

HIV Proviral DNA: Clinical Applications, Drug Resistance, and the HIV Reservoir

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Abstract: Antiretroviral therapy has rendered HIV a manageable chronic infection, but integrated proviral DNA within long-lived CD4+ memory T cells persists indefinitely and constitutes both a barrier to cure and an archive of the patient's virologic history. Genotypic resistance testing performed on proviral DNA from peripheral blood mononuclear cells can recover drug resistance mutations no longer detectable in plasma, characterize viral subtype, and provide an estimate of reservoir burden. This narrative review summarizes the biology of the proviral reservoir, compares Sanger and next-generation sequencing platforms, and addresses interpretive pitfalls including apolipoprotein B mRNA editing enzyme catalytic polypeptide-like 3 (APOBEC3)-mediated G-to-A hypermutation and the predominance of replication-defective proviruses. The clinical weight of an archived mutation varies by antiretroviral class, tracking inversely with genetic barrier. The CD4/CD8 ratio is discussed as an accessible correlate of reservoir burden and immune dysregulation. Judicious use requires clear articulation of how a result will alter management.

Keywords: proviral DNA; archived drug resistance; next-generation sequencing; latent reservoir; long-acting antiretroviral therapy

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Highlights

- HIV proviral DNA may provide critical information on HIV subtypes, archived drug resistance mutations (DRMs), and the size of the viral reservoir.
- Next-generation sequencing (NGS) can detect minority HIV variants at frequencies below 1%, enabling more complete characterization of archived resistance in the proviral reservoir.
- Proviral DNA genotyping before initiating long-acting injectable cabotegravir/rilpivirine (CAB/RPV) is increasingly recommended by the European AIDS Clinical Society and the British HIV Association guidelines, as archived rilpivirine resistance strongly predicts virologic failure.

- The CD4/CD8 ratio serves as a surrogate marker of the HIV reservoir burden.
- Early ART initiation, particularly during acute HIV infection, is the most effective strategy for limiting proviral DNA reservoir size and preserving immune architecture (higher CD4/CD8 ratios).

Introduction

Antiretroviral therapy (ART) has transformed HIV infection from a fatal disease into a manageable chronic condition. However, a fundamental barrier to cure and to optimizing long-term therapy remains: HIV integrates into the genome of host CD4+ T lymphocytes, creating a latent proviral reservoir that persists despite decades of suppressive treatment [1]. The detection and characterization of this proviral DNA, HIV genetic material embedded within peripheral blood mononuclear cells, has emerged as a clinically meaningful tool for guiding therapeutic decisions. This review examines the biology of HIV proviral DNA, available sequencing methodologies, class-specific implications for drug resistance testing, clinical applications in the era of long-acting injectables, and the relationship between the HIV reservoir and immune reconstitution as reflected by the CD4/CD8 ratio. Infectious disease clinicians treating patients with HIV require an understanding of the potential, and pitfalls, of this technology.

What Is HIV Proviral DNA?

HIV proviral DNA refers to the double-stranded viral DNA present in peripheral blood lymphocytes following reverse transcription and integration into the host genome. Unlike plasma HIV RNA, which reflects active viral replication, proviral DNA represents the archived genetic record of a patient's complete HIV history, including viruses that were present during prior treatment failures, viruses that have since been suppressed below detectable limits, and mutant variants that were never dominant but remain stored in long-lived memory T cells [1].

Clinically, proviral DNA testing yields three categories of information. It carries information regarding HIV subtype and phylogenetic classification, particularly relevant when prior subtyping is unavailable or uncertain (e.g., distinguishing subtype A1 from A6, which carry different resistance profiles). Additionally, it provides clues of archived DRMs accumulated during prior virologic failures. Finally, it quantitatively estimates the size of the latent HIV reservoir, which correlates with long-term immune outcomes [2].

Testing Methodologies

Sanger (Dideoxy-Terminator) Sequencing

As the traditional platform for HIV genotyping, Sanger sequencing reads one consensus sequence at a time by synthesizing DNA in the presence of chain-terminating dideoxynucleotides. It is well established, widely available, and relatively low-cost per run. Its principal limitation in the proviral context is sensitivity: Sanger sequencing reliably detects mutant variants only when they constitute at least 15–20% of the total viral population. This threshold means that minority resistance variants, particularly those archived at low frequencies following distant treatment failures, may be missed entirely. This could underestimate the burden of clinically relevant resistance [2].

Next-Generation Sequencing (NGS)

NGS, also called deep sequencing or massively parallel sequencing, reads millions of DNA fragments simultaneously, generating hundreds of thousands of reads per sample [2]. In the context of proviral

DNA, this approach offers transformative advantages. Minority variants present at frequencies below 1% can be detected. The full diversity of viral quasispecies within the reservoir can be characterized. Clonal expansion of infected CD4+ T cells can be tracked. These capabilities make NGS particularly well suited to identifying archived DRMs that were dominant only transiently, during prior failure episodes, but have since been archived in long-lived memory cells [2].

APOBEC3-Induced Hypermutation: A Diagnostic Pitfall

A critical interpretive challenge in proviral DNA sequencing is the contribution of APOBEC3 host enzymes. APOBEC3 proteins act as innate antiviral defenses by introducing G-to-A hypermutation into reverse-transcribed viral DNA. Because these APOBEC-induced mutations can appear in codons known to confer drug resistance, mimicking DRMs, they may produce false-positive resistance findings if not recognized. Heavily hypermutated proviral sequences can generally be flagged by the pattern and degree of G-to-A substitutions; distinguishing genuine resistance from APOBEC editing artifact is essential for accurate clinical interpretation [2].

Drug Resistance by Antiretroviral Class

The clinical significance of archived DRMs detected by proviral DNA genotyping is not uniform across antiretroviral classes. Each class's genetic barrier to resistance, for example, the number and nature of mutations required to confer clinically meaningful resistance, determines how much weight a proviral finding should carry in therapeutic decision-making [2].

Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)

NNRTIs carry the highest clinical impact from proviral DNA genotyping findings, a consequence of their low genetic barrier to resistance. A single mutation, such as K103N, is sufficient to confer high-level resistance to first-generation NNRTIs (efavirenz, nevirapine). Crucially, K103N persists indefinitely in the proviral reservoir and cannot be overcome by standard NNRTI dosing [2]. Similarly, second-generation NNRTI rilpivirine is susceptible to multiple archived mutations including K101E, K101P, E138A/G/K/Q/R, V179L, Y181C, Y188L, H221Y, F227C, and M230I [2,3].

Current clinical guidance advises proviral DNA genotyping before initiating any rilpivirine-based regimen in patients with prior NNRTI exposure, as even archived resistance undetectable by plasma RNA testing can compromise outcomes [2].

Integrase Strand Transfer Inhibitors (INSTIs)

Second-generation INSTIs, dolutegravir (DTG) and bictegravir (BIC), have high genetic barriers, and single archived mutations rarely translate to virologic failure in these agents. However, the Q148 pathway mutations, particularly when combined with secondary mutations such as G140S, can compromise even DTG and BIC. Proviral DNA integrase genotyping is most clinically relevant in patients with documented prior exposure to first-generation INSTIs raltegravir or elvitegravir, where resistance pathway mutations may be archived [2].

Nucleoside/Nucleotide Reverse Transcriptase Inhibitors (NRTIs)

The M184V/I mutation (conferring resistance to lamivudine/3TC and emtricitabine/FTC) is among the most commonly archived mutations in treated populations [2]. Its clinical significance within dual-therapy regimens remains a subject of active debate. The VOLVER clinical trial (GESIDA

11820) evaluated dolutegravir/lamivudine (DTG/3TC) maintenance therapy and examined the impact of proviral-DNA M184V/I on 96-week outcomes. Paradoxically, M184V impairs viral replicative fitness, raising the possibility that it may be clinically tolerated in the setting of high-barrier INSTI combinations [4].

Thymidine analog mutations arising from historical zidovudine or stavudine exposure remain highly relevant in salvage therapy planning and should be sought in patients with prior NRTI failures [2].

Protease Inhibitors (PIs)

Boosted PI regimens (darunavir/ritonavir or darunavir/cobicistat) have inherently high genetic barriers to resistance, requiring the accumulation of multiple PI-associated mutations for meaningful resistance. While archived PI mutations carry importance in salvage planning for patients with extensive prior PI exposure, they are less critical than NNRTI or INSTI mutations when selecting a first-line boosted PI for a treatment-naïve or early-failure patient [2].

Applying clinical decisions based on identified mutations can be challenging. A common conclusion in the proviral DNA literature is that DNA testing may overestimate resistance, especially if the genetic material identified represents large internal deletions in nonviable viruses. These researchers caution that DNA resistance testing may prove to be “too restrictive” when considering options for therapy [2].

Proviral DNA and Long-Acting Injectables

The approval of long-acting injectable cabotegravir/rilpivirine (CAB/RPV) represents one of the most significant advances in HIV treatment in the past decade, offering patients a monthly or bimonthly alternative to daily oral therapy [4,5]. However, this regimen contains rilpivirine, an NNRTI with a low genetic barrier, making it particularly vulnerable to archived NNRTI resistance [2,3].

The ATLAS and FLAIR trials demonstrated robust efficacy of CAB/RPV in virologically suppressed adults but also identified key predictors of virologic failure: baseline NNRTI resistance mutations, BMI > 30 kg/m², and HIV subtype A6 [4,5]. Importantly, patients carrying archived RPV resistance mutations in proviral DNA, even when undetectable by conventional plasma RNA genotyping, showed significantly higher rates of virologic failure on CAB/RPV [3,5,6].

Analysis of virologic failures in post-approval settings has further characterized the interplay between drug levels, resistance, and viral subtypes. Mazzitelli et al. examined virologic failure patterns among patients receiving CAB/RPV, finding that resistance selection and pharmacokinetic factors contribute to breakthrough in a complex, multifactorial manner [3]. Separately, Cutrell et al. identified that baseline resistance represented the dominant modifiable risk factor amenable to pre-switch screening [6].

In response to this evidence, both the EACS and the BHIVA now increasingly recommend proviral DNA resistance testing before switching to CAB/RPV in any patient with prior NNRTI exposure, with the goal of ruling out archived rilpivirine resistance that could render the long-acting regimen ineffective from initiation [2,3,6].

Clinical Framework for Proviral DNA Testing

Effective use of proviral DNA testing requires integration of clinical context to establish pretest probability and to guide interpretation of results [2]. The following factors should inform the clinical decision to pursue proviral DNA genotyping:

- Prior treatment history: Prior NNRTI use substantially raises the pretest probability of archived NNRTI resistance. Prior INSTI (particularly first-generation) exposure warrants integrase proviral genotyping [2].
- Viral load history: Patients with documented “blips” (transient low-level viremia) during suppressive therapy carry higher likelihood of archived resistance [2]. A lower CD4 nadir suggests a larger proviral reservoir and a longer historical window during which resistant variants could have been generated [1].
- Prior HIV RNA resistance test results: Historical genotyping reports should be reviewed before ordering proviral testing; proviral DNA adds most value when prior resistance data are unavailable or incomplete [2].
- Impact on clinical decision: The critical question for ordering proviral DNA testing is whether a positive or negative result will change the treatment choice; if the decision is already determined regardless of result, testing may not add value [2].

The CD4/CD8 Ratio as a Measure of the HIV Reservoir

Pathophysiology of Ratio Inversion

In immunocompetent individuals, the CD4/CD8 T cell ratio is typically 1.5–2.5, reflecting homeostatic balance between helper and cytotoxic T cell populations [1]. HIV infection disrupts this architecture through two simultaneous mechanisms: CD4+ T cells, the primary HIV target and reservoir, are progressively depleted, while CD8+ T cells expand in response to chronic immune activation driven by persistent antigen exposure from the viral reservoir [1]. The result is a characteristically inverted ratio (<1.0) that persists on ART in many patients and serves as a fingerprint of reservoir-driven immune dysregulation [1].

Correlation with Proviral DNA Levels

The CD4/CD8 ratio correlates inversely with proviral DNA levels even after controlling for absolute CD4 count and duration of viral suppression: patients with lower ratios carry higher proviral DNA burdens. The nadir CD4 count exerts a strong influence on both parameters; patients who experienced profound immunosuppression before ART initiation tend to harbor both larger reservoirs and more persistently inverted ratios, reflecting shared biological determinants of immune damage [1].

Four Mechanisms Sustaining Ratio Dysregulation on ART

Despite effective viral suppression, several reservoir-dependent mechanisms perpetuate CD4/CD8 ratio inversion [1]:

- Reservoir-driven chronic immune activation: Low-level transcriptional activity from the proviral reservoir and sporadic viral protein production continuously stimulate CD8+ T cells, maintaining chronic activation even in the absence of detectable viremia [1].
- Clonal expansion of infected CD4 cells: Latently infected CD4+ T cells undergo homeostatic proliferation to maintain the CD4 pool, simultaneously replicating the integrated proviral DNA. The reservoir thus perpetuates itself while the immune system remains under chronic stress [1].
- Residual CD4 functional defects: Despite numerical CD4 count recovery, CD4 functional capacity remains impaired, with reduced proliferative capacity, altered cytokine production, and accelerated senescence [1].
- CD8 exhaustion and senescence: Chronically activated CD8 cells eventually exhaust and accumulate as senescent cells, distorting the ratio even as active viral replication is suppressed [1].

Interpretation of the CD4/CD8 Ratio in Treated HIV

CD4/CD8 Ratio	Interpretation in Treated HIV
>1.5	Approaching normal immune architecture; typically seen in patients treated during acute HIV infection [1]
>1.0	Associated with lower reservoir burden and better immune restoration [1]
0.5–1.0	Partial immune recovery with residual immune activation [1]
<0.5	Significant immune dysregulation; higher reservoir burden; elevated non-AIDS complication risk [1]

Complications of Persistent Ratio Inversion in HIV-Infected Individuals

The failure to normalize the CD4/CD8 ratio despite virologic suppression has been linked to a spectrum of non-AIDS complications driven by reservoir-mediated chronic inflammation. These include cardiovascular disease (via endothelial activation and chronic systemic inflammation), non-AIDS malignancies (from impaired immune surveillance mediated by exhausted CD8 cells), HIV-associated neurocognitive disorders (driven by central nervous system reservoir and neuroinflammation), osteoporosis (via bone resorption driven by chronic immune activation), and premature immunosenescence with accelerated T cell aging. Collectively, these complications contribute to excess mortality risk in virologically suppressed patients with persistent ratio inversion [1]. Clinically, few options exist for the ID physician to mitigate these risks.

Early ART Initiation and Reservoir Limitation

The most effective intervention for limiting proviral reservoir size and preserving immune architecture is early ART initiation, optimally during acute HIV infection. Patients treated during primary infection consistently achieve lower proviral DNA levels, higher and more normalized CD4/CD8 ratios, and better long-term immune restoration compared to those treated during established chronic infection. This observation underscores the importance of universal early treatment and rapid ART initiation programs, as the window for limiting reservoir seeding closes within weeks of initial infection [1].

Summary and Future Directions

HIV proviral DNA testing is an increasingly discussed tool in the infectious disease literature, with clinical utility extending beyond simple resistance testing to include reservoir quantification, subtype determination, and prediction of long-acting ART outcomes. Its current limitation lies in availability and efficacy. Proviral DNA sequencing remains largely confined to research settings and specialized reference laboratories, limiting routine clinical access [2].

As long-acting injectables become more widely used, the clinical need for pre-switch proviral genotyping will intensify [2,4,5]. Guidelines from EACS and BHIVA already reflect this trajectory, and it is anticipated that proviral DNA testing will become standard practice before initiating CAB/RPV and potentially other future long-acting combinations [2,3]. Understanding the utility and limitations of this technology is paramount.

The correlation between CD4/CD8 ratio and proviral DNA levels offers clinicians a broadly accessible, low-cost surrogate for reservoir burden. Tracking this ratio alongside proviral quantitative data may provide a more nuanced picture of immune recovery and reservoir control than absolute

CD4 count alone, informing both individual patient prognosis and the design of future reservoir reduction strategies [1].

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