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MAJOR ARTICLE

HIV-1 DNA Testing in Viremic Patients Identifies More Drug Resistance Than HIV-1 RNA Testing

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Background. The Department of Health and Human Services HIV-1 Treatment Guidelines recommend drug resistance testing in HIV-1 RNA to guide the selection of antiretroviral therapy in patients with viremia. However, resistance-associated mutations (RAMs) in HIV-1 RNA may reflect only the patient's current regimen and can be lost during prolonged absence of therapy. We determined if HIV-1 DNA testing can provide drug resistance information beyond that identified in contemporaneous plasma virus.

Methods. This was a retrospective database review of results obtained for patients with viremia for whom commercial HIV-1 RNA and HIV-1 DNA drug resistance testing was ordered on the same day. Resistance-associated mutations and drug susceptibility calls were compared between paired tests, and the effect of HIV-1 viral load (VL) on test concordance was assessed using Spearmen's rho correlation.

Results. Among 124 paired tests, more RAMs were identified in HIV-1 DNA in 63 (50.8%) cases, and in HIV-1 RNA in 11 (8.87%) cases. HIV-1 DNA testing captured all contemporaneous plasma virus RAMs in 101/117 (86.3%) cases and identified additional RAMs in 63/117 (53.8%) cases. There was a significant positive correlation between the viral load at the time of resistance testing and the percentage of plasma virus RAMs detected in HIV-1 DNA ($r_s = 0.317$; $P < .001$). In 67 test pairs demonstrating pan-sensitive plasma virus, resistance in HIV-1 DNA was seen in 13 (19.4%) cases.

Conclusions. HIV-1 DNA testing identified more resistance than HIV-1 RNA testing in most patients with viremia and may be informative in patients whose plasma virus reverts to wild-type following therapy discontinuation.

Keywords. proviral HIV-1; HIV-1 DNA; PBMC compartment; HIV-1 drug resistance.

In patients with HIV-1 viremia, the Department of Health and Human Services (DHHS) HIV-1 Treatment Guidelines recommend drug resistance testing on plasma virus (HIV-1 RNA) to identify appropriate antiretroviral therapy (ART) [1]. However, plasma virus testing preferentially identifies mutations that confer resistance to the patient's current failing regimen. Additional resistance, reflective of prior therapy failures or transmitted resistance-associated mutations (RAMs), may be harbored in proviral HIV-1 DNA within latently infected peripheral blood mononuclear cells (PBMCs), and such archived variants may reemerge under appropriate drug pressure [2, 3]. Moreover, RAMs are gradually lost from plasma virus in the absence of selective drug pressure, as occurs in patients who discontinue ART [4–6]. For these reasons, plasma virus testing in patients with viremia may not yield sufficient information to construct suppressive treatment regimens.

Unlike actively replicating virus, HIV-1 DNA provirus within PBMCs is not subject to selective pressures exerted by the patient's treatment regimens. As such, the HIV-1 DNA reservoir is comprised of a collection of provirus genomes encoding virus variants that replicated in the host during states of viremia. Drug resistance in the proviral reservoir has been shown to predict virologic rebound [7]; highlighting this phenomenon is a post hoc analysis of a recent phase 3 study investigating the long-acting injectable cabotegravir + rilpivirine regimen, where baseline proviral rilpivirine RAMs were associated with virologic failure [8].

Prior studies have demonstrated different degrees of utility of HIV-1 DNA testing for the identification of drug resistance in various clinical contexts [7–50]. However, these studies were performed using noncommercial research assays, which may not be comparable to tests used for patient management in the United States. We sought to determine if commercial proviral HIV-1 DNA testing can provide drug resistance information in patients with viremia beyond that found in plasma virus. To this end, we compared resistance derived from plasma virus and PBMC compartment samples obtained at the same time and evaluated the effect of viral load (VL) on result concordance.

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METHODS

This was a retrospective review of paired HIV-1 RNA and HIV-1 DNA drug resistance tests performed on samples that were collected on the same day from each patient between February 2015 and December 2019. The tests were performed in a Clinical Laboratory Improvement Amendments–certified laboratory (Monogram Biosciences, South San Francisco, CA, USA), and all results were de-identified.

Resistance-associated major and minor mutations to nucleos(t)ide and non-nucleoside reverse transcriptase inhibitors (NRTIs and NNRTIs), protease inhibitors (PIs), and integrase inhibitors (INIs) were identified by Sanger (PhenoSense GT *Plus* Integrase, Monogram Biosciences) or next-generation (GenoSure Archive, Monogram Biosciences) sequencing as previously described [51]. Triplicate nested polymerase chain reaction amplification of the HIV-1 pol region was done after genomic DNA was extracted from whole-blood EDTA samples. Amplicons were pooled, purified, and prepared using the Nextera XT library and index kits, which was followed by 2×150 base paired-end sequencing on the Illumina MiSeq platform. Paired-end reads were joined, and the reads were aligned to the NL43 GenBank: KM390026.1 reference sequence in a codon-aware manner. Quality metrics were in place to determine alignment coverage >1000× at all positions and >Q30 Phred score at all positions. A naïve Bayes classification model was used to individually evaluate for evidence of APOBEC-induced G to A hypermutation. Hypermutated reads were excluded, and the remaining reads were assessed for variant frequency. The reporting threshold was set at 10% to minimize the impact of APOBEC-induced hypermutation false positivity. For drug resistance mutations occurring at positions unlikely to undergo APOBEC-induced changes, the minimum reporting threshold was 3%. Genotype-based antiviral (ARV) susceptibility assessments were made using a proprietary algorithm that incorporates clinical trial data for each drug and >120 000 matched genotype-phenotype results. Viral load measurements occurred no more than 5 days before or 3 days after resistance testing and were performed using the Cobas AmpliPrep/Cobas TaqMan HIV-1 assay.

Data were summarized using descriptive statistics; for data with non-normal distributions, medians were reported. The effect of VL on test concordance was evaluated by Spearman's rho correlation. Comparisons of nonparametric data were performed using Mann-Whitney *U* testing, and a 2-tailed *P* value <.05 was considered significant.

RESULTS

We identified 106 patients with HIV-1 viremia for whom drug resistance tests in plasma virus (HIV-1 RNA) and PBMC proviral DNA (HIV-1 DNA) were performed on samples collected on the same day, for a total of 124 paired tests; 10.4% of patients were male, and 89.6% were female, as most of the data were linked to a women's health clinic. Most patients (90.6%) were infected with HIV-1 subtype B. The median VL was 17 250 c/mL at the time of resistance testing (Table 1), with 2 measurements <500 c/mL.

HIV-1 RNA testing demonstrated resistance to ≥1 ARV on 45.2% of reports, and HIV-1 DNA testing on 51.6% of reports. A greater number of resistance calls was reported across PBMC samples vs plasma samples (n = 300 vs n = 231); the same was true for the number of RAMs identified in PBMC samples vs plasma samples (n = 702 vs n = 603) (Table 2).

Ninety-two percent of the 603 total RAMs identified in plasma virus samples were also identified in PBMC samples, and 88.3% of 231 resistance calls reported for plasma virus were also reported for virus in the PBMC compartment. In contrast, only 79.1% of RAMs and 72.3% of resistance calls reported for virus in the PBMC compartment were also reported for plasma virus (Figure 1; Supplementary Figures 1 and 2).

In pairwise comparisons, more RAMs were detected in HIV-1 DNA than HIV-1 RNA in 63/124 (50.8%) cases; 67/124 (54.0%) HIV-1 DNA tests detected unique RAMs (ie, RAMs found only in HIV-1 DNA in a test pair), with an average of 2.19 unique RAMs per report. Conversely, 11/124 (8.87%) HIV-1 RNA tests detected more RAMs than the paired HIV-1 DNA tests; 11/124 (8.87%) HIV-1 RNA tests detected unique RAMs (ie, RAMs found only in HIV-1 RNA in a test pair), with an average of 2.88 RAMs per report. Consequently, a greater number of ARV resistance calls were reported for HIV-1 DNA in 31/124 (25%) test pairs vs only 6/124 (4.8%) test pairs for HIV-1 RNA (Supplemental Table 1). These findings demonstrate that a larger fraction of resistance information in this cohort resides within the PBMC compartment, which was inaccessible via plasma virus testing.

Table 1. Patient and Virus Characteristics

Characteristic	No. (% or Range)
Male	11 (10.4)
Female	95 (89.6)
Mean age, y	36 (17–71)
<20	4 (3.23)
20–30	29 (23.4)
30–40	60 (48.4)
41–50	23 (18.5)
>50	8 (6.45)
HIV-1 subtype	
B	96 (90.6)
AG	4 (3.77)
C	2 (1.89)
A1	1 (0.943)
Complex	1 (0.943)
D	1 (0.943)
G	1 (0.943)
Median viral load at resistance testing, c/mL	17 250 (150–2 980 000)

Table 2. Resistance Calls and Drug Resistance Mutation Characteristics

Resistance Calls, No. (% or Range)	Plasma Virus (HIV-1 RNA)	PBMCs (HIV-1 DNA)
Resistance calls across cohort	231	300
NRTI	77 (33.3)	101 (33.6)
NNRTI	110 (47.6)	130 (43.3)
PI	20 (8.67)	39 (13.0)
INSTI	24 (10.4)	30 (10.0)
Resistance calls per report		
None	68 (54.8)	60 (48.4)
1-class	40 (32.3)	42 (33.9)
2-class	8 (6.45)	13 (10.5)
3-class	8 (6.45)	9 (7.26)
4-class	0	0
Average resistance calls per report	1.86 (0–10)	2.42 (0–16)
RAMs across cohort	603	702
NRTI	88 (14.6)	112 (16.0)
NNRTI	146 (24.2)	172 (24.5)
PI	335 (55.6)	380 (54.1)
INSTI	34 (5.64)	38 (5.41)
RAMs per report		
None	7 (5.65)	3 (2.42)
1-class	38 (30.6)	28 (22.6)
2-class	47 (37.9)	51 (41.1)
3-class	24 (19.4)	36 (29.0)
4-class	8 (6.54)	6 (4.84)
Average RAMs per report	4.86 (0–15)	5.66 (0–15)

Abbreviations: INSTI, integrase strand transfer inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleos(t)ide reverse transcriptase inhibitor; PBMCs, peripheral blood mononuclear cells; PI, protease inhibitor; RAMs, resistance-associated mutations.

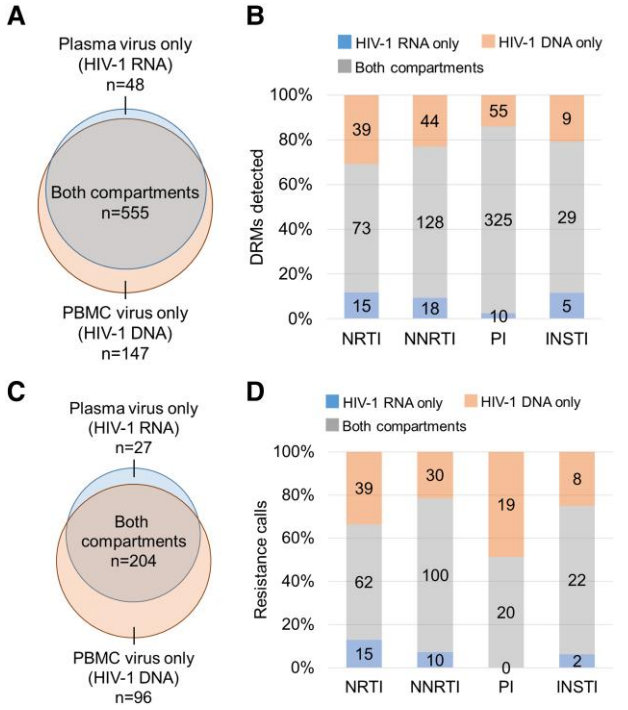

Figure 1. Overlap of RAMs detected across the entire cohort (*A*) by drug class (*B*) and of resistance calls made across the entire cohort (*C*) by drug class (*D*). Abbreviations: DRM, drug resistance mutation; INSTI, integrase strand transfer inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleos(t)ide reverse transcriptase inhibitor; PI, protease inhibitor; RAMs, resistance-associated mutations.

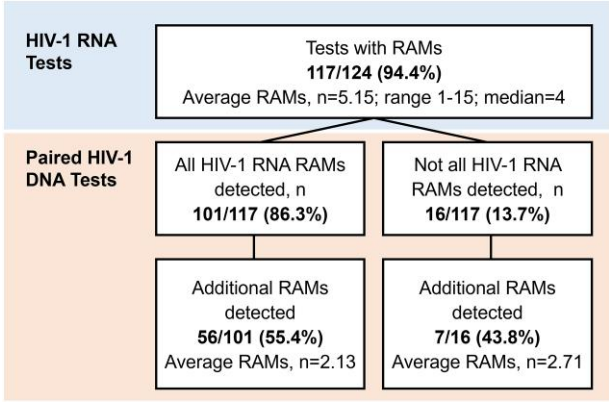

Figure 2. Detection of plasma virus (HIV-1 RNA) RAMs in the paired PBMC compartment (HIV-1 DNA) tests. Abbreviations: PBMC, peripheral blood mononuclear cell; RAMs, resistance-associated mutations.

We investigated whether resistance in the circulating plasma virus can be detected in the PBMC compartment during viremia. Among 117 HIV-1 RNA tests where ≥1 RAM was detected, paired HIV-1 DNA testing identified all of the RAMs in 101 (86.3%) cases and detected an average of 2.13 additional RAMs in 56 (55.4%) of these cases. In 16 cases where HIV-1 DNA testing missed ≥1 HIV-1 RNA RAM, an average of 2.71 additional RAMs were identified on 7 paired HIV-1 DNA tests (Figure 2). Overall, these data show that HIV-1 DNA testing captured all RAMs present in contemporaneous plasma virus in most cases and detected additional RAMs in approximately half of the cases.

The VL at time of resistance testing correlated with the percentage of plasma virus RAMs detected in the PBMC compartment ($r_s = 0.317$; $P < .001$) (Figure 3*A*), with a significant difference between patients with VL >10 000 c/mL vs those with VL <10 000 c/mL (mean, 98.8% vs 86.3%; $P = .007$) (Figure 3*B*). Additionally, the median VL of samples where ≥1 plasma virus RAM was missed by PBMC testing was significantly lower than the median VL of samples where all plasma virus RAMs were found in PBMCs (4970 c/mL vs 22 300 c/mL; $P = .001$) (Figure 3*C*). These observations are in line with prior findings [39,52] and suggest that virus variants may not become archived in the PBMC compartment efficiently until they reach a certain VL threshold.

In contrast, VL at time of resistance testing did not correlate with detection rates of PBMC compartment RAMs in plasma virus ($r_s = .073$; $P = .43$), and there was no difference between the median VLs of samples with and those without unique

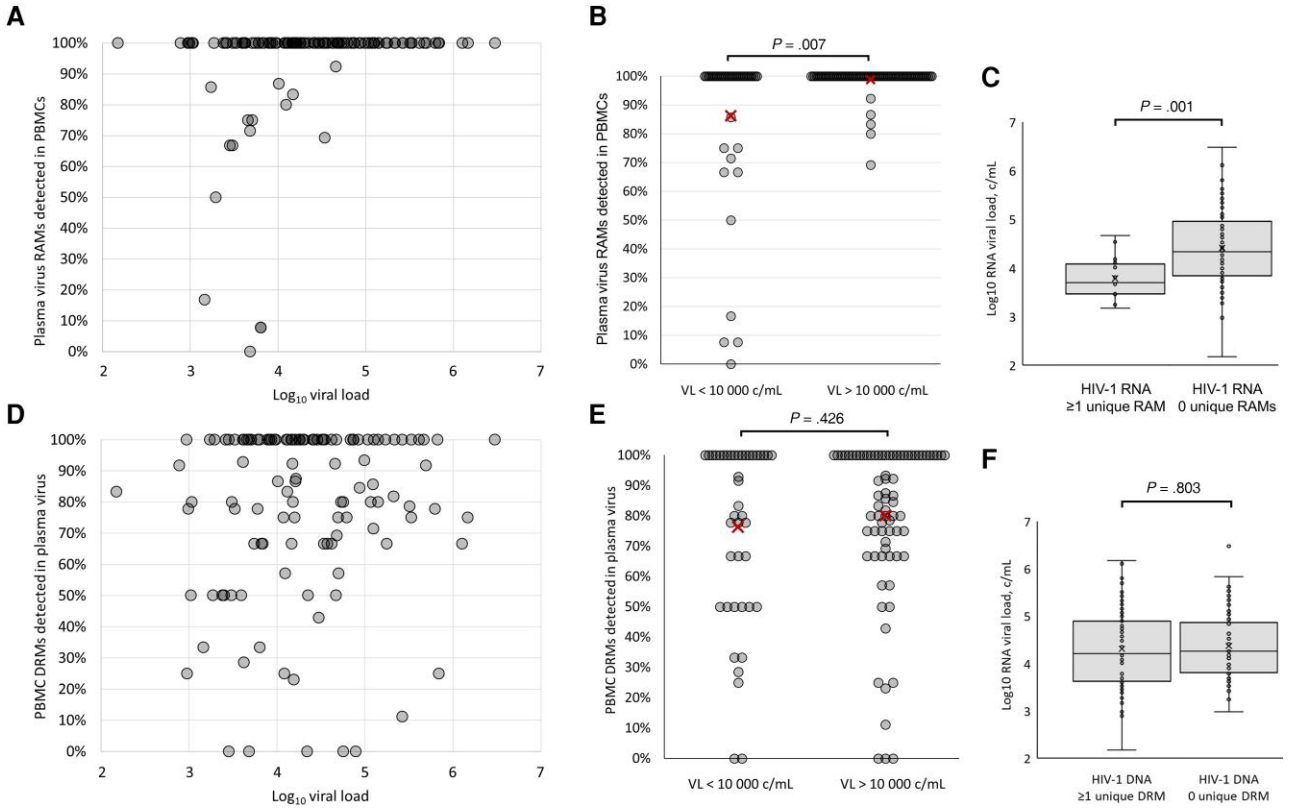

Figure 3. *A*, Association between viral load at the time of resistance testing and the percentage of plasma virus RAMs detected in PBMCs. *B*, Significant difference in the percentage of plasma virus RAMs detected in PBMCs by viral load at the time of testing. *C*, Significant difference in viral loads between test pairs without and with unique HIV-1 RNA RAMs. *D*, No correlation between viral load at the time of resistance testing and the percentage of PBMC RAMs detected in plasma virus. *E*, No significant difference in the percentage of PBMC RAMs detected in plasma virus by viral load at the time of testing. *F*, No significant difference in viral loads between test pairs with and without unique HIV-1 DNA RAMs. X, median. Abbreviations: DRMs, drug resistance mutations; PBMC, peripheral blood mononuclear cell; RAMs, resistance-associated mutations; VL, viral load.

PBMC RAMs (16 200 c/mL vs 18 100 c/mL; $P = .80$) (Figure 3*D*–*F*). Therefore, VL was not associated with emergence of variants from the PBMC compartment. These data also suggest that, despite efficient archiving of contemporaneous variants circulating during high-level viremia, viral genomes that were previously archived in PBMCs can also be identified with HIV-1 DNA testing.

Drug resistance can be lost from plasma virus in the absence of selective drug pressure, as occurs in patients who discontinue ART, or in newly diagnosed patients with transmitted RAMs for whom treatment is delayed. We evaluated whether HIV-1 DNA testing can identify drug resistance in the subset of patients with pan-sensitive plasma virus. Across 67 test pairs for 65 patients, 61 RAMs were found in PBMCs but not in plasma virus (Figure 4*A*–*D*) and were associated with resistance to ≥1 ARV in 13 cases (19.4%) (Figure 4*E*).

The median VL was not significantly different between HIV-1 DNA tests reporting resistance to ≥1 ARV and those reporting pan-sensitivity (23 200 c/mL vs 22 100 c/mL; $P = .88$) (Supplementary Figure 3*A*), and VL at the time of testing did not correlate with the number of ARVs to which resistance was reported ($r_s = -0.026$; $P = .83$) (Supplementary Figure 3*B*). These data demonstrate that PBMC testing identified drug resistance in viremic patients with pan-sensitive plasma virus in a manner independent of viral load.

DISCUSSION

The Centers for Disease Control and Prevention estimates that approximately one-third of people diagnosed with HIV in the United States were not virologically suppressed in 2019. Resistance to ARVs is a well-described cause of virologic failure and may contribute to low suppression rates. In this cohort, simultaneous plasma virus and PBMC testing was performed with intent to obtain as comprehensive a resistance profile as possible. Analysis of the results showed that plasma virus testing in patients with viremia frequently may not fully capture total drug resistance. Evaluation of proviral DNA in the PBMC compartment revealed archived RAMs that were absent from circulation in 63/117 (53.8%) cases.

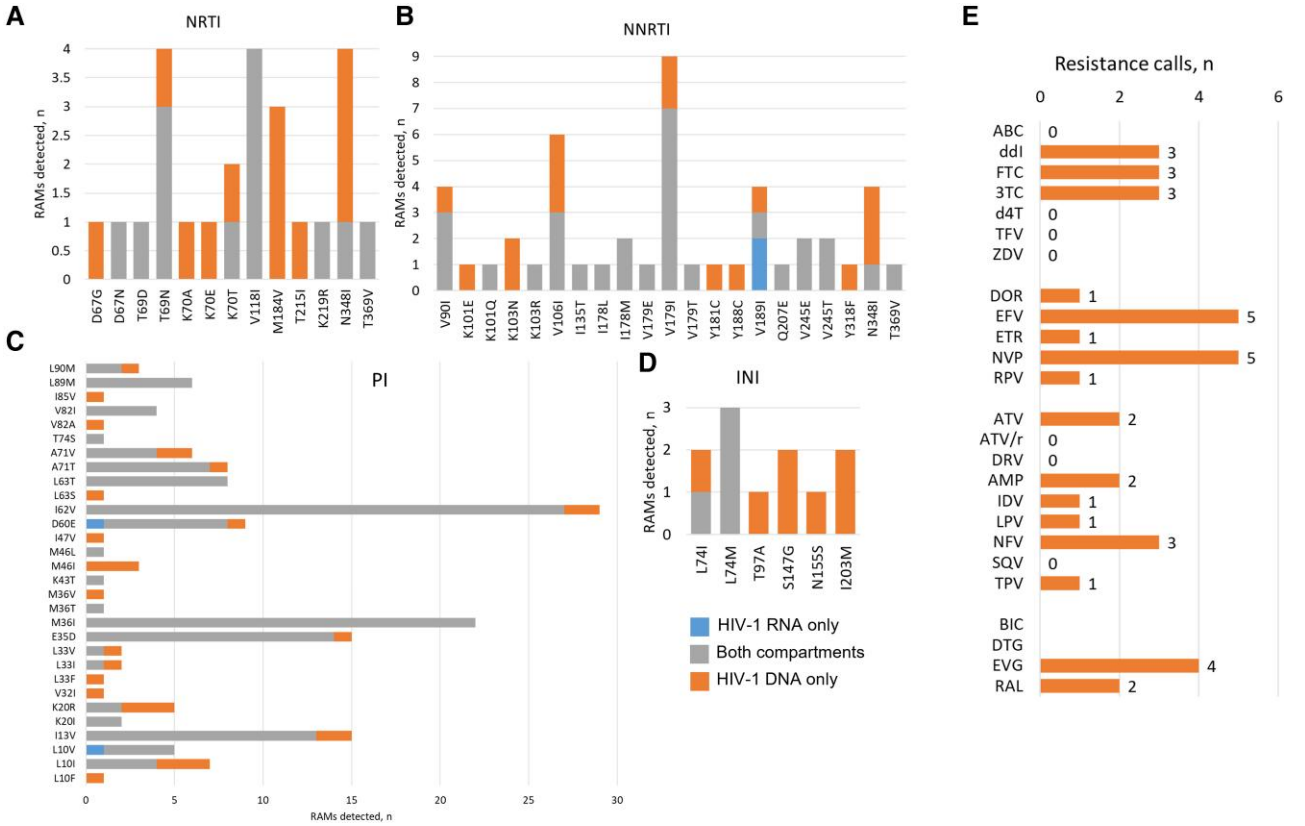

Figure 4. Mutations associated with resistance to NRTIs (*A*), NNRTIs (*B*), PIs (*C*), or INIs (*D*) detected in HIV-1 RNA only (blue bars), HIV-1 DNA only (orange bars), or both (gray bars) among patients with pan-sensitive plasma virus. *E*, Resistance to ARVs detected in HIV-1 DNA across 67 test pairs from 65 patients with pan-sensitive plasma virus. Abbreviations: ARVs, antivirals; INIs, integrase inhibitors; NNRTIs, non-nucleoside reverse transcriptase inhibitors; NRTIs, nucleos(t)ide reverse transcriptase inhibitors; PIs, protease inhibitors.

The loss of circulating drug-resistant virus occurs during the absence of selective drug pressure and poses a challenge to constructing suppressive regimens, especially among patients lost to care for extended periods. For this reason, the DHHS HIV treatment guidelines recommend that plasma virus drug resistance testing be performed while the patient is on the failing regimen or within 4 weeks of treatment discontinuation. The subgroup of patients in our cohort with wild-type plasma virus may represent treatment-naïve patients who acquired wild-type virus; treatment-naïve patients whose virus had RAMs that were lost due to delay in therapy; or treatment-experienced patients whose virus reverted to wild-type in the absence of ART. Because this was a retrospective database analysis, we did not have the necessary information to distinguish between these groups. Nonetheless, our analysis showed that PBMC testing identified drug resistance in 19.4% of patients with pan-sensitive plasma virus. These findings are in line with previous evidence showing that RAMs can be identified in plasma virus earlier than in PBMCs but persist longer in the latter compartment [49, 53–55].

The ability to detect plasma virus RAMs in PBMCs was associated with VL at the time of testing, with a greater percentage of HIV-1 RAMs detected in PBMCs among patients with VLs >10 000 c/mL than those with VLs <10 000 c/mL. These data corroborate previous findings [39] and suggest that the efficacy of viral archiving depends on the viral load reached by a particular variant.

M184V was the only major plasma virus NRTI RAM that was not captured in all paired PBMC tests; this occurred in 5 of 13 paired tests, all 5 of which were performed at VLs <10 000 c/mL and/or were present as mixtures with M184I, which precedes M184V [56], or with M184M. The existence of M184 mixtures in plasma, along with lower VLs, suggests that these patients were experiencing early virologic failure. Combined with the known fitness costs of the mutation and its rapid reversion [57–60], these observations suggest that the M184V variant may not have reached sufficient circulating levels to become archived in PBMCs at detectable frequencies.

Previous work demonstrated that pools of integrated viral genomes can expand through homeostatic T-cell proliferation, or contact with T-cell turnover [61, 62]. Therefore, persistence of RAMs in the PBMC compartment is likely subject to reservoir dynamics, with proliferation or contraction of T cells

encoding a drug resistance variant, increasing or decreasing the likelihood of its detection. However, failure to detect a previously documented RAM does not preclude its existence in a compartment inaccessible to blood draws, such as the central nervous system or the gut-associated lymphoid tissue.

A recent retrospective study investigated PBMC RAMs in 16 patients who maintained virologic suppression despite low genotypic susceptibility scores (GSS) to their current regimens, as predicted by historical plasma virus genotypes. Interestingly, PBMC testing predicted higher GSS, reflecting susceptibility to the patients' current regimens. The authors concluded that clearance of RAMs from the latent reservoir may explain successful virologic suppression in this cohort [63]. Larger studies are needed to confirm these findings and determine if failure to detect historical RAMs in the PBMC compartment may indicate renewed susceptibility to the associated ARVs. However, the dispersion of latently infected cells, the stochasticity of HIV-1 reservoir dynamics, and the ethical considerations of recycling ARVs to which patients have documented resistance may raise questions regarding the feasibility of such studies.

Our study has several limitations. First, we did not have historical plasma virus drug resistance records for our cohort. Therefore, we could not determine if the HIV-1 DNA tests provided information beyond historical records, nor could we assess the concordance between these sets of data. Studies evaluating concordance between drug resistance in historical HIV-1 RNA and HIV-1 DNA have reported values ranging from 28% to 100% [8, 10, 64–69]. These studies were performed in cohorts with different baseline characteristics and used various sample preparation methods, sequencing platforms, and analytical pipelines, making it difficult to compare the findings and attribute concordance rates to any particular factor.

In the characterization of the commercial proviral HIV-1 DNA assay used for the present study, 85% of historical plasma virus RAMs and 89% of resistance calls were captured in the PBMC compartment in 48 highly treatment-experienced patients with virologic suppression [68]. Similar results were reported in 2 subsequent studies using the same assay, where 93.7% and 88.9% of historical drug resistance mutations were detected in PBMCs in 140 and 9 patients with virologic suppression, respectively [67, 69]. In contrast, a recent analysis reported detection of only 50% of the historical plasma virus M184I/V in PBMCs [70]—a much lower proportion than reported elsewhere for these mutations [67, 68]. Given our present findings, an investigation of historical VLs reached by M184I/V variants in each study may help to elucidate these discrepancies.

In previous work, HIV-1 DNA testing identified resistance beyond that found in historical plasma virus resistance records [67, 69]. Mutations that appear incongruent with previously documented plasma virus resistance could reflect the following: incomplete or inaccurate historical data; failure to capture plasma virus resistance due to reversion of mutations to wild-type; platform differences in RAM detection limits among commercial assays; laboratory differences in data processing and RAM reporting thresholds; and failure to identify mutations that confer drug resistance due to immature genotypic data for newly approved drugs. RAMs uniquely identified in the PBMC compartment in our cohort may reflect all of these factors.

Another limitation of our study is the failure to document whether the simultaneous utilization of HIV-1 RNA and DNA testing altered clinical decision-making, as well as the virologic outcomes of these decisions. Ideally, such a testing approach would need to be prospectively evaluated. In prior studies among virologically suppressed patients, HIV-1 DNA testing resulted in a regimen change in 56% and 80% of cohorts, with 85% and 96% of patients maintaining virologic suppression, respectively [67, 69]. Because studies evaluating the use of HIV-1 DNA characteristically involve virologically suppressed patients, our study is considered exploratory in nature. It would be valuable to determine whether our viremic cohort achieved high suppression rates as well.

Lastly, plasma virus tests were performed using Sanger sequencing, while proviral HIV-1 DNA tests were run on a next-generation sequencing (NGS) platform. It is possible that the greater ability to detect drug resistance by proviral HIV-1 DNA testing reflects the lower RAM detection thresholds enabled by NGS. However, the HIV-1 DNA test reports RAMs at a sensitivity level equivalent to Sanger sequencing [71], minimizing the effects of sequencing platforms on result concordance.

Despite these limitations, our work provides evidence that proviral HIV-1 DNA testing can identify drug resistance in patients with viremia beyond that found in plasma virus. This may be particularly helpful in constructing suppressive regimens for patients whose plasma virus loses RAMs in the absence of selective drug pressure, as can be encountered among patients who fall out of care. In such patients, who may comprise as many as 40% of persons with diagnosed HIV [72], clinical management can be challenging because historical resistance data are frequently unavailable.

We also show that testing a single compartment—either plasma or PBMC—may not capture the full extent of HIV-1 drug resistance that exists in viremic patients. In clinical settings where achieving rapid virologic suppression is paramount, the analysis of both HIV-1 RNA and HIV-1 DNA may offer a more comprehensive drug resistance profile.

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Author contributions. C.M.W. and H.A.H. devised the study; D.C. conducted the data analysis; all authors contributed to data interpretation and development of the manuscript.

Patient consent. This retrospective database study of de-identified test results does not necessitate patient consent.

Data availability. Data are available upon request.

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