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The anti-HIV activity of Entecavir: a multicentre evaluation of lamivudine-experienced and -naïve patients

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Abstract

Background—Entecavir, an antiviral with potent anti-hepatitis B virus (HBV) activity, was recently shown to have anti-HIV activity in three patients and the ability to select for the lamivudine-resistant HIV polymerase mutation M184V in a patient with prior antiretroviral therapy (ART).

Objectives—To further characterize entecavir's anti-HIV activity and identify risk factors for selection of the M184V.

Design—Retrospective cohort study.

Methods—We evaluated HIV and HBV virological characteristics in 17 HIV-HBV co-infected patients (10 ART-naïve and 7-ART experienced) prior to and while receiving entecavir monotherapy. Descriptive statistics were used to assess changes in HIV RNA and HBV DNA. Variables associated with development of the M184V were also determined.

Results—Of the 17 patients, 13 (76%) demonstrated a reduction in HIV RNA of $\geq 0.5 \log_{10}$ copies/ml. Of the remaining four patients, two had the M184V detected prior to entecavir and the other two had wild type HIV. The median reduction in HIV RNA for the cohort was $1.2 \log_{10}$ copies/ml, which

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was similar in ART-naïve and -experienced patients. The M184V mutation emerged in six patients receiving entecavir, including three ART-naïve patients. No other HIV mutations were consistently detected. Risk factors for emergence of the M184V mutation were a decline in HBV DNA ($P=0.04$) and duration of entecavir use ($P=0.05$).

Conclusions—Entecavir monotherapy in HIV-HBV co-infected patients has significant HIV activity and can result in the development of the M184V variant, even in ART-naïve patients. Entecavir should not be used in this setting in the absence of concomitant ART.

Keywords

HIV; HBV; co-infection; entecavir; polymerase mutation; anti-HIV activity

Introduction

Approximately 400 million people globally are chronically infected with the hepatitis B virus (HBV) whilst nearly 40 million people are infected with human immunodeficiency virus (HIV) [1,2]. HIV-HBV co-infection is frequently observed due to shared routes of transmission, with reported figures indicating that approximately 10% of HIV-infected patients are chronically infected with HBV [3-7].

Current HIV guidelines recommend delaying antiretroviral therapy (ART) until CD4 cell counts fall to 200-350 cells/mm³ due to issues of long-term side effects and the risk of developing drug-resistant HIV [8]. Some HIV-HBV co-infected patients therefore require treatment for chronic HBV but not for HIV. This group is difficult to treat because many of the anti-HBV agents have activity against HIV and therefore cannot be used without a full anti-HIV regimen.

Entecavir is a guanosine analogue approved for the treatment of chronic hepatitis B [9-11]. On the basis of *in vitro* studies indicating its lack of anti-HIV activity [12], entecavir was previously recommended in multiple guidelines as an agent for treatment of HBV when HIV therapy was not indicated [1,8,13,14] (available at <http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf>). We recently demonstrated that entecavir has *in vitro* and *in vivo* anti-HIV activity [15]. In that report, 1 of the 2 patients with samples available for testing selected for the lamivudine-resistant HIV variant M184V while on entecavir monotherapy. This patient had received lamivudine in the past, so it is not known if this mutation was selected from a previously archived virus or was selected *de novo*. In order to better characterize the anti-HIV activity of entecavir and to determine the risk factors associated with development of the M184V, we established a multi-centre, international cohort study of 17 HIV-HBV co-infected patients who received entecavir in the absence of other antiretroviral agents.

Subjects and Methods

Study participants

The study was a retrospective case review of seventeen HIV-HBV co-infected patients who received entecavir as the only active HIV or HBV agent at multiple sites in Australia and the United States. Inclusion criteria were: 1) HIV antibody positive at least one year prior to entecavir, 2) positive for HBsAg on at least two occasions six months apart, and 3) entecavir treatment without concomitant anti-HIV or anti-HBV therapy for at least 14 days. Approval for this study was obtained from the Bayside Health Human Research and Ethics Committee, Melbourne, Australia. Oral consent was obtained from the study participants.

Data abstraction

HIV RNA, HBV DNA, and HIV resistance test results were abstracted from medical records prior to receiving entecavir (data dated between July 2004 and July 2007). The timepoints selected for analysis were: 1) last available sample prior to introduction of entecavir, 2) last available sample while receiving entecavir and 3) interim samples while receiving entecavir. In addition, all available results of HIV resistance tests prior to entecavir were collected. The time on entecavir to the first sample available for quantification of HIV RNA ranged from 17 to 166 days (median=54 days) and to the last sample available for quantification of HIV RNA ranged from 38 to 470 days (median = 144 days).

HIV RNA quantification, HIV resistance testing, and HBV DNA quantification

HIV RNA levels, HBV DNA levels, and HIV resistance were all determined at the participating sites' clinical laboratories using standard assays except where noted in the text. In particular, HIV RNA was quantified by the following assays: Roche Cobas HIV-1 Monitor Version 1.5 (lower limit of detection (LLOD) 400 copies/ml; Roche, Branchburg, NJ), Roche Cobas HIV-1 Monitor Version 1.5-Ultrasetitive (LLOD=50 copies/ml), Roche Cobas AmpliPrep/ TaqMan (LLOD=40 copies/ml) and Bayer Versant HIV-1 RNA 3.0 (LLOD=50 copies/ml; Bayer HealthCare, Tarrytown, NY). When the HIV RNA result was >100,000 copies, the samples were diluted and re-assayed. HBV DNA was quantified by Roche Cobas TaqMan HBV (LLOD=170 copies/ml; 30 IU/ml), Roche Cobas Amplicor HBV Monitor (LLOD=200 copies/ml; 35 IU/ml), or Bayer Versant HBV DNA 3.0 (LLOD=2000copies/ml; 357IU/mL). All HBV DNA results were converted from copies/ml to IU/ml, using a conversion factor of 5.6 or 5.8, depending on the particular assay used. Samples with an initial HBV DNA result over the upper limit of detection were diluted 1:1000 and retested.

Sequencing of HIV RT was performed with either Viroseq HIV-1 Genotyping system version 2.0 (Celera Diagnostics, Alameda, CA), Phenosense/GT (Monogram Biosciences, San Francisco, CA), vircoTYPE HIV-1 (Virco, Bridgewater, NJ) or Trugene (Bayer HealthCare). Clonal analysis was used to assess for HIV resistance mutations in three treatment-experienced patients (13,15 and 17), and was performed as previously described [15].

Statistical methods

Statistical analyses were performed using SPSS for Windows Release 14.0.02. The pre-entecavir values were the last available sample tested prior to initiation of entecavir. For HBV DNA this was a median of 36 days prior to entecavir (range 0-118 days). For HIV RNA the median was 34 days with a range of 0-131 days. Declines in HIV RNA and HBV DNA were calculated by subtracting the value prior to ETV and the lowest value on ETV (referred to as the nadir). Therefore, in some patients, there was a difference between time to nadir HBV DNA or HIV RNA and time of total ETV exposure. For the patients with a <0.3 log reduction in HIV RNA, the nadir was defined as the last available HIV RNA value on ETV. When the HIV RNA or HBV DNA was below the LLOD, a value midway between zero and the LLOD for that assay was used. Continuous variables were compared using the Mann-Whitney test. Categorical variables were compared using Pearson Chi-Square or Fischer's Exact test. The relation between decline in HBV DNA and total duration on entecavir was investigated using Spearman's Rank Order of Correlation. All tests were two-tailed and results were considered significant at $P < 0.05$.

Results

Demographic & clinical features

A total of 17 patients fulfilled the study criteria including 10 who were naïve to ART (ART-naïve) and 7 who had previously received ART that included lamivudine (ART-experienced). One ART-naïve and two ART-experienced patients were previously described (Patients 10, 15 and 17) [15]. The median age of the cohort was 36 years with the majority being men of either Caucasian or African American ethnicity (Table 1). The most common risk factor for HIV and HBV acquisition was men having sex with men. The median duration of known HIV infection was 8 years, which was shorter in the ART-naïve compared to the ART-experienced (3.5 years and 15 years, respectively). The median nadir CD4 cell count was 276 cells/ul (range 30-447). The ART-naïve patients had a higher median CD4 cell count of 356 cells/ul (range 50-450) compared to the ART-experienced patients, who had a median CD4 cell count of 263 cells/ul (range 9-288). The frequency of prior AIDS-defining illnesses was similar between ART-naïve and -experienced patients. The vast majority of patients were HBeAg positive with 25% co-infected with Hepatitis D virus (HDV) and 12% co-infected with hepatitis C virus (HCV). The only baseline characteristic that was significantly different between the groups was the increased duration of time on entecavir (defined as total time to the last sample within the reporting period) with a median of 24.6 weeks in the ART-naïve group and 47.2 weeks in the ART-experienced group ($p=0.03$).

Virological response to entecavir

HBV—The median pre-entecavir HBV DNA for the whole cohort was 6.4×10^7 IU/ml (range 4.3×10^5 - 4.3×10^9 IU/ml), and the median on-entecavir HBV DNA nadir was 4.5×10^3 IU/ml (range 9.0×10^0 - 5.4×10^6 IU/ml) (Table 2). This represented a median reduction of HBV DNA of 3.6 log₁₀ IU/ml after a median of 152 days of ETV (range 31-391 days). The mean HBV DNA reduction for the cohort was 4.1 log₁₀ IU/ml. The changes in HBV DNA are summarized in Table 2 and Figure 1.

The changes in HBV DNA were not significantly different between the ART-naïve and ART-experienced patients. In the ART-naïve patients, the median and mean declines in HBV DNA were 3.4 log₁₀ IU/ml (range 1.6-7.3) and 4.2 log₁₀ IU/ml, respectively after a median of 159 days of ETV (range 31-391) (Figure 2a). In the ART-experienced patients, the median and mean declines in HBV DNA were 4.5 log₁₀ IU/ml (range 0.5-6.1) and 4.0 log₁₀ IU/ml, respectively after a median of 144 days of ETV (range 51-303 days).

HIV—Thirteen of the 17 (76%) patients had a reduction in HIV RNA of at least 0.5 log₁₀ copies/ml, including 8/10 (80%) of ART-naïve patients and 5/7 (71%) ART-experienced patients. Of the four patients with < 0.5 log copies/ml decline in HIV RNA, the two ART-experienced patients had the M184V mutation prior to ETV (Patients 11 and 12), which is known to confer resistance to ETV. The other two patients had wild-type virus (Patients 4 and 6).

The median pre-ETV HIV RNA in the whole cohort was 2.6×10^4 copies/ml (range 7.8×10^3 - 2.9×10^6) while the median on-entecavir HIV RNA nadir was lower at 2.3×10^3 copies/ml (range <50 - 3.0×10^5). This represented a median and mean overall decline in HIV RNA on ETV of 1.2 and 1.0 log₁₀ copies/ml, respectively. The median time to nadir HIV RNA was 96 days of ETV (range 28-291). For the patients with a <0.3 log reduction in HIV RNA, time to nadir was defined as the time to the last available HIV RNA value on ETV. Changes in HIV RNA are summarized in Table 3 and Figure 1.

The declines in HIV RNA were similar regardless of prior ART. In the 10 ART-naïve patients, the median and mean reduction in HIV RNA was 1.3 log₁₀ (range +0.5 - -2.3) and 1.1 log₁₀ copies/ml, respectively after a median of 113 days of entecavir (range 28-291). The seven ART-experienced patients had a median reduction in HIV RNA of 1.1 log₁₀ copies/ml (range +0.5-2.3 log₁₀) after a median of 96 days of ETV (range 51-215), and the mean reduction was 0.9 log₁₀ copies/ml.

Four of these 13 patients (31%) with at least a 0.5 log decline in HIV RNA also had a rebound of HIV RNA of $\geq 0.5 \log_{10}$ copies/ml after achieving their nadir. These were all patients in the ART-experienced group of whom three had the M184V mutation detected at the time of HIV RNA rebound (Patients 14, 15, and 16).

Of the seven ART-experienced patients, two (29%) had the M184V mutation detected on HIV genotypic analysis prior to entecavir (Patients 11 and 12). Of the remaining five patients, three (60%) developed the M184V on entecavir therapy (Patients 13, 14, and 15) after a median of 98 days and subsequently had viral rebound. Thus, only two ART-experienced patients (Patients 16 and 17) did not select the M184V. Patient 16 had received lamivudine therapy twice: briefly in 2001 as part of an ART regimen with didanosine and nevirapine, which was discontinued due to a hepatitis flare; then again in 2006, in combination with tenofovir and lopinavir/ritonavir, which was also discontinued due to hepatic flare after approximately nine weeks of therapy. Patient 16 did not have plasma available pre-entecavir for HIV RT sequencing, and after receiving 11 months of entecavir monotherapy the HIV genotype did not show selection of the M184V mutation. Patient 17 had received lamivudine therapy from 1996 to 2000, in combination with zidovudine. His antiretrovirals were discontinued in 2000 due to non-compliance. HIV RT sequencing on this patient's pre-entecavir serum sample only showed the K103N. The M184V mutation was not selected after seven months of entecavir therapy.

Seven of the ten ART-naïve patients had a pre-ETV HIV RT genotype, and none of them had the M184V mutation. However, on ETV monotherapy, 2/7 (29%) of them (Patients 5 and 9) selected for the M184V after a median of 132 days on therapy. Of the three other ART-naïve patients without a pre-ETV HIV RT genotype, one (Patient 8) had the M184V mutation detected after 260 days of entecavir.

In order to determine the factors associated with the development of the M184V mutation, we used univariate analysis to compare those who did (n=6) and did not (n=7) select the variant on ETV (Table 3). A total of 13 patients had both pre- and on-entecavir HIV RT resistance testing results available. A longer duration of ETV was associated with an increased risk for developing the M184V (46.0 versus 25.2 weeks, respectively, $P=0.05$). In addition, HBV DNA reduction (to nadir) was significantly associated with development of the M184V ($P=0.04$). Those who selected the M184V had a median reduction in HBV DNA of 6.1 log₁₀ IU/ml compared to 3.0 log₁₀ IU/ml in those who did not select for the M184V. To determine whether these two factors were correlated, we used Spearman's Rank Order of Correlation. This analysis demonstrated a positive correlation between log₁₀ decrease in HBV DNA and time on-ETV (weeks) in the entire cohort ($r=0.537$, $p=0.03$).

Discussion

This study confirms the anti-HIV antiviral activity of ETV when used as monotherapy in an HIV-infected population [15]. Patients receiving ETV monotherapy had a median 1.0 log reduction in HIV RNA. These findings are in agreement with recent studies demonstrating that entecavir triphosphate can be incorporated by purified HIV RT in cell free *in vitro* reactions. Consistent with this anti-HIV activity, the M184V variants, which have reduced susceptibility

to ETV, emerged during ETV monotherapy in patients who were ART-experienced and ART-naïve [16].

The anti-HIV efficacy of ETV was comparable to other nucleoside analogues such as zidovudine and lamivudine when used as monotherapy [17,18]. In the present study, the anti-HIV efficacy of ETV was similar in magnitude in both the ART-naïve and ART-experienced groups suggesting that prior HIV therapy does not impair the anti-HIV activity of ETV in this population. Interestingly, one patient had a decline in HIV RNA despite a history of the M184V mutation prior to entecavir initiation. This may have been because the predominant genotype at the time entecavir was started was a wild type form that was susceptible to entecavir. Alternatively, the decline in viremia may reflect selection for viruses containing the M184V mutation which has a deleterious effect on viral fitness [19,20]. A recent study also showed that the M184V virus could still incorporate substrate but at a slower rate, with lower binding affinity and lower incorporation efficiency of ETV triphosphate compared to wild type virus [16]; thus, potentially also explaining some activity of entecavir against the M184V strain.

Entecavir use led to the selection of the M184V mutation in almost half the patients who did not have this mutation pre-ETV (5 of 12) and in one additional lamivudine-naïve patient who did not have a genotype prior to entecavir. In contrast to the original report, which demonstrated selection of the M184V in a lamivudine-experienced patient, we demonstrated the ability of ETV to select for resistance in a third of the ART-naïve patients, which is consistent with a recent case report of M184V selection by ETV in an ART-naïve patient [21]. While it is possible that the ART-naïve patients may have been initially infected with the M184V variant and consequently harboured archived mutations, it is highly unlikely that this would occur at a high frequency [22]. Furthermore one lamivudine-naïve patient (patient #5) demonstrated the M184V mutation despite acquiring HIV well before the availability of lamivudine. In comparison, a larger proportion (60%) of the ART-experienced patients who did not have the M184V pre-ETV developed this mutation on entecavir. This could be due to ease of selecting a pre-existing archived mutant compared to de novo selection. An alternative explanation is that those who were ART-experienced had a longer median duration of ETV and therefore had more time to select for the mutation. It was also notable that in the patients who experienced HIV virological rebound of 0.5 log₁₀ or greater while still receiving ETV, the majority had the M184V mutation at baseline. All of these patients had profound simultaneous reductions in HBV DNA suggesting adherence to therapy. The fact that not all patients selected for the M184V may be due to the limited replication advantage of the M184V variants, which is a consequence of the unusual dose response curve of ETV [23].

We found that selection of HIV resistance by ETV was associated with a greater reduction of HBV DNA and longer duration on entecavir. These variables were shown to be co-dependent and are consistent with the longer duration of ETV use resulting in a more profound HBV DNA drop. It is also possible that the reduction in HBV DNA may have had an indirect effect on HIV RNA via non-specific mechanisms such as enhanced mitogen and antigen-specific T-cell immunity as previously described following lamivudine treatment of HBV mono-infection [24].

In several patients without the M184V mutation, ETV administration did not result in a significant HIV RNA decline despite HBV DNA reductions. It is notable that these patients had the lowest HBV DNA reduction and a similar duration on entecavir to those who did achieve a reduction in HIV RNA. It is possible that other HIV mutations prevented ETV from having an anti-HIV effect. An alternative explanation is potential inter-individual differences in entecavir pharmacokinetics or intracellular phosphorylation.

This study has several limitations. First, it was a small, retrospective study; however, we believe that this is likely to be the largest cohort used to examine the anti-HIV effect of ETV. Second, given it was a multicentre study using different assays to measure both HIV RNA and HBV DNA, the log drop of both HIV RNA and HBV DNA may be an underestimate of the true log drop given that the most sensitive assay was not used in all patients. In addition, the duration of ETV treatment and timing of samples was variable with some patients only having HIV RNA and HBV DNA determined at one timepoint on ETV. We may therefore have overestimated the time to HIV nadir and underestimated the true HIV nadir and incidence of HIV virological rebound with selection of resistance on ETV. Finally, we did not consistently detect any other known drug-resistant HIV mutations besides the M184V. In one patient (Patient 14) the K70R was detected with the M184V post entecavir. This is a thymidine analogue mutation and it would be highly unusual for a guanosine analogue to select this out. It is thus unlikely that entecavir resulted in its emergence. However, since the follow-up was limited we cannot determine that no other mutations would develop with longer duration of therapy.

Despite the limitations of the study, these findings clearly demonstrate that ETV has significant anti-HIV activity and can select for the M184V mutation *de novo*. Therefore, its use as a single agent for HBV treatment in HIV-HBV co-infected patients should be avoided. It should however still be acceptable to use entecavir in a situation where HIV is well controlled by other ART and regular HIV monitoring is performed. Further, these data also support the routine assessment for undiagnosed HIV infection in all HBV-infected patients, especially before ETV is prescribed given that it is now commonly recommended as first line therapy for chronic HBV in this setting [25-27].

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Figure 1. Changes in HIV RNA and HBV DNA in ART-naïve (Patients 1-10) and ART-experienced (patients 11-17) HIV-HBV co-infected patients

Changes in HIV RNA (black) and HBV DNA (blue) are shown prior to and following entecavir. The time of collection of plasma for sequencing of HIV RT is shown as a black arrow.

Table 1**demographics**

Characteristic	Total	ART-naïve	ART-experienced
Number	17	10	7
Age, median (IQR), years	36 (32.5-41.5)	37.5 (32.3-44.5)	35 (32-41)
Gender; M, F	16, 1	9, 1	7, 0
Ethnicity, %			
Asian	11.8 (2/17)	20.0 (2/10)	0
Caucasian	41.2 (7/17)	50 (5/10)	28.6 (2/7)
Hispanic	5.9 (1/17)	10 (1/10)	0
African-American	29.4 (5/17)	20.0 (2/10)	42.9 (3/7)
Indigenous Australian	5.9 (1/17)	0	14.3 (1/7)
Southern European	5.9 (1/17)	0	14.3 (1/7)
Mode of HIV acquisition, %			
Sexual	58.8 (10/17)	70.0 (7/10)	42.9 (3/7)
IDU	17.6 (3/17)	20.0 (2/10)	14.3 (1/7)
Blood transfusion	11.8 (2/17)	0	28.6 (3/7)
Unknown	11.8 (2/17)	10.0 (1/10)	14.3 (1/7)
Nadir CD4 count, median (IQR) cells/uL	276 (30-447) (n=16)	356 (50-450) (n=9)	263 (9-288) (n=7)
Duration of known HIV infection, median (IQR), years	8.0 (2.3-16.5)	3.5 (1.0-10.0)	15 (8.0-20.0)
Prior AIDS-defining illness, %	29.4 (5/17)	30.0 (3/10)	28.6 (2/7)
HBV eAg +ve, %	94.1 (16/17)	90.0 (9/10)	100 (7/7)
HBV eAb +ve, %	29.4 (5/17)	30 (3/10)	28.6 (2/7)
HDV Ab +ve, %	25.0 (3/12)	16.7 (1/6)	33.3 (2/6)
HCV Ab +ve, %	11.8 (2/17)	10.0 (1/10)	14.3 (1/7)
Duration of ETV monotherapy, median (IQR) weeks	37.2 (21.2-47.6)	24.6 (12.6-38.9)	47.2 (40.0-52.0)

Table 2

Changes in HBV DNA prior to and on entecavir monotherapy

	Pre-ETV HBV DNA, IU/mL	On-ETV nadir HBV DNA, IU/mL	Duration on-ETV to nadir result, days	Log drop HBV DNA, IU/mL
ART-naïve				
1	1.1×10^7	* $<3.6 \times 10^2$	91	4.8
2	4.8×10^7	1.9×10^4	207	3.4
3	3.9×10^8	6.4×10^5	44	2.8
4	1.3×10^7	1.4×10^4	152	3.0
5	1.8×10^7	1.0×10^4	201	3.3
6	1.8×10^7	4.7×10^5	31	1.6
7	1.9×10^8	* 1.8×10^1	391	7.3
8	3.6×10^8	3.7×10^2	166	6.0
9	3.8×10^7	* $<6.0 \times 10^1$	165	6.1
10	1×10^9	9.6×10^5	35	3.0
Median	4.3×10^7	1.2×10^4	159	3.4
IQR	$1.7 \times 10^7 - 3.6 \times 10^8$	$1.2 \times 10^2 - 5.1 \times 10^5$	42 - 203	2.9 - 6.3
ART-experienced				
11	1.8×10^7	5.4×10^6	114	0.5
12	6.4×10^7	3.0×10^5	51	2.3
13	4.0×10^8	3.5×10^2	303	6.1
14	4.3×10^5	* 2.9×10^1	84	4.2
15	4.3×10^9	4.5×10^3	215	6.0
16	1.1×10^8	7.3×10^2	228	5.2
17	4.3×10^6	9.8×10^1	144	4.6
Median	6.4×10^7	7.3×10^2	144	4.5
IQR	$4.3 \times 10^6 - 4.0 \times 10^8$	$9.8 \times 10^1 - 3.0 \times 10^5$	84 - 228	2.3 - 6.0
Total				
Median	6.4×10^7	4.5×10^3	152	3.6
IQR	$1.6 \times 10^7 - 3.8 \times 10^8$	$1.4 \times 10^2 - 3.9 \times 10^5$	68 - 211	2.9 - 6.1

* below the limit of detection. ETV-entecavir; IQR = interquartile range

Table 3
Changes in HIV RNA and mutations in HIV reverse transcriptase (RT) prior to and on entecavir monotherapy

HIV RNA			HIV RT mutations					LMV/FTC ever
Pre-ETV, HIV RNA, Copies/mL	On-ETV HIV RNA (nadir), Copies/mL	Duration on ETV to nadir, days	Log drop HIV RNA, copies/ml	End-ETV ¹ , HIV RNA, copies/mL	Log increase HIV RNA, (from nadir)	Total duration on ETV, days ²	Pre-ETV	On-ETV
ART- naïve								
1	1.2 × 10 ⁴	91	1.3	-	-	-	L210FS, T215C, G333E	NA
2	7.3 × 10 ⁴	207	2.0	-	-	-	wt	wt
3	3.0 × 10 ⁶	44	1.5	-	-	-	wt	wt
4	1.4 × 10 ⁴	134	+0.5	-	-	-	wt	wt
5	5.5 × 10 ⁴	202	0.7	-	-	-	G333E	M184V, G333E
6	8.4 × 10 ³	38	+0.1	1.0 × 10 ⁴	0.2	38	wt	wt
7	7.8 × 10 ³	291	2.3	*7.5 × 10 ¹	0.1	418	NA	NA
8	6.1 × 10 ⁴	166	0.8	1.7 × 10 ⁴	0.2	260	NA	M184M/V, R211K, 214F
9	2.6 × 10 ⁴	54	1.4	3.0 × 10 ³	0.4	116	R211K	M184V, R211K, 214F
10	4.0 × 10 ⁴	28	1.2	4.0 × 10 ³	0.2	44	NA	wt
Median	3.3 × 10 ⁴	113	1.3					
IQR	1.1 × 10 ⁴ – 6.5 × 10 ⁴	40 - 203	0.6 – 1.6					
ART- experienced								
11	1.0 × 10 ⁴	114	+0.1	-	-	-	M184M/V	M184M/V
12	3.3 × 10 ⁵	51	0.03	-	-	-	M184V	M184M/V
13	2.7 × 10 ⁴	79	0.5	1.9 × 10 ⁴	0.3	316	wt (clonal analysis)	M184V
14	2.1 × 10 ⁴	166	1.2	8.3 × 10 ³	0.8	470	wt	K70R, M184V
15	3.4 × 10 ⁴	215	1.4	5.4 × 10 ³	0.6	307	wt (clonal analysis)	M184V (clonal analysis)
16	2.1 × 10 ⁴	91	1.1	1.0 × 10 ⁴	0.8	228	NA	wt
17	3.1 × 10 ⁴	96	2.3	6.9 × 10 ³	1.7	144	K103N	K103N (clonal analysis)
Median	2.7 × 10 ⁴	96	1.1					

HIV RNA				HIV RT mutations				LMV/FTC ever
Pre-ETV, HIV RNA, Copies/mL	On-ETV HIV RNA (nadir), Copies/mL	Duration on ETV to nadir, days	Log drop HIV RNA, copies/ml	End-ETV ^I , HIV RNA, copies/mL	Log increase HIV RNA, (from nadir)	Total duration on ETV, days ^I	Pre-ETV	On-ETV
IQR	2.1 × 10 ⁴ –3.4 × 10 ⁴	79 - 166	0.03 – 1.4					
Total cohort								
Median	2.7 × 10 ⁴	96	1.2					
IQR	1.3 × 10 ⁴ – 5.8 × 10 ⁴	65 - 184	0.3 – 1.5					

^I if different from nadir result.

* below the limit of detection; NA: not available, wt: wild type, ETV=entecavir; LMV=lamivudine; FTC=entricitabine; IQR=interquartile range; RT = reverse transcriptase