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MAJOR ARTICLE

Learning From Full Characterization of HIV Proviruses in People Receiving Long-Acting Cabotegravir/Rilpivirine With a History of Replication on the Antiretroviral Classes

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Background. To better understand factors associated with virologic response, we retrospectively characterized the HIV proviruses of 7 people with HIV who received long-acting cabotegravir/rilpivirine (CAB/RPV-LA) and were selected according to the following criteria: virologic control achieved despite a history of viral replication on 1 or both corresponding antiretroviral classes (n = 6) and virologic failure (VF) after CAB/RPV-LA initiation (n = 1).

Methods. Last available blood samples before the initiation of CAB/RPV-LA were analyzed retrospectively. Near full-length HIV DNA genome haplotypes were inferred from Nanopore sequencing by the in vivo Genome Diversity Analyzer to search for archived drug resistance mutations (DRMs) and evaluate the frequency and intactness of proviruses harboring DRMs.

Results. Archived DRMs including G-to-A mutations were found in samples from 3 patients who maintained virologic control. Genomes harboring DRMs were majorly in minority variants (<20%) and were defective in all cases except for 1 participant. In this participant, intact genomes with the H221Y mutation on reverse transcriptase were detected representing 11 copies per 10^6 peripheral blood mononuclear cells. The other mutations observed in the participants of the study resulted most likely from hypermutations. The patient with VF presented archived mutations, all associated with defects. Other factors could explain this VF.

Conclusions. Our findings highlight the difficulty in interpreting the clinical significance of DRMs when detected in proviral DNA and the need to filter out hypermutated sequences. Detected DRMs could be harbored by defective archived genomes unlikely to contribute to treatment failure.

Keywords. Drug Resistance Mutations; HIV; HIV DNA genotyping; long-acting antiretrovirals; near-full genome sequencing.

Maintaining high levels of adherence to antiretroviral treatment (ART) is crucial for achieving viroimmunologic control in people with HIV-1 (PWH) [1, 2]. However, many PWH face challenges with lifelong adherence to treatment [1]. Consequently, various strategies have been developed to improve adherence [2, 3]. Long-acting (LA) intramuscular formulations of cabotegravir (CAB) and rilpivirine (RPV; henceforth, CAB/RPV-LA) became the first LA regimen for treatment of HIV-1 in adults to be approved by multiple health authorities. Currently, CAB/RPV-LA is recommended for PWH who have been virologically suppressed during ART for at least 6 months and have no history of viral resistance or virologic failure (VF) while taking a nonnucleoside reverse transcriptase inhibitor (NNRTI) or an integrase strand transfer inhibitor (INSTI) [4].

Although phase 3 studies (FLAIR, ATLAS, ATLAS-2M, SOLAR) have demonstrated the long-term efficacy of this combination, a minority of participants (around 1%) experienced VF during these trials [5–9], which is often associated with the emergence of drug resistance mutations (DRMs) to 1 or both antiretroviral classes [10]. Four factors have been identified from data from FLAIR, ATLAS, or ATLAS-2M as potential factors associated with VF: archived DRM conferring resistance to RPV at baseline (on peripheral blood mononuclear cells withdrawn at the moment of CAB/RPV-LA initiation), HIV-1 subtype A6/A1, body mass index >30 kg/m^2, and low RPV trough concentrations at week 8 [11]. However, the small number of participants with VF in the phase 3 studies is not sufficient to draw definitive conclusions. In addition, none of the previously mentioned risk factors were present in the participants with VF from the SOLAR study;

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particularly, baseline DRMs to RPV were not found [6]. In contrast, Kityo et al recently reported on archived DRMs in the CARES trial. The authors reported DRMs associated with intermediate- to high-level resistance to CAB and RPV in the peripheral blood mononuclear cells (PBMCs) of 11% and 8% of PWH at baseline, respectively, among patients with sustained virologic control under CAB/RPV-LA and without a history of VF. To explain these discrepancies, some of the described DRMs in the CARES trial could have either arose from APOBEC-induced hypermutations (apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like) or been present on defective genomes (eg, largely deleted sequences) [12]. Thus, additional studies and real-life data are required to better understand the factors influencing virologic response. Therefore, we retrospectively characterized HIV proviruses from PWH taking CAB/RPV-LA.

MATERIALS AND METHODS

Participant Inclusion Criteria

Among PWH receiving CAB/RPV-LA and followed in the University Hospital of Orléans (Orléans, France), we investigated the cases of 2 groups: (1) those who had a history of replication during an NNRTI and/or INSTI regimen and sustained virologic control under this combination and (2) those who presented with VF during CAB/RPV-LA as defined by 2 consecutive positive viral loads (ie, HIV RNA >50 copies/mL).

Near HIV Full-length Genome Sequencing With Oxford Nanopore Technology Sequencing

Last available frozen blood samples before the switch to CAB/RPV-LA were analyzed retrospectively for all participants. A blood sample taken 1 year after CAB/RPV-LA VF was also analyzed in case of VF. Near full-length HIV genome (NFG) Nanopore single-genome sequencing was performed on HIV DNA after limiting dilution to search for archived DRMs, link them, and evaluate the intactness of proviruses harboring these DRMs. DNA extraction was performed with a NucleoSpin Blood Kit (MACHEREY-NAGEL). A limited dilution of each extract was determined to allow the study of viral haplotypes and avoid the overrepresentation of largely deleted fragments. We selected the dilution based on the number of HIV DNA copies per well that is associated to a polymerase chain reaction (PCR) positivity rate of no more than 30%. All available extracts were used for the limited dilutions. HIV DNA was amplified first with a PCR, followed by a nested PCR as previously described [13]. PCR products were purified by a QIAquick PCR Purification Kit (Qiagen). For DNA end repair and adaptor ligation, the NEB NextARTIC SARS-CoV-2 Companion Kit (Oxford Nanopore Technologies) was used. Cleanup was performed with custom AMPure beads (Beckman Coulter). The library was prepared with the SQK-LSK-109 kit. Sequencing was launched on a FLO-MIN106 (R9.4.1) Flow Cell (Oxford Nanopore Technologies). A dehosting step was performed via a VIRiONT pipeline [14]. A custom-designed bioinformatic pipeline adapted from the in vivo Genome Diversity Analyzer algorithm was developed to recover representative haplotypes [15] per the following initial criteria: total reads >30, minimum length >1000 base pairs (bp), minimum frequency >0.01 (1%), and mean depth >100 reads. Putative haplotypes were analyzed for the search of DRMs by Stanford's HIVdb Program: Sequence Analysis [16]. Reported mutations in the reverse transcriptase (RT) and integrase genes were interpreted according to the algorithm of the French ANRS-MIE (National Agency for AIDS Research–Emergent Infectious Diseases; version 34, November 2023) [17]. The criteria for defining genetically defective and intact haplotypes were as described previously [13]. Briefly, large deletions were defined as haplotypes <8400 bp. For contigs >8400 bp, APOBEC-induced hypermutations were evaluated according to the Los Alamos HIV database Hypermut2 program [18], and premature stop codons were identified in nonhypermutated haplotypes with the Gene Cutter tool from the Los Alamos HIV database [19]. Psi/MSD defects were defined as a deletion or a point mutation in the well-conserved region encoding for the packaging signal Psi and major splice donor site (MSD). They were identified by aligning haplotype sequences with the HXB2 reference sequence via MEGA11 [20] and by comparison with HIV compendium sequences.

Mutational Load and Intact Mutational Load of Archived HIV Genome

Total HIV DNA load in peripheral blood mononuclear cells was quantified as previously described [21]. The mutational load of archived DRMs was calculated by multiplying the frequency of each DRM with the total HIV DNA load. The intact mutational load for each DRM was determined by multiplying the frequency of intact genomes harboring each DRM with the total HIV DNA load.

RESULTS

Patients' Characteristics

Seven PWH (ID1–ID7) were examined according to the inclusion criteria. ID1 to ID6 had a history of VF under an NNRTI and/or INSTI agent (Table 1, Supplementary Figure 1) and sustained virologic control under CAB/RPV-LA for >22 months. ID7 experienced a confirmed VF 3 months after treatment switch (HIV-RNA, 11 900 copies/mL).

PWH clinical and virologic characteristics are presented in Table 2. All individuals (ID1–ID7) had received multiple lines of therapy for many years before CAB/RPV-LA (Supplementary Figure 1). HIV RNA at the time of CAB/RPV-LA initiation was <50 copies for all participants except ID6, with a median 63 months (IQR, 27–77) of HIV RNA <50 copies/mL. HIV RNA was <50 copies/mL in the plasma at 1 month of CAB/RPV-LA

Table 1. Past Documented Virologic Failure and/or DRMs on NNRTI and/or INSTI for Participants ID1–ID6

	NNRTI			INSTI			Past Documented DRMs		
Participant	Duration of the Replication, mo[a]	No. of VF Episodes	Zenith Viral Load, Copies/mL	Duration of the Replication, mo[a]	No. of VF Episodes	Zenith Viral Load, Copies/mL	NNRTI	INSTI	GSS Score to CAB/RPV-LA
ID1	3	1	10 708	0.5	1	168	K103H/N/S/T,[b] Y188H	NG	2
ID2	19	2	2402	28	4	704 000	K101E, G190A,[b] H221Y	N155H + 97A (proviral DNA),[c] E138K (proviral DNA)[c]	0
ID3	12	1	2260	0	0	NA	NG	NG	2
ID4	0	0	NA	1	1	4010	H221Y (proviral DNA)	T97A[d]	1
ID5	0	1	NA	2	2	153 848	NF	NF	2
ID6	5	1	43 022	0	0	NA	NF	NF	2

Abbreviations: ANRS, National Agency for AIDS Research; CAB, cabotegravir; DRM, drug resistance mutation; GSS, genotypic sensitivity score; INSTI, integrase strand transfer inhibitor; NA, not applicable; NF, not found; NG, no genotyping data; NNRTI, nonnucleoside reverse transcriptase inhibitor; RPV, rilpivirine; VF, virologic failure.

[a]Calculated as the sum of the times during which HIV RNA was ≥50 copies/mL under the class.

[b]According to the ANRS algorithm, these mutations do not confer resistance to RPV but to other agents of the class of NNRTI.

[c]Mutations found retrospectively on stored blood cells.

[d]According to the ANRS algorithm, these mutations do not confer resistance to CAB but to other agents of the class of INSTI.

treatment for all. Patients were all adherent to treatment and received on-time CAB/RPV-LA injections.

Risk Factors of VF for PWH With Virologic Response. In addition to a history of replication on an INSTI and/or an NNRTI agent, for ID1, ID2, and ID4 who sustained virologic control, prior DRMs to the classes were found on viral DNA or viral RNA during a previous VF under an NNRTI or INSTI agent. The 3 other participants (ID3, ID5, ID6) had no previously documented DRMs to NNRTIs and INSTIs (Table 1, Supplementary Figure 1).

ID2 exhibited additional VF risk factors, such as high body mass index (≥30) and low RPV trough concentration at week 4 below the Q1C trough threshold defined from phase 3 trials (32 ng/mL). ID1, ID2, and ID3 exhibited lower CAB trough concentrations than the threshold (1120 ng/mL) [22].

Characteristics of PWH With VF Under CAB/RPV-LA. ID7 had a previous viral replication episode (197 copies/mL) under INSTI without confirmed VF or documented DRMs to INSTIs (Supplementary Figure 1). This participant had low RPV and CAB trough concentrations at 1 and 3 months (Table 2). RPV trough concentrations achieved satisfying levels following the third injection (47 ng/mL). In addition, he had a body mass index of 30.3, another risk factor associated with VF. At the time of VF, a Q148R/Q (double population) on integrase was observed in plasma HIV RNA. Following the third injection of CAB/RPV-LA, his plasma viral load was reduced to 27 copies/mL.

Archived DRMs on HIV DNA With NFG sequencing on the Last Blood Samples Before CAB/RPV-LA Initiation and Evaluation of Their Frequency and the Intactness of Proviruses Harboring Them

To complete the genotypic data collected during participants' follow-up, NFG sequencing was performed retrospectively. The analyzed samples predate the switch to injectable regimen by a period ranging from 1 month to 8 years (Table 2). An overall 251 HIV DNA haplotypes were obtained with NFG sequencing, with a median 43 haplotypes (IQR, 23–47) per participant with a median coverage of 8823 reads (IQR, 1692–25 774). The characteristics of sequences harboring DRMs including G-to-A mutations are depicted in Figure 1 and Tables 3 and 4. Of note, all G-to-A DRMs (RT: G190E, E138K, M230I; integrase: E138K, G140S, G140R, R263K) detected for all participants were associated with APOBEC hypermutations.

Among participants with virologic control, no DRMs were found for ID2, ID3, and ID6 (Tables 3 and 4). For others (ID1, ID4, and ID5), DRMs to 1 or 2 classes concerned mostly minority variants (ie, <20%) except for M230I on RT for ID5 (21% with NFG). Among these mutations, only H221Y (in ID4, 14%) on RT was previously described during participants' follow-up (Tables 1 and 3). All the mutations detected with NFG for ID1 and ID5 were present on defective genomes (mainly on hypermutated and largely deleted genomes; Figure 1). Overall, only ID4 harbored an intact archived HIV genome with DRMs: 20% of the sequences harboring H221Y were considered intact proviruses, with an intact mutational load of 11 copies per 10^6 peripheral blood mononuclear cells.

In the participant who experienced VF (ID7), DRMs to INSTIs and NNRTIs were found archived in peripheral blood

Table 2. Clinical and Virologic Characteristics Among Patients ID1–ID7

			HIV-1		ART Before CAB/RPV-LA			CAB/RPV-LA				Trough Concentration at Week 4	
ID	Sex	BMI, kg/m^2	DNA, log cp/10^6 PBMCs	Subtype	Duration, y	Last	Analyzed Sample Date (Viral Load, cp/mL)	Initiation Date	HIV RNA Undetectability Before, mo	Oral Lead-in	HIV Viral Load at Initiation of CAB/RPV-LA, cp/mL	RPV	CAB
ID1	F	27.6	2.46	CRF02_AG	17	BIC + TAF + FTC + RPV	Jan 2014 (<20)	Feb 2022	60	Yes	<20	43	1060
ID2	M	**32.2**[a]	2.65	CRF02_AG	17	EVGc + FTC + TAF	Jan 2014 (1360)	Feb 2022	4	No	<20	**18**[a]	751
ID3	F	28.1	2.75	C	24	FTC + TAF + RPV	Aug 2018 (30)	Mar 2022	108	No	<20	35	462
ID4	M	27.4	2.63	B	24	DTG + 3TC	Sep 2020 (<20)	Jun 2022	66	No	<20	53	1600
ID5	M	22.9	2.33	B	22	BIC + TAF + FTC + RPV	Nov 2021 (60)	Jan 2022	0	No	68	44	1820
ID6	M	22.9	2.71	B	14	DTG + RPV	May 2021 (<20)	Apr 2022	82	No	<20	97	2600
ID7	M	**30.25**[a]	3.06	B	8	BIC + TAF + FTC	Jan 2022 (22)	Apr 2022	16	No	<20	**17**[a]	776

Abbreviations: 3TC, lamivudine; ART, antiretroviral treatment; BIC, bictegravir; BMI, body mass index; CAB, cabotegravir; cp, copies; DTG, dolutegravir; EVGc, elvitegravir-cobicistat; F, female; FTC, emtricitabine; LA, long-acting; M, male; mo, months PBMC, peripheral blood mononuclear cell; RPV, rilpivirine; TAF, tenofovir alafenamide; y, years.

[a]Risk factors for virologic failure during CAB/RPV-LA according to Cutrell et al [11].

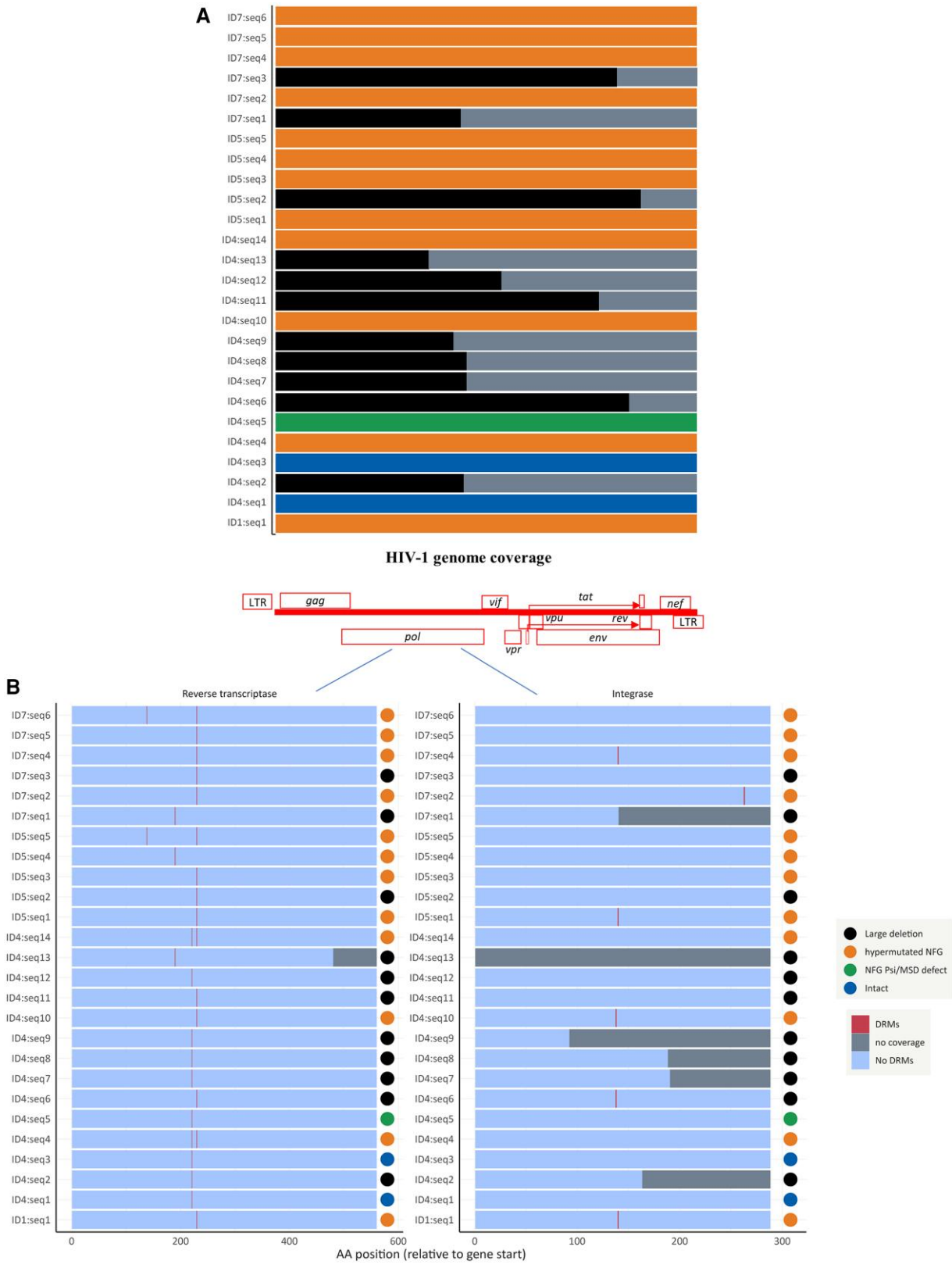

Figure 1. *A*, Characteristics and coverage of haplotypes harboring DRMs including G-to-A mutations with near full-length genome sequencing. *B*, The position of DRMs on reverse transcriptase and integrase on the last available blood sample before CAB/RPV-LA switch. G-to-A DRMs (reverse transcriptase: G190E, E138K, M230I; integrase: E138K, G140S, G140R, R263K) were associated with APOBEC hypermutations. Only haplotypes harboring DRMs are depicted in the figure. Each line with the details concerning reverse transcriptase and integrase is presented at the bottom, corresponding to each line of full-length genome at the top. APOBEC, apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like; CAB, cabotegravir; DRM, drug resistance mutation; LA, long-acting; MSD, major splice donor site; NFG, near full-length genome; RPV, rilpivirine.

Table 3. Archived NNRTI DRMs on the Last Available Blood Sample Before CAB/RPV-LA Initiation With Near Full-length Genome Sequencing for Patients ID1–ID7

		Results With Near Full-length Genome Sequencing			Percentage of Defective Genomes Harboring DRMs				
ID	NNRTI DRM	DRM Frequency, %	No. of Contigs Analyzed	Mutational Load, Copies/10^6 PBMCs	Large Deletions, Contigs <8400 bp	Contigs >8400 bp With Premature Stop Codon	Contigs >8400 bp With Only Psi/MSD Defect in the 5′ LTR	Percentage of Intact Sequences Harboring DRMs	Intact Mutational Load, Copies/10^6 PBMCs
ID1	M230I[a]	2	46	6	0	100	0	0	0
ID2	NF	0	27	0	NA	NA	NA	NA	0
ID3	NF	0	11	0	NA	NA	NA	NA	NA
ID4	G190E[a]	2	58	7	100	0	0	0	0
	H221Y	14		59	50	20	10	**20**	11
	M230I[a]	9		37	30	70	0	0	0
ID5	E138K[a]	5	19	11	0	100	0	0	0
	G190E[a]	5		11	0	100	0	0	0
	M230I[a]	21		45	20	80	0	0	0
ID6	NF	0	43	0	NA	NA	NA	NA	0
ID7	E138K[a]	2	47	24	0	100	0	0	0
	G190E[a]	2		24	100	0	0	0	0
	M230I[a]	11		121	20	80	0	0	0

Bold indicates the only DRM present on an intact genome.

Abbreviations: bp, base pairs; CAB, cabotegravir; DRM, drug resistance mutation; LA, long-acting; LTR, long terminal repeat; MSD, major splice donor site; NNRTI, nonnucleoside reverse transcriptase inhibitor; NA, not applicable; NF, not found; No, number; PBMC, peripheral blood mononuclear cell; RPV, rilpivirine.

[a]G-to-A DRMs.

Table 4. Archived INSTI DRMs on the Last Available Blood Sample Before CAB/RPV-LA Initiation With Near Full-length Genome Sequencing for Patients ID1–ID7

		Results With Near Full-length Genome Sequencing (Nanopore)			Percentage of Defective Genomes Harboring DRMs					
ID	INSTI DRMs	DRM Frequency, %	No. of Contigs Analyzed	Mutational Load, Copies/10^6 PBMCs	Large Deletions, Contigs <8400 bp	Contigs >8400 bp With Premature Stop Codon	Contigs >8400 bp With Only Psi/MSD Defect in the 5′ LTR	Percentage of Intact Sequences Harboring DRMs	Intact Mutational Load, Copies/10^6 PBMCs	GSS Score to CAB/RPV-LA Based on NGS Results
ID1	G140R[a]	2	46	6	0	100	0	0	0	0
ID2	NF	0	27	0	NA	NA	NA	NA	0	2
ID3	NF	0	11	0	NA	NA	NA	NA	0	2
ID4	E138K[a]	3	58	15	50	50	0	0	0	0
ID5	G140S[a]	5	19	11	0	100	0	0	0	0
ID6	NF	0	43	0	NA	NA	NA	NA	0	2
ID7	G140S[a]	2	47	24	0	100	0	0	0	
	R263K[a]	2	47	24	0	100	0	NA	0	

Abbreviations: bp, base pairs; CAB, cabotegravir; DRM, drug resistance mutation; GSS, genotypic sensitivity score; INSTI, integrase strand transfer inhibitor; LA, long-acting; LTR, long terminal repeat; MSD, major splice donor site; NA, not applicable; NF, not found; NGS, next-generation sequencing; No, number; PBMC, peripheral blood mononuclear cell; RPV, rilpivirine.

[a]G-to-A DRMs.

prior to the initiation of CAB/RPV-LA (Tables 3 and 4). These DRMs were G-to-A mutations with a frequency ranging from 2% to 11%. They were associated with hypermutations and exclusively present on defective genomes. DRMs' persistence in the archived proviruses of this patient was evaluated 1 year after CAB/RPV-LA interruption (HIV RNA <50 copies/mL). In total, 57 HIV DNA haplotypes were obtained with NFG. Only 1 contig harbored M230I on RT and no DRM to INSTIs. This sequence was hypermutated. Notably, the Q148R on integrase that was found in plasma (as a double

population with Sanger sequencing) at the time of VF was not found.

DISCUSSION

To our knowledge, this is the first study characterizing the intactness or defectiveness of archived proviruses harboring DRMs in PWH receiving CAB/RPV-LA despite past documented viral replication to NNRTIs and/or INSTIs. The limited number of cases with VF from clinical trials and the scarcity of real-life data make it hard to conclude on the potential predictive role of different baseline factors on virologic response. Thus, it is critical to understand the predictive factors of virologic response [23] and to understand how PWH harboring archived DRMs in the blood proviruses can have sustained virologic control under CAB/RPV-LA, as recently presented [12].

Among the 6 participants who had a history of replication during an NNRTI and/or INSTI regimen and sustained virologic control under this combination, 3 had archived DRMs to RPV and/or CAB (including G-to-A mutations) in the last available blood sample prior to CAB/RPV-LA initiation. Their genotypic sensitivity score was then 0 or 1 to this combination. These DRMs were found archived before the initiation of CAB/RPV-LA but were not identified during previous VF to the classes. This could be due to their low frequency, making them undetectable by Sanger sequencing, or because the timing of sequencing was not optimal for detecting resistant variants under ART pressure. G-to-A DRMs detected in our study most likely resulted from the cytidine deaminase APOBEC3G enzyme, which will result in defective proviruses [24–26]. Conversely, some DRMs identified during the past VF were no longer detected several months or years after. While it is thought that HIV DRMs are chronically archived in HIV reservoir, the kinetics of the persistence and recirculation of cells harboring archived proviruses with DRMs remains an unresolved question. Recent data have shown a progressive clearance of M184V in blood over 5 years [27]. In our study, 1 previously described DRM persisted in the last available sample before CAB/RPV-LA initiation.

Notably, only 1 participant (ID4) with virologic control during CAB/RPV-LA presented with some intact proviruses harboring H221Y, which is associated with resistance to RPV [28]. This represented a low intact mutational load. All other DRMs were present on defective proviruses (largely deleted, hypermutated, or with Psi/MSD defects). This is concordant with previous data reporting that the majority of proviral DNA is defective [29, 30]. Most described DRMs were G-to-A mutations and were associated to hypermutations. In a study of 1126 PWH with virologic control, APOBEC-related DRMs to the current regimen were found in 17% of participants [31]. Detecting G-to-A DRMs on hypermutated genomes is rather common in routine assays, and some quality control systems allow the elimination of hypermutated genomes to avoid their misinterpretation. In addition, studies describing other antiretroviral strategies have already highlighted the fact that the presence of archived DRMs will not necessarily lead to VF [32, 33], and several examples of ART recycling cases have been reported in literature [34–36]. In the CARES trial, major DRMs to CAB/RPV-LA were found in a significant percentage of participants who maintained virologic control under this combination, and some of these DRMs were G-to-A DRMs [12]. In a similar fashion, Cutrell et al demonstrated that although baseline RPV DRMs significantly increase the risk of VF to CAB/RPV-LA, some participants in phase 3 trials presented with archived DRMs to RPV on HIV DNA at baseline and maintained virologic control [11]. As shown in our study, these archived DRMs could be either harbored by defective genomes or present at a low intact mutational viral load with resistance to 1 antiretroviral (RPV for ID4) that the other antiretroviral can control alone (CAB for ID4).

In hindsight, our results highlight the difficulty in interpreting the potential impact of DRMs on HIV DNA genotypes. Our results also underscore the value of full genome sequencing to evaluate the intactness of proviral sequences harboring 1 or more DRMs. This is particularly important as other sequencing approaches, whether Sanger or next-generation sequencing amplicon methods, cannot detect all types of defects (eg, large deletion or Psi/MSD defects) or the coexistence of ≥2 DRMs on a single genome, which is sometimes necessary to assess reduced susceptibility to ART. However, this technique remains expensive and time-consuming.

The participant from our study who experienced VF 3 months after CAB/RPV-LA initiation did not have any DRMs present on intact HIV DNA proviruses in blood 3 months before CAB/RPV-LA initiation. He had a history of viral replication under triple therapy with bictegravir without available genotyping data. Archived DRMs to INSTIs on intact proviruses could have persisted in other tissues, such as lymph nodes or gut-associated lymphoid tissue. Another possibility could be the complementation of archived defective proviruses with DRMs with an intact HIV genome. However, these assumptions are not the most likely because this participant successfully regained control of viral replication following the third injection of CAB/RPV-LA. Factors other than DRMs could explain the VF for this participant: high body mass index and low RPV and CAB trough concentrations at week 4 [37]. He had a higher total HIV DNA level than the patients with virologic control. To the best of our knowledge, there are no studies that investigated the potential predictive role of high total HIV DNA load on VF under CAB/LA-RPV, as it has been shown for some monotherapy strategies [38, 39].

One of the limitations of our study is that we analyzed a single blood sample collected at various time points before the initiation of CAB/RPV-LA. This "one-shot" approach assessing the circulating viral reservoir may not fully capture the viral

diversity present within the organism. Despite the limited volume of blood analyzed, several hundreds of copies of HIV DNA were analyzed for each participant by our assay, given the HIV DNA loads.

Our findings indicate that the virologic response to CAB/RPV-LA despite a history of DRMs and/or replication with other agents of the corresponding antiretroviral classes could be related to the fact that these DRMs may predominantly or exclusively be archived in defective proviruses. Our results highlight the difficulty in interpreting HIV DNA genotyping assays and the unknown clinical significance of mutations detected in proviral DNA. Larger studies are needed to investigate the impact of NFG sequencing in characterizing the intactness of archived genomes harboring DRMs.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Data availability. Nucleotide sequences are accessible at https://doi.org/10.5061/dryad.9w0vt4bqp.

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