The RT M184V resistance mutation clearance in the reservoir is mainly related to CD4 nadir and viral load zenith independently of therapeutic regimen type

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Running title: M184V mutation clearance is related to nadir and zenith.

Synopsis

Objectives: Resistance associated mutations (RAMs) are archived in the HIV reservoir and can re-emerge with an inappropriate ART use limiting treatment options. However, recent studies, using ultra deep sequencing (UDS), showed a decrease of quasispecies harbouring RAMs, suggesting that recycling some antiretrovirals could be considered. The aim of this study was to characterize, in HIV treated PLWHIV, the M184V mutation decrease kinetics in proviral DNA and associated factors of M184V mutation clearance over time.

Methods: UDS was performed on HIV DNA from blood cells at different time points to quantify the percentage of M184V positive quasispecies. The sequence reads were analysed with a minimum coverage set at 50 and an ambiguity filter at 5% or 2%.

Results: At 2.5 years after the first time-point the M184V-lost was observed in 50% of PLWHIV. Moreover univariate analyses highlight that a higher nadir CD4 count and a lower zenith HIV1 RNA viral load were correlated with a faster clearance of the mutation. In multivariate analysis, a higher zenith was negatively associated with the M184V clearance at the 5% threshold. Interestingly, the Lamivudine/Emtricitabine presence in the ART therapy regiment during the five years was not associated with the persistence of the M184V.

Conclusions: Our study provides new information concerning the clearance speed of M184V mutation over time in PLWHIV with fully suppressed viremia, open the discussion about duration needed to consider a Lamivudine/Emtricitabine recycling and reinforce the association of the nadir and zenith values with the M184V mutation clearance.

Introduction

With the extension of life expectancy of people leaving with HIV (PLWHIV), characterization of resistance-associated-mutations (RAMs) and optimization of ART are a key challenge considering theirs resistance and toxicities past histories. The RAMs lead to different drug-resistance, are archived in the HIV reservoir, at least for years and can re-emerge with an inappropriate ART use limiting treatment options.[1] Studies using ultra deep sequencing (UDS) demonstrated the decrease in the proportion of viral variants harbouring RAMs in the HIV reservoir over time in PLWHIV with sustained viral suppression and recent studies suggested that a recycling of some antiretrovirals could be feasible in some cases, despite the presence of past archived RAMs.[2,3] The M184V RAM induced resistance to two largely used ART, Emtricitabine and Lamivudine.[4] Moreover, ART, including Emtricitabine/Lamivudine are the most currently recommended by the ART guidelines[5] that reinforces the interest of the possible recycling of these molecules.

The aim of this study was to characterize, in treated PLWHIV, the kinetics of M184V mutation decrease in proviral DNA and to determine associated factors with M184V mutation clearance over time.

Materials and methods

To characterize the kinetic of the M184V clearance in HIV reservoir, we retrospectively selected 22 PLWHIV receiving care. Biological and clinical data were available for all PLWHIV witch have signed an informed consent to have their medical information stored and their use has been approved by a local ethics committee (n°20231013133851). All PLWHIV had an HIV RNA <50 copies/mL for at least 5 years and a M184V resistance mutation documented in past RNA genotypes. UDS was

performed from HIV-DNA from frozen blood samples at least one time/per year over 5 years to quantify the proportion of M184V-positive quasispecies, following the guidelines of the French National Agency for Research on AIDS and Emerging Infectious Diseases (ANRS-MIE) consensus, using Illumina technology as previously described (Genbank sequences ID PP726027-PP726106).[6,7] For the UDS, the sequence reads were analysed with the Geneious software with a minimum coverage set at 50 and an ambiguity filter at 5% or at 2%. A Kaplan-Meier survival model and cox regression (univariate and multivariate analysis) were realized with 5% and 2% thresholds.

Results

The 22 PLWHIV were 18 males and 4 females, with a median age of 56 years [IQR 49-65] and a duration of virological suppression median at the first time-point (D0) (HIV-RNA< 50 copies/mL) of 7.7 years [IQR 7.0-10.0]. They present a median CD4 cell count of 560/mm^3 [IQR 465-807] at D0, a median nadir of 164/mm^3 [IQR 77-259] and a median HIV viral load zenith of 4.99 log_{10} copies/mL [IQR 4.20-5.56]. At D0, all the PLWHIV presented a M184V detected in the HIV reservoir. Using an ambiguity filter at 2% or 5%, we obtained a median survival of 2.5 years (the M184V was not detected for 50% of PLWHIV at 2.5 years) with no significant difference with the two thresholds (p=0.88) (Fig1 A-B). Time points of M184V clearance for each patient are shown in table S1.

Univariate analyses, for the two threshold, highlight that a higher nadir and a lower zenith were correlated with a faster clearance of the mutation (table.1). Indeed, sex, CD4 cells count during the time-course and type of ART therapy were not associated with a faster clearance of the M184V. Moreover, multivariate analysis with the 5% threshold shown that a higher zenith was negatively associated with the M184V

clearance. Interestingly, the Lamivudine/Emtricitabine presence in the ART line therapy during the five years was not associated with the persistence of the M184V.

Discussion

Several others studies raised the question of the risk of virological failure with the re-used of ART despite past RAMs and suggested that a longer time of virological suppression lead to a lesser risk of virological rebound despite past RAMs. Considering the significance of Lamivudine and Emtricitabine in main antiretroviral regimens, it is crucial to establish the M184V mutation dynamic in the HIV reservoir of PLWHIV who are virally suppressed for a prolonged period. Several studies have also investigated the dynamics and effect of past RAM in ART efficiency. Indeed, the LAMRES study showed that presence of past detected M184V <3.5 years significantly affect the probability of virological rebound and blips under Dolutegravir/Lamivudine ART-line.[8] Moreover, the presence of an NRTI mutation, notably the M184V, before ART switch may lead to a higher risk of dolutegravir resistance mutation in dual ART treatment.[9,10] However, Prospective open labelled studies show that Dolutegravir/Lamivudine effectively maintained virological suppression in PLWHIV with past history of lamivudine resistance.[2,3] Another study showed that the percentage of women with a detectable Nevirapine resistance declines over time and also observed a declining risk of virological failure associated with Nevirapine-containing ART with time since the first exposure of Nevirapine ART.[11] Martin-Carbonero team's shown that the switch to the regimen of Bictegravir, Emtricitabine and tenofovir alafenamide is effective even in PLWHIV with pre-existing nucleos(t)ide reverse transcriptase inhibitor RAM detected in a median of 8.8 years.[12] Altogether, these results suggesting that a longer delay after the last RAM detection is needed to minimize the risk of virological failure.

Interestingly, our results showed that the presence of Lamivudine/Emtricitabine in the ART regimen during the follow-up was not associated with the M184V maintenance. Taken with our previous study, its seems that the Lamivudine/Emtricitabine presence has an impact only on the past ART line regimen under virological replication.[7]

Moreover, our results reinforce, as others studies, the fact that to evaluate the presence of RAMs quasispecies in order of ART recycling, the use of UDS is preferable for DNA genotyping due to its better sensitivity.[13] Indeed, the use of UDS increased the proportion of PLWHIV with detected RAMs compared to the Sanger sequencing, which failed to detect variants in less than 15%–25% of the total population.[14] Moreover, literature not provide an agreement on the optimal threshold for detecting RAMs using UDS and the possibility that reducing the threshold lead to the risk of including sequencing errors in the result. In our study, we did not observe a significant difference between the 2% and the 5% threshold.

Our study provides new information concerning the clearance speed of M184V mutation over time in PLWHIV with fully suppressed viremia and open the discussion about duration needed to consider a Lamivudine/Emtricitabine recycling.

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Transparency declaration

The authors declare that they have no conflict of interest.

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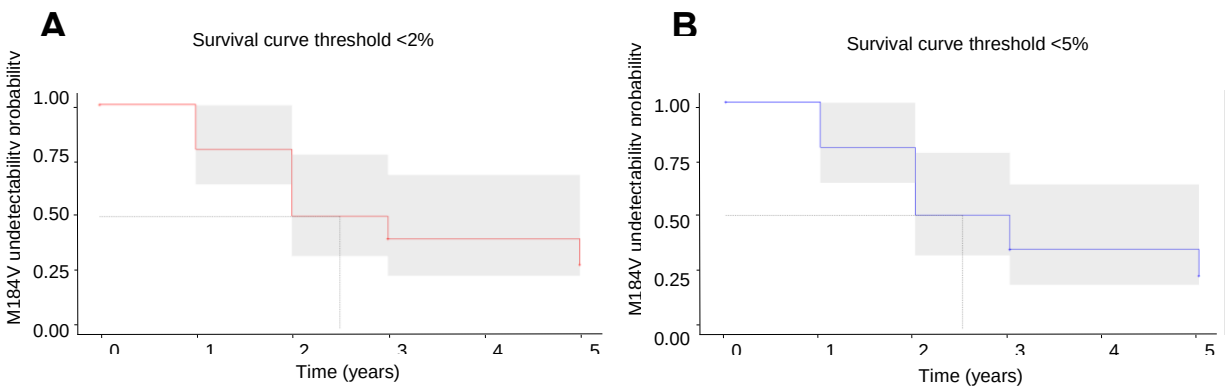

Figure1. M184V resistance mutation dynamics in HIV infected patients. Survival curves for the percentage of patients with a M184V undetectable over time with a threshold at < 2% (A) or at <5% (B). No statistical difference between the two UDS threshold (p=0.88).

	2% Univariate analysis			5% Univariate analysis	
Characteristic	**OR [95% CI]**	**p value**		**OR [95% CI]**	**p value**
CD4	0.907 [0.541-1.519]	0.709		0.882 [0.533-1.458]	0.624
Emtricitabine_Lamivudine	1.554 [0.539-4.48]	0.414		1.318 [0.474-3.663]	0.597
Type_therap (dual vs triple)	0.74 [0.256-2.142]	0.579		0.764 [0.268-2.176]	0.615
Sex	2.741 [0.811-9.26]	0.105		2.595 [0.781-8.619]	0.12
Nadir	1.661 [1.004-2.749]	**0.048**		1.569 [0.962-2.559]	0.071
Zenith	0.4 [0.209-0.765]	**0.006**		0.398 [0.209-0.758]	**0.005**
	2% Multivariate analysis			**5% Multivariate analysis**	
Characteristic	**OR [95% CI]**	**p value**		**OR [95% CI]**	**p value**
Emtricitabine _Lamivudine	1.093 [0.73-1.637]	0.664		1.054 [0.717-1.548]	0.79
Sex	1.125 [0.826-1.534]	0.455		1.119 [0.839-1.491]	0.445
Nadir	1.121 [0.909-1.382]	0.284		1.066 [0.876-1.297]	0.524
Zenith	0.842 [0.689-1.03]	0.094		0.82 [0.68-0.99]	**0.039**

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Table1. Factors associated with the M184V clearance.
Univariate and multivariate logistic regression were realized for the two UDS thresholds.