HIV-1 DNA ultra-deep sequencing analysis at initiation of the dual therapy dolutegravir + lamivudine in the maintenance DOLULAM pilot study

Charlotte Charpentier1*, Brigitte Montes2, Marine Perrier1, Nadia Meftah3 and Jacques Reynes4,5

1IAME, UMR 1137, INSERM, Université Paris Diderot, Sorbonne Paris Cité, AP-HP, Laboratoire de Virologie, Hôpital Bichat, AP-HP, Paris, France; 2Laboratoire de Virologie, CHU de Montpellier, Montpellier, France; 3COREVIH, CHU de Montpellier, Montpellier, France; 4Département de Maladies infectieuses et tropicales, CHU de Montpellier, Montpellier, France; 5Unité Mixte Internationale 233 IRD-U1175 INSERM-Université de Montpellier, Montpellier, France

*Corresponding author. Hôpital Bichat-Claude Bernard, Laboratoire de Virologie, 46 Rue Henri Huchard, 75018 Paris, France. Tel: +33-1-40-25-61-50; Fax: +33-1-40-25-67-69; E-mail: charlotte.charpentier@aphp.fr

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Background: The DOLULAM study assessed the efficacy of dolutegravir + lamivudine dual therapy to maintain virological suppression in heavily treatment-experienced HIV-1-infected adults. No virological failure occurred during the first year of the dual therapy.

Objectives: A virological substudy was conducted to assess the prevalence of M184I/V mutations at dual therapy initiation using historical DNA/RNA genotypes and baseline DNA genotype obtained by next-generation sequencing (NGS).

Methods: HIV-1 RT sequences were obtained from DNA and/or historical RNA using Sanger technology. HIV-1 DNA RT and integrase NGS was performed using Illumina technology.

Results: Among the 27 patients enrolled in the DOLULAM study, historical HIV DNA and RNA Sanger sequences were available in 14 and 18 patients, respectively. At the initiation of DOLULAM, DNA NGS genotypes showed that 45% and 21% of the patients harboured minority resistant variants (MRV) in RT and integrase, respectively. Combining all available genotype data, an M184I/V was observed in 17 of 27 (63%) of the patients. Most M184V were detected in historical RNA genotypes (n=8 of 11), whereas M184I were exclusively detected in DNA genotypes (n=10, including 7 as MRV). Ten patients displayed defective viral genomes in cellular reservoirs, all including M184I and stop codons. At the time of DOLULAM initiation, M184V was observed in DNA NGS in five patients, including one as MRV.

Conclusions: These first NGS data on HIV DNA at initiation of a switch study showed (i) a high proportion of patients harbouring defective viral genomes, whose mutation M184I is a marker, and (ii) a low number of patients in whom M184V remained as a major viral variant in PBMCs.

Introduction

In clinical routine, a high proportion of HIV-infected patients with previous virological failure (VF) occurring in the pre-HAART era are now well-controlled for several years, since the introduction of new antiretroviral drugs, and they need to switch from HAART to prevent toxicity and/or for simplification. The M184I/V mutation is the most prevalent resistance-associated mutation (RAM), present in 25% of samples in ART-treated patients experiencing VF in the most recent epidemiological surveillance survey in ART-treated patients in France.1 Few data are available regarding the impact of historical M184I/V resistance mutations, in highly pre-treated patients virologically suppressed for several years, on efficacy in maintaining virological suppression in a dual therapy switch study based on lamivudine.

Regarding the PI/ritonavir (PI/r) + lamivudine trials, they had all excluded patients with previous VF or previous genotypic resistance to lamivudine, except the Agence Nationale de recherche sur le SIDA et les hépatites virales (ANRS) 12286/MOBIDIP trial,2 a second-line switch ART trial exclusively conducted in resource-limited settings.

The DOLULAM pilot study assessed the efficacy of a dual therapy including an integrase inhibitor (dolutegravir) and lamivudine to maintain virological suppression in highly treatment-experienced...
patients with problems of tolerability of previous regimen. Lack of resistance to integrase inhibitors was a requirement, but some patients had previous VF with the selection of M184I/V mutations during their therapeutic history. Princesp study results showed that no VF, i.e., two consecutive viral loads (VLs) >50 copies/mL, occurred during the first year of the dual therapy dolutegravir + lamivudine.\

The aim of this DOLULAM substudy was to assess the prevalence of the M184I/V mutations using historical DNA/RNA resistance genotypes and DNA genotype obtained by next-generation sequencing (NGS) at the initiation of the dual therapy dolutegravir + lamivudine.3

Methods

Study population and ethics

The DOLULAM study is a prospective, monocentric, cohort study, as previously described. Twenty-seven highly ART-experienced patients were switched to a dual combination of dolutegravir (50 mg once daily) + lamivudine (300 mg once daily). In all patients, the decision to switch therapy, under the direct responsibility and information of an HIV specialist, was taken for clinically relevant reasons such as drug adverse events (e.g., intestinal discomfort), altered laboratory tests (e.g. dyslipidaemia, proteinuria), prevention of drug-to-drug interaction, or simplification of regimen.

For inclusion in this cohort, patients had to meet the following eligibility criteria: (i) stable ART regimen for at least 12 months; (ii) plasma VL <50 copies/mL for >12 months; and (iii) no genotypic resistance to integrase inhibitors. Nine patients (33%) have been previously exposed to raltegravir. At initiation of the DOLULAM study, median duration of virological suppression (i.e. VL <50 copies/mL) was 6.4 years (IQR = 4.6–10.1). Most of the patients (n = 24 of 27, 89%) were infected with subtype B. A VF was defined as a confirmed VL >50 copies/mL with a retest within the 4 following weeks. All participants signed an informed consent for the anonymous use of their clinical and biological data.

Historical HIV-1 resistance genotypes

All historical resistance genotypes performed during patient’s therapeutic history were collected. These genotypes were obtained from PBMCs and/or from plasma. All historical HIV-1 RT and integrase sequences were obtained using Sanger sequencing technology using ANRS procedures (www.hivfrenchresistance.org). All historical HIV-1 RT and integrase sequences were re-interpreted using the latest ANRS resistance interpretation algorithm (version 26, September 2016).

HIV-1 DNA resistance genotype at initiation of the DOLULAM study

HIV-1 DNA sequencing of RT and integrase regions at the initiation of the DOLULAM study was performed using both Sanger and NGS sequencing technologies. RT and integrase NGS was performed using Illumina® technology, according to manufacturer recommendations. RT and integrase reads were analysed using the SmartGene® NGS HIV-1 module prototype (SmartGene, Zug, Switzerland). This software was specifically developed for the detection of HIV minority resistant variants (MRV). Only mutations >1% were retained. RT and integrase RAMs were identified using the ANRS resistance interpretation’s algorithm.

Hypermutation analysis

Hypermutation was assessed using the Hypermut 2.0 program (https://www.hivlani.gov/content/sequence/HYPERMUT/hypermut.html), using HXB2 sequence as reference. This program performed a Fisher’s exact test comparing the number of G-to-A changes in the trinucleotidic APOBEC3F/3G context [GGD or GAD (D = A, G or T)] versus the control context (GYN or GRC, Y = C or T, R = A or G, N = all), and a sequence with P < 0.05 was considered as hypermutated.

The following drug resistance mutations E138K, M184I, M230I in RT and E138K, G140S, R263K in integrase, all result from a G-to-A mutation in the APOBEC3F/3G context. Thus, they were considered as APOBEC-induced mutations. We defined as defective viral genome all hypermutated sequences and those containing at least one stop codon.

Results

Analysis of HIV-1 genotypes obtained using Sanger technology

At the initiation of the DOLULAM study, the DNA Sanger genotype showed that 18 patients (67%) harboured viruses with at least one RAM, interpreted as resistance to at least one antiretroviral drug in 14 cases (52%). The most prevalent NRTI RAMs were thymidine analogue mutations (n = 10 of 18, 56%). The M184V mutation was present in viruses from six patients (22%), including four M184V and two M184I (Table 1).

Historical HIV-1 DNA and RNA RT resistance genotypes were also available in 14 and 18 patients, respectively. Last historical DNA and RNA genotypes were obtained a median of 3.4 years (IQR = 1.1–4.2) and 7.9 years (IQR = 6.1–10.8) before initiation of the dual therapy dolutegravir + lamivudine, respectively.

Two additional patients harbouring viruses with RAMs (n = 20) were identified when using historical DNA RT genotypes. We also observed a higher number of RAMs in three patients, including M184I in one case.

Historical RNA RT genotypes showed a higher number of RAMs in plasma viruses from nine patients, including M184V in six cases.

Taking into account all available Sanger resistance genotypes, an M184I/V mutation was detected in viruses from 12 patients, leading to an overall proportion of 44% (n = 12 of 27). One patient displayed both M184I and M184V mutated viruses. In addition, viral genomes defective in the RT gene were present as majority variants in cellular reservoirs from four patients (n = 4 of 27, 15%), including two that contain hypermutated viral sequences.

A Sanger DNA integrase genotype was available at initiation of the DOLULAM study in 20 patients, showing polymorphisms associated with resistance in two cases (L74I, n = 2). In addition, historical integrase resistance genotypes were available (n = 3 in DNA and n = 2 in RNA) showing no major integrase inhibitor RAMs.

Analysis of HIV-1 DNA NGS at initiation of DOLULAM

Twenty-five of the 27 DNA samples available at the initiation of DOLULAM could be assessed with NGS technology, this being successful in 22 and in 19 samples for RT and integrase genes, respectively. HIV-1 DNA NGS performed at the initiation of the DOLULAM dual therapy showed that 45% (n = 10 of 22) and 21% (n = 4 of 19) patients harboured MRV in RT and integrase regions, respectively (Table 1). MRV were present at the median proportion of 9.6% (IQR = 5.2–11.8). The most prevalent MRV in RT were M184I (n = 7, 32%), M230I (n = 4, 18%) and E138K (n = 3, 14%). The most prevalent MRV in the integrase was E138K (n = 2, 11%).

The analysis of NGS RT DNA data at the initiation of DOLULAM does not lead to a higher number of patients harbouring viruses
Table 1. Description of RAMs in patients included in the DOLULAM study using all available genotypes

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>RAL pre-exposure</th>
<th>Codon 184 at initiation of DOLULAM (DNA Sanger)</th>
<th>DNA NGS at initiation of DOLULAM (% of variants)</th>
<th>Other RAMs combining all available genotype data (% of variants)</th>
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<td>M/V</td>
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NA, not available; RAL, raltegravir.
RAMs found only by NGS technology are underlined.
with RAMs. However, a higher number of RAMs was observed in proviruses from 10 patients, when using NGS, including the M184I mutation in seven cases and M184V in one case. Among the 18 supplementary RAMs detected by NGS, 14 (78%) were APOBEC-induced mutations [M230I \(n=4\), E138K \(n=3\) and M184I \(n=7\)].

Using NGS technology showed that 10 patients harboured RT defective viral genomes in cellular reservoirs and all harboured the M184I mutation. RT defective viral genomes were present in minority proportion in cellular reservoirs in seven cases.

NGS integrase DNA data analysis showed that four of the five RAMs detected as MRV were APOBEC-induced mutations (E138K, \(n=2\); G140S, \(n=1\); and R263K, \(n=1\)). No difference was observed in the prevalence of integrase MRV between patients pre-exposed to raltegravir and those naive of integrase inhibitors (2 of 9 and 2 of 10, respectively).

**Description of M184I/V mutations**

Combining all available genotype data, an M184I/V mutation was observed in 17 of 27 patients (63%) of the DOLULAM study (Figure 1). The two M184I and M184V mutations were detected during the therapeutic history of three patients. Most of the M184V mutations \((n=8\) of 11) were detected in historical RNA resistance genotypes. On the contrary, all 10 M184I mutations were exclusively detected in DNA genotypes, including seven as MRV. Among the 11 cases of historical M184V, this mutation was still detectable in five patients, including one as MRV, in the DNA genotype performed at the initiation of the DOLULAM dual therapy.

No correlation was found between the duration of the virological suppression and the proportion of M184I/V variants at the time of initiation of DOLULAM.

No impact of the presence of the M184I/V mutation on the virological outcome could be assessed, since no VF occurred during the first year of the dual therapy dolutegravir + lamivudine. Regarding the two patients who experienced a viral blip during the first year of the DOLULAM study (52 and 66 copies/mL at week 12 and 36, respectively), both harboured virus with RAMs in DNA genotype performed at initiation of the DOLULAM dual therapy. One had an M184V-mutated virus in majority proportion, and one had an M184I-mutated virus, which is an RT-defective MRV only detected by NGS.

**Discussion**

In this first study assessing NGS on HIV-1 DNA at initiation of a switch study in highly ART-experienced patients, we showed: (i) a high proportion of patients harbouring defective viral genomes in cellular reservoirs and all harboured the M184I mutation. RT defective viral genomes were present in minority proportion in cellular reservoirs in seven cases.

NGS integrase DNA data analysis showed that four of the five RAMs detected as MRV were APOBEC-induced mutations (E138K, \(n=2\); G140S, \(n=1\); and R263K, \(n=1\)). No difference was observed in the prevalence of integrase MRV between patients pre-exposed to raltegravir and those naive of integrase inhibitors (2 of 9 and 2 of 10, respectively).

Several studies have shown that the use of NGS technology, allowing the detection of MRV over 1%, leads to a two to three factor increase in the prevalence of transmitted drug resistance in ART-naive patients.\(^6\)\(^7\) Similarly, a higher number of RAMs was detected at the time of VF when NGS technology was used.\(^8\) However, very few data are available regarding the prevalence of MRV in the cellular reservoir of patients with VL < 50 copies/mL. A recent study based on longitudinal NGS analysis of proviral RT and protease issued from virologically suppressed patients showed that, despite virological control, the diversity of the viral quasispecies continued...
to evolve. In our study, we showed a 3-fold increase in the prevalence of the M184I/V mutations according to genotypes taken into account and sequencing technology used. Thus, the M184I/V mutation was present in viruses from six patients (22%) using only the Sanger DNA genotype at the initiation of the DOLULAM study, increasing to 12 patients (44%) when adding data provided by historical DNA and RNA Sanger genotypes, to finally reach 17 patients (63%) when adding data provided by NGS technology performed at the initiation of DOLULAM. Both M184I and M184V mutations displayed a high level of resistance to lamivudine/emtricitabine; however, their kinetics of appearance is different. Thus, during the development of resistance, the M184I mutation appeared first, being rapidly subsequently replaced by the M184V mutation.13,11 This transitional state of the M184I mutant results from its drastically decreased viral replicative capacity.10,11

In the ART-experienced virologically suppressed patients of the DOLULAM study, we showed a high prevalence of MRV in cellular reservoir with 45% of the patients having MRV in the RT gene and 21% in the integrase gene. Interestingly, most of these MRV (78%) consist of APOBEC-induced mutations such as M184I and E138K. A previous study has shown that the M184I and E138K mutations may pre-exist in the cellular reservoir at a high frequency prior to drug exposure, because of APOBEC3 editing.12 Thus, APOBEC3-driven mutagenesis can contribute to the generation of specific RAMs such as M184I or E138K.

In our study, we showed that RT-defective viral genomes were the major viral variants present in the cellular reservoir of four patients. The use of NGS technology led to the detection of six additional patients harbouring also RT-defective viral genomes in the cellular reservoir, but as MRV, all displaying at least one APOBEC-induced mutation and one stop codon. It has been previously described that long-term virologically suppressed patients have more frequently defective viral genomes than ART-naive patients.13

Patients enrolled in the DOLULAM study had a long duration of virological suppression of 6.4 years in median. However, there was no association between the presence of defective viral genomes and the duration of the virological suppression, and may be assessment of the proportion of defective viral genomes within the cellular reservoir would be more informative.

Despite the high prevalence of M184I/V mutations in the therapeutic history of patients enrolled in the DOLULAM study, no VF occurred during the first year of the dolutegravir + lamivudine maintenance study. Another study showed the lack of impact of the mutation M184V on the maintenance of a dual therapy strategy combining PI/r + lamivudine.2 Thus, the recent MOBIDIP study compared two maintenance treatments with PI in monotherapy or dual therapy plus lamivudine in a group of virologically suppressed patients on second-line ART conducted in resource-limited settings.2 Unexpectedly, the maintenance with PI/r + lamivudine was associated with a high rate of success despite the presence of the M184V mutation in nearly all patients, while PI/r monotherapy cannot be recommended, as the efficacy of this strategy was statistically inferior to the dual therapy, PI/r + lamivudine.2 It has been well-described that the M184V mutation significantly decreased the in vitro viral replicative capacity,10 and this mechanism can play a role in the specific characteristics of the M184V mutation. In addition, recently, in vitro culture selection experiments have shown that the presence of the M184V or K65R mutation prevented the selection of dolutegravir resistance mutation.14 Finally, in the DOLULAM study, lamivudine was combined with dolutegravir, a drug with the highest genetic barrier to resistance among the integrase inhibitor drug class, since at this time only one case report of selection of an integrase inhibitor RAM in a patient experiencing a VF has been described in ART-naive patients initiating a first-line dolutegravir-based regimen.15

Several observational cohorts and a randomized clinical trial assessed the efficacy of switching to a non-conventional strategy of dolutegravir monotherapy in virologically suppressed patients.16–18 All studies showed a prevalence of VF around 10% with a selection of integrase RAMs in almost all cases.16–18 Of note most of the patients enrolled in these studies had been pre-exposed to integrase inhibitors (raltegravir or elvitegravir).16–18 Thus, even with no previous VF, pre-exposure to integrase inhibitors seems a deleterious factor to the virological response to a dolutegravir monotherapy strategy. Interestingly, in our study, NGS of integrase gene in cellular reservoir at the time of DOLULAM initiation showed no difference in the proportion of patients with MRV between previously raltegravir-exposed patients and integrase inhibitor-naive patients. Although this is on a small sample size, this is the first time such findings are described.

The present study has its limitations. The sample size is small, impeding the statistical power; and the NGS Illumina technology has a short sequencing length preventing quantification of the proportion of the defective viral genomes within the cellular reservoir. An M184I/V mutation was detected at least once in RNA/DNA genotypes in 63% of the DOLULAM patients; however, no deleterious impact of their presence was observed on the efficacy of the dolutegravir + lamivudine dual therapy to maintain virological suppression. We showed a differential profile between the two mutations: the M184I mutation was exclusively present in the defective viral genomes present in the cellular reservoir, whereas the M184V mutation was mainly detected at the time of previous VF in historical RNA genotypes. In addition, the latter remained detectable in the cellular reservoir at the time of DOLULAM initiation in less than half of the patients, even in minority proportion. These elements most likely contribute to the limited impact of the presence of the M184I/V mutations on the virological suppression maintenance observed in the DOLULAM study.

Acknowledgements
We dedicate this work to the memory of Dr Mark Wainberg.

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Transparency declarations
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References


