



## Prevalence of M184V and K65R in proviral DNA from PBMCs in HIV-infected youths with lamivudine/emtricitabine exposure

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**Objectives:** We analysed the prevalence of M184V/I and/or K65R/E/N mutations archived in proviral DNA (pDNA) in youths with perinatal HIV, virological control and who previously carried these resistance mutations in historic plasma samples.

**Methods:** We included vertically HIV-infected youths/young adults aged  $\geq 10$  years in the Madrid Cohort of HIV-1 Infected Children and Adolescents, exposed to lamivudine and/or emtricitabine, with M184V/I and/or K65R/E/N in historic plasma samples, on antiretroviral therapy (ART), virologically suppressed (HIV-1 RNA  $< 50$  copies/mL), and with available PBMCs in the Spanish HIV BioBank. Genomic DNA was extracted from PBMCs and HIV-1 RT gene was amplified and sequenced for resistance testing by Stanford HIV Resistance tool.

**Results:** Among the 225 patients under follow-up in the study cohort, 13 (5.8%) met selection criteria, and RT sequences were recovered in 12 (92.3%) of them. All but one were Spaniards, carrying subtype B, with a median age at PBMCs sampling of 21.3 years (IQR: 15.6–23.1) with 4 years (IQR 2.1–6.5) of suppressed viral load (VL). Nine (75%) youths did not present M184V/I in pDNA after at least 1 year of viral suppression. In December 2019, the remaining three subjects carrying M184V/I in pDNA maintained suppressed viraemia, and two still used emtricitabine in ART.

**Conclusions:** The prevalence of resistance mutations to lamivudine and emtricitabine in pDNA in a cohort of youths perinatally infected with HIV who remain with undetectable VL, previously lamivudine and/or emtricitabine experienced, was infrequent. Our results indicate that ART including lamivudine or emtricitabine may also be safe and successful in youths with perinatal HIV with previous experience of and resistances to these drugs detected in plasma.

## Introduction

Vertically HIV-infected patients who have survived and who are currently adolescents and young adults have been exposed to various ART regimens during their lifetime and treated with sub-optimal ART regimens. As a consequence, they have a higher risk of selecting and accumulating resistance mutations, among others, M184V/I and/or K65R/E/N in the HIV reverse transcriptase.<sup>1,2</sup> Treatment of these patients is often challenging; the use of combinations including multiple drugs increases drug-related toxicity and increases adherence problems, making it difficult to achieve and maintain virological suppression.

Simplification of ART improves adherence and quality of life in virologically suppressed patients, and is a key aspect in the management of the HIV-infected youth population. Dual integrase-inhibitors-based therapy with dolutegravir (DTG) plus lamivudine (3TC) can be a very attractive antiretroviral treatment strategy, showing durable efficacy, safety, low toxicity, and in a single tablet facilitating adherence. Dolutegravir/lamivudine dual therapy has been shown non-inferior to dolutegravir/tenofovir disoproxil fumarate/emtricitabine in treatment-naïve adult patients and also as switch therapy.<sup>3,4</sup> However, the development of resistance mutations is a concern, since in patients with previous treatments that have included lamivudine or emtricitabine (FTC), there could be a functional monotherapy concealed in simplification to two-drug combinations.<sup>5-7</sup> Furthermore, patients with a long history of ART exposure, such as vertically HIV-infected adolescents who have received many regimens throughout their lives, often present resistance mutations to these drugs (M184V/I and/or K65R/E/N),<sup>1,2</sup> and it is unknown whether these simplification strategies are safe in patients with many lines of treatment and previous failures, e.g. youths with perinatal HIV. Some studies in adults have found that M184V/I, as a unique resistance mutation, did not increase the risk of virological failure in an initial follow-up period in well-controlled patients who switched to a regimen of the dual combination of dolutegravir/lamivudine.<sup>8,9</sup> There are, however, no studies performed in vertically HIV-infected patients.

The objective of our study was to determine the prevalence of resistance mutations to lamivudine and emtricitabine in proviral DNA (pDNA) in youths with perinatal HIV under ART who were lamivudine and/or emtricitabine experienced, virologically suppressed for at least 1 year before sampling and carrying M184V/I and/or K65R/E/N in their historic plasma genotypes. We also reported their virological situation at 31 December 2019.

## Patients and methods

### Ethics

The study was conducted according to the Declaration of Helsinki and was approved by the Clinical Research Ethical Committee of the Hospital Universitario Gregorio Marañón (06/2019). Written informed consent was obtained from all participants, as well as from the parents/guardians of children <12 years old.

### Study population

The study was performed within Madrid Cohort of HIV-1-Infected Children and Adolescents, belonging to the Paediatric Cohort of the Spanish National AIDS Network (CoRISpe), which actively collaborates with the Spanish HIV BioBank.<sup>10,11</sup> Inclusion criteria included vertically HIV-infected youths and

young adults aged 10 years and older at patient selection time, under ART and virologically suppressed with HIV-1 RNA <50 copies/mL for  $\geq 1$  year before sampling, lamivudine-and/or emtricitabine-experienced, and carrying M184V/I and/or K65R/E/N in their historic plasma samples, as indicated in Figure 1. We considered three timepoints: point 1 referred to the collection time of historic plasma carrying M184V; point PBMCs referred to PBMCs sampling time; and 31 December 2019 was the last evaluation. Clinical, epidemiological, immunological and virological data, and all available resistance genotypes were collected for each patient.

### Nucleic acid extraction and HIV-1 RT amplification

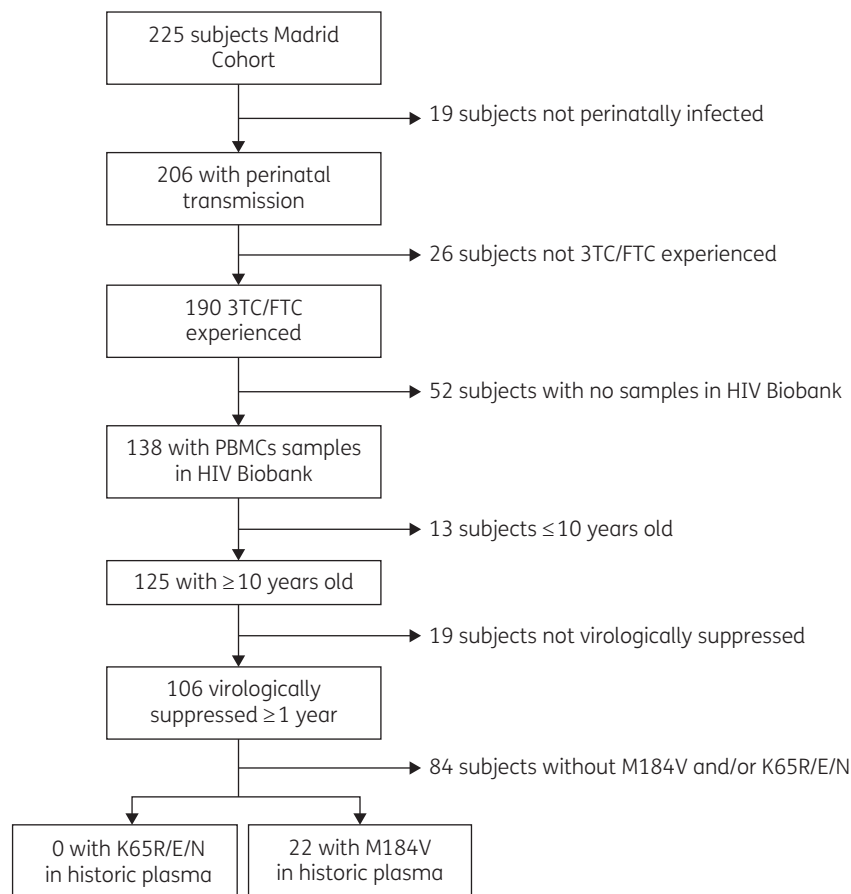
To analyse the prevalence of resistance changes M184V/I and/or K65R/E/N to lamivudine and emtricitabine in pDNA of study population, genomic DNA was extracted from available PBMCs obtained from the Spanish HIV BioBank using QIAamp<sup>®</sup> DNA Blood Mini Kit (QIAGEN) according to manufacturer's protocol. Partial reverse transcriptase (RT, codons 1-335) from HIV-1 *pol* was amplified by RT-PCR and nested-PCR from extracted DNA using primers designed by the WHO ([https://www.who.int/hiv/pub/drugresistance/dried\\_blood\\_spots/en/](https://www.who.int/hiv/pub/drugresistance/dried_blood_spots/en/)). PCR amplicons were purified using the Illustra<sup>™</sup> ExoProStar 1-Step<sup>™</sup> (GE Healthcare Life Sciences, UK) and sequenced by Macrogen Inc. (Geumchun-gu, Korea) to obtain consensus RT sequences. The presence of M184V/I and K65R/E/N was analysed by the HIVdb Program Genotypic Resistance Interpretation Algorithm v8.9-1 (Stanford University, USA). Genotyping testing in plasma was performed under similar conditions during routine clinical assessment of patients by using Viroseq HIV-1 Genotyping (Abbott).

### Statistical analysis

Categorical variables were expressed as counts and proportions and comparisons between them were assessed using the Fisher test. Quantitative variables were reported by medians and IQR and compared using the Mann-Whitney *U* test. All analyses were performed by using GraphPad Prism version 8.0.1 (San Diego, California, USA). Two-sided *P* values of <0.05 were considered statistically significant.

## Results

Among the 225 patients under follow-up in the Madrid study cohort at study time, 22 (9.8%) met the inclusion criteria. Five males and four females, all born in Spain except three patients who came from sub-Saharan Africa, North Africa and Latin America, were excluded because they did not have available PBMCs in the Spanish HIV BioBank. Thirteen patients (59.1%) had available PBMCs. We were able to amplify HIV and obtain the consensus RT sequence from pDNA in 12 (92.3%) of these 13 patients (Table 1). Among them, five were males (P4, P6, P8, P9, P10) and seven were females (P1, P2, P3, P5, P7, P11, P12). Most were born from Spanish parents and carried HIV-1 subtype B, except one (P12) born to sub-Saharan parents with CRF02\_AG recombinant. Only one patient had AIDS stage (P9). All carried M184V mutation in their historic plasma (point 1 in Table 1), but no K65R/N/E mutations. The median time between the start of the lamivudine/emtricitabine regimen and the first detection of historical M184V mutation in plasma was 3.8 years (IQR: 1.8-6.1). At PBMC sampling 9 out of 12 subjects were taking regimens including lamivudine or emtricitabine, and 2 out of 9 carried M184V in pDNA. The subjects' median age was 21.3 years (IQR: 15.6-23.1), with a median of 906 CD4 T lymphocytes (IQR: 617-1212) and all presented suppressed viral load (VL). PBMCs were collected between 2010 and 2018, after a median of 8 years (IQR: 4.6-10.9) from first M184V detection in



**Figure 1.** Flow chart of patients included in the study.

plasma genotype and after a median of 4 years (IQR: 2.1–6.5) of virological suppression. At last evaluation (31 December 2019) all patients had VL <50 copies/mL, with a median time of 7.2 years (IQR: 6.1–8.6) of undetectable VL from PBMCs sampling. None of the 12 patients presented K65R/N/E in their pDNA. Nine (75%) subjects did not present M184V/I in pDNA after at least 1 year of viral suppression.

No statistically significant differences were found between patients with or without M184V in pDNA (Table 1). Youths with and without M184V at pDNA showed similar time (median years) under ART to PBMCs collection [14.3 (IQR: 13.4–15.8) versus 13.7 (IQR: 10.8–14.7),  $P=0.7$ ]. However, youths with M184V tended to have more time with undetectable VL since M184V appearance in the last positive historical genotype in plasma to PBMCs sampling [5.1 (IQR: 3.3–7.7) versus 3.6 (IQR: 2.1–6.2),  $P=0.9$ ] and tended to have greater time under lamivudine or emtricitabine exposure before historical genotype in plasma [6.1 (IQR: 4–6.2) versus 2.4 (IQR: 1.8–5.8),  $P=0.6$ ] but lower time under lamivudine or emtricitabine from the first M184V detection in plasma to PBMCs sampling [1.4 (IQR: 0.9–3.3) versus 4.4 (IQR: 2.2–7.6),  $P=0.3$ ].

## Discussion

This is the first study analysing the existence of lamivudine- or emtricitabine resistance-conferring mutations in pDNA (M184V/I

and/or K65R/E/N) in historic plasma genotypes in a cohort of vertically HIV-infected patients under ART after at least 1 year of virological suppression. We observed long viral suppression [4 years median (IQR: 2.1–6.5)] in the study patients with historic resistance to lamivudine in plasma, as previously reported in adult cohorts.<sup>8,9</sup> We also emphasize the absence of detection of M184V by population sequencing of PBMCs after years of complete virological suppression. Only 3/12 patients (25%) with pDNA sequence under study presented the M184V mutation in their pDNA and none presented K65R/E/N.

Previous studies have suggested that a prolonged time with undetectable VL is associated with clearance of M184V in PBMCs in some adult cohorts.<sup>4,12,13</sup> However, the nine youths in our study without M184V in their pDNA presented lower time under undetectable VL than those with M184V. We also observed that these three youths had more time exposed to lamivudine/emtricitabine before the first detection of M184V in their historic plasma, but less time exposed to these drugs from the first detection in plasma to the PBMCs sampling, compared with the nine youths without M184V in their pDNA. It may be possible that the loss of M184V in PBMCs might be associated with the longer time under lamivudine/emtricitabine exposure, although this should be confirmed in larger study cohorts.

It is of interest to confirm whether lamivudine or emtricitabine could have any role in viral replication control in youths with

**Table 1.** Main clinical features of the 12 vertically HIV-infected patients included in the study

	With M184V in pDNA						Without M184V in pDNA						P value
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	
Timepoint PBMCs													
Time on ART	17.2	14.3	12.4	9.8	18.8	14.7	8.8	13.7	13.8	17.9	13.5	10.8	0.7
Time from point 1	11	5.1	1.5	8.8	7.6	1.8	8.3	13.4	2.9	12.1	6.6	10.8	0.5
Time with undetectable VL since point 1	10.2	5.1	1.5	6.2	7.5	1.8	2.1	3	1.9	3.6	4.4	9	0.9
Total time with 3TC/FTC exposure	6.6	6.9	7.5	1.1	15	6.8	5.9	6.7	12.9	10.2	12	10.8	0.6
Time with 3TC/FTC exposure before point 1	6.2	1.8	6.1	1.1	7.4	5.1	2.4	2.3	10.7	1.8	5.8	0	0.6
Time with 3TC/FTC exposure since point 1	0.4	5.1	1.4	0	7.6	1.7	3.5	4.4	2.2	8.4	6.2	10.8	0.3
ART regimen	RPV/DTG	FTC/TDF/EFV	FTC/TDF/EFV	ZDV/ABC/ LPV/r	FTC/TDF/ DRV/r	FTC/TDF/ DRV/r	3TC/ABC/ ATV/r	FTC/TDF/ DRV/r	3TC/ABC/ EFV	DRV/r	FTC/TDF/ LPV/r	3TC/ABC/ EFV	-
Duration ART regimen	3.5	2.5	1.1	3.7	3.8	1.7	3.6	3.1	2.1	2.8	4.6	1.2	0.4
31 December 2019													
Time from PBMC sampling	1.2	8.5	9.2	7.5	5	8.2	8.8	6.5	6.8	6.7	9.2	1.7	0.7
ART regimen	RPV/DTG	FTC/TAF/ RPV	FTC/TAF/ RPV	3TC/ABC/ DTG	3TC/ABC/ DTG	3TC/ABC/ DTG	3TC/ABC/ DTG	3TC/ABC/ DTG	3TC/DRV/c	DRV/c	DRV/r	3TC/ABC/ DTG	-

All times and ages are expressed in years. Point 1 refers to collection time of historic plasma carrying M184V. Timepoint PBMCs refers to PBMCs sampling time. 31 December 2019, last evaluation; total time 3TC/FTC exposure refers to cumulative exposure to 3TC/FTC. P, patient; ART, antiretroviral therapy. VL, viral load (RNA-HIV-1 copies/mL); pDNA, proviral DNA. 3TC, lamivudine; FTC, emtricitabine; ABC, abacavir; RPV, rilpivirine; TDF, tenofovir disoproxil fumarate; TAF, tenofovir alafenamide; EFV, efavirenz; ZDV, zidovudine; LPV/r, lopinavir/ritonavir; DRV/r, darunavir/ritonavir; ATV/r, atazanavir/ritonavir; DRV/c, darunavir/cobicistat; DTG, dolutegravir.

perinatal HIV with previously documented resistance to these drugs in their historical genotype in plasma, since 9 (75%) out of 12 patients were still using lamivudine or emtricitabine in their ART regimen on 31 December 2019, maintaining undetectable VL. A previous study showed an increase in VL after discontinuing lamivudine despite the presence of the M184V mutation in plasma, suggesting a residual antiviral effect of this drug.<sup>14</sup> Other studies conducted in HIV-infected adults have shown that lamivudine remained effective for virological suppression despite the existence of documented genotypic mutations.<sup>15,16</sup>

Studies in adults have gone a step further and have used dual therapies based on lamivudine and an integrase inhibitor as suppressive simplification regimens in patients with archived mutations in the historical genotype. In a pilot clinical trial (ART-PRO) performed in an HIV-infected adult cohort, dolutegravir/lamivudine demonstrated efficacy for maintenance of HIV viral suppression at 48 weeks in 21 patients with archived lamivudine-resistant viruses present in pDNA at low frequency, since they were detected by next generation sequencing, but not by population sequencing. However, some patients included in ART-PRO had a low minority of M184V detectable by ultra-deep sequencing, although the long-term clinical impact of this low burden of archived resistance is unknown and deserves further study.<sup>8,9</sup>

ART switching is a therapeutic strategy used to improve quality of life and avoid toxicity in patients under ART. Some publications have showed that lamivudine/emtricitabine-based regimens might work in the presence of M184V in historical Sanger baseline sequences. In the MOBIDIP cohort, dual maintenance therapy with boosted protease inhibitor plus lamivudine was associated with a high rate of success, despite the presence of M184V at first-line treatment failure. However, boosted protease inhibitor monotherapy should not be recommended for these patients.<sup>17</sup> Other studies showed that switch regimens, as bictegravir/emtricitabine/tenofovir alafenamide and abacavir/lamivudine/dolutegravir triple therapy and ART-PRO for dual therapy, were successful in the absence of historical M184V in baseline PBMCs.<sup>18,19</sup> The choice of ART in the clinic has always been based on the study of genotype mutations by Sanger sequencing. Some studies have showed that choosing treatment based on ultradeep mutations has a limited impact on virological outcomes.<sup>20</sup> We do not know whether in perinatally infected youths with accumulated resistances and in whom adherence is sometimes compromised, treatment based on PBMCs resistance may be helpful or not, compromising the success of virological control and future treatments in this vulnerable population. In clinical practice, to improve adherence and to avoid toxicity, there are studies showing that switching to a dual therapy strategy is effective for maintaining viral suppression in this population.<sup>21</sup> In our study cohort, it can be seen that previously archived mutations such as M184V are lost over time and after virological suppression. pDNA measurement may then be a useful decision-making tool for switching to simpler and less-toxic treatment regimens.

Our study has some limitations. The first one was the sample size, since only 12 subjects met all the inclusion criteria for the study due to PBMCs availability. Secondly, we used historical plasma genotypes and PBMCs samples in the study. Frozen samples might just by chance have PBMCs that do not contain archived mutations, in contrast with plasma genotyping, performed in the setting of viral replication. Thirdly, only one patient switched to a

lamivudine/emtricitabine dual regimen, and none switched to dolutegravir/lamivudine/emtricitabine. However, our study, together with the one published in adult cohorts on this same topic, may open the possibility of evaluating the use of regimens that include lamivudine or emtricitabine, especially dolutegravir-based regimens, in the treatment of vertically HIV-1-infected patients with a long period of infection and previous experience of and genotypic resistance to lamivudine/emtricitabine.

To sum up, in the study cohort, most (9/12) vertically HIV-infected youths with previous M184-bearing resistant viruses in plasma, did not present this mutation in pDNA after a long period of virological suppression. These results suggest that measurement of pDNA may be useful to detect the presence of M184V/I mutation in vertically HIV-infected youths with this mutation previously detected in plasma. Our results also suggest that pDNA measurement may also be useful to select possible simplification therapies including lamivudine or emtricitabine in vertically HIV-infected patients with previous experience of and resistance to these drugs.

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## Transparency declarations

None to declare.

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