



Genotypic Resistance Testing of HIV-1 DNA in Peripheral Blood Mononuclear Cells

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SUMMARY HIV-1 DNA exists in nonintegrated linear and circular episomal forms and as integrated proviruses. In patients with plasma viremia, most peripheral blood mononuclear cell (PBMC) HIV-1 DNA consists of recently produced nonintegrated virus DNA while in patients with prolonged virological suppression (VS) on antiretroviral therapy (ART), most PBMC HIV-1 DNA consists of proviral DNA produced months to years earlier. Drug-resistance mutations (DRMs) in PBMCs are more likely to coexist with ancestral wild-type virus populations than they are in plasma, explaining why next-generation sequencing is particularly useful for the detection of PBMC-associated DRMs. In patients with ongoing high levels of active virus replication, the DRMs detected in PBMCs and in plasma are usually highly concordant. However, in patients with lower levels of virus replication, it may take several months for plasma virus DRMs to reach detectable levels in PBMCs. This time lag explains why, in patients with VS, PBMC genotypic resistance testing (GRT) is less sensitive than historical plasma virus GRT, if previous episodes of virological failure and emergent DRMs were either not prolonged or not associated with high levels of plasma viremia. Despite the increasing use of PBMC GRT in patients with VS, few studies have examined the predictive value of DRMs on the response to a simplified ART regimen. In this review, we summarize what is known about PBMC HIV-1 DNA dynamics, particularly in patients with suppressed plasma viremia, the methods used for PBMC HIV-1 GRT, and the scenarios in which PBMC GRT has been used clinically.

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INTRODUCTION

People with HIV-1 who have attained virological suppression (VS) on antiretroviral therapy (ART) often require changes to their therapy to avoid drug toxicity, intolerance, drug-drug interactions, and to improve adherence. In these patients, standard genotypic resistance testing (GRT) of plasma virus is not possible. HIV-1 DNA GRT of peripheral blood mononuclear cells (PBMCs) has increasingly been studied for the optimization of ART in patients with VS or low plasma virus levels, and in other scenarios in which plasma virus GRT might be insensitive for detecting drug-resistance mutations (DRMs). PBMC GRT results, however, are difficult to interpret because there are fewer data demonstrating the predictive value of PBMC GRT compared with plasma virus GRT.

Retrospective studies have shown that the presence of HIV-1 DRMs in plasma virus before the start of a new ART regimen are independent predictors of the virologic response to that regimen (1–8). Prospective and retrospective studies have shown that patients whose care providers have access to plasma virus GRT respond better to therapy than those whose providers do not (9–13). The accumulation of such retrospective and prospective data has led to the routine use of plasma GRT in the management of people with HIV-1 (14–16). Whereas it is unlikely that prospective clinical trials will be performed to evaluate the use of PBMC GRT, a critical mass of retrospective data that provides insight into scenarios in which PBMC GRT may be useful could be applied to clinical practice.

The topic of PBMC GRT intersects with recent research conducted to understand the replication-competent HIV-1 reservoir. Therefore, this review will begin with several sections summarizing recent studies on PBMC HIV-1 dynamics particularly in patients with suppressed plasma viremia, as understanding this foundational topic is important for the optimal use of PBMC GRT in clinical decision-making.

HIV DNA POPULATIONS

HIV-1 DNA exists in nonintegrated linear and circular episomal forms and as integrated proviruses. The biological significance of the nonintegrated forms of HIV-1 DNA is poorly understood. Both nonintegrated linear and episomal forms have short half-lives ranging from several days to weeks, although episomal forms can persist for longer periods of time (17–22). Consequently, within several months of VS, PBMC HIV-1 DNA consists primarily of integrated proviral DNA (23–27). Although PBMC HIV-1 GRT is often referred to as proviral DNA sequencing, PBMC GRT assays rarely distinguish between integrated and nonintegrated HIV-1 DNA. Additionally, the terms proviral DNA compartment and latent HIV reservoir have been used interchangeably because there is no way to determine which parts of the proviral DNA compartment are responsible for virological rebound in patients who discontinue ART.

The vast majority (>90%) of HIV-1-infected CD4⁺ T cells from peripheral blood and lymph node tissue contain only one integrated HIV-1 DNA molecule (28, 29). In the absence of therapy, HIV-1 DNA is present at a level ranging between 2.5 to 3.5 log copies/10⁶ PBMCs (20). Elite controllers and the rare posttreatment controllers who maintain VS off ART have been shown to average approximately 1.5 log copies/10⁶ PBMCs (20, 30–32). In research studies, GRT can be performed using sorted CD4⁺ lymphocytes. However, in clinical settings, GRT is performed using the complete PBMC population, which includes multiple cell types not infected by HIV-1, including monocytes, B lymphocytes, CD8⁺ T lymphocytes, and natural killer cells.

Complete proviral DNA genomic sequencing has revealed that approximately 90% to 95% of integrated HIV-1 genomes have major sequence abnormalities incompatible with viral replication, including large deletions and apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC)-mediated G-to-A hypermutation (23, 33, 34). The deletions result from replication errors as the reverse transcriptase (RT)

enzyme switches between paired RNA template genomes during reverse transcription. G-to-A hypermutation often results in lethal mutations, such as premature stop codons and mutations at highly conserved positions.

The frequency of replication-competent viruses that can be cultured in quantitative virus outgrowth assays (QVOAs) is nearly 100-fold lower than the number of nondefective proviral genomes (33). However, QVOAs provide only a minimal estimate of the number of nondefective proviral genomes because the single round of T cell stimulation performed in these assays is insufficient to activate all replication-competent proviruses and because not all activated proviruses are capable of establishing a spreading infection (33, 35). Thus, only a small proportion of the proviruses that appear genetically intact contribute to the replication-competent viral reservoir. Many additional proviruses may be epigenetically silenced as a result of repressive chromatin states (23) and transcriptional interference (36, 37). Moreover, some proviruses without obvious genetic defects may contain subtle genetic changes associated with reduced replication capacity.

PROVIRAL DNA DYNAMICS DURING ACTIVE VIRAL REPLICATION

Although activated CD4⁺ lymphocytes are most vulnerable to HIV-1 infection, they are also the cells most vulnerable to being eliminated following infection as a result of virus cytopathic effect, cytotoxic T cell (CTL) killing, or apoptosis. In contrast, memory CD4⁺ lymphocytes, the main reservoirs of latent virus, are less vulnerable to infection (38, 39). Based on these observations, it has been hypothesized that the latent virus reservoir is established when HIV-1 infects activated CD4⁺ T lymphocytes that transition to memory CD4⁺ lymphocytes before they are destroyed in what has been termed an “effector-to-memory transition” (23, 39).

In patients with acute infection, proviral DNA levels and sequence diversity increase during the first few years after infection (40–43). In the absence of ART, proviral viral DNA levels eventually reach an equilibrium between processes that increase and decrease their levels. Proviral DNA levels are increased when CD4⁺ lymphocytes are infected during periods of active virus replication and during clonal proliferation of latently infected cells. Clonal proliferation (Sidebar) has been reported primarily in patients with VS because proviral DNA sequence homogeneity is most readily detected in patients in whom active viral replication is not occurring (44–47). Proviral DNA levels decrease when viruses reactivate, resulting either in viral cytopathic effects or host cytolytic effects (48, 49). Nonviral causes of cell death, such as senescence, are also likely to reduce proviral DNA levels (50).

In untreated patients, the proviral DNA reservoir undergoes continuous evolution (51–54). In several studies, the plasma virus quasispecies in ART-naive patients at multiple time points over periods ranging from 3 to 10 years before ART initiation were compared with the PBMC virus quasispecies following ART-induced VS. One study specifically sequenced the replication-competent proviral DNA reservoir (51) while others examined the nondefective PBMC HIV-1 DNA population (52–54). In two studies, most proviral DNA sequences were closely related to the pretherapy sequences obtained in the months prior to ART initiation (51, 52), while in three other studies a significant proportion of proviral DNA sequences was closely related to earlier pretherapy sequences (53–55). Together, these studies indicate that in the absence of ART, the proviral DNA population is constantly evolving. The likelihood of detecting older circulating variants within this population is not a linear function of time likely because of the stochasticity with which proviral DNA sequences are eliminated by virus activation and CTL killing as opposed to being amplified by clonal proliferation.

PROVIRAL DNA DYNAMICS FOLLOWING ART INITIATION

In most patients initiating highly effective ART regimens, CD4⁺ lymphocytes no longer become infected (51, 52, 56–60). PBMC DNA levels decline approximately 0.5 to 1.0 log₁₀ copies/10⁶ cells within weeks to months because of a decline in linear and, to

some extent, episomal DNA but then plateau after one to 2 years (19, 43, 61–63). Following ART initiation, the turnover of the proviral DNA reservoir also slows. The estimated half-life of approximately 1 year observed for this compartment in untreated patients (51–54) lengthens to approximately 3 to 4 years in virologically suppressed patients (64–66). This slower turnover is believed to result from the progressive increase over time in the proportion of latently infected cells undergoing clonal proliferation (67) and from changes in the immunological environment, as both immune activation and CD4⁺ lymphocyte killing are reduced following VS (68).

In virologically suppressed patients, clonal proliferation of latently infected cells helps to stabilize the size of the proviral DNA reservoir, even though CD4⁺ lymphocytes are no longer being infected. Clonally expanded cells often eventually dominate the latent reservoir (67–72). Although clonal expansion of memory CD4⁺ lymphocytes containing both replication-competent and defective proviruses have been reported (23, 46, 69, 70, 73), there is likely to be a progressive enrichment of defective viruses since these are less likely to reactivate and lead to viral or cytotoxic T-cell killing (46, 71, 74, 75).

In patients with active virus replication and those with stable VS, the PBMC HIV-1 DNA population appears to be in equilibrium with the plasma virus RNA population and with the lymphoid HIV-1 DNA population (59, 60, 76, 77). Several studies have also shown that in patients who discontinue therapy, the rebounding plasma viral RNA population is closely related to the nondefective PBMC HIV-1 DNA population (55, 71, 78).

PBMC GRT METHODS

To perform PBMC GRT, DNA is extracted either from whole blood or, following density gradient centrifugation, from the fraction of the blood that contains lymphocytes and monocytes. The extracted DNA is then amplified using nested PCR (PCR). The number of viral genomes able to be sampled depends on the PBMC virus load, the number of PBMCs from which DNA is extracted, and the efficiency of DNA extraction and PCR. The number of sampled genomes may not be linearly related to the number of PBMCs used in an assay because the efficiency of PCR has been reported to be reduced when the amount of DNA in the assay is increased (79). Although PBMC HIV DNA levels are predictors of the response to therapy independent of the CD4⁺ count and plasma VL (20, 80, 81), these levels are rarely quantified when PBMC HIV GRT is performed. Additionally, in most publications, the number of PBMCs used for GRT is not reported.

PBMC HIV-1 DNA GRT has been performed using both dideoxy-terminator Sanger sequencing and next-generation sequencing (NGS) technologies. In a clinical sample, Sanger sequencing can detect the presence of mutations at levels of approximately 20% in the viral population (82–84). In contrast, the proportion at which variants can be detected by NGS depends on the selected threshold. Although mutation-detection thresholds as low as 1% have been used in research studies, most clinical applications of NGS have used thresholds between 5% and 10% in part to avoid the increased risk of sequence artifact that occurs at lower thresholds (85–87). Indeed, the widely used commercial PBMC GRT assay developed by Monogram Biosciences called the GenoSure Archive assay uses NGS with a mutation-detection threshold of about 10% (88–91).

There appears to be greater intrasample stochastic variation in the detection of DRMs in PBMC than in plasma, probably because DRMs are more likely to coexist with ancestral wild-type virus populations in PBMCs than in plasma (92–94). Indeed, a recent study in which two to four GenoSure Archive assays were performed on 90 samples from 70 patients with VS indicated that the mean reproducibility at detecting DRMs was approximately 80% (93). In another study of 55 patients who underwent three PBMC GRTs at different times also using the GenoSure Archive assay, the overall re-detection rate for 178 DRMs detected at the first time point was also 80% (89). Sampling bias is likely to affect re-detection rates particularly when the copy number of DRMs is low.

The interpretation of PBMC GRT results requires an approach that considers the near universal presence of APOBEC-mediated G-to-A hypermutation at some level within PBMC HIV-1 DNA (Sidebar). APOBEC3F and 3G are host enzymes that cripple viral genomes by indiscriminately mutating the dinucleotides GG→AG and GA→AA, respectively (95). DRMs resulting from G-to-A hypermutation (rather than drug selection pressure) are likely to be present in nonfunctional viruses that also contain stop codons and other crippling mutations. Ignoring all DRMs that could have resulted from a GG→AG or GA→AA change, however, would reduce the sensitivity for detecting HIV drug resistance (HIVDR), as nearly 20 DRMs arise in one of these two dual nucleotide contexts: most commonly the nucleoside RT inhibitor (NRTI)-resistance mutation M184I, the protease inhibitor (PI)-resistance mutation M46I, the nonnucleoside RT inhibitor (NNRTI)-resistance mutations E138K and G190S, and the integrase strand transfer inhibitor (INSTI)-resistance mutations G118R, G140S, and R263K. Several of the DRMs that are commonly caused by G-to-A hypermutation, such as the rilpivirine-associated mutation M230I and the cabotegravir-associated mutation G140R, are extremely rare even in the presence of selective drug pressure (96–98). Two approaches to minimize reporting of APOBEC-mediated DRMs can be applied using NGS: (i) excluding sequence reads that appear to be hypermutated; and (ii) determining the proportion of sequences that appear to be hypermutated and then reporting only those APOBEC-context DRMs that occur above this proportion (99).

PBMC DNA GRT DURING ACTIVE REPLICATION

Several studies have compared the results of PBMC and plasma virus GRT in persons with detectable viremia. Table 1 shows data from six of the largest studies of ART-naive individuals undergoing simultaneous PBMC and plasma virus GRT. There was complete concordance between PBMC and plasma for the one study in which all patients were acutely infected (100). In four studies, DRMs were more likely to be detected only by PBMC GRT (98, 101–103). However, in one of these four studies, eight of the nine additional DRMs detected by PBMC GRT occurred in an APOBEC dinucleotide context, including four instances of the otherwise extremely rare DRM M230I (98). The finding that PBMC GRT was usually more sensitive than plasma virus GRT at detecting DRMs in ART-naive persons with transmitted drug resistance (TDR) is consistent with the established observation that many transmitted DRMs are out-competed in plasma by more fit wild-type revertants (104, 105).

Table 2 shows data from 13 of the largest studies of ART-experienced persons undergoing simultaneous PBMC and plasma virus GRT. In contrast to the scenario in ART-naive patients, PBMC GRT can be less sensitive than plasma virus GRT for detecting DRMs in ART-experienced patients because there is often a lag of weeks to months before circulating DRMs reach sufficiently high levels in PBMCs to become detectable. This lag is especially prolonged in patients with low plasma HIV-1 RNA levels. Indeed, three smaller studies (not included in Table 2) that reported the results of simultaneous PBMC and plasma virus GRT at multiple time points supported this observation. In one of these studies in which nine patients received nonsuppressive therapy with a lamivudine-containing regimen, the lamivudine-resistance mutation M184V required 24 weeks to reach levels in PBMC between 10% and 80% (106). In a second study of 107 sequential samples from 22 patients with multiple virological failures (VFs) on a PI-containing regimen, 53 of 58 PI-associated DRMs emerged first in plasma (107). Furthermore, plasma virus GRT detected these DRMs 425 days earlier than PBMC GRT when plasma HIV-1 RNA levels were <4.0 log copies/mL compared with 225 days when levels were ≥4.0 log copies/mL (107). In a third study in which NGS was used to detect INSTI-associated DRMs in four patients with VF on a raltegravir-containing regimen, DRMs were detected by NGS at levels of 0%, 1%, 11%, and 36% in PBMCs versus 78% to 100% in plasma between 6 to 12 months following VF (108).

TABLE 1 Comparison of HIV-1 PBMC and plasma virus GRT in ART-naive patients

| Study author and year | No. of patients | Patient characteristics | Genes | Sequencing method | No. of patients with DRMs and no. of DRMs ^a | No. of DRMs detected by plasma and PBMC GRT | No. of DRMs detected by plasma GRT alone | No. of DRMs detected by PBMC GRT alone |
|------------------------------|-----------------|--|-------|--------------------------------|--|---|--|--|
| Ghosh 2006 (100) | 44 | Acutely infected; known transmitted drug resistance. | PR/RT | Sanger sequencing | 44 patients with 150 DRMs | 150 (100%) | 0 (0%) | 0 (0%) |
| Vicenti 2007 (219) | 169 | Most chronically infected. | PR/RT | Sanger sequencing (in GenBank) | 24 patients with 65 DRMs | 53 (81%) | 5 (8%) | 7 (11%) |
| Parisi 2007 (101) | 288 | 80% chronically and 20% recently infected. | PR/RT | Sanger sequencing | 80 patients with 170 DRMs | 71 (42%) | 39 (23%) | 60 (35%) |
| Bon 2007 (102) | 31 | Most chronically infected. | PR/RT | Sanger sequencing | 17 patients with 30 DRMs | 16 (53%) | 0 (0%) | 14 (47%) |
| Kabamba-Mukadi 2010 (103) | 44 | Most chronically infected. | PR/RT | Sanger sequencing | 8 patients with 11 DRMs | 4 (36%) | 1 (9%) | 6 (55%) |
| Huruy 2018 (98) ^b | 41 | Most chronically infected. | PR/RT | Sanger sequencing (in GenBank) | 11 patients with 15 DRMs | 4 (27%) | 2 (13%) | 9 (60%) |

^aWhenever possible the drug-resistance mutations (DRMs) included the predominantly nonpolymorphic mutations used by the Stanford HIV Drug Resistance Database (HIVDB) sequence interpretation program. Data for Parisi 2007 were obtained based on the presence of 8 major NRTI DRMs, 4 major NNRTI DRMs, and 6 major PI DRMs.

^bAlthough the sequences did not have evidence for G-to-A hypermutation, 8 of the 9 DRMs detected only in PBMCs in Huruy 2018 occurred in an APOBEC context, including the otherwise rare mutation M230I which was detected in 4 patients. One study that performed 454 NGS on plasma and PBMC samples from 20 patients with evidence for TDR by Sanger sequencing and which reported extensive G-to-A hypermutation in the PBMC sequences is not shown (220). Studies comparing the prevalence of DRMs in plasma and PBMCs using point-mutation assays are not shown (221, 222).

TABLE 2 Comparison of HIV-1 PBMC and plasma virus GRT in ART-experienced patients

| Study author and year | No. of patients | Patient characteristics | Genes | Sequencing Method ^a | Total no. of DRMs ^b | No. of DRMs detected by | |
|---------------------------------|-----------------|-----------------------------|----------|--|--------------------------------|-------------------------|------------|
| | | | | | | plasma and PBMC GRT | plasma GRT |
| Devereux 2000 (223) | 12 | Multiple past ART regimens. | PR/RT | Sanger sequencing (in GenBank) | 44 | 39 (89%) | 1 (2%) |
| Venturi 2002 (116) | 58 | First ART VF. | PR/RT | Sanger sequencing (in GenBank) | 129 | 78 (60.5%) | 44 (34.1%) |
| Chew 2005 (224) | 34 | ART history not described. | PR/RT | Sanger sequencing | 127 | 83 (65%) | 35 (28%) |
| Turriziani 2007 (225) | 95 | Mean ART history: 7 yrs. | PR/RT | Sanger sequencing | 85 patients had 676 DRMs | 483 (71%) | 122 (18%) |
| Saracino 2008 (226) | 73 | First and second ART VF. | PR/RT | Sanger sequencing (in GenBank) | 438 | 179 (41%) | 235 (54%) |
| Diallo 2012 (227) | 76 | Median ART history: 3 yrs. | PR/RT | Sanger sequencing (in GenBank) | 416 | 380 (91%) | 15 (4%) |
| Derache 2015 (228) | 18 | Median ART history: 4 yrs. | PR/RT | Sanger sequencing (in GenBank) | 8 patients had 31 DRMs | 25 (81%) | 6 (19%) |
| Lubke 2015 (229) | 30 | ART history not described. | PR/RT | Sanger sequencing | 25 patients had 169 DRMs | 128 (76%) | 39 (23%) |
| Michelini 2016 (164) | 12 | Median ART history: 12 yrs. | PR/RT | Sanger sequencing | 136 | 90 (66%) | 18 (13%) |
| Sotillo 2018 (109) ^c | 12 | ART history not described. | PR/RT | Sanger historical sequencing, single genome sequencing | 79 | 38 (48%) | 29 (37%) |
| Milne 2019 (230) ^d | 26 | History of PMTCT. | RT | NGS at a 10% mutation-detection threshold for PBMC | 13 patients had 29 DRMs | 25 (86%) | 1 (4%) |
| Curanovic 2020 (90) | 89 | ART history not described. | PR/RT/IN | NGS at a 10% mutation-detection threshold | 676 | 505 (75%) | 43 (6%) |
| Armenia 2022 (110) | 20 | Multiple past ART regimens. | PR/RT/IN | NGS at a 1% mutation-detection threshold | 255 | 169 (66%) | 16 (6%) |

^aNGS was performed using Illumina technology. Curanovic 2020 used the Monogram Biosciences GenoSure Archive assay.

^bFor those studies in GenBank or for which mutations are present in tables, DRM totals are based on those DRMs used by the Stanford HIV Drug Resistance Database interpretation program. A subset of samples in two studies (90, 225) included several highly polymorphic mutations comprising approximately 10% and 30% of mutations in these studies, respectively.

^cIn Sotillo 2018, 5 of the 12 patients had undetectable VL at the time of PBMC GRT.

^dPrevention of mother-to-child transmission (PMTCT) with single-dose nevirapine with or without zidovudine plus lamivudine. Additional studies that contained fewer patients (106, 108, 231), that did not report DRMs (232), or that compared plasma virus with cultured PBMCs (233) are not included.

However, in studies of highly treatment-experienced patients, PBMC GRT can be nearly as sensitive or more sensitive than simultaneous plasma virus GRT for detecting DRMs, particularly when NGS is used (90, 109, 110). In one of these studies, which employed a mutation-detection threshold of 5%, many of the PBMC GRT DRMs were detected on historical plasma virus GRTs but not in simultaneously performed plasma virus GRT even at the same threshold (110) (Table 2).

In one clinical trial that is not shown in Table 2, 492 ART-naive patients were randomized to undergo PBMC GRT using an oligonucleotide ligation assay to detect three NNRTI and two NRTI mutations, and 495 were randomized to undergo standard-of-care which did not involve GRT (111). Participants randomized to the GRT arm initiated ART with a ritonavir-boosted lopinavir (lopinavir/r)-containing regimen if they were found to have any of the five tested mutations. The remaining participants in the GRT arm and all of the participants in the standard-of-care arm were randomized to a first line NNRTI-containing regimen. PBMC GRT was found to reduce the risk of VF at 12 months in the subset of participants with TDR. Although simultaneous plasma sequencing was not performed, this trial indicates that there are scenarios in which clinical investigators have chosen to perform PBMC GRT on ART-naive patients possibly because of the potential increased sensitivity of detecting DRMs in PBMC in patients with TDR or because PBMC samples are easier to transport.

ART Discontinuation

In ART-experienced patients who develop VF as a result of HIVDR and discontinue therapy, plasma viruses containing DRMs are usually rapidly outcompeted by ancestral wild-type viruses established in viral reservoirs prior to ART initiation because wild-type viruses replicate better in the absence of ART (112–118). Indeed, by 2 to 3 months after ART discontinuation of therapy, approximately 60% to 90% of nonpolymorphic DRMs are no longer detectable in plasma by Sanger sequencing (112, 114, 116, 117). Limited data suggest DRMs may be detectable for a slightly longer period of time by PBMC compared with plasma virus GRT using Sanger sequencing (112, 114–117). Indeed, in one study of 66 patients who had wild-type plasma viremia after discontinuing ART, only 20% had DRMs detectable by PBMC GRT using NGS at a mutation-detection threshold of 10% (91). In this study, the time since the patients had discontinued therapy was not reported. One likely reason the sensitivity of PBMC GRT might show little improvement over that of plasma virus GRT in this scenario is because in the absence of therapy, there is a rapid accumulation of recently produced wild-type linear and episomal viral DNA within PBMC to some extent obscuring the proviral DNA population. Moreover, if the time since discontinuing therapy was more than several months, the proviral DNA population may have also undergone significant turnover.

Low-Level Viremia

A confirmed plasma HIV-1 RNA level between 200 and 1,000 copies/mL is associated with an increased risk of further elevations in virus load and the emergence of HIVDR (119–128). An increasing number of studies have also reported that patients with persistent plasma HIV-1 RNA levels between 50 to 200 copies/mL, referred to as low-level viremia, are also at an increased risk of VF (123, 125, 129–132).

Low-level viremia in patients receiving ART may result from two distinct processes that have different implications for patient management. In some patients, low-level viremia results from ongoing viral replication and is associated with the risk of emergent HIVDR and further elevations in virus load (133). In other patients, it results from the activation of virus in latently infected, clonally proliferated cells (134–136). Although neither plasma virus nor PBMC GRT can definitively distinguish between these two processes, the detection of HIVDR by GRT by either method could prompt a change in therapy to reduce the risk of further elevations in virus load (137–139). The proportion of patients for whom plasma virus GRT is successful is reduced at plasma virus levels below 1,000 copies/mL and is usually less than 70% at levels below 200 copies/mL (137–143).

Most studies of PBMC GRT in patients with low-level viremia pooled GRT results from patients who had low-level viremia with those who had complete VS (144–149). Only small numbers of PBMC GRT results in patients with low-level viremia have been correlated with either historical (145, 146, 148, 149) or simultaneous plasma virus GRT results (145). In these studies, approximately half of the mutations identified by historical plasma virus GRT and a slightly higher proportion of the mutations identified by simultaneous plasma virus GRT were detected by PBMC GRT (145, 146, 148, 149). In addition, in one of the smaller studies, PBMC GRT did not detect DRMs associated with patients' most recent regimens, suggesting that if DRMs had developed, they were not yet detectable in PBMCs (147). These findings are consistent with the time lag of weeks to months before emergent DRMs become detectable by PBMC GRT in patients with low plasma virus levels (107, 108). Nonetheless, detecting DRMs in unintegrated viral DNA may be possible as these are more likely to reflect recently circulating viruses (20, 150–152).

PBMC DNA GRT DURING STABLE VIROLOGICAL SUPPRESSION

In patients with stable VS, several studies have assessed the concordance between PBMC and historical plasma virus GRT. Table 3 shows those studies that compared the frequency with which PBMC and historical plasma virus GRT detected DRMs that were detected by either approach (94, 97, 146, 149, 153–155). Table 4 shows those studies that compared the frequency with which PBMC GRT detected M184V/I in patients for whom this mutation was previously detected by historical plasma virus GRT (156–161). In the studies shown in Table 3, PBMC GRT was less sensitive than historical plasma virus GRT for detecting HIV-1 DRMs in five of seven studies (94, 97, 146, 149, 153). The sensitivity of PBMC GRT was higher in patients with a greater number of past VFs and with VFs of longer duration (146, 154, 161), as well as in patients with a higher plasma VL at the time of VF and a higher PBMC VL at the time of PBMC GRT (161). The one study in which PBMC GRT detected more DRMs than historical plasma virus GRT was a clinical trial involving patients who had not experienced VF and who therefore had TDR for which PBMC GRT is more sensitive than plasma virus GRT (155).

In the studies shown in Table 4, PBMC GRT using Sanger sequencing detected M184V/I in about 25% of those in whom it had been detected by historical plasma virus GRT. In contrast, when PBMC GRT was performed using NGS, M184V/I was detected in 40% to 90% of patients, with higher sensitivities reported at the lower mutation-detection thresholds.

Sequential PBMC GRT in the setting of stable VS has been described in at least four studies (Table 5). In one study, there was a marked reduction in the detection of DRMs in PBMCs over a period of 5 years (162). In this study, the number of NRTI-associated DRMs decreased from 40 to 14 in 10 patients receiving an NRTI-sparing suppressive regimen and the number of NNRTI-associated DRMs decreased from 10 to 0 in 11 patients receiving an NNRTI-sparing regimen. The reduced detectability of DRMs in this population may reflect the fact that in nine of the patients with clearance of PBMC DRMs, there were periods of residual viremia that may have generated new linear and episomal DNA DRMs that obscured persistent proviral DNA DRMs. In two of the remaining three studies, there were modest reductions in the detectability of PBMC DRMs over a period of 24 to 72 months (163, 164). In a fourth study of 20 heavily treated patients who possessed a mean of 12 RT and protease DRMs detected by PBMC GRT prior to VS, there was little change in the number of DRMs after 18 months of VS (165).

Genotypic testing has also been performed to determine HIV-1 tropism and the likely response to the CCR5 inhibitor maraviroc. Such testing involves sequencing the HIV-1 envelope gp120 V3 loop often in combination with other parts of gp120 (166). The detection of envelope variants that are associated with CXCR4 tropism is a contraindication to prescribing maraviroc, even if these variants represent only a small proportion of a patient's virus population. Although phenotyping is more sensitive for detecting minor populations of CXCR4 tropic viruses, NGS GRT is more widely available and is considered an acceptable substitute for phenotypic testing (166). The determination of tropism by simultaneous plasma virus and PBMC sequencing have been

TABLE 3 Comparison of HIV-1 PBMC GRT with historical plasma GRT in ART-experienced patients with VS^a

| Study author and year | No. of patients | Characteristics | ART history (yrs) | VS duration (yrs) ^b | Genes | Sequencing method ^c | Plasma DRMs ^d | PBMC DRMs ^e |
|---------------------------------------|-----------------|--|-------------------|--------------------------------|----------|---|--------------------------|------------------------|
| Verhofstede 2004 (154) | 11 | History of HIVDR on suboptimal ART. | 8.5 | 5 | RT | Sanger sequencing of 3 to 8 limiting-dilution clones (in GenBank) | 47 (96%) | 45 (92%) |
| Wirden 2011 (149) | 151 | Heavily treated; VS (70%) or LLV (30%); median 3 historical GRTs. | NA | NA | PR/RT | Sanger sequencing | NA (94%) | NA (61%) |
| DeLaugerre 2012 (153) ^f | 121 | Heavily treated patients in the ANRS EASIER Trial; median 4 historical GRTs. | 13.6 | 0.25 | PR/RT | Sanger sequencing | 1408 (NA) | 889 (NA) |
| Zaccarelli 2016 (146) | 149 | VS (~60%) or LLV (~40%) with ≥ 2 historical GRTs. | 8 | NA | PR/RT/IN | Sanger sequencing | 677 (94%) | 304 (42%) |
| Lambert-Niclot 2016 (97) ^g | 114 | History of VF. | 3.1 | ≥ 1 | RT | Sanger sequencing | 101 (NA) | 71 (NA) |
| Porter 2016 (155) | 51 | Clinical trial participants with transmitted K103N without a history of VF. | 2.4 | ≥ 0.5 | RT | NGS at a 10% mutation-detection threshold | 46 (69%) | 62 (92%) |
| Hoffmann 2022 (94) ^h | 96 | Patients with history of DRMs associated with ≥ 3 ART classes. | NA | 9 | PR/RT/IN | NGS at a 15% mutation-detection threshold | 872 (93%) | 610 (65%) |
| | | | | | | NGS at a 1% mutation detection threshold | 872 (84%) | 831 (80%) |

^aNA, details not available or not provided in the study.

^bFor all studies, VS was defined as a plasma HIV-1 RNA level <50 copies/mL except for DeLaugerre 2012 which included patients with levels <400 copies/mL.

^cIllumina NGS technology was used for Porter 2016 and Hoffmann 2022. The Monogram Biosciences GenoSure Archive assay, which uses a mutation-detection threshold of 10%, was used for Porter 2016.

^dNumber of DRMs detected in plasma with the percentage indicating the number of DRMs detected in plasma/(number of DRMs detected in plasma + number of DRMs detected in PBMCs).

^eNumber of DRMs detected in PBMC with percentage indicating the number of DRMs detected in PBMC/(number of DRMs detected in PBMC + number of DRMs detected in plasma). The plasma DRMs percentage was below 100% when PBMC GRT detected DRMs that were not present by historical plasma virus GRT.

^fSuccessful genotyping of both PR and RT in 121 patients; RT alone in 128 patients; and PR alone in 156 patients. A total of 34 nonpolymorphic RT and PR mutations were evaluated.

^gOnly DRMs associated with tenofovir, emtricitabine, and rilpivirine were reported.

^hPBMC NGS was performed at two time points (2017, 2020) and compared to historical plasma GRT; percentage is based on total number of DRMs identified at all time points assessed. In an additional study, PBMC GRT and historical plasma virus GRT were compared for their ability to detect resistance to seven ARVs but data on DRMs were not provided (148).

TABLE 4 HIV-1 PBMC GRT in ART-experienced patients with VS and M184V/I by historical plasma virus GRT^a

| Study author and year | No. of patients with past M184V/I ^b | Patient characteristics | ART history (yrs) | VS duration (yrs) ^c | Sequencing method ^d | Detection of M184V/I by PBMC GRT |
|----------------------------|--|---|-------------------|--------------------------------|--|--|
| Charpentier 2017 (156) | 8 of 18 tested | Heavily treated; DOLULAM cohort; 18 of 27 had undergone historical plasma virus GRT. | 18 | 4.3 | NGS at mutation-detection threshold of 1% | 38% |
| DeMiguel 2020 (157) | 21 of 41 | ART-PRO single-arm trial. | 18 | 6 | NGS at 20%, 10%, 5%, 1% mutation-detection thresholds | 33%, 52%, 67%, and 95% at 20%, 10%, 5%, and 1% mutation-detection thresholds, respectively. |
| Margot 2020 (158) | 84 | GS-US-282-1824 EVG/c/F/TAF trial participants with history of M184V/I. | 17 | 7 | NGS at 10% mutation-detection threshold | 48% |
| Montejano 2021 (159) | 52 | ART-experienced GEN-PRO cohort with history of M184V/I. | 19 | 8.7 | Sanger sequencing NGS at 20%, 10%, 5%, 1% mutation-detection thresholds | 27% 48%, 65%, 75%, and 88% at 20%, 10%, 5%, and 1% mutation-detection thresholds, respectively. |
| Jimenez de Ory 2021 (160) | 12 | Perinatally infected youth with history of M184V. | 13.5 | 4 | Sanger sequencing | 25% |
| Delaugerre 2021 (161, 234) | 242 of 252 | MOBIDIP participants with history NRTI/NNRTI resistance on WHO 1 st -line ART with VS on 2 nd -line PI-based ART. | 7.5 | 3 | NGS at 5% and 1% mutation-detection thresholds | 72% and 81% at 5% and 1% mutation-detection thresholds, respectively. |

^aHistorical plasma virus GRT was performed using Sanger sequencing in all cases.

^bJimenez De Ory 2021 did not include M184I.

^cPlasma HIV-1 RNA level <50 copies/mL and for ≥6 months.

^dIllumina technology was used for all NGS studies. Margot 2020 used the Monogram Biosciences GenoSure Archive assay.

TABLE 5 Studies Tracking DRMs at multiple time points by PBMCGRT in patients with VS

| Study author and year | No. of patients | Patient characteristics | VS (yrs) | Genes | Sequencing method ^a | No. of baseline DRMs ^b | No. of follow-up DRMs ^b | Notes |
|--------------------------------|-----------------|--|----------|-------|--|-----------------------------------|------------------------------------|---|
| Falasca 2013 (165) | 20 | VS after salvage ART with a DRV/r-based regimen. | 1.5 | PR/RT | Sanger sequencing | ~240 | ~240 | In 6 patients, the no. of DRMs increased. In 3 patients, the no. of DRMs decreased. The specific DRMs were not reported. |
| Gantner 2016 (163) | 10 | VS after salvage ART (INNOVE and ANRS 123 ETOILE studies). | 2–6 | PR/RT | NGS at a mutation-detection threshold of 1% | 69 | 44 | In 3 patients, all 23 baseline DRMs were no longer detected after 48–72 mo. In 1 patient, 6 new DRMs were detected. No meaningful change observed in 6 patients. |
| Michelini 2016 (164) | 12 | VS after salvage ART. | 2 | PR/RT | Sanger sequencing | 118 | 100 | In 6 patients, 22 baseline DRMs were no longer detected. In 3 patients, 4 new DRMs were detected. |
| Nouchi 2018 (162) ^c | 21 | VS on an NRTI- (<i>n</i> = 10) or NNRTI-sparing (<i>n</i> = 11) regimen. | 5 | RT | Sanger sequencing and NGS at a 1% mutation-detection threshold | 57 | 21 | In 10 patients receiving an NRTI-sparing regimen, the no. of detectable NRTI DRMs with a proportion > 10% decreased from 40 to 14. In 11 patients receiving an NNRTI-sparing regimen, the no. of detectable nonpolymorphic NNRTI DRMs with a proportion > 10% decreased from 10 to 0. |

^a454 technology was used for Gantner 2016 and Illumina NGS technology was used for Nouchi 2018.

^bIn studies for which mutations were present in tables, the DRM totals were based on those DRMs used by the Stanford HIV Drug Resistance Database interpretation program.

^cIn Nouchi 2018, residual viremia was found at least once during the 5 years in 13 patients of whom 9 showed progressive clearance of archived DRMs. DRV/r, ritonavir-boosted darunavir.

about 85% concordant (167–169). Furthermore, the determination of tropism by PBMC sequencing has been used as an inclusion criterion for clinical trials that have involved switching patients with VS to a maraviroc-containing regimen (170).

CLINICAL SIGNIFICANCE OF PBMC DNA GRT DURING STABLE VIROLOGICAL SUPPRESSION

The first clinical trial which involved changing therapy in patients with VS randomized patients with plasma HIV-1 RNA levels below 200 copies/mL for 16 to 24 weeks who were receiving zidovudine/lamivudine/indinavir to either zidovudine/lamivudine alone, indinavir alone, or continued three-drug therapy (171). Patients who were randomized to zidovudine/lamivudine or indinavir were more likely to experience VF than those who continued three drug-therapy (23% versus 4%; $P < 0.001$). Although PBMC GRT was not performed in this trial, one of two other subsequent studies that involved switching patients with VS on a PI-containing regimen to a triple NRTI-containing regimen containing abacavir/lamivudine/zidovudine reported that the detection of NRTI-associated mutations by PBMC GRT increased the risk of VF (172, 173) (Table 6).

In 2010, the SWITCHMRK I and II trials reported that patients with VS (defined as having a plasma HIV-1 RNA level <50 or <75 copies/mL for three or more months) on a lopinavir/r-containing regimen who substituted raltegravir for lopinavir/r had a 6.2% increased risk of VF at week 24 (95% CI: 1.3%–11.2%) compared to those who continued lopinavir/r (174). Although baseline PBMC GRT was not performed in this trial, the finding that the increased risk of VF was confined to patients who had a history of VF led to the hypothesis that pretherapy NRTI-resistance was likely responsible for the increased risk of VF in the raltegravir arms.

During the past 10 years, there have been many clinical trials in patients with stable VS that involved changing therapy from a PI-containing regimen to an NNRTI- or INSTI-containing regimen. The inclusion criteria of most of these trials were designed to minimize the risk of VF and emergent HIVDR. Indeed, tenofovir/emtricitabine/rilpivirine (155), dolutegravir/lamivudine (175–180), dolutegravir/rilpivirine (181), and long-acting cabotegravir/rilpivirine (182, 183) were usually studied in patients without a history of VF or DRMs associated with lamivudine, rilpivirine, or INSTI resistance. Although VFs were rare with these regimens, the few patients with VF and emergent HIVDR appeared to be more likely to have had preexisting DRMs detectable by PBMC GRT than those without VF (155, 184, 185) (Table 6). In contrast, several three-drug regimens, including tenofovir/emtricitabine/bictegravir (186), tenofovir/emtricitabine/elvitegravir/cobicistat (187), and abacavir/lamivudine/dolutegravir (188) were shown to be efficacious at maintaining VS even in patients with a history of lamivudine resistance.

During the past 5 years, there have been four clinical trials in which dolutegravir monotherapy has been investigated for ART simplification. In a meta-analysis of these trials, the risk of VF among patients randomized to dolutegravir monotherapy was 7% (16/227) compared with 0% (0/189; $P < 0.001$) for patients continuing their previous ART regimen (189). Among 15 patients who underwent integrase sequencing at the time of VF, seven (3.1% of the total) had newly developed INSTI-resistance mutations. Four factors were identified as risk factors for VF, including a PBMC virus level ≥ 2.7 copies/mL, a $CD4^+$ count <350 copies/mL, a plasma HIV-1 RNA PCR signal at baseline despite the absence of quantifiable RNA levels and having begun ART more than 3 months after HIV-1 infection (189). Baseline PBMC GRT was not performed in any of the trials in this meta-analysis.

Although dolutegravir monotherapy is not considered an acceptable option for maintaining VS in patients with stable VS (14), dolutegravir/lamivudine has been widely studied to maintain VS even in patients with a history of the lamivudine-resistance mutations M184V/I by historical plasma virus GRT or by preswitch PBMC GRT. Table 7 summarizes the findings from two clinical trials and eight retrospective cohort studies of dolutegravir/lamivudine simplification in about 3,700 patients of whom approximately 480 had M184V/I detected by historical plasma virus GRT and 40 had M184V/I detected by PBMC GRT just prior to starting dolutegravir/lamivudine (156,

TABLE 6 Studies describing the predictive value of PBMC GRT in patients with VS switching ART regimens

| Study author and year | No. of patients | Patient characteristics | Sequencing method ^a | PBMC GRT results and virologic response |
|-----------------------|-----------------|---|--|---|
| Opravil 2002 (173) | 84 | Patients with VS on a PI-containing ART regimen who were switched to a triple NRTI regimen (ABC/3TC/AZT). | Sanger sequencing (PR/RT) | 13 (15%) patients experienced VF. 6/13 had ≥ 1 ABC-resistance mutation. It was not possible to determine whether these mutations increased the risk of VF. |
| Pellegrin 2003 (172) | 55 | Patients with VS on a PI-containing ART regimen who were switched to a triple NRTI regimen (ABC/3TC/AZT). | Sanger sequencing (PR/RT) | 9/15 (60%) patients with and 8/34 (24%) without ≥ 1 ABC-resistance mutations experienced VF. The no. of ABC-resistance mutation ($P = 0.009$) and the PBMC virus load ($P = 0.006$) were each associated with an increased risk of VF. |
| Porter 2016 (155) | 51 | Patients with VS and no history of VF or historical plasma virus resistance to TDF, FTC, or RPV who switched from a PI-containing regimen to TDF/FTC/RPV. Subset of 24 patients with and 27 without K103N history who had stored PBMC samples (SPIRIT trial). | NGS at 10% mutation-detection threshold (PR/RT) | Of 4 patients with VF and emergent HIVDR, 1 had Y181C and M184I detected by PBMC GRT at baseline. E138A ($n = 4$), E138G ($n = 1$), E138K ($n = 1$), and E138Q ($n = 1$) were present at baseline in patients who did not develop VF. |
| Armenia 2018 (204) | 227 | ART-experienced patients with a history of VS for a median of 4 yrs who underwent PBMC GRT and switched ART. | Sanger sequencing (PR/RT/IN) | 24 mo after switch, 28% of patients with intermediate or full resistance to one of the drugs used at the time of the switch experienced VF compared with 14% lacking genotypic resistance. |
| Ellis 2020 (205) | 83 | Clinic patients undergoing PBMC GRT. 66 switched ART and 59 had postswitch follow-up. 76% had VS and 15% had LLV. 46% had been on ≥ 2 regimens; 34% lacked a complete ART history. Only 9 had historical plasma virus GRTs. | NGS at 10% mutation-detection threshold (PR/RT/IN) | Among the 24 patients not switching ART, 4 chose not to switch because of the presence of multiclass resistance by PBMC GRT. In a logistic regression model, switching therapy did not appear to increase the risk of subsequent VF. |
| Meybeck 2020 (148) | 185 | Clinic patients undergoing PBMC GRT. 73 switched ART most commonly to an INSTI- (70%) or RPV- (8%) based regimen. The mean no. of previous ART regimens was 5.4. Approx. one-half had historical plasma virus GRTs. | NA | After 6 mo, those who switched therapy had a lower risk of VF than those who continued their original regimen (19% vs. 5%). Other predictors of VF were higher PBMC virus load, lower CD4 nadir, and shorter time of VS. |
| Rodriguez 2021 (206) | 72 | STRUCTR trial participants with VS while on EVG/c/TAF/FTC or EVG/c/TDF/FTC. Patients were switched to ABC/3TC/DTG if PBMC GRT did not detect DRMs associated with this regimen. | NGS at 10% mutation-detection threshold (PR/RT/IN) | 9 of 72 screening patients were found to have resistance to ABC and/or 3TC and therefore did not switch therapy. 50 patients were switched to ABC/3TC/DTG. 44 of 44 evaluable patients maintained VS at wk 48. |
| Overton 2021 (184) | 1045 | Of 1,045 ATLAS-2M participants, 10 had confirmed VF. Patients had been VS for ≥ 6 mo and had no history of VF or baseline NNRTI or INSTI resistance by plasma virus GRT associated with cabotegravir or RPV, the drugs used for ART simplification. | NGS at 10% mutation-detection threshold (PR/RT/IN) | 5 of 10 patients with confirmed VF had NNRTI resistance by PBMC GRT that was not detected by historical plasma virus GRT, including 3 with major NNRTI-resistance DRMs (Y181C+H221Y; Y188L; Y188L) and 2 with E138A. |

^a Illumina was used for NGS in each study. ABC, abacavir; 3TC, lamivudine; AZT, zidovudine; TDF, tenofovir DF; FTC, emtricitabine; RPV, rilpivirine; EVG/c, elvitegravir/cobicistat; DTG, dolutegravir; NA, details not available or not provided in the study.

TABLE 7 Studies of dolutegravir (DTG)/lamivudine (3TC) in patients with and without M184V/I by historical plasma virus GRT^a

| Study author and year | No. of patients | Patient characteristics | ART (yrs) | VS (yrs) | RT184 genotypes | Outcome |
|---|-----------------|--|-----------|----------|---|---|
| Charpentier 2017 (156) | 27 | Heavily treated; DOLULAM substudy; no INSTI DRMs. | 18 | 6 | 8 of 18 with historical plasma virus GRT had M184V. 3 of these 8 and 7 additional patients had M184V/I detected by PBMC GRT. | No cases of VF occurred during the first yr of ART. |
| Gagliardini 2018 (191) | 436 | Retrospective study of patients switching to DTG/3TC (n = 126) or bPI/3TC (n = 310). | 7.8 | 4.4 | 87 (20%) had M184V by historical plasma virus GRT, including 21 of those switched to DTG/3TC. | VF occurred in 12% with and 8% without M184V. GRT at VF was available only for 8 patients who switched to bPI/3TC. |
| De Miguel 2020 (157, 235) | 41 | Single-arm pilot trial; ART-PRO; INSTI-naïve patients switched to DTG/3TC. | 18 | 6 | 21 had history of plasma virus M184V/I of whom 7 (33%), 11 (52%), 14 (67%), and 20 (95%) had M184V/I detected by PBMC GRT at 20%, 10%, 5%, and 1% mutation-detection thresholds. | No patient developed protocol-defined VF or emergent DRMs. |
| Galizzi 2020 (192) | 374 | Retrospective study of VS patients switching to DTG/3TC (n = 307) or DTG/RPV (n = 67). 45% had previous VF. | 15 | 5.1 | 220 patients had a historical plasma virus GRT, including 174 in the 3TC group and 46 in the RPV group. 60 (27%) had a history of M184V/I and 35 had a history of an RPV-resistance mutation. | In the entire 3TC group, 17 patients developed VF after a median of 1.7 yrs. 0/8 patients with GRT at VF developed new INSTI resistance DRMs. |
| Blick 2021 (199) | 100 | SOLAR 3D open label trial. | 22 | 12 | 50 had history of plasma virus M184V/I which was a stratification criterion. | At wk 48, 1 patient in the M184V/I arm and 3 in the non-M184V/I arm had RNA \geq 50 copies/mL. None had confirmed VF or emergent DRMs. |
| Borghetti 2021 (190, 193, 194) | 669 | Retrospective study of patients switching to DTG/3TC during VS. 14 patients had previous VF on an INSTI regimen. | 12 | 8 | 48 had history of plasma virus M184V/I. | 23 cases of VF after a median follow-up period of 1.9 yrs. No patients developed HIVDR. A history of M184V/I and a history of VF on an INSTI regimen were associated with an increased risk of VF. |
| Cicullo 2021 (195, 236) | 785 | Retrospective study of patients switching to DTG/3TC during VS. | 11.5 | 2.5 | 33 had history of plasma virus M184V/I. | 18 cases of VF after a median follow-up period of 2.2 yrs. No patients developed HIVDR. A history of M184V/I combined with a shorter period of VS (<7.3 yrs) was associated with an increased risk of VF. |
| Santoro 2022 (198) | 712 | Retrospective study of patients switching to DTG/3TC during VS (LAMRES cohort). | 7 | 5.2 | 60 (8.4%) had history of past M184V before DTG/3TC switch: 48 (80.0%) only in HIV-RNA GRTs, 10 (16.7%) exclusively in HIV-DNA, and 2 (3.3%) in both HIV-DNA and -RNA. | 22 cases of VF after 1 yr of follow-up. 1 patient developed M184V after 4 mo on DTG/3TC. There was an increased risk of VF in those with M184V and a duration of VS \leq 3.5 yrs (M184V + VS \leq 3.5 yrs = 22.7%; M184V + VS > 3.5 yrs = 7.8%; no mutation + VS \leq 3.5 yrs = 9.0%; no mutation + VS > 3.5 yrs = 4.9%). |
| Deschavres 2021, Hocqueloux 2021 (196, 237) | 695 | Retrospective study of patients (Dat'AIDS cohort) switching to DTG/3TC or DTG/FTC. | 10.6 | 6.3 | 105 had historical plasma virus M184V/I a median of 10 yrs before switching therapy. | 15 cases of VF after a median follow-up of 1.2 yrs. No patients developed HIVDR. |
| Underwood 2022 (180) | 192 | Post-hoc analysis to determine proportion of baseline samples with PBMC DRMs (SALSA); patients had VS for \geq 6 mo with no history of VF. | 5.5 | NA | 5 patients in DTG/3TC arm had M184V by PBMC GRT at a mutation-detection threshold of 15%. | None of the 5 patients with baseline M184V by PBMC GRT developed VF. |

^aPatients were HBsAg-negative. Except for Underwood 2022, past VF was allowed and had occurred in a significant proportion of persons in all studies. VF and/or treatment discontinuation were considered outcome variables. Several of the above studies may contain overlapping patients as they are from the same medical centers. bPI, boosted darunavir, lopinavir, or atazanavir; RPV, rilpivirine. NA, details not available or not provided in the study.

157, 180, 190–198). In one open-label trial of 100 patients, in which 50 were stratified based on a history of M184V/I, switching to dolutegravir/lamivudine was shown to be equally effective in those with and without M184V/I (199). In another clinical trial of 493 patients with VS randomized to receive dolutegravir/lamivudine or to continue three-drug ART, a *post hoc* analysis of preswitch PBMC GRT demonstrated the presence of M184V in 5 of the 192 patients randomized to dolutegravir/lamivudine (180). In the eight retrospective studies, treatment discontinuation rates over a follow-up period of one or more years were consistently below 5% to 10%, and protocol-defined VF rates were even lower, regardless of the patient's M184V/I history. Although a history of M184V/I in combination with a shorter time of VS was associated with an increased risk of VF (193, 195), there were no reports of emergent INSTI resistance in any of these studies.

It has been hypothesized that in persons with a history of M184V/I or with M184V/I detectable by PBMC GRT, the combination of dolutegravir/lamivudine is more active than dolutegravir monotherapy because M184V/I is associated with reduced virus replication (200–202). Additionally, the success of dolutegravir/lamivudine despite genotypic evidence for lamivudine resistance may be because the risk of reactivation of viruses containing M184V/I is low in patients with prolonged VS and that the number of reactivating viruses with M184V/I may be insufficient to overcome the high genetic barrier to dolutegravir resistance.

Another study which highlighted the importance of baseline PBMC virus load prior to switching to a regimen with a low genetic barrier to resistance was the TRULIGHT trial (203). It found that in patients with VS with an HIV-1 DNA level $<2.7 \log_{10}$ copies/ 10^6 PBMC and no history of VF, a regimen containing tenofovir/emtricitabine alone was noninferior at week 48 to a regimen containing tenofovir/emtricitabine plus a third antiretroviral agent (203). Nonetheless, VF was more common in the tenofovir/emtricitabine arm with 6 of 113 patients developing VF compared with 2 of 110 in the control arm. In the tenofovir/emtricitabine arm, one person developed the DRM K65R and one developed the DRM M184V. In contrast, no DRMs developed in the control arm (203). Baseline PBMC GRT was not performed in this trial.

Three additional studies provide anecdotal evidence that PBMC GRT might be useful in clinical practice. One involved 227 heavily treated clinic patients with VS for a median of 4 years who underwent PBMC GRT prior to ART simplification (204). In this analysis, 28% of patients with intermediate or full resistance to one of the drugs used at the time of the switch were likely to experience VF compared with 14% lacking genotypic resistance. In two other clinic-based studies, the use of PBMC GRT was reported to assist clinicians in switching therapy in patients with VS, but the predictive value of DRMs detected by PBMC GRT was not reported (205, 206) (Table 6).

The success of certain regimens, such as dolutegravir or boosted darunavir in combination with lamivudine despite the presence of lamivudine resistance by PBMC GRT, suggests that regimen-specific interpretations may be necessary as regimens anchored by a single fully active drug with a high genetic barrier to resistance may be successful despite the presence of resistance to one or more companion drugs. The success of such regimens, particularly in patients with prolonged VS or low PBMC HIV-1 DNA levels may be part of a spectrum that includes posttreatment controllers who experience prolonged periods of VS following the discontinuation of ART (30, 207–209).

CONCLUSIONS

The main scenario for which PBMC GRT is likely to be most useful in patient management is for modifying therapy in patients with VS on a stable ART regimen. However, there are three additional scenarios in which PBMC GRT has also been studied, albeit less often: (i) modifying therapy in patients with persistent low-level viremia; (ii) detecting DRMs in patients with VF on an ART regimen who recently discontinued therapy; and (iii) detecting DRMs in patients who may have been infected with a drug-resistant virus. In each of these scenarios, there are limitations to the sensitivity of

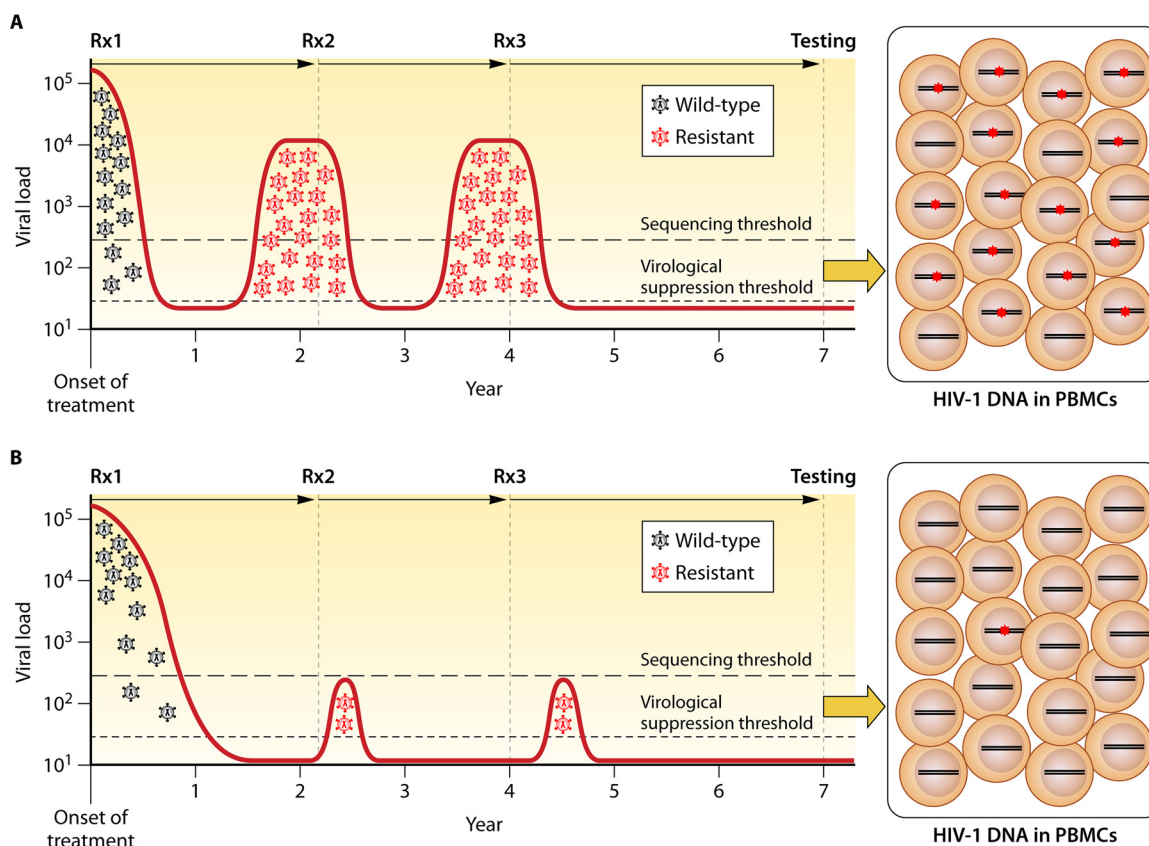


FIG 1 Sensitivity of peripheral blood mononuclear cell (PBMC) genotypic resistance testing (GRT) in patients with plasma virus suppression while receiving antiretroviral therapy (ART). In the first scenario (A), in which previous episodes of virological failure (VF) were prolonged and associated with high plasma HIV-1 RNA levels, the likelihood of detecting drug-resistance mutations (DRMs) in proviral DNA will be high. In the second scenario (B), in which previous episodes of VF were short and associated with low plasma HIV-1 RNA levels, the likelihood of detecting DRMs in proviral DNA will be low. Rx1, Rx2, and Rx3 followed by an arrow indicate periods of antiviral therapy. Proviral DNA containing DRMs are indicated by red asterisks in the boxes at the right side of the figure.

PBMC GRT that must be appreciated to appropriately use PBMC GRTs and to interpret their results.

NGS is preferable to Sanger sequencing for PBMC GRT because it has greater sensitivity for detecting DRMs present in a mixed virus population and because it can be used to reduce the risk of reporting DRMs resulting from G-to-A hypermutation. However, when a low mutation-detection threshold is used, the specific threshold at which each mutation is detected should be reported, as mutations detected at low levels are at increased risk of being PCR or sequence artifact. Moreover, viruses containing mutations present at low levels may be less likely to reactivate than those present at high levels.

In patients with VS, PBMC GRT will often not detect DRMs that were once detected by historical plasma virus GRT. This is particularly the case if previous episodes of VF and emergent drug resistance were either not prolonged or not associated with high levels of plasma viremia (Fig. 1). In contrast, in patients who have had multiple VFs but who infrequently underwent genotypic testing during past episodes of VF, PBMC GRT may be more sensitive than historical plasma virus GRTs. However, in either case, the results of PBMC GRT should be interpreted in conjunction with the results of historical plasma virus GRTs and careful consideration of the drug resistance likely to have emerged during past episodes of VF.

In patients with persistent low-level viremia, PBMC GRT is likely to be insensitive at detecting emergent drug resistance because it can take several months for newly emergent DRMs to become detectable (Fig. 2). Moreover, there are few studies of PBMC GRT in this scenario. If the plasma virus level is high enough, plasma virus GRT

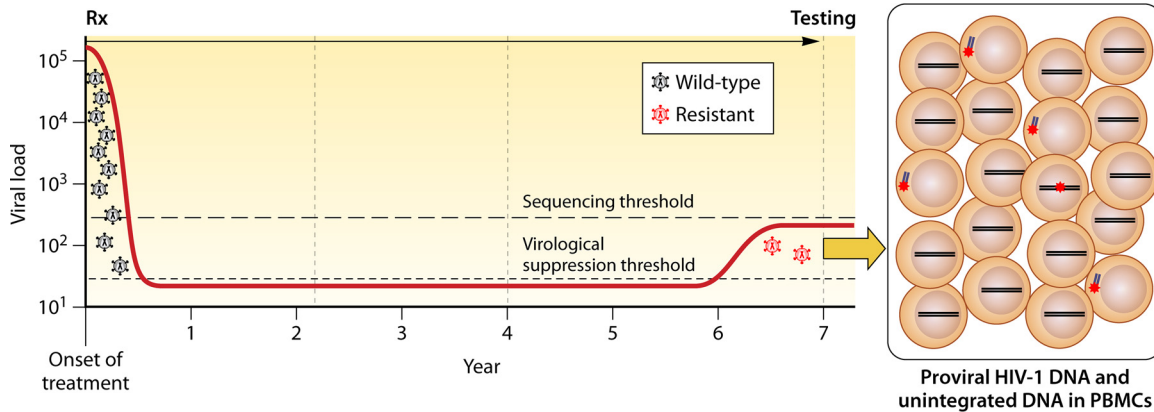


FIG 2 Sensitivity of PMBC GRT in patients with the recent development of low-level viremia while receiving ART. Rx followed by an arrow indicates the period of antiviral therapy. The dashed line indicates a threshold of between 200 and 500 copies/mL below which plasma virus GRT is often not successful. The likelihood of detecting DRMs in proviral DNA is likely to be low. Detecting DRMs in unintegrated linear and episomal viral DNA may be possible because these are more likely to reflect recently circulating viruses. Proviral and unintegrated viral DNA containing DRMs are indicated by red asterisks. In the boxed figure on the right, unintegrated linear viral DNA is shown outside the nucleus. Episomal DNA is not shown; its dynamics are likely to be closer to unintegrated linear DNA than proviral DNA.

should be initially attempted. PBMC GRT may be useful because unintegrated linear and episomal DNA are in equilibrium with plasma virus and may be detectable, although there are no published studies to support this conjecture.

Plasma virus GRT is most valuable when performed while a patient experiencing VF is still taking antiretroviral drugs or within 4 weeks after discontinuing therapy (14). If a patient has been off therapy for a longer period and plasma virus GRT does not explain the cause of VF, PBMC GRT may be considered (Fig. 3). However, the sensitivity of PBMC GRT in this scenario may not be high if the duration of viremia while receiving ART was not prolonged. Moreover, within several months following ART discontinuation, there will be an accumulation of recently produced nonintegrated HIV-1 DNA genomes and a partial turnover of the proviral DNA population.

PBMC GRT may be more sensitive than plasma virus GRT at detecting transmitted DRMs, as viruses containing these DRMs are often outcompeted in plasma by viruses containing wild-type revertants. Nonetheless, such testing has rarely been performed and the added clinical benefit of such testing is not known.

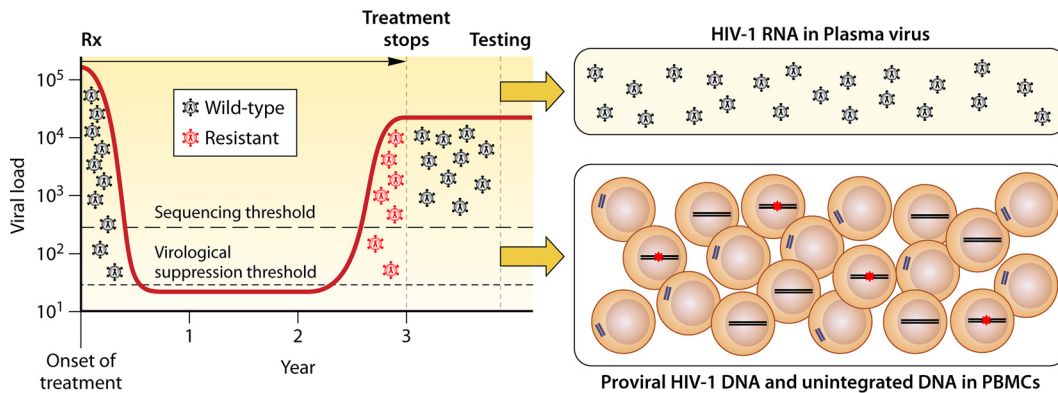


FIG 3 Sensitivity of plasma virus and PMBC GRT in patients who discontinue ART following VF and emergent HIV drug resistance (HIVDR). Rx followed by an arrow indicates the period of antiviral therapy. Most DRMs will no longer be detectable by plasma virus GRT within 2 to 3 months following ART discontinuation because plasma viruses containing DRMs are often rapidly outcompeted by ancestral wild-type viruses established in viral reservoirs prior to ART initiation. Although recently emergent DRMs may have begun to seed the proviral DNA reservoir, their levels in this compartment will be low unless VF has been prolonged. In addition, the presence of unintegrated linear and episomal viral DNA, which are in equilibrium with circulating plasma virus, may also reduce the proportion of viral DNA molecules containing DRMs. In the boxed figure on the right, proviral DNA containing DRMs are indicated by red asterisks. Unintegrated linear viral DNA is shown outside the nucleus. Episomal DNA is not shown; its dynamics are likely to be closer to unintegrated linear DNA than proviral DNA.

Limitations to the sensitivity of PBMC GRT are described above, however, there may also be limitations to its specificity. Although most laboratories performing PBMC GRT employ algorithms to minimize the reporting of DRMs resulting from APOBEC-mediated G-to-A hypermutation, these algorithms are imperfect. In addition, mutations present at low-levels within PBMC may represent PCR or sequence artifact. Finally, while there are many studies demonstrating the clinical relevance of DRMs detected by plasma virus GRT, there are few such studies for PBMC GRT at this time. Nonetheless, ART modifications in patients with sustained VS are being increasingly studied in clinical trials and performed in clinical practice. As premodification PBMC GRT will be increasingly performed prospectively or on stored samples in clinical trials, there will be an increased opportunity to determine the predictive value of PBMC GRT on the response to a new ART regimen.

Sidebars

Clonal expansion. Around 2005, several groups observed that low-level viremia in patients receiving ART was often composed of viruses with identical *env* sequences (134, 135). The levels of viremia observed suggested that multiple cells from a clonal lineage were synchronously producing virions because single cells would be incapable of producing the observed quantities of viruses (210). Although clonal expansion of proviral DNA-containing cells may also be occurring in patients with ongoing virus replication, this phenomenon only became noticeable in patients with VS. This is in part because CD4⁺ lymphocytes are no longer becoming newly infected with diverse viral variants and in part because clonally proliferating cells appear to account for a higher proportion of circulating virus the longer a patient maintains VS (67, 210). To prove that identical viral sequences were due to the proliferation of infected cells expressing circulating viruses, several groups developed assays to demonstrate these identical viral sequences were being produced by proviruses integrated in the same human genomic location (72, 210–212).

APOBEC-mediated G-to-A hypermutation. APOBEC3F and 3G are host cytidine deaminases that inhibit viral infections by causing extensive cytidine (C) to uridine (U) editing of negative-strand viral RNA in newly infected cells. This results in guanosine (G) to adenosine (A) hypermutations in plus-stranded cDNA (95). APOBEC 3F and 3G act in specific dinucleotide contexts. APOBEC3F causes GA to be mutated to AA and APOBEC3G causes GG to be mutated to GA. The HIV-1 Vif protein targets host APOBEC molecules for proteasomal destruction and usually blocks the actions of APOBEC3F and 3G. Nonetheless, for unknown reasons, APOBEC-mediated viral editing is frequently successful and results in defective nonviable integrated hypermutated genomes. These genomes often contain stop codons due to APOBEC3G editing of tryptophan (W): TGG → TAG or TGG → TGA or active site mutations in PR (D25N), RT (D110N, D185N, and D186N), and IN (D64N, D116N, and E152K) resulting from APOBEC3F editing of aspartic acid GAC/T (D) → AAC/T (N) or glutamic acid GAA/G (E) → AAA/G (K). PBMC sequences can be considered hypermutated if they have a global excess of G to A changes in the appropriate dinucleotide context (213) or if they contain two to three or more signature APOBEC mutations in a single sequence (99). In either scenario, any DRM that can arise in an APOBEC dinucleotide context should be regarded with caution. The list of such mutations includes D30N, M46I, G48S, and G73S in PR; D67N, E138K, M184I, G190ES, and M230I in RT; and G118R, E138K, G140RS, G163KR, D232N, and R263K in IN.

Tropism. In addition to attaching to the CD4⁺ receptor, HIV-1 must bind one of two coreceptors, CCR5 or CXCR4. Most patients are primarily infected by CCR5-tropic HIV-1 variants, with CXCR4-tropic variants emerging during disease progression (128, 214). The CCR5 inhibitor maraviroc is inactive against CXCR4 variants. In patients receiving CCR5 inhibitors, the most common mechanism of VF is the expansion of preexisting CXCR4 tropic viruses intrinsically resistant to CCR5 inhibitors (215). CXCR4 tropism is most reliably detected phenotypically using pseudotyped viruses containing the HIV-1 *env* gene (216). It can also be detected genotypically as certain positively charged residues at positions 11

and 25 of the V3 loop of gp120 and several less common combinations of mutations primarily, but not exclusively, within the V3 loop are associated with CXCR4 tropism (217).

Next-generation sequencing and low-abundance variants. Dideoxynucleotide Sanger sequencing of nonclonal PCR products (direct PCR sequencing) of plasma viral cDNA has been the standard approach to HIV-1 GRT for more than 25 years. Sanger sequencing usually detects HIV-1 variants present in proportions above 20% with a range of 10% to 30% depending on the nucleotide context (82–84). Next-generation sequencing (NGS) technologies have been used in research studies to detect variants present in proportions as low as 1% (218). However, mutation-detection thresholds of $\geq 5.0\%$ have been recommended for use in clinical settings to reduce the risk of experimental artifact and increase reproducibility (15, 85, 86, 99). NGS is not necessarily considered superior to Sanger sequencing for plasma virus GRT. This is because the number of additional DRMs detected by plasma virus GRT at mutation-detection thresholds between 5% to 20% is not very high, as most DRMs emerge rapidly in plasma in patients experiencing VF (15, 87). However, the ability to detect variants below 20% is more important for PBMC GRT because the emergence of DRMs in PBMCs is slower than in plasma.

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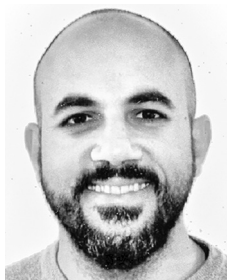
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