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# Cathepsin A levels in CD4+ T cells from HIV-positive patients

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***ABSTRACT:***

Tenofovir (TFV) is an acyclic nucleotide analog of dAMP, a potent inhibitor of human immunodeficiency virus type 1 (HIV-1) replication, although with low cellular permeability and oral bioavailability. In order to improve its pharmacological profile, prodrugs of TFV have been synthesized, such as tenofovir disoproxil fumarate (TDF) and tenofovir alafenamide fumarate (TAF). The pharmacological advantages provided by TAF are attributed to its activation mechanism, which is related to the serine protease cathepsin A (CTSA), a component of a high-molecular weight complex of lysosomal-galactosidase and N-acetyl neuraminidase. CTSA is expressed by a broad range of human tissues, including kidney, liver, macrophages, platelets, and testis. In the present study, we have determined the level of CTSA expression in CD4+ T cells from HIV-positive patients, correlating it to the viral set point, and its modulation upon antiretroviral treatment initiation.

Our data show CTSA is constitutively expressed in CD4+ T cells from high-viremic patients, low viremic patients, elite controllers (EC) and healthy donors, with a non-significant trend towards higher viral set point, being CTSA lower in the CD4+ T cells from EC. ART initiation did not change the expression levels of CTSA.

***Keywords:*** HIV, Tenofovir, TFV, TDF, TAF, Cathepsin A, CTSA, CD4 T cells, Elite controllers.

## INTRODUCTION

Tenofovir {9-R-[(2-phosphonomethoxy)propyl]adenine} (TFV), is an acyclic nucleotide analog of dAMP, a potent inhibitor of human immunodeficiency virus type 1 (HIV-1) replication[1]. TFV is phosphorylated within the cell by AMP kinase and nucleoside diphosphate kinase to the active molecule, tenofovir diphosphate[2,3], which potently inhibits HIV-1 reverse transcriptase[4,5]. In order to increase the cellular permeability and oral bioavailability of TFV, prodrugs of TFV have been synthesized. Tenofovir disoproxil fumarate (TDF) is a bis-isopropoxycarbonyloxymethyl ester of TFV approved for the treatment of HIV. The oral administration of TDF results in high systemic levels of TFV[6] and it is well tolerated, with a good long-term toxicity profile, and uncommon development of resistance[1,7]; however, the rapid systemic degradation of TDF to TFV limits its uptake into target cells.

Tenofovir alafenamide fumarate (TAF) is a next generation prodrug of TFV with good oral bioavailability and increased plasma stability compared to TDF[8,9]. Studies in HIV-infected patients have shown improved viral suppression at significantly lower doses of TAF compared to TDF[10]. The pharmacological advantages provided by TAF are attributed to its activation mechanism, which is related to the serine protease cathepsin A (CTSA)[11,12].

CTSA is a component of a high-molecular weight complex of lysosomal-galactosidase and N-acetyl neuraminidase[13]. CTSA protects glycosidases from proteolysis in the lysosomes[13-15] and it seems to exert deaminase and esterase activities at neutral pH, and carboxypeptidase activity at pH 5.0 to 5.5[16-19]. Also, CTSA shows

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high affinity for charged residues in the P1 position of peptide substrates with hydrophobic amino acid residues in this position[20,21]. CTSA is expressed by a broad range of human tissues, including kidney, liver, macrophages, platelets, and testis[22] and, therefore, it may be a useful target enzyme for the activation of prodrugs active against pathogens that exhibit diverse tissue tropism.

In the present study, we aimed at determining the level of CTSA in the CD4+ T cells from HIV-positive patients and controls, correlating it to the viral set point and clinical status, as well as evaluating its modulation upon antiretroviral treatment.

## MATERIALS AND METHODS

### Bioinformatic analysis

The microarray dataset used to determine the transcriptional levels of CTSA in the CD4+ T cells from HIV-positive patients (HIV), including a subgroup of Elite Controllers (ECs), was obtained from the NCBI Gene Expression Omnibus databank (GEO, http://www.ncbi.nlm.nih.gov/geo/), under accession number GSE18233. The EC group, defined in Rotger et al 2010, included 16 patients. Complete demographic data of all patients are available in the relative publications[2,3]. For the present analysis, drug-naive patients were firstly selected and stratified using the viral set point as parameter. HIV patients were divided in low-, medium- and high-viremic, which corresponded to the 33$^{th}$ (mean Log(copies/ml)=2.715 ± 0.361), the 67$^{th}$ (mean Log(copies/ml)=4.091±0.283) and the 99$^{th}$ (mean Log(copies/ml)=4.991 ± 0.298) percentile, respectively. Eight samples from 3 healthy controls served as control population. In addition, when available, CTSA expression levels were determined in the patients before and after receiving antiretroviral treatment (ART).

To generate the dataset, CD4+ T cells were positively selected from frozen PBMCs using magnetically labeled CD4 microbeads and subsequent column purification. Gene expression levels were assessed on total RNA using the Human-6 v3 Expression BeadChips (Illumina). Data pre-processing, including variance-stabilizing transformation and robust-spline normalization, were applied to the raw data.

### Statistical Analysis

Data are presented as normalized expression levels in scatter plots, showing mean value across samples with 95% confidence interval. Statistical analysis of CTSA levels in HIV-positive patients and control donors was performed using either One-Way ANOVA followed by Bonferroni post-hoc or unpaired Student's *t*-test. When CTSA levels were compared before and after ART treatment, a paired Student's *t*-test was applied. Correlation analysis between the viral set point and CTSA levels was performed using a two-tailed Spearman correlation test. A $p$ value <0.05 was considered to be statistically significant.

## Results

### *CTSA levels in HIV-positive patients*

We wanted to evaluate whether altered levels of CTSA levels could be detected in HIV-infected patients as compared to healthy donors. The high-viremic group included 40 patients, the medium-viremic group included 41 patients and the low-viremic group 40 patients. CTSA expression levels were 8.307±0.2988, 8.162±0.2699, 8.181±0.2776, respectively. CTSA levels were 8.093±0.3051 in the EC group and 8.107±0.2745 in the healthy donors (Figure 1A).

A significant trend of increase could be observed in the high-viremic patients as compared to the EC patients (16% of increase, $p$=0.0219 by unpaired Student's *t*-test), although it did not pass the Bonferroni post-hoc correction (Figure 1A).

Correlation analysis showed a trend of positive correlation between the viral set point and the CTSA levels ($p$=0.0541 by two-tailed Spearman test) (Figure 1B).

### *CTSA levels after ART*

Next, we wanted to determine whether successfully ART treatment was associated to a modulation in the CTSA levels in sorted CD4 + T cells from HIV patients.

A trend of reduction could be observed after ART treatment, the levels being 8.217±0.2757 before treatment and 8.165±0.2195 post-ART ($p$=0.3604 by Paired Student's *t*-test) (Figure 1C).

## DISCUSSION

The present investigation demonstrates that CTSA is constitutively expressed in the CD4+ T cells from HIV-positive patients (both high or elite controllers) and healthy controls. ART initiation did not change the values of CTSA in HIV-1-infected patients.

TAF activation mechanism, which is related to the serine protease cathepsin A (CTSA), seems to be broadly present in several human organs, tissues and cells at a constitutional level.

The anti-HIV activity of the acyclic nucleoside phosphonate tenofovir (TFV) was first reported by Balzarini and collegues[24]. Further studies have shown that the pharmacologically active diphosphate metabolite (TFV-DP) potently inhibits the HIV reverse transcriptase. TFV-DP is a poor substrate and inhibitor of the mitochondrial DNA polymerase g[25,26]. In addition, TFV-DP also has a long intracellular half-life in PBMCs. Despite this, TFV in parent form cannot be orally administered, because of its poor membrane permeability[27].

The disoproxil prodrug was found to have improved cell permeability, anti-HIV activity and oral bioavailability[27] and therefore, TFV disoproxil, formulated as fumarate salt (TDF), was ultimately approved by the US Food and Drug Administration in 2001, and the European Medicines Evaluation Agency in 2002. However,

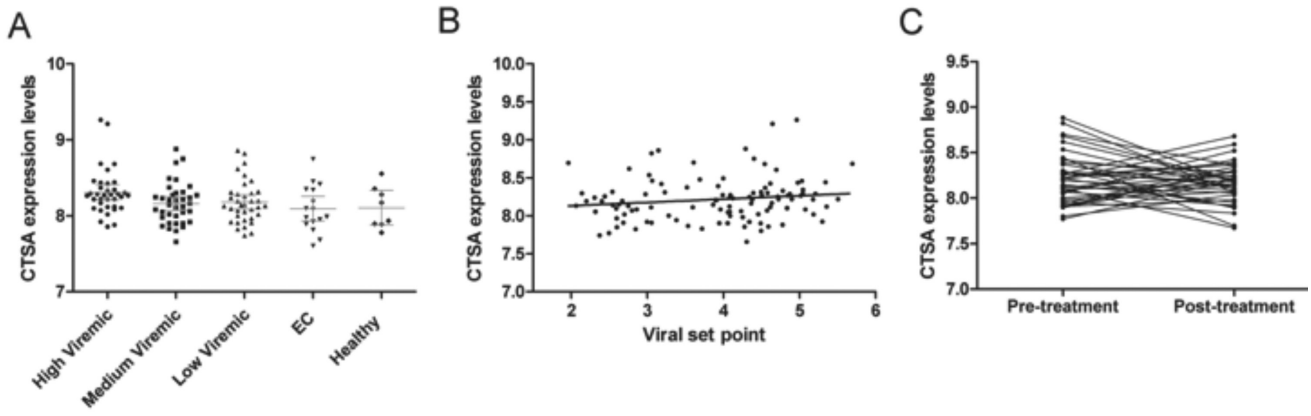


**Figure 1.** *A*, CTSA expression in HIV-1 infected individuals and healthy controls; *B*, Correlation between CTSA levels and viral set point; *C*, CTSA expression before and after ART initiation.

while TDF therapy is generally well tolerated, its use is associated with side effects on renal function and bone mineral density. TAF has been identified as an alternative TFV prodrug to TDF[9]. *In vivo* stability and selective intracellular cleavage of TAF allows for prolonged systemic exposure to intact prodrug and the accumulation of higher intracellular levels of the active metabolite TFV-DP as compared to TDF. The lysosomal CTSA has been identified as the primary hydrolase catalyzing the initial step in intracellular activation of TAF in cells[12]. CTSA is a ubiquitously expressed enzyme[28] that it is not adversely affected by concomitant agents including HIV protease inhibitors and other HCV protease inhibitors[29], with the exception of telaprevir and boceprevir.

## CONCLUSIONS

In the present study we have determined the level of CTSA expression in the CD4+ T cells from HIV patients, correlating it to the viral set point, and its modulation upon antiretroviral treatment. Our data show a positive trend of correlation between CTSA expression level and the viral set point, being lower in the CD4+ T cells from EC patients and healthy donors as compared to the CD4+ T cells from high-viremic patients.

### Conflict of Interests:

The Authors declare that they have no conflict of interests.

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