# HIGH FREQUENCY OF CD8 ESCAPE MUTANTS IN ELITE CONTROLLERS AS NEW OBSTACLE FOR HIV CURE

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Maria Angeles Navarrete-Muñoz [1,2], Marcial García [1,2], Ricardo Ramos [3], Africa Holguín [4], Clara Restrepo [1,2], Alfonso Cabello [5], Juan Carlos López-Bernaldo [6], Francisco de la Hera [5], Carlos Barros [7], Manuel Fernández-Guerrero [5], Vicente Estrada [8], Miguel Górgolas [5], José M Benito [1,2], **Norma Rallón** [1,2]

[1] Instituto de Investigación Sanitaria-Fundación Jiménez Díaz, Universidad Autónoma de Madrid (IIS-FJD, UAM), Madrid, Spain; [2] Hospital Universitario Rey Juan Carlos, Móstoles, Spain; [3] Unidad de Genómica Parque Científico de Madrid, Madrid, Spain; [4] Instituto Ramón y Cajal de Investigación Sanitaria, Madrid, Spain; [5] Hospital Universitario Fundación Jiménez Díaz, Madrid, Spain; [6] Hospital General Universitario Gregorio Marañon, Madrid, Spain; [7] Hospital Universitario de Móstoles, Móstoles, Spain; [8] Hospital Universitario Clínico San Carlos, Madrid, Spain

Contact: normaibon@yahoo.com / jbenito1@hotmail.com

## I. Background

- The shock and kill strategy to purge the HIV reservoir has failed likely as a consequence of several obstacles.
- The existence of escape mutations in regions of the HIV proviral sequence coding for epitopes of CD8 immune response has been postulated as one of the reasons for this failure.
- Herein, we have analyzed the frequency of mutations in HIV-Gag epitopes of CD8 response in proviral HIV sequences obtained from one of the most important cellular compartment of the HIV reservoir, the resting memory CD4 T cells (Trm), in two different groups of HIV patients with complete viral suppression: patients on successful cART and elite controller patients.

## II. Methods

- Seventeen HIV patients with undetectable plasma HIV-RNA were included: 7 elite controllers (EC) and 10 non-controller patients on successful cART (TX). A visual scheme of the methodology employed is shown in **Figure 1.**
- Trm (defined as CD4+CD45RO+CD25-CD69-HLADR-) cells were isolated from peripheral blood mononuclear cells (PBMCs) using an immunomagnetic separation technique.
- Total genomic DNA from purified Trm cells was extracted and the entire HIV-Gag gene was amplified by nested-PCR.
- A pair-end sequencing run was performed on a MiSeq system (Illumina). Read set qualities were assessed using FastQC and trimmed of adapters and low quality data with Cutadapt and Prinseq. Trimmed fastq files were mapped and aligned to the consensus HXB2 sequence using Bowtie2. Genetic variation analysis was accomplished with the VarScan software.
- HLA alleles class I (A, B and C) typing was performed using the HLA-SSO LIFECODES methodology based on the amplification of DNA by PCRSSO (PCR with sequence specific oligonucleotides) and subsequent hybridization of the PCR product to SSO probes.
- Optimal HIV-Gag epitopes of CD8 immune response were predicted for each patient based on HLA class I haplotype (A, B, C), according to a list of the best-defined HIV-1 CTL epitopes in the Los Alamos Immunology Database. Sequences of HIV-Gag epitopes obtained from the autologous virus were compared to sequences of the optimal epitopes and the prevalence of mutated epitopes, as well as the prevalence of mutated aminoacids per epitope were calculated for each patient. The Los Alamos database Analyze Align tool was used to generate sequence LOGOs.
- Impact of epitopes mutations on HLA recognition was evaluated using artificial neural networks integrated in NetMHC 4.0 Server (DTU Health Tech, University of Denmark).

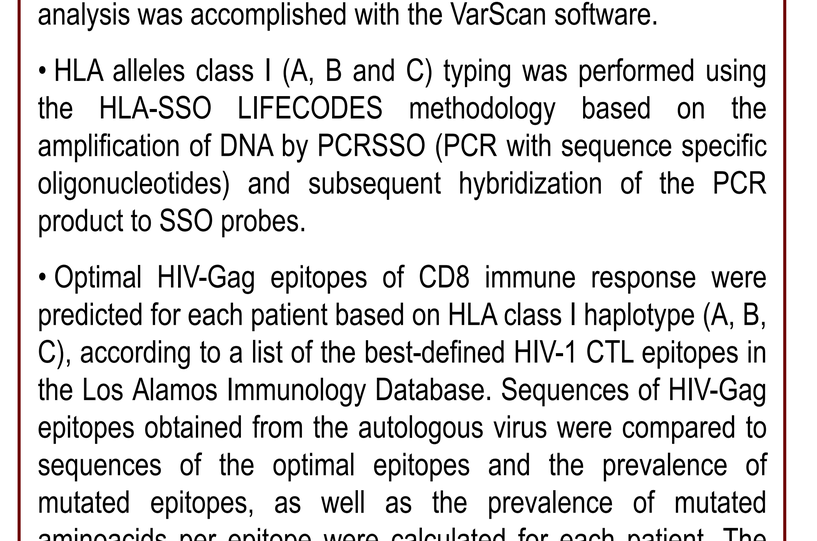



**Figure 1.** Scheme of the methodology employed.

## III. Results

**Table 1.** Characteristics of patients included in the study.

| Characteristic | Study Group | | p-value |
|---|---|---|---|
| | EC | TX | |
| n | 7 | 10 | - |
| Age (Years) | 42 [32-46] | 47 [44-51] | 0.070 |
| Male (%) | 57 | 90 | 0.116 |
| CD4 counts (cells/μL) | 744 [696-957] | 988 [585-1469] | 0.526 |
| Time since HIV diagnosis(Years) | 5 [4-12] | 13 [11-16] | 0.011 |
| Lenght of EC status (Years) | 5 [2-7] | NA | - |
| Lenght of treatment (Years) | NA | 12 [9-16] | - |

Data are expressed as Median [IQR], except sex, expressed as %; *p*-value: comparison between EC and TX group (Mann-Whitney U test); NA: not apply.

- There were no significant differences between EC and TX patients in terms of age, gender and CD4 counts at the moment of inclusion in the study (**Table 1**).
- The median follow-up of EC patients maintaining EC status was 5[2–7] years.
- TX patients had been on cART for a median of 12[9-16] years.

- The whole HIV-Gag sequence was successfully amplified and sequenced in all patients.
- Based on the HLA haplotype of each patient, the median number of HIV-Gag CD8 epitopes predicted for EC and TX patients were 7[4-7] and 7[6-12], respectively (p=0.233) (**Table 2**) .
- Of note, the frequency (%) of mutated HIV-Gag CD8 epitopes was 75[46-100] and 54[48-74] in EC and TX, respectively (p=0.432), and the frequency (%) of mutated aminoacids per HIV-Gag CD8 epitope was 18[13-22] and 15[11-19] in EC and TX, respectively (p=0.407) (**Table 2**). Examples of the frequency of mutations in some of the most immunogenic HIV-Gag CD8 epitopes in autologous sequences obtained from Trm cells of EC and TX patients are shown using sequence LOGOs representations (**Figure 2**).
- Lastly, the frequency (%) of mutated HIV-Gag CD8 epitopes with a significant impact in reducing HLA recognition was also similar in EC and TX groups (50[33-50] in EC and 41[19-52] in TX, p=0.552). (**Table 2**).

**Table 2.** Number of HIV-Gag CD8 epitopes predicted, frequency of mutated epitopes, frequency of mutated aminoacids per epitope in autologous HIV-Gag sequences in EC and TX patients and frequency of mutations with negative impact on epitope recognition.

| | Study Group | | p-value |
|---|---|---|---|
| | EC | TX | |
| Number of HIV-Gag CD8 epitopes predicted | 7[4-7] | 7[6-12] | 0.233 |
| Mutated HIV-Gag CD8 epitopes in autologous sequences (%) | 75[46-100] | 54[48-74] | 0.432 |
| Mutated aminoacids per HIV-Gag CD8 epitope (%) | 18[13-22] | 15[11-19] | 0.407 |
| HIV-Gag CD8 epitopes with mutations impairing HLA recognition (%) | 50[33-50] | 41[19-52] | 0.552 |

Data are expressed as Median [IQR]; *p*-value: comparison between EC and TX group (Mann-Whitney U test).

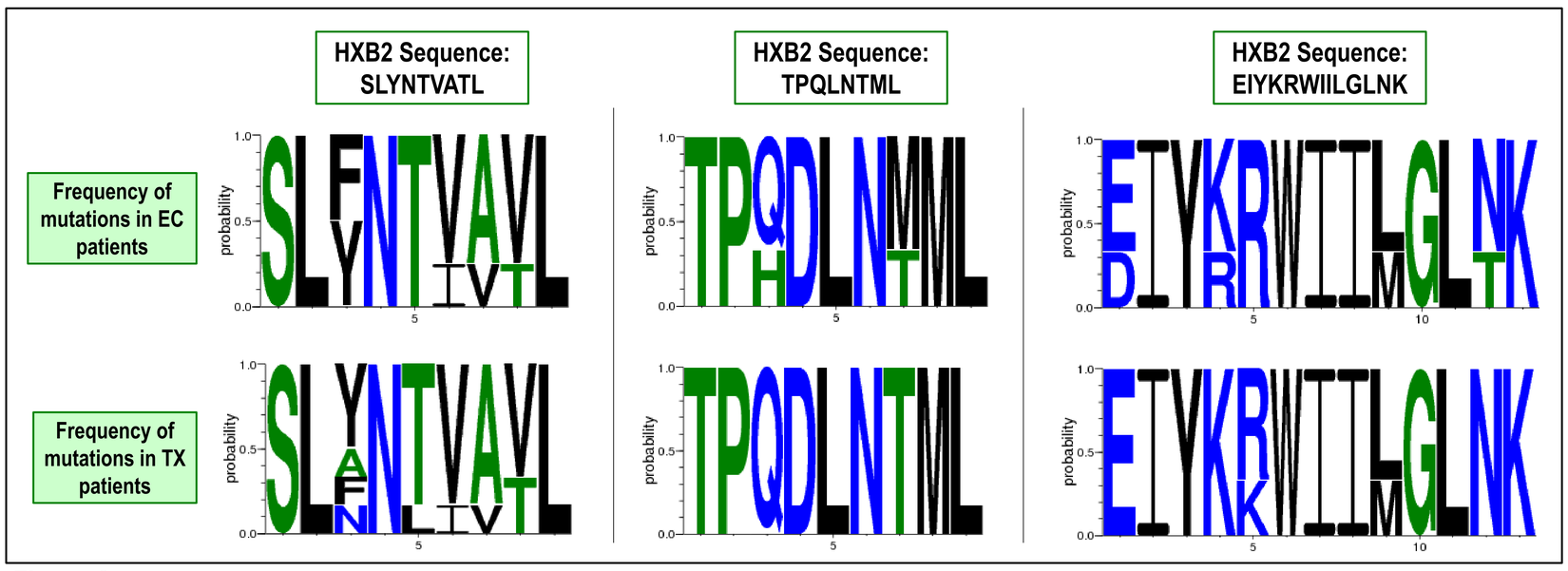



**Figure 2.** Sequence LOGOs of amino acids sequences in different HIV-Gag CD8 epitopes, showing frequencies of mutated amino acids in some of the most immunogenic HIV-Gag CD8 in autologous HIV-Gag sequences obtained from Trm cells of EC and TX patients.

## IV. Conclusions

✓ Our results show a high prevalence of mutations in HIV-Gag epitopes of CD8 T-cell response not only in the HIV reservoir of patients with successful cART-mediated control, but also in patients with spontaneous HIV control in whom control is reached at an early stage of infection.

✓Many of these mutations have a potential negative impact on epitope recognition.

✓These findings support the role of existence of escape mutations as another obstacle to purge the HIV reservoir. This could provide a proof of concept challenging the current HIV cure strategies based on reservoir reactivation.

## V. Funding / Acknowledgements

We would like to thank all patients who participated in the study. This study has been funded by projects PI16/01769 and RD16/0025/0013 integrated in the State Plan for Scientific and Technical Research and Innovation and co-funded by ISCIII-Sub-Directorate General for Research Assessment and Promotion and European Regional Development Fund (ERDF). N Rallón is a Miguel Servet II investigator from the ISCIII (CPII19/00025), Madrid, Spain. MA Navarrete-Muñoz is a predoctoral student funded by IND2018/BMD-9651 project. M García is co-funded by RD16/0025/0013 project and an Intramural Research Scholarship from IIS-FJD. C Restrepo is funded by project RD16/0025/0013.