

Statistical analysis was performed by IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp, Chicago, Armonk, NY, USA). All tests were two-tailed and p-values <0.05 were considered significant. Kaplan-Meier and Cox regression analyses were used to analyze the time to achieve the first value of CD4+ T cell count ≥500 cells/mm^3 [10, 18]. Preliminary analysis found the proportional hazards assumption was violated if all data was combined, so we split the follow-up period in two stretch (<48 months and ≥48 months) to analyze the likelihood of achieving the first value of CD4+ count ≥500 cells/mm^3. Thus, the 48 months cut-off was chosen post-hoc (a posteriori). Cox regression analysis was adjusted by baseline characteristics such as gender, age, hepatitis C virus (HCV) infection, AIDS, CD4+ T cells/mm^3, HIV-RNA, NRTIs with mitochondrial toxicity (Zidovudine, Didanosine, Stavudine) in the initial cART regimen, and mitochondrial haplogroup J [10]. In addition, Hardy-Weinberg equilibrium (HWE) and pair-wise linkage disequilibrium (LD) analysis were computed by Haploview 4.2 software.

RESULTS

Characteristics of the study population

Table 1 shows the characteristics of 151 HIV-infected patients who self-identified as "white" European. The median age was 40 years, 77.5% were male, 62.9% had had prior AIDS-defining conditions, and 31.1% had HCV infection. When the cART was started, 63.6% of the patients were taking protease inhibitor (PI)-based cART and 26.5% nonnucleoside reverse transcriptase inhibitor (NNRTI)-based cART regimens.

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

	All patients
No.	151
Male	117 (77.5%)
Age (years)	40 (34; 49)
Clinical category C (CDC)	95 (62.9%)
Intravenous drug use (IDU)	48 (31.8%)
HCV infection	47 (31.1%)
HIV markers	
CD4+ T cells/mm^3	60 (25; 125)
Log_{10} plasma HIV-RNA (copies/mL)	5.11 (4.36; 5.46)
Time on follow-up (months)	43.3 (21.5; 74.6)
First cART regimen	
Nucleoside analogue (NRTI)	
Zidovudine	53 (35.1%)
Zalcitabine	4 (2.6%)
Didanosine	31 (20.5%)
Stavudine	45 (29.8%)
Lamivudine	112 (74.2%)
Abacavir	15 (9.9%)
Tenofovir	32 (21.2%)
Emtricitabine	26 (17.2%)
Protease inhibitor (PI)	
Ritonavir	59 (39.1%)
Nelfinavir	8 (5.3%)
Lopinavir	40 (26.5%)
Saquinavir	4 (2.6%)
Indinavir	44 (29.1%)
Amprenavir	3 (2%)
Atazanavir	3 (2%)
Non-Nucleoside analogue (NNRTI)	
Nevirapine	17 (11.3%)
Efavirenz	25 (16.6%)
Others	
Enfuvirtide	1 (0.7%)
Raltegravir	3 (2%)
cART protocols	
3 NRTI	3 (2%)
2 NRTI + 1 PI	48 (31.8%)
2 NRTI + 1 NNRTI	33 (21.9%)

	All patients
2 NRTI + 2 PI	48 (31.8%)
3 NRTI + 1 NNRTI	7 (4.6%)
Others	12 (7.9%)

Values are expressed as median (percentile 25 - percentile 75) and absolute count (percentage). Abbreviations: 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.

Characteristics of *IL7RA* polymorphisms

Table 2 shows the characteristics of *IL7RA* polymorphisms, which had a minimum allele frequency (MAF)>5%, displayed missing values <5%, and were in HWE (p>0.05). These frequencies in our dataset were in accordance with data listed on the NCBI SNP database for rs10491434 polymorphism (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=rs10491434) and rs6897932 polymorphism (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=rs6897932).

Additionally, a strong LD (non-random association of alleles at different loci) among *IL7RA* SNPs was found (D´≥0.92), meaning that there is no evidence for recombination between these SNPs. However, the R-squared among *IL7RA* SNPs was low (R-squared=0.32), meaning that the *IL7RA* SNPs did not provide exactly the same information and it could not be substituted one for another.

Table 2. Summary of allele and genotype frequencies, and Hardy Weinberg Equilibrium (HWE) for *IL7RA* polymorphisms in HIV/HCV coinfected patients.

	Frequencies				HWE
***IL7RA* SNPs**	**Alleles**		**Genotype**		**p-value**
rs10491434	T	0.72	C/C	0.08	
	C	0.28	T/C	0.40	0.840
	-	-	T/T	0.52	
rs6897932	C	0.76	C/C	0.58	
	T	0.24	C/T	0.35	0.666
	-	-	T/T	0.07	

IL7RA polymorphisms and time to achieve CD4+ T cell values ≥500 cells/mm^3

More than half of the patients reached CD4+ T cell values ≥500 cells/mm^3 [crude probability= 57.6% (87/151)]. The Kaplan-Meier analysis shows the estimated probability of achieving the first value of CD4+ T cell ≥500 cells/mm^3 according to *IL7RA* genotypes (**Figure 1**). Patients with rs10491434 TT or rs6897932 TT genotypes had a faster CD4+ T cell recovery than patients with rs10491434 CT/CC or rs6897932 CC/CT genotypes (F**igure 1A (**p=0.062) and (**Figure 1B** (p=0.028)).

Table 3 shows the adjusted odds of achieving the first value of CD4+ T cell values ≥500 cells/ mm^3 according to *IL7RA* genotypes. In the first place it is noteworthy that the **Figure 1** shows two different trends of CD4+ T cell recovery. During the first 48 months, 60/151 (39.7%) of the patients reached CD4+ T cell values ≥500 cells/mm^3, and no differences were observed between *IL7RA* genotypes. After the first 48 months of follow-up, 27/151 (17.8%) of the patients reached CD4+ T cell values ≥500 cells/mm^3**,** with a different pattern of CD4+ T cell

recovery depending on *IL7RA* genotype. During the first 48 months, there was no evidence for a difference between the groups. However, after the first 48 months of follow-up, patients with rs10491434 TT genotype and rs6897932 TT genotype were more likely of achieving CD4+ value ≥500 cells/mm^3 than patients with rs10491434 CT/CC genotype (adjusted hazard ratio (aHR)=3.59; p=0.005) and patients with rs6897932 CC/CT genotype (aHR=11.7; p<0.001) (**Table 3**).

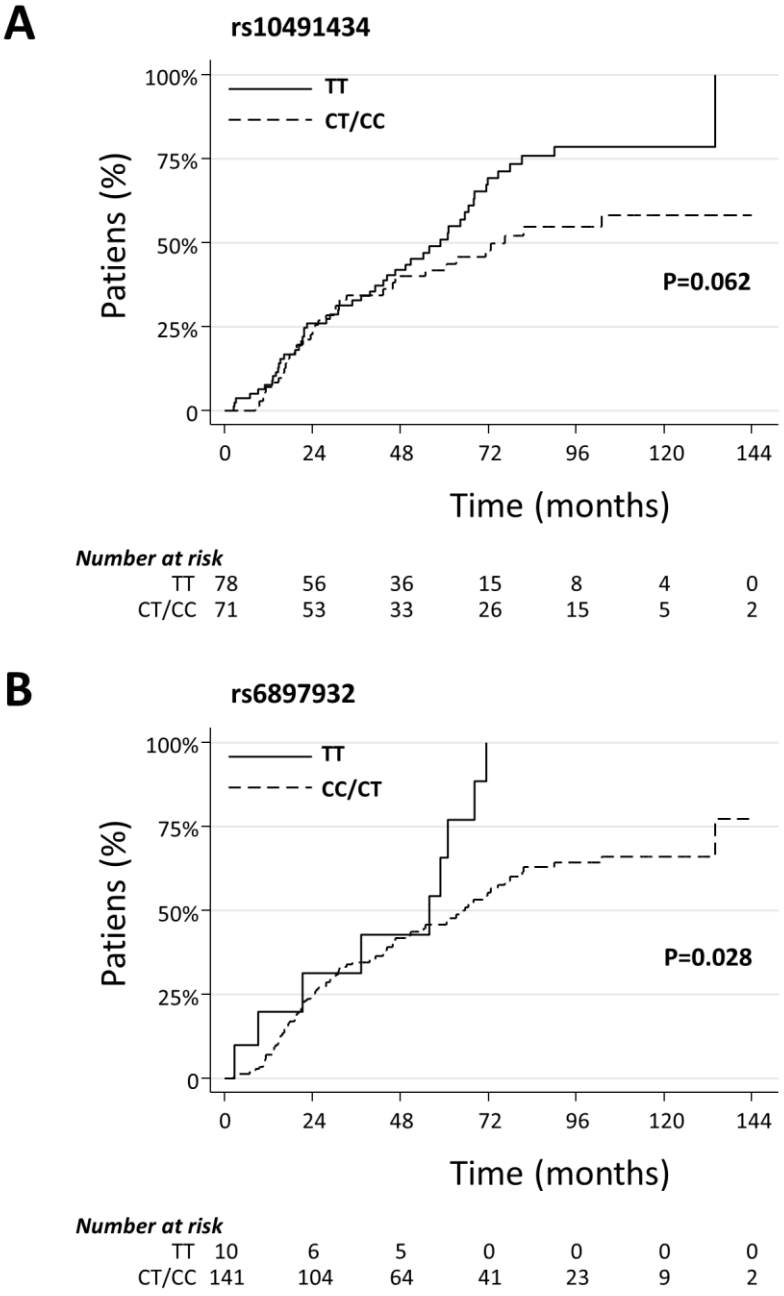

Figure 1. Kaplan-Meier curves of achieving the first CD4+ T cell count value ≥500 cells/mm^3 according to IL7RA polymorphisms in HIV-infected patients on cART. Month 0=beginning of HAART.

Table 3. Likelihood of achieving the first value of CD4+ T cell values ≥500 cells/ mm^3 according to *IL7RA* genotypes in HIV-infected patients on cART.

***IL7RA* polymorphism**	**CT/CC**	**TT**	**p-value [(a)]**	**aHR (95%CI) [(b)]**	**p-value [(b)]**
rs10491434 (n=149)	35/71 (49.3%)	50/78 (64.1%)	0.068		
The first 48 months	27/71 (38.0%)	31/78 (39.7%)	0.830	0.82 (0.47; 1.44)	0.506
After the first 48 months	8/33 (24.2%)	19/36 (52.8%)	**0.015**	3.59 (1.48; 8.69)	**0.005**
	CC/CT	**TT**	**p-value [(a)]**	**aHR (95%CI) [(b)]**	**p-value [(b)]**
rs6897932 (n=151)	78/141 (55.3%)	9/10 (90.0%)	0.032		
The first 48 months	56/141 (39.7%)	4/10 (40.0%)	0.986	1.02 (0.35; 2.87)	0.982
After the first 48 months	22/64 (34.4%)	5/5 (100%)	**0.004**	11.7 (3.63; 41.1)	**<0.001**

(a), p-values were calculated by Chi-squared test; (b), Data and p-values were calculated by Cox regression test, which was adjusted by baseline characteristics (gender, age, hepatitis C virus (HCV) infection, AIDS, CD4+ T cells/mm^3, HIV-RNA, NRTIs with mitochondrial toxicity (Zidovudine, Didanosine, Stavudine) in the initial cART regimen, and mitochondrial haplogroup J). Statistically significant differences are shown in bold.
Abbreviations: 95%CI, 95% of confidence interval; aHR, adjusted hazard ratio; p-value, level of significance.

DISCUSSION

This study assessed the influence of the *IL7RA* genotypes on time to reach CD4+ T-cell counts ≥500 cells/mm^3 in a cohort of patients starting cART in advanced HIV disease (CD4+ T-cell counts <200 cells/mm^3). The major finding was that both rs10491434 TT and rs6897932 TT genotypes were associated with higher proportion of patients with CD4+ T-cell recovery among HIV-infected patients with severe immunodeficiency after 48 months on cART. Our data are in concordance with the previous studies performed in white subjects infected with HIV [15, 16], but with some special features that will be discussed below.

The *IL7RA* rs10491434 polymorphism has been previously associated with rapid decline of CD4+ T cell count during HIV infection [14]. The rs10491434 polymorphism is located within the 3′UTR of *IL7RA* gene, which may influence methylation at the nearby CpG sequences [17].The rs10491434 C variant seems to be associated with higher methylation at CpG sites near the rs10491434 SNP, which might decrease the CD127 expression; while the rs10491434 T allele seems to be associated with lower methylation at nearby CpG sites and, thus, it might increase the CD127 expression. In our study, patients with rs10491434 TT genotype (major allele homozygous) had higher proportion of patients with CD4+ T-cell recovery after 48 months on cART than those with rs10491434 CC/CT genotype (presence of minor allele), but this effect of rs10491434 SNP on CD4+ T cell recovery was observed after 48 months cART, possibly when changes in methylation were large enough to have a physiological effect. To our knowledge, it is the first time that the association *IL7RA* rs10491434 polymorphism and CD4+ T cell recovery in HIV infected patients on cART is described.

The *IL7RA* rs6897932 polymorphism, a missense variant located within the alternatively spliced exon 6 of IL7RA, causes a substitution of threonine with isoleucine in the CD127 transmembrane domain that may influence the amount of sCD127 (soluble isoform) and CD127 (membrane-bound isoform) by putatively disrupting an exonic splicing motif [19]. In this scenario, rs6897932 CC genotype has been associated to higher plasma levels of soluble CD127 (sCD127) in Caucasian HIV-infected patients [20, 21], being able to bind to circulating IL-7 and decrease the IL-7 bioavailability, limiting its effects [22]. By contrast, rs6897932 TT genotype is related to lower plasma levels of soluble CD127 (sCD127) [20, 21]. In a recent article, rs6897932 T allele has been related to faster CD4+ T cell recovery in European whites HIV infected patients on cART [15]. However, in that paper, Hartling et al. found that the effect of *IL7RA* rs6897932 polymorphism on CD4+ T cell count recovery is observed after the first 6 months of follow-up, and then it was maintained over time for a long time period. In our study, patients with rs6897932 TT genotype (minor allele homozygous) had higher proportion of patients with CD4+ T-cell recovery in HIV-infected on after 48 months on cART. However, no differences were observed in a first phase. Only after 48 months on cART, significant differences on CD4+ T cell recovery were found between rs6897932 genotypes. The differences between study designs could be responsible for the different findings between the article of Hartling et al. and our data, since Hartling et al. used the change of CD4+ T cells in absolute value while our outcome variable was to achieve the first value CD4+ T cell count ≥500 cells/mm^3. Note that we were not able to try to reproduce the findings of Hartling et al. due to the low sample size of our study. Moreover, we should not rule out the differences in both cohorts, since they may have an influence on these different findings. For example, Hartling et al. used patients from a prospective cohort (2007-2013) with more current cARTs than in our retrospective study (1996-2010), which included more patients with severe immunodeficiency who were treated in the earlier cART era. Apart of these differences, we do not offer any plausible biological reason for explaining the differences between our findings and that of Hartling et al.

Note that we did not have any direct functional measurements of both *IL7RA* rs10491434 and rs6897932 polymorphisms in these subjects to provide additional data on the potential mechanism. Furthermore, at present, over 100 SNPs are known in *IL7RA* according to Haploview. Thus, the effect found in rs6897932 and rs10491434 SNPs on CD4+ T cell recovery might be questionable since these two *IL7RA* SNPs would be in LD with other *IL7RA* SNPs.

In HIV-infected patients on cART, the restoration of CD4+ T cells is predominantly driven by increases in CD4+CD127+ T cells [23]. Thus, it seems to be an advantage to have a low level of IL7RA (sCD127) for the CD4+ T cell recovery after initiation of cART, because the IL-7 bioavailability is increased. IL-7 is a key factor for CD4+ T cell recovery [12]. On the one hand, IL-7 stimulates the thymopoeisis and the de-novo production of naïve T cells, particularly in the second phase of CD4+ T cell recovery. Our data show an impact of IL7RA polymorphisms on the second phase of CD4+ T cell recovery after long-term cART. Moreover, IL-7 is an important antiapoptotic factor (Bcl-2) for memory CD4+T cells expressing IL7RA, which is essential for the maintenance of the memory T cell pool.

There are several factors that may influence the extent of CD4 recovery in patients on cART, but we have not studied them in detail in this article. (1) This is a retrospective cohort that contains a limited number of patients, which could limit achieving statistically significant p-values and it might lead to potentially large percentage fluctuations. (2) The cART modality has an influence on CD4+ T cell recovery [24, 25]. 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. 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. (3) HCV infection has also an influence on CD4+ T cell recovery [26, 27]. In our study, around 30% of the patients had HCV infection at baseline and no patient was treated in the analyzed follow-up period. However, HCV infection was added to Cox Regression analysis to adjust the HR values. (4) Hypersplenism, a condition particularly important in patients with cirrhosis due to chronic hepatitis C may influence in CD4+ T cell recovery [28]. In our cohort, around 30% of the patients had HCV infection at baseline. However, according to our estimates, we think that only about 7% of the patients had cirrhosis in 2002 and 12% of the patients had it 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 which should have little effect on our results.

In summary, the *IL7RA* polymorphisms seem to predict the CD4+ T cell recovery in HIV-infected patients who started cART with severe immunodeficiency, in the second phase of CD4+ T cell recovery after long-term cART.

FUNDING:

This work has been supported by grants given by Fondo de Investigación de Sanidad en España (FIS) [Spanish Health Founds for Research] [grant numbers PI11/01556, PI14/01094, PI11/00245, PI14CIII/00011], Red Española de Investigación en SIDA (RIS) [Spanish Network for AIDS Research] [grant RD12/0017/0024 and RD12/0017/0004], and "Fundación para la Investigación y la Prevención del Sida en España" (FIPSE) [grant number 361020/10].

This work has been (partially) funded by the RD12/0017 project as part of the Plan Nacional R + D + I and cofinanced by ISCIII- Subdirección General de Evaluación y el Fondo Europeo de Desarrollo Regional (FEDER).

DPT, MGF, MAJS, MGA, and PGB are supported by *Instituto de Salud Carlos III* [grant numbers CM12/00043, RD12/0017/0024, CD13/00013, CD12/00442, and FI12/00036, respectively]. JB is an investigator from the Programa de Intensificación de la Actividad Investigadora en el Sistema Nacional de Salud (I3SNS).

COMPETING INTERESTS:

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

ETHICAL APPROVAL:

The study was approved by the Research Ethic Committee of the Instituto de Salud Carlos III (ISCIII). This study was conducted in accordance with the Declaration of Helsinki and patients gave their written consent for the study.

ACKNOWLEDGEMENTS:

The authors thank the Spanish National Genotyping Center (CeGen) for providing SNP genotyping services (http://www.cegen.org).

AUTHOR'S CONTRIBUTIONS:

JB and SR participated in the study concept and design.

JB, DM, CD, PC and TAE participated in patient selection, collection of samples and acquisition of data. MGF, MAJS, DPT, MGA, and PGB 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.

REFERENCES

1. Saag MS, Holodniy M, Kuritzkes DR, O'Brien WA, Coombs R, Poscher ME*, et al.* HIV viral load markers in clinical practice. *Nat Med* 1996,**2**:625-629.

2. O'Brien WA, Hartigan PM, Martin D, Esinhart J, Hill A, Benoit S*, et al.* Changes in plasma HIV-1 RNA and CD4+ lymphocyte counts and the risk of progression to AIDS. Veterans Affairs Cooperative Study Group on AIDS. *N Engl J Med* 1996,**334**:426-431.

3. Kaplan JE, Benson C, Holmes KH, Brooks JT, Pau A, Masur H. Guidelines for prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. *MMWR Recomm Rep* 2009,**58**:1-207; quiz CE201-204.

4. Panel de expertos de Gesida y Plan Nacional sobre el Sida. National consensus document by GESIDA/National Aids Plan on antiretroviral treatment in adults infected by the human immunodeficiency virus (January 2011 update). *Enferm Infecc Microbiol Clin* 2011,**29**:209 e201-103.

5. Thompson MA, Aberg JA, Hoy JF, Telenti A, Benson C, Cahn P*, et al.* Antiretroviral treatment of adult HIV infection: 2012 recommendations of the International Antiviral Society-USA panel. *JAMA* 2012,**308**:387-402.

6. Gutierrez F, Padilla S, Masia M, Iribarren JA, Moreno S, Viciana P*, et al.* Clinical outcome of HIV-infected patients with sustained virologic response to antiretroviral therapy: long-term follow-up of a multicenter cohort. *PLoS One* 2006,**1**:e89.

7. Kelley CF, Kitchen CM, Hunt PW, Rodriguez B, Hecht FM, Kitahata M*, et al.* Incomplete peripheral CD4+ cell count restoration in HIV-infected patients receiving long-term antiretroviral treatment. *Clin Infect Dis* 2009,**48**:787-794.

8. Helleberg M, Kronborg G, Larsen CS, Pedersen G, Pedersen C, Obel N*, et al.* Poor CD4 response despite viral suppression is associated with increased non-AIDS-related mortality among HIV patients and their parents. *AIDS* 2013,**27**:1021-1026.

9. Baker JV, Peng G, Rapkin J, Krason D, Reilly C, Cavert WP*, et al.* Poor initial CD4+ recovery with antiretroviral therapy prolongs immune depletion and increases risk for AIDS and non-AIDS diseases. *J Acquir Immune Defic Syndr* 2008,**48**:541-546.

10. Guzman-Fulgencio M, Berenguer J, Micheloud D, Fernandez-Rodriguez A, Garcia-Alvarez M, Jimenez-Sousa MA*, et al.* European mitochondrial haplogroups are associated with CD4+ T cell recovery in HIV-infected patients on combination antiretroviral therapy. *J Antimicrob Chemother* 2013,**68**:2349-2357.

11. Massanella M, Negredo E, Clotet B, Blanco J. Immunodiscordant responses to HAART--mechanisms and consequences. *Expert Rev Clin Immunol* 2013,**9**:1135-1149.

12. Corbeau P, Reynes J. Immune reconstitution under antiretroviral therapy: the new challenge in HIV-1 infection. *Blood* 2011,**117**:5582-5590.

13. Capitini CM, Chisti AA, Mackall CL. Modulating T-cell homeostasis with IL-7: preclinical and clinical studies. *J Intern Med* 2009,**266**:141-153.

14. Limou S, Melica G, Coulonges C, Lelievre JD, Do H, McGinn S*, et al.* Identification of IL7RA risk alleles for rapid progression during HIV-1 infection: a comprehensive study in the GRIV cohort. *Current HIV Research* 2012,**10**:143-150.

15. Hartling HJ, Thorner LW, Erikstrup C, Harritshoj LH, Kronborg G, Pedersen C*, et al.* Polymorphism in interleukin-7 receptor alpha gene is associated with faster CD4(+) T-cell recovery after initiation of combination antiretroviral therapy. *AIDS* 2014,**28**:1739-1748.

16. Rajasuriar R, Booth D, Solomon A, Chua K, Spelman T, Gouillou M*, et al.* Biological determinants of immune reconstitution in HIV-infected patients receiving antiretroviral therapy: the role of interleukin 7 and interleukin 7 receptor alpha and microbial translocation. *J Infect Dis* 2010,**202**:1254-1264.

17. Hutchinson JN, Raj T, Fagerness J, Stahl E, Viloria FT, Gimelbrant A*, et al.* Allele-specific methylation occurs at genetic variants associated with complex disease. *PLoS One* 2014,**9**:e98464.

18. Bewick V, Cheek L, Ball J. Statistics review 12: Survival analysis. *Critical Care* 2004,**8**:389-394.

19. Gregory SG, Schmidt S, Seth P, Oksenberg JR, Hart J, Prokop A*, et al.* Interleukin 7 receptor alpha chain (IL7R) shows allelic and functional association with multiple sclerosis. *Nat Genet* 2007,**39**:1083-1091.

20. Kreft KL, Verbraak E, Wierenga-Wolf AF, van Meurs M, Oostra BA, Laman JD*, et al.* Decreased systemic IL-7 and soluble IL-7Ralpha in multiple sclerosis patients. *Genes Immun* 2012,**13**:587-592.

21. Rajasuriar R, Booth DR, Gouillou M, Spelman T, James I, Solomon A*, et al.* The role of SNPs in the alpha-chain of the IL-7R gene in CD4+ T-cell recovery in HIV-infected African patients receiving suppressive cART. *Genes Immun* 2012,**13**:83-93.

22. Crawley AM, Faucher S, Angel JB. Soluble IL-7R alpha (sCD127) inhibits IL-7 activity and is increased in HIV infection. *J Immunol* 2010,**184**:4679-4687.

23. Hodge JN, Srinivasula S, Hu Z, Read SW, Porter BO, Kim I*, et al.* Decreases in IL-7 levels during antiretroviral treatment of HIV infection suggest a primary mechanism of receptor-mediated clearance. *Blood* 2011,**118**:3244-3253.

24. Spaulding A, Rutherford GW, Siegfried N. Tenofovir or zidovudine in three-drug combination therapy with one nucleoside reverse transcriptase inhibitor and one non-nucleoside reverse transcriptase inhibitor for initial treatment of HIV infection in antiretroviral-naive individuals. *Cochrane Database Syst Rev* 2010:CD008740.

25. Negredo E, Molto J, Burger D, Viciana P, Ribera E, Paredes R*, et al.* Unexpected CD4 cell count decline in patients receiving didanosine and tenofovir-based regimens despite undetectable viral load. *AIDS* 2004,**18**:459-463.

26. Motta D, Brianese N, Foca E, Nasta P, Maggiolo F, Fabbiani M*, et al.* Virological effectiveness and CD4+ T-cell increase over early and late courses in HIV infected patients on antiretroviral therapy: focus on HCV and anchor class received. *AIDS Res Ther* 2012,**9**:18.

27. Potter M, Odueyungbo A, Yang H, Saeed S, Klein MB. Impact of hepatitis C viral replication on CD4+ T-lymphocyte progression in HIV-HCV coinfection before and after antiretroviral therapy. *AIDS* 2010,**24**:1857-1865.

28. McGovern BH, Golan Y, Lopez M, Pratt D, Lawton A, Moore G*, et al.* The impact of cirrhosis on CD4+ T cell counts in HIV-seronegative patients. *Clin Infect Dis* 2007,**44**:431-437.