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

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

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24 **Synopsis**

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

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

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

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

47

48 **Introduction**

49 With the extension of life expectancy of people leaving with HIV (PLWHIV),
50 characterization of resistance-associated-mutations (RAMs) and optimization of ART
51 are a key challenge considering their resistance and toxicities past histories. The
52 RAMs lead to different drug-resistance, are archived in the HIV reservoir, at least for
53 years and can re-emerge with an inappropriate ART use limiting treatment options.¹
54 Studies using ultra deep sequencing (UDS) demonstrated the decrease in the
55 proportion of viral variants harbouring RAMs in the HIV reservoir over time in PLWHIV
56 with sustained viral suppression and recent studies suggested that a recycling of some
57 antiretrovirals could be feasible in some cases, despite the presence of past archived
58 RAMs.^{2,3} The M184V RAM induced resistance to two largely used ART, Emtricitabine
59 and Lamivudine.⁴ Moreover, ART, including Emtricitabine/Lamivudine are the most
60 currently recommended by the ART guidelines⁵ that reinforces the interest of the
61 possible recycling of these molecules.

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

65

66 **Materials and methods**

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

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

82

83 **Results**

84 The 22 PLWHIV were 18 males and 4 females, with a median age of 56 years [IQR
85 49-65] and a duration of virological suppression median at the first time-point (D0)
86 (HIV-RNA < 50 copies/mL) of 7.7 years [IQR 7.0-10.0]. They present a median CD4
87 cell count of 560/mm³ [IQR 465-807] at D0, a median nadir of 164/mm³ [IQR 77-259]
88 and a median HIV viral load zenith of 4.99 log₁₀ copies/mL [IQR 4.20-5.56]. At D0, all
89 the PLWHIV presented a M184V detected in the HIV reservoir. Using an ambiguity
90 filter at 2% or 5%, we obtained a median survival of 2.5 years (the M184V was not
91 detected for 50% of PLWHIV at 2.5 years) with no significant difference with the two
92 thresholds (p=0.88) (Fig1 A-B). Time points of M184V clearance for each patient are
93 shown in table S1.

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

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

101 **Discussion**

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

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

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

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

139
140 This study was previously presented as oral presentation at European meeting on
141 HIV & Hepatitis 2023, Rome, Italy, abstract number 7.

142
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147

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151 pharmacology network).

152 **Transparency declaration**

153 The authors declare that they have no conflict of interest.

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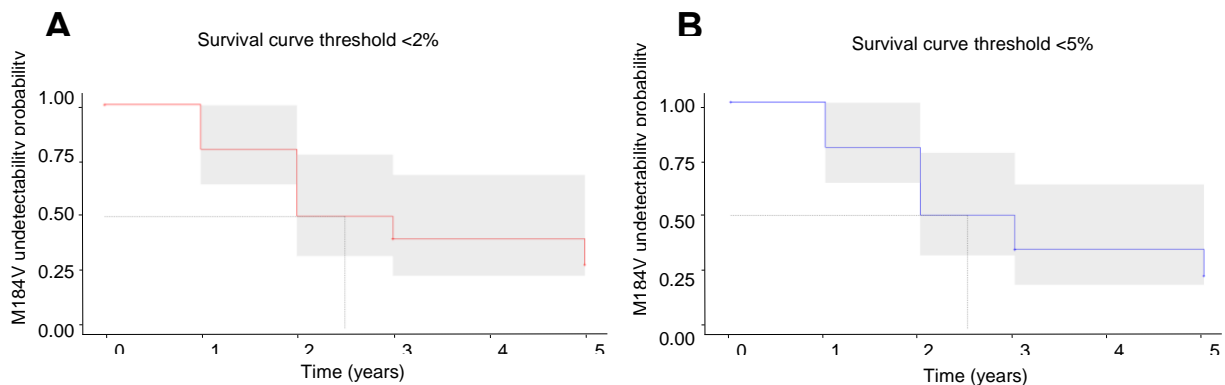
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Figure1. M184V resistance mutation dynamics in HIV infected patients.
 Survival curves for the percentage of patients with a M184V undetectable over
 time with a threshold at < 2% (A) or at <5% (B). No statistical difference
 between the two UDS threshold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