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Drug Resistance Mutation Profiles in Patients with Virologic Failure

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Abstract

Virologic failure on antiretroviral therapy is, in most cases, accompanied by a recognizable pattern of HIV-1 drug resistance mutations whose composition depends on which drug class failed and how long failure went undetected. This article synthesizes the literature on these mutation profiles across the four major antiretroviral classes in current use. Nucleoside reverse transcriptase inhibitor resistance follows two largely mutually exclusive pathways, thymidine analogue mutations and the K65R/M184V pathway, whose specific combination determines residual backbone activity. Non-nucleoside reverse transcriptase inhibitor resistance arises from single, low-genetic-barrier mutations such as K103N and Y181C that rapidly foreclose the class. Protease inhibitor resistance, particularly to boosted darunavir, instead requires the combinatorial accumulation of individually modest mutations, conferring a high genetic barrier. Integrase strand transfer inhibitor resistance, most consequential now that dolutegravir anchors first-line therapy worldwide, is generally rare in treatment-naïve patients with an intact nucleoside backbone but emerges disproportionately when that backbone is already compromised, a pattern increasingly documented in programmatic cohorts in sub-Saharan Africa. We also examine how next-generation sequencing is revealing clinically relevant minority-variant resistance below the detection threshold of conventional genotyping, and where programmatic surveillance, detection technology, and treatment-switching protocols remain unresolved.

Keywords: HIV drug resistance; virologic failure; antiretroviral therapy; integrase inhibitor resistance; genotypic resistance testing

1. Introduction

Antiretroviral therapy (ART) has turned HIV-1 infection from a near-certain death sentence into a manageable chronic condition, but that success rests on a fragile premise: that the drugs keep working. They do not always keep working. A meaningful fraction of people on treatment experience virologic failure — persistently detectable HIV-1 RNA despite ongoing therapy — and when clinicians investigate why, the answer is very often written into the virus's own genome, in the form of resistance-associated mutations selected under the selective pressure of an incompletely suppressive drug regimen. Understanding which mutations arise, in which genes, under which drugs, and in which order, is not an academic exercise. It is what determines whether a second-line or third-line regimen will actually work, whether a national treatment program needs to change its standard first-line combination, and whether an individual patient's virus has become resistant to an entire drug class or only to one agent within it.

The clinical and public-health stakes of this question have shifted substantially over the past decade. Two developments in particular have reshaped what "drug resistance mutation profile" means in 2026. First, the global rollout of dolutegravir-based first-line therapy, driven by its favorable tolerability, high genetic barrier relative to older integrase inhibitors, and low cost, has changed the mutational landscape clinicians need to recognize: the mutations that matter most for the majority of people starting ART today are integrase strand transfer inhibitor (INSTI) mutations layered onto an already-resistant nucleoside reverse transcriptase inhibitor (NRTI) backbone in many treatment-experienced patients, rather than the non-nucleoside reverse transcriptase inhibitor (NNRTI) mutations that dominated the efavirenz and nevirapine era [1,2]. Second, next-generation sequencing (NGS) has moved from a research tool into something approaching routine clinical and surveillance use, revealing that a substantial amount of clinically relevant resistance exists below the roughly 20% variant-frequency detection threshold of conventional Sanger sequencing, with direct implications for how "resistant" or "susceptible" a given regimen should be considered at the time of a treatment decision [3,4].

This article synthesizes the literature on HIV-1 drug resistance mutation profiles in patients experiencing

virologic failure. It is not a report of new laboratory or clinical data; it is a synthesis of what a substantial and still-growing body of published work — clinical trials, programmatic cohort studies, in vitro selection experiments, and genotype-database analyses — has established about how resistance arises, which mutations characterize failure on each major antiretroviral drug class, how these patterns differ between well-resourced clinical trial settings and the routine programmatic settings where the majority of the world's people with HIV actually receive care, and what the unresolved problems are. We organize the discussion around the four major drug classes still in first-line and second-line use worldwide — NRTIs, NNRTIs, protease inhibitors (PIs), and integrase strand transfer inhibitors — followed by a dedicated treatment of the programmatic and surveillance literature from low- and middle-income countries (LMICs), where the burden of HIV and the burden of drug resistance are both concentrated, and a discussion of how sequencing technology is changing what "detecting resistance" even means.

A note on scope: this is a synthesis grounded in the published literature on acquired drug resistance following virologic failure, with secondary attention to pretreatment (transmitted) resistance where it bears directly on the same mutational profiles. It does not attempt original mutation-frequency analysis, phylogenetics, or structural modeling; where we report a specific percentage or fold-resistance figure, it is one a cited source itself reports, and we have tried to be explicit about which population and which assay produced it, because these numbers vary enormously by cohort, region, and detection threshold and are easy to over-generalize.

2. The Genetics of HIV-1 Drug Resistance: Concepts and Classification

HIV-1's reverse transcriptase lacks proofreading activity, and the resulting high mutation rate, combined with a large within-host viral population and a short replication cycle, means that essentially every possible single point mutation in the genome is generated many times over in an untreated, actively replicating infection. Most of these mutations are neutral or deleterious to viral fitness and are lost. Under antiretroviral selection pressure, however, mutations that reduce a drug's ability to bind its target — reverse transcriptase, protease, or integrase

— confer a replicative advantage whenever drug levels are present but not fully suppressive, and that advantage is what drives resistant variants from a minority quasispecies present at low frequency into the dominant, detectable viral population.

Table 1: Major HIV-1 drug resistance mutation pathways by antiretroviral drug class, their principal biochemical mechanism, and their characteristic genetic barrier, as characterized in the mutation-selection and resistance-mechanism literature. [7,6,10,11,5,13,14].

Drug class	Representative mutation(s)	Principal mechanism	Genetic barrier
NRTI -- thymidine analogues	TAMs: 41L, 67N, 70R, 210W, 215Y/F, 219Q/E	Enhanced excision of incorporated chain-terminating nucleotide; confers broad NRTI-class cross-resistance	High (sequential accumulation required) [7]
NRTI -- tenofovir/abacavir pathway	K65R	Reduced nucleotide incorporation efficiency; largely antagonistic with TAM pathway	Low-to-moderate (single mutation) [6]
NRTI -- lamivudine/emtricitabine	M184V/I	Steric interference with incorporation; increases fidelity and reduces processivity; resensitizes to AZT/TDF	Low (single mutation) [6]
NNRTI (first-generation)	K103N, Y181C, G190A	Altered allosteric binding-pocket geometry, reducing NNRTI binding without impairing catalysis	Low (single mutation, minimal fitness cost) [10,11]
Protease inhibitor (darunavir)	V32I plus accessory protease mutations	Combinatorial accumulation of low-impact substitutions altering protease-inhibitor binding	High (multiple mutations required) [5]
Integrase strand transfer inhibitor (dolutegravir)	R263K	Reduced strand-transfer efficiency; carries a measurable replicative fitness cost	High in treatment-naïve patients with intact NRTI backbone [13,14]

Resistance mutations differ substantially in what virologists call genetic barrier: the number of nucleotide changes, and the fitness cost of each, required to produce a clinically meaningful reduction in drug susceptibility. A single mutation such as M184V, which confers high-level resistance to lamivudine and emtricitabine, exemplifies a low genetic barrier — it arises quickly, often within weeks of virologic failure on a lamivudine-containing regimen, because a single nucleotide change is sufficient and the mutation itself, while it reduces reverse transcriptase's catalytic efficiency, does not impose a prohibitive fitness cost. By

contrast, resistance to boosted darunavir requires the sequential accumulation of multiple protease mutations, each individually contributing only a small increment of reduced susceptibility, which is precisely why darunavir retains activity against most PI-experienced viruses and why treatment-emergent darunavir resistance in PI-naïve patients remains uncommon even after virologic failure [5]. Genetic barrier is not a fixed property of a drug in isolation; it depends on the specific mutational context already present in the virus, which is why a mutation that would ordinarily require several further steps to become clinically significant can sometimes appear rapidly in a background that already carries partner mutations.

A second organizing concept is cross-resistance versus class-specific resistance. Some mutations narrowly reduce susceptibility to a single drug; others confer resistance across an entire drug class, or in some cases across two classes with structurally related resistance mechanisms. Thymidine analogue mutations (TAMs), for instance, arise under selection by zidovudine or stavudine but reduce susceptibility to essentially the entire NRTI class through a shared biochemical mechanism (excision of the incorporated chain-terminating nucleotide), while M184V, despite being selected by lamivudine or emtricitabine specifically, paradoxically partially resensitizes TAM-containing viruses to zidovudine and tenofovir — an interaction that has real clinical implications for how NRTI backbones are sequenced [6,7]. Table 1 summarizes the major mutation pathways discussed in this article across the four drug classes, their principal biochemical mechanisms, and their characteristic genetic barriers, drawing on the mutation-selection and resistance-mechanism literature synthesized in the sections that follow.

Interpreting a genotype in a clinical setting is itself a nontrivial exercise that has produced its own subfield. The Stanford HIV Drug Resistance Database (HIVdb) algorithm, first developed in the early 2000s and continuously updated since, assigns each observed mutation a penalty score against each individual antiretroviral drug and sums these scores, with adjustments for known mutation interactions, to produce a susceptibility category ranging from susceptible to high-level resistance; it remains the most widely used interpretation system in both clinical

practice and research, alongside the French ANRS and Belgian Rega algorithms, which use broadly similar rules-based approaches but differ in some specific mutation weightings [8]. These algorithms exist because raw mutation lists are not directly actionable — clinicians and program managers need a translation layer from genotype to predicted phenotype, and the fact that three independently maintained algorithms broadly agree on the clinically important calls, while disagreeing at the margins, is itself informative about where the genuine uncertainty in resistance interpretation lies.

3. Nucleoside and Nucleotide Reverse Transcriptase Inhibitor Resistance

NRTIs remain the backbone of essentially every first-line and second-line antiretroviral regimen in use today, which makes NRTI resistance mutations the most frequently encountered resistance pattern in patients experiencing virologic failure worldwide, essentially regardless of which third agent (NNRTI, PI, or INSTI) is paired with the backbone. Two broad mutational pathways dominate the literature: the TAM pathway and the non-TAM pathway typified by K65R and M184V.

The TAM pathway — comprising mutations at reverse transcriptase codons 41, 67, 70, 210, 215, and 219 — was characterized initially in the context of zidovudine monotherapy and confers resistance through enhanced ATP- or pyrophosphate-mediated excision of the chain-terminating nucleotide analogue after it has been incorporated into the growing DNA chain, effectively allowing reverse transcriptase to "back up" and remove the drug before it permanently terminates synthesis. TAMs accumulate in a roughly ordered fashion under continued thymidine-analogue pressure, and the number of TAMs present correlates with the degree of cross-resistance to the entire NRTI class, including tenofovir, though the specific TAM combination matters: TAM-2 pathway mutations (67N, 70R, 219Q/E, sometimes with 215F/Y) confer broader cross-class resistance than the TAM-1 pathway (41L, 210W, 215Y) at equivalent TAM counts [7].

K65R, by contrast, arises under selection by tenofovir, abacavir, didanosine, or stavudine and works through a fundamentally different mechanism: it reduces the efficiency of nucleotide incorporation itself rather than promoting excision, and — notably — it is largely mutually exclusive with the TAM pathway in a single

viral genome, because K65R partially reverses the TAM-associated excision phenotype and the two mutation sets are, in a biochemical sense, in tension with one another. This K65R–TAM antagonism, characterized in detail by Parikh and colleagues, has direct clinical relevance to NRTI sequencing decisions: a patient whose virus already carries multiple TAMs is relatively protected from also acquiring K65R, which means tenofovir may retain partial activity in that specific genetic background even after documented failure of a thymidine-analogue-containing regimen, a nuance easily lost if a genotype report is read only at the level of a resistance summary score rather than the specific mutation combination present [6,7]. This interaction is also central to why the World Health Organization–recommended second-line strategy of "recycling" tenofovir and lamivudine alongside a new third agent after first-line failure — rather than automatically switching the entire NRTI backbone — has performed better in trials than initial concerns about tenofovir cross-resistance would have predicted, a point we return to below in the discussion of the NADIA trial [2,9].

M184V (and its less common variant M184I), selected by lamivudine or emtricitabine, is in many respects the single most consequential NRTI mutation in the modern treatment landscape, precisely because both drugs remain part of essentially every first-line regimen recommended globally, including the tenofovir–lamivudine–dolutegravir (TLD) combination now used by the large majority of people on ART worldwide. M184V confers high-level, single-mutation resistance to lamivudine and emtricitabine — a low genetic barrier mutation in the truest sense — but it also modestly increases susceptibility to zidovudine, tenofovir, and stavudine, reduces overall reverse transcriptase processivity and hence viral replicative capacity, and improves polymerase fidelity, which has led to ongoing interest in whether maintaining lamivudine in a regimen even after M184V has emerged provides a net benefit through fitness-cost pressure rather than a net cost through loss of a fully active drug [6]. In the dolutegravir era this question has taken on new urgency, because M184V is frequently the only NRTI-class mutation present at first-line failure on TLD, and whether the lamivudine component still contributes meaningfully to a switched or intensified regimen in that setting is a live clinical question rather than a settled

one.

4. Non-Nucleoside Reverse Transcriptase Inhibitor Resistance

NNRTI resistance follows a different biochemical logic from NRTI resistance: rather than interfering with nucleotide incorporation directly, NNRTIs bind an allosteric pocket near the reverse transcriptase active site, and resistance mutations act by altering the shape, size, or chemistry of that pocket in ways that reduce drug binding without substantially impairing the enzyme's normal catalytic function. Because the NNRTI binding pocket is a relatively small, well-defined structural feature, single point mutations can produce large reductions in susceptibility, and first-generation NNRTIs — nevirapine and efavirenz — have a correspondingly low genetic barrier to resistance, a property that mattered enormously during the era when NNRTI-based first-line therapy was the global standard, and one of the principal reasons the field ultimately moved away from NNRTI-based first-line regimens toward dolutegravir.

K103N is the archetypal efavirenz- and nevirapine-selected mutation: it confers high-level resistance to both drugs without meaningfully compromising viral replicative capacity, and its emergence essentially forecloses the entire first-generation NNRTI class. Y181C, more characteristically selected by nevirapine, and G190A, which can arise under either drug, are similarly consequential, and longitudinal genotyping of patients on prolonged nevirapine-containing therapy has shown that these three mutations — K103N, Y181C, and G190A — accumulate over time on treatment in a pattern that is broadly, though not perfectly, sequential, with K103N tending to emerge earliest and the accumulation of additional mutations associated with longer cumulative exposure to failing therapy [10]. A Bayesian comparative analysis of resistance patterns selected specifically by nevirapine versus efavirenz in patients failing first-line therapy found that, while both drugs commonly select K103N, the two drugs differ measurably in the relative frequency of several other mutations, including a higher frequency of Y181C under nevirapine selection — a finding with practical implications for choosing which NNRTI to retain (or avoid) when a patient has documented exposure to only one drug in the class and clinicians are weighing whether any residual within-class activity might remain [11].

Etravirine and rilpivirine, the second-generation NNRTIs, were designed specifically to retain activity against many first-generation-resistant viruses by tolerating some conformational flexibility in the binding pocket, and single mutations that confer high-level resistance to efavirenz or nevirapine often leave etravirine and rilpivirine substantially or fully active. However, this advantage erodes as mutations accumulate: a virus carrying multiple NNRTI mutations selected sequentially over a long treatment history — a pattern common in patients who cycled through first-generation NNRTI-based first-line therapy for years before genotype-guided switching became available — frequently proves cross-resistant to the entire class, including its second-generation members. This accumulation phenomenon is one of the clearest illustrations in HIV drug resistance of why the duration of unrecognized virologic failure matters as much as the fact of failure itself: a genotype obtained promptly after failure is detected typically shows a simpler, more tractable mutation profile than one obtained after months or years of undetected failure on a failing regimen, precisely because each additional month of ongoing low-level replication under residual drug pressure is an opportunity for further mutations to accumulate.

5. Protease Inhibitor Resistance

Protease inhibitors occupy a distinctive position in the drug-resistance landscape because of the unusually high genetic barrier of the current-generation, ritonavir-boosted agents, darunavir in particular. Where a single point mutation can abolish susceptibility to an NNRTI, clinically meaningful darunavir resistance in a PI-naïve patient is uncommon even after documented virologic failure, because the mutations required to substantially reduce darunavir susceptibility are individually low-impact and must accumulate in combination, with each additional mutation imposing its own fitness cost on an enzyme (HIV-1 protease) that is under tight structural constraint because it must correctly process the Gag and Gag-Pol polyproteins for viral maturation to succeed at all.

Detailed structural and virologic characterization of this genetic barrier has identified the V32I substitution as a pivotal, if individually rare, event: V32I occurs infrequently under darunavir selection pressure, but once it does occur, it substantially predisposes the virus

to accumulate further protease mutations and develop clinically significant darunavir resistance — a "gatekeeper" mutation in the sense that its presence, rather than its absence, is the more informative signal for predicting subsequent resistance evolution [5]. This kind of finding illustrates a broader point about protease inhibitor resistance that distinguishes it from the NRTI and NNRTI literatures discussed above: because no single mutation is normally sufficient, understanding PI resistance requires attention to mutation combinations and their order of acquisition, not just to the presence or absence of individual substitutions, and genotype interpretation algorithms for PIs are correspondingly more combination-sensitive than their NRTI or NNRTI counterparts.

The practical consequence of this high genetic barrier is that boosted darunavir remains one of the most durable options available for patients who have already failed multiple prior regimens and accumulated substantial resistance to other classes, which is precisely the role it plays in second-line and third-line treatment strategies in both well-resourced and resource-limited settings. The NADIA trial's head-to-head comparison of dolutegravir versus darunavir, each paired with a recycled or switched NRTI backbone, as second-line therapy after first-line NNRTI failure in sub-Saharan Africa found both regimens to be effective, which is itself informative about the PI class's continued durability even as global policy has moved toward integrase-inhibitor-based regimens for most patients [2,9]. A subsequent randomized comparison, the D2EFT study, similarly evaluated dolutegravir plus boosted darunavir against standard-of-care regimens after first-line NNRTI failure, reinforcing that boosted darunavir continues to be treated in the current clinical trial literature as a reference-standard, high-barrier option against which newer regimens are benchmarked [12].

6. Integrase Strand Transfer Inhibitor Resistance and the Dolutegravir Era

No development in the past decade has reshaped the practical landscape of HIV drug resistance mutation profiles more than the near-universal shift to dolutegravir-based first-line therapy. Dolutegravir's appeal rests substantially on its resistance profile: compared with the first-generation integrase inhibitor raltegravir and the second-generation agent elvitegravir,

dolutegravir has a markedly higher genetic barrier to resistance, particularly in treatment-naïve patients, where treatment-emergent INSTI resistance mutations have proven rare in clinical trials and in the large programmatic rollouts that followed [1,2].

The mutation R263K, characterized in cell-culture selection experiments by Quashie and colleagues, illustrates why. R263K was the primary mutation selected by dolutegravir *in vitro*, but it confers only low-level resistance on its own and, notably, imposes a measurable fitness cost on the virus — a combination that helps explain dolutegravir's comparatively low rate of treatment-emergent resistance in first-line use, since a mutation that both underperforms at reducing susceptibility and impairs replication is a poor candidate to be selected to fixation under ongoing therapy [13]. Subsequent work extended this picture by showing that R263K in HIV-1 integrase also impairs the subsequent emergence of resistance mutations against reverse transcriptase inhibitors when a virus already carrying R263K is placed under new selective pressure from an NRTI or NNRTI, a cross-class interaction that appears to reflect a broader fitness constraint imposed by R263K on the overall replication complex rather than a direct biochemical interaction between the integrase and reverse transcriptase enzymes [14].

This favorable picture, however, applies most cleanly to treatment-naïve patients starting dolutegravir with an otherwise fully active regimen. The picture is materially different in patients who initiate or switch to dolutegravir with pre-existing NRTI resistance already present in the backbone — precisely the situation facing the large population of patients worldwide who were switched from an NNRTI-based first-line regimen to dolutegravir-based TLD as a programmatic policy change, often without a genotype to confirm the NRTI backbone was still fully active. Early case reports documented emergence of INSTI resistance mutations during dolutegravir-containing triple therapy specifically in patients with pre-existing M184V or M184I, suggesting that a compromised NRTI backbone removes some of the protection that an otherwise fully active two-drug NRTI combination normally provides against integrase resistance evolving under monotherapy-like functional pressure from dolutegravir alone [15]. A systematic review of the genetic mechanisms of dolutegravir resistance across both in

vitro passage experiments and reported clinical cases of virologic failure with INSTI resistance reached a broadly consistent conclusion: dolutegravir resistance, while still uncommon in absolute terms, occurs disproportionately in patients with reduced NRTI backbone activity, reinforcing that INSTI resistance in the dolutegravir era should not be assessed in isolation from the NRTI mutations already present [16].

This is exactly the scenario that large programmatic cohort studies in sub-Saharan Africa have now begun to characterize directly. A study of virologic failure and drug resistance after programmatic switching to dolutegravir-based first-line therapy in Malawi and Zambia — real-world public-sector treatment programs rather than a controlled trial — found that most patients switching to dolutegravir maintained viral suppression, but that among those who did experience virologic failure after the switch, resistance mutations, including emergent INSTI mutations, were identified in a meaningful subset, with pre-switch viral load and pre-existing NRTI resistance among the factors associated with post-switch failure [17]. A parallel review of adherence, resistance, and viral suppression on dolutegravir across sub-Saharan African cohorts more broadly, written explicitly with an eye toward the implications of the TLD rollout era, similarly concluded that while population-level outcomes on dolutegravir have been favorable, the emergence of resistance in the subset of patients who do fail is a real and monitorable phenomenon rather than a theoretical risk, and argued for sustained viral load monitoring and, where feasible, targeted genotyping of patients failing dolutegravir-based regimens rather than assuming the drug's high genetic barrier makes such monitoring unnecessary [18].

7. Programmatic and Surveillance Data From Low- and Middle-Income Countries

The majority of people living with HIV worldwide receive care in low- and middle-income countries, and the resistance patterns observed in these programmatic settings differ in important ways from those seen in well-resourced clinical trial cohorts — not because the underlying virology is different, but because the surrounding conditions (drug supply consistency, viral load monitoring frequency and turnaround time, adherence support infrastructure, and the historical sequence of regimens a national program has used)

shape which mutations accumulate and how long they go undetected before a treatment change occurs.

A systematic review and meta-regression analysis of pretreatment (transmitted) HIV-1 drug resistance among people initiating or re-initiating first-line ART in LMICs found that pretreatment resistance to NNRTIs in particular had risen over the preceding decade in several regions, reaching levels in some countries that exceeded the World Health Organization's threshold for reconsidering NNRTI-based first-line therapy at a national level — one of the concrete pieces of evidence that motivated the global shift toward dolutegravir-based first-line regimens, since a drug class with a low genetic barrier is a poor fit for a setting where an increasing fraction of newly diagnosed patients are already infected with a partially resistant virus before they ever start treatment [19]. A related large multicentre retrospective cohort study characterizing the global epidemiology of drug resistance after failure of WHO-recommended first-line regimens — spanning cohorts across sub-Saharan Africa, Asia, and Latin America — documented that NNRTI and NRTI resistance mutations were common at the point of first-line failure across essentially all regions studied, with cross-resistance patterns that materially narrowed second-line treatment options in settings where regimen choice was already constrained by drug availability [20].

These programmatic surveillance findings matter clinically in ways that trial data alone cannot fully capture, because programmatic cohorts include the patients who are hardest to keep in care, hardest to monitor closely, and most likely to experience long, undetected periods of virologic failure before a regimen change occurs — precisely the conditions under which mutation profiles become more complex and cross-resistant, as discussed above in the NNRTI section. A cross-sectional analysis of pretreatment HIV drug resistance among adults initiating or re-initiating first-line therapy in Zimbabwe, conducted specifically to inform the pace of the national transition to dolutegravir-based regimens, is illustrative of the kind of locally grounded evidence programs have needed to make this transition well: it combined pretreatment resistance prevalence data with programmatic considerations about how quickly a dolutegravir-based regimen could and should be prioritized for patients at highest risk of NNRTI-based first-line failure [21]. Work

of this kind — locally generated, programmatically actionable resistance surveillance rather than only globally pooled meta-analyses — has become an increasingly important complement to the large multicountry reviews, because the specific mutation prevalence and the specific programmatic bottlenecks driving virologic failure vary enough from country to country that global averages, while useful for setting overall policy direction, are not always sufficient to guide a given national program's implementation choices.

8. Detection Methods: Genotypic Testing, Next-Generation Sequencing, and Minority Variants

Everything discussed above depends on the ability to actually detect the mutations in question, and the technology used to do so has itself been a moving target over the period this literature covers. Conventional Sanger population sequencing, the long-standing clinical and surveillance standard, reliably detects mutations present in roughly 20% or more of the circulating viral population within a sample but is comparatively insensitive to minority variants present below that threshold — variants that may nonetheless be clinically relevant, either because they represent an emerging resistant population on the verge of becoming dominant, or because they reflect an archived resistant variant from a prior treatment episode that has receded to low frequency but can rapidly re-expand under renewed selective pressure from the same or a related drug.

Next-generation sequencing changes this picture substantially by sequencing many thousands of individual viral templates from a single sample, which allows detection of resistant variants at frequencies well below the Sanger threshold. A systematic review and meta-analysis of HIV-1 drug resistance detected by NGS specifically among antiretroviral-naïve individuals — that is, characterizing pretreatment resistance rather than acquired resistance after failure — pooled data across 39 eligible studies covering more than 15,000 treatment-naïve patients and found that the estimated prevalence of pretreatment drug resistance was strongly dependent on the variant-frequency detection threshold applied: prevalence estimates ranged from roughly 30% when variants present at just 1% of the viral population were counted, down to approximately 11% when only

variants at 20% or higher were counted — the range within which conventional Sanger sequencing operates [3]. This threshold sensitivity is not a minor technical footnote; it means that two studies reporting very different "pretreatment resistance prevalence" figures for ostensibly similar populations may simply be using different detection thresholds rather than describing genuinely different epidemiological realities, a source of literature heterogeneity that any synthesis of pretreatment resistance prevalence needs to account for explicitly.

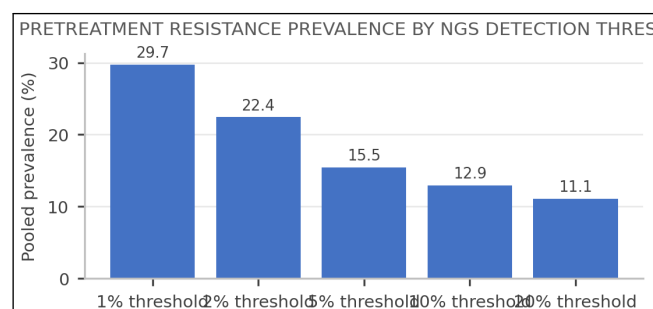


Figure 1: Pooled prevalence of pretreatment HIV-1 drug resistance detected by next-generation sequencing among antiretroviral-naïve individuals, as a function of the minority-variant detection threshold applied, across 39 studies and over 15,000 patients -- illustrating how much of the apparent resistance burden is invisible to conventional Sanger sequencing, which operates near the 20% threshold [3].

The same threshold sensitivity is evident, and arguably more clinically consequential, in treatment-experienced patients with virologic failure. A study applying NGS alongside conventional Sanger sequencing among individuals on first-line ART found that NGS detected drug resistance mutations, including an expanded profile of minority-variant mutations not identified by Sanger sequencing, in a notably higher proportion of samples from patients experiencing treatment failure than Sanger sequencing alone identified — 77.8% by NGS compared with 71.1% by Sanger sequencing among the same failing-treatment samples in that cohort — with the additional mutations detected by NGS specifically enriching for lower-frequency variants that a purely Sanger-based genotype would have reported as wild-type or "no mutations detected" [4]. Figure 1 summarizes the pretreatment-resistance prevalence estimates by detection threshold reported by Ouyang and colleagues, illustrating how sharply the apparent burden of resistance changes depending on the sensitivity of the assay used to look for it.

Despite this demonstrated added sensitivity, NGS has not yet displaced Sanger sequencing as the dominant

technology in either routine clinical genotyping or large-scale programmatic surveillance, for reasons that are mostly practical rather than scientific: NGS requires more specialized laboratory infrastructure, higher per-sample cost in most settings, and — critically — an unresolved clinical question about how to act on a minority variant detected at, say, 3% frequency, given that the clinical significance of low-frequency resistant variants depends heavily on which drug, which mutation, and which clinical context is under consideration, and the evidence base for translating a specific minority-variant frequency into a specific treatment recommendation remains considerably thinner than the evidence base for interpreting a standard Sanger-detected mutation. The Stanford HIVdb interpretation system and its counterpart algorithms, built and validated primarily on Sanger-era genotype-phenotype correlations, are only gradually being extended and revalidated to handle the richer minority-variant data NGS provides, and this validation gap is itself one of the more concrete open problems in the field, discussed further below [8].

9. Open Challenges

Several gaps recur across the literature synthesized in this article and are worth stating plainly. First, the relationship between INSTI resistance and NRTI backbone integrity in the dolutegravir era remains incompletely characterized at a population level. The case reports and mechanistic work showing that dolutegravir resistance emerges disproportionately against a background of pre-existing NRTI resistance are compelling, but the large programmatic cohorts needed to quantify this risk precisely — and to determine whether pre-switch genotyping to confirm NRTI backbone activity is cost-effective at scale in resource-limited settings — are still emerging, and the Malawi–Zambia programmatic switching data represent an early rather than a mature evidence base on this specific question [15,17].

Second, the clinical actionability of NGS-detected minority variants remains genuinely unresolved. The technical capability to detect resistant variants well below the Sanger threshold has clearly outpaced the clinical evidence needed to say what a physician should actually do differently — which regimen to avoid, or which to prefer — on the basis of a minority-variant finding at a given frequency, and until interpretation

algorithms and, ideally, prospective outcome data catch up with the sequencing capability, NGS's main proven value remains in surveillance and research contexts rather than routine individual patient management [3,4].

Third, resistance surveillance infrastructure remains geographically uneven in a way that directly limits how well the field can characterize the current global mutation landscape. The multicentre and meta-regression studies discussed here draw disproportionately on cohorts where genotyping infrastructure already existed, which likely underrepresents the settings with the least monitoring capacity — plausibly also the settings where undetected virologic failure persists longest and where the most complex, heavily cross-resistant mutation profiles are most likely to accumulate before being identified at all [19,20].

Fourth, and more mundane but no less real: comparability across studies is hampered by inconsistent reporting of detection thresholds, assay platforms, and mutation-scoring algorithms, which makes pooling prevalence estimates across the literature (as any synthesis, including this one, inevitably must) a genuinely difficult exercise, and one where apparent disagreement between studies sometimes reflects methodological differences rather than true epidemiological variation. We do not think any of these gaps undermine the core, well-replicated findings summarized in this article — that NRTI, NNRTI, PI, and INSTI resistance each follow well-characterized, biochemically grounded mutational pathways, and that the practical burden of resistance is shaped as much by program-level factors (monitoring frequency, time to detecting failure, backbone integrity at the time of a regimen switch) as by the intrinsic genetic barrier of any single drug — but they are the honest state of the evidence, and studies claiming precise, generalizable prevalence figures for any specific resistance mutation profile should be read with these caveats in mind.

10. Conclusion

The literature reviewed here supports a reasonably specific set of conclusions about drug resistance mutation profiles in patients with virologic failure. NRTI resistance, whether through the TAM pathway or the K65R/M184V pathway, remains the most consistently encountered resistance signature across drug classes

because NRTIs anchor nearly every regimen in use, and the specific combination of mutations present — not merely their number — determines how much residual activity a backbone retains [7,6]. NNRTI resistance follows a lower genetic barrier and a more rapidly foreclosing pattern than resistance to any other class, which is precisely why the field has moved away from NNRTI-based first-line therapy even as the accumulated mutation data from decades of nevirapine- and efavirenz-based treatment remain clinically relevant for the very large number of patients who were treated with those regimens historically [10,11]. Protease inhibitor resistance, by contrast, exemplifies a high genetic barrier built from the combinatorial accumulation of individually modest mutations, which is what makes boosted darunavir a durable option even deep into a treatment-experienced patient's regimen history [5,9].

Integrase strand transfer inhibitor resistance, and specifically dolutegravir resistance, is the newest and in some ways the most consequential piece of this picture, precisely because dolutegravir now anchors first-line therapy for the majority of people with HIV globally. The mechanistic and clinical-trial literature is reassuring about dolutegravir's genetic barrier in treatment-naïve patients with an intact NRTI backbone [1,13], but the emerging programmatic literature on switching treatment-experienced patients to dolutegravir-based regimens, often without confirming NRTI backbone activity beforehand, describes a genuinely different and less thoroughly characterized risk profile [15,17,16]. Making sense of any of this pattern, in any individual patient or any national program, increasingly depends on sequencing technology that can see below the traditional detection threshold — and the growing NGS literature makes clear that a meaningful amount of clinically relevant resistance has been sitting just below that threshold all along, detectable in aggregate but not yet fully actionable at the level of an individual treatment decision [3,4]. None of this argues that the current generation of antiretroviral therapy is fragile; if anything, the consistency of the mutational logic across four structurally distinct drug classes, replicated across in vitro, clinical-trial, and programmatic-cohort literatures, is strong evidence that HIV-1 drug resistance is a well-understood biological phenomenon. What remains unfinished is translating that understanding fully into the surveillance infrastructure, sequencing capacity, and treatment-switching protocols needed to

act on it consistently in the settings where the largest number of people with HIV actually receive their care.

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