HIV-1 Drug Resistance Mutations: Potential Applications for Point-of-Care Genotypic Resistance Testing

Running Title: HIV-1 Drug Resistance Mutations

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SUMMARY

The increasing prevalence of acquired and transmitted HIV-1 drug resistance is an obstacle to successful antiretroviral therapy (ART) in the low- and middle-income countries (LMICs) hardest hit by the HIV-1 pandemic. Genotypic drug resistance testing could facilitate the choice of initial ART in areas with rising transmitted drug resistance (TDR) and enable care-providers to determine which patients with virological failure (VF) on a 1st- or 2nd-line ART regimen require a change in treatment. An inexpensive near point-of-care (POC) genotypic resistance test would be useful in settings where the resources, capacity, and infrastructure to perform standard genotypic drug resistance testing are limited. Such a test would be particularly useful in conjunction with the POC HIV-1 viral load tests that are currently being introduced. A POC genotypic resistance test is likely to involve the use of allele-specific point mutation assays for detecting drug-resistance mutations (DRMs). This document proposes a set of DRMs for POC genotypic resistance testing in LMIC settings and outlines how such an assay could be used to optimize ART. Considering the technical challenges associated with the inclusion of each additional DRM in a point-mutation assay, we organized DRMs into a core group of essential tier 1 DRMs and additional tiers of incremental clinical usefulness.

INTRODUCTION

The global scale-up of antiretroviral therapy (ART) has dramatically reduced HIV-1-associated mortality, mother-to-child HIV-1 transmission, and adult HIV-1 incidence (1-4). These public health accomplishments are the result of the widespread administration of standardized 1st-line regimens containing two NRTIs plus an NNRTI, followed by a LPV/r-based regimen in those patients who subsequently develop virological failure (VF) (5, 6). However, the margin of long-term ART success is compromised by the development of acquired drug resistance (ADR) and transmitted drug resistance (TDR) (7, 8).

Between 10% and 30% of patients receiving a 1st-line NRTI/NNRTI-containing treatment regimen will develop VF at some point during their treatment (9-11); the majority of these patients are expected to have NRTI- and/or NNRTI-resistant viruses (7, 11-13). As the number of patients with ADR has increased so has the proportion of newly infected patients with TDR (7, 14-16). In many regions, the proportion of patients with transmitted NNRTI resistance has been increasing since ART scale-up (7, 14, 15). In recent studies, TDR levels above five percent were reported in about one-fourth of the surveys conducted in Sub-Saharan Africa and South/Southeast Asia and more than one-half of the surveys conducted in the Latin America/Caribbean region (7, 14, 15, 17, 18).

In upper-income countries, genotypic HIV-1 drug resistance testing is used to guide the selection of initial ART and subsequent treatments in patients with VF. However, the resources and capacity to perform standard genotypic resistance testing in the low- and middle-income countries (LMICs) for individual patient management are limited or concentrated in a few central laboratories. A point-of-care (POC) genotypic resistance test would avoid the logistical challenges and delays associated with centralized genotypic resistance testing. Assuming a clinic has the ability to act upon a genotypic resistance test result, such testing may strengthen the provider and patient relationship and support efforts to maximize retention on ART. Even in the context of a public health approach to ART, where few standardized regimens are available, a reliable and inexpensive POC genotypic resistance test would enable HIV-1 care providers to make informed treatment decisions for three categories of patients: (1)

ART-naïve patients starting therapy; (2) patients with VF on an initial NRTI/NNRTI-containing regimen; and (3) patients with persistently detectable viremia on a 1st- or 2nd-line PI-containing regimen.

ART-Naïve Patients Starting Therapy

Should population -levels of TDR continue to increase, the inability to predict which patients will respond to an initial NRTI/NNRTI-containing regimen would undermine confidence in the treatability of HIV-1 in LMICs and weaken the HIV care continuum. In regions where surveillance indicates elevated levels of drug resistance in patients beginning ART, pre-therapy POC genotypic resistance testing would identify those patients who should receive standard 1st-line therapy and those who should instead receive a boosted PI-containing regimen. Genotypic resistance testing would likely be particularly useful in the management of the increasing proportion of patients presenting for care for whom the past ART history is uncertain and to ensure that HIV-infected pregnant women with drug-resistant viruses receive the optimal regimen to prevent mother-to-child transmission.

Patients with VF on an Initial NRTI/NNRTI-Containing Regimen

Coupling genotypic resistance testing with viral load testing would make it possible to determine which patients with VF also have ADR. As the number of patients undergoing POC viral load monitoring increases (19, 20), POC genotypic resistance tests will help HIV care providers determine which patients require further adherence support and which patients should switch regimens (15, 21, 22).

Patients with Detectable Viremia on a 1st- or 2nd-Line PI-Containing Regimen

PIs are the main component of 2nd-line therapy in LMICs. PIs are also recommended as 1st-line therapy in women previously treated with single-dose NVP to prevent mother-to-child transmission and in infants less than three years regardless of their perinatal NVP exposure status (23-27). The absence of genotypic resistance in patients with detectable viremia on a PI-containing regimen is an indication for adherence counseling rather than a treatment change. The presence of genotypic PI resistance in patients

with persistently detectable viremia could prompt consideration of a 3rd-line regimen in those regions in which this is an option.

<p style="text-align:center">HIV-1 DRM CLASSIFICATION</p>

The NRTIs, NNRTIs, and PIs are the ARV classes used in most LMICs. Although the integrase inhibitors (INIs) are highly effective, safe, and well tolerated, they have been used primarily in upper-income countries. Should INIs become affordable, they will also play a pivotal role in ART in LMICs (28). All NRTI and NNRTI drug-resistance mutations (DRMs) are in the RT gene but there is practically no cross-resistance between these two drug classes.

A DRM can be characterized according to the following five criteria: (1) ***Polymorphism frequency***: its prevalence in virus isolates from ART-naïve patients in regions with low-levels of TDR; (2) ***Treatment prevalence***: Its prevalence in virus isolates from patients receiving ART. (3) ***Primacy***: its relative prevalence in the presence or absence of other DRMs; (4) ***In vitro phenotype***: its contribution to reduced *in vitro* susceptibility either alone or in combination with other DRMs; (5) ***Association with VF***: its association with a reduced virological response to an ARV in a new treatment regimen.

The Stanford HIV Drug Resistance Database (HIVDB) has an online genotypic resistance interpretation program to help clinicians and laboratories interpret HIV-1 genotypic resistance tests (http://hivdb.stanford.edu). The program accepts submitted RT, protease and/or integrase sequences and returns a list of penalty scores for each DRM in the sequence and an estimate of reduced susceptibility for each ARV obtained by adding the penalty scores for each DRM. The DRM penalty scores (http://hivdb.stanford.edu/DR/) are based upon the five criteria described in the previous paragraph and upon the consensus about the clinical significance of a DRM as reflected by experts such as the IAS-USA Drug Resistance Mutations Group (29). A penalty score of 15 to 29 predicts low-level resistance; a score of 30 to 59 predicts intermediate resistance; and a score of 60 or above predicts high-level resistance.

In this document, NRTI and PI DRMs with a score of 30 or more and NNRTI DRMs with a score of 60 or more are referred to as major DRMs. A lower score cut-off is used for the NRTIs and PIs because

high-level NRTI and PI resistance usually results from the accumulation of multiple DRMs associated with low-level and intermediate resistance rather than from a single DRM associated with high-level resistance. Tables 1, 2 and 3 contain the HIVDB DRM penalty scores and summarize the polymorphism frequency, treatment prevalence, primacy, and *in vitro* phenotype of the NRTI, NNRTI and PI DRMs.

Polymorphism Frequency

Most DRMs are nonpolymorphic in that they do not occur in the absence of selective drug pressure. Some DRMs, however, are polymorphic and may occur naturally in ARV-naïve patients. Nonpolymorphic DRMs may reduce susceptibility either alone or in combination with other DRMs; polymorphic DRMs are usually accessory. The fourth column of Tables 1, 2 and 3 indicates the polymorphism rates of the NRTI, NNRTI and PI DRMs. Nonpolymorphic DRMs used for TDR surveillance (surveillance DRMs; SDRMs) are indicated by a check in the SDRM column (30).

Treatment Prevalence

The development of a mutation during ARV therapy is Darwinian evidence that the mutation is associated with resistance to the ARV that selected the mutation. Assays that include a sufficient number of common nonpolymorphic DRMs will be specific and sensitive for detecting TDR and ADR. The fifth column of Tables 1, 2 and 3 indicates the prevalence of NRTI, NNRTI and PI DRMs in pooled sequences from NRTI-, NNRTI- and PI-experienced patients in HIVDB.

PI DRMs develop much less often in patients receiving a potent ritonavir-boosted PI-containing regimen such as LPV/r, ATV/r, and DRV/r than do NRTI and NNRTI DRMs in patients receiving NRTI/NNRTI-containing regimens (31-36). The reduced risk of resistance associated with boosted PIs is likely due to the narrow drug concentration range in which PI levels are both low enough to allow virus replication and high enough to exert selective drug pressure (37). Indeed, most patients without PI DRMs who experience VF while on an initial PI-containing regimen achieve virologic suppression with

improved adherence (38). Nonetheless, the possibility that mutations outside of protease may also be primary causes of VF is an area of active investigation (39, 40).

Several DRMs preferentially occur in certain HIV-1 subtypes. The NNRTI DRM V106M occurs more often in subtype C viruses from patients treated with NVP or EFV because V106M requires a single base-pair change in subtype C viruses – GTG (V) => ATG (M) – but a two base-pair change in all other subtypes – GTA (V) => ATG (M) (41, 42). By a similar mechanism, CRF01_AE viruses preferentially develop the NRTI DRM V75M (43), subtype G viruses preferentially develop the PI DRM V82M (44), and subtype A viruses from the former Soviet Union (A_{FSU}) preferentially develop the NNRTI DRM G190S (45). By a different mechanism, subtype C viruses are predisposed to develop the NRTI DRM K65R (46).

Primacy

HIV-1 strains from patients with VF often contain more than one DRM associated with resistance to an ARV they are receiving. Usually, the first or primary DRM reduces ARV susceptibility and subsequent DRMs either reduce susceptibility or compensate for reduced fitness associated with the primary DRM (47). The order in which DRMs develop depends on the ART regimen. For example, M184V causes high-level lamivudine (3TC)- and emtricitabine (FTC) resistance and develops rapidly in patients with VF while receiving one of these NRTIs. K65R, L74V and T215Y are primary DRMs associated with reduced susceptibility to ARVs other than 3TC or FTC. These DRMs usually follow M184V because 3TC and FTC are essential components of most NRTI-containing regimens. The sixth column of Tables 1, 2 and 3 indicates the prevalence with which each DRM occurs in the absence of other major DRMs.

In Vitro Susceptibility

The clinical significance of reductions in *in vitro* susceptibility often varies among ARVs belonging to the same or different ARV classes. For example, the dynamic susceptibility range between

wild type and the most NRTI-resistant viruses can be as low as 5-fold for tenofovir (TDF) and abacavir (ABC) but above 200-fold for zidovudine (AZT), 3TC and FTC (48-50). Similar but less pronounced dynamic susceptibility range differences exist in the NNRTI, PI and INI classes (51-53).

The difference in ARV susceptibility between a wild type laboratory clone and one containing a DRM yields an unbiased assessment of that DRM's phenotypic effect. However, the number of DRMs studied with the same susceptibility assay is limited. The contribution of a DRM to reduced ARV susceptibility can also be studied in clinical isolates using regression analyses in which the presence or absence of a DRM is an explanatory variable and the fold reduction in susceptibility is the outcome variable. The regression coefficients obtained from these models indicate the relative contribution of a DRM to reduced ARV susceptibility while attempting to control for the other DRMs in a virus sequence.

Columns 7 to 10 in Tables 1, 2 and 3 indicate the estimated fold reduction in susceptibility to the NRTIs 3TC, ABC, AZT and TDF; the NNRTIs nevirapine (NVP), efavirenz (EFV), etravirine (ETR) and rilpivirine (RPV); and the PIs ATV, darunavir (DRV) and LPV. For the same virus, FTC susceptibility levels are highly similar to 3TC susceptibility levels. The estimates in these tables were derived using regression models similar to those recently described (49, 51, 52). The dataset used in these regression models can be downloaded from http://hivdb.stanford.edu/pages/genopheno.dataset.html.

Clinical or Virological Response to ARV Therapy

In some regions, genotypic resistance tests are routinely performed prior to treatment to guide initial ARV therapy choices. This makes it difficult to examine the effect of a pre-existing DRM on the response to an initial ARV regimen. To do so, it is necessary to rely on the few studies in which HIV-1 was sequenced from cryopreserved blood samples obtained prior to the initiation of therapy from patients for whom genotypic resistance testing was not used to guide initial treatment decisions (54-59). These studies suggest that pre-therapy DRMs pose a higher risk to the success of 1st-line NRTI/NNRTI-containing regimens than to boosted PI-containing regimens. This conclusion is supported by additional studies in which ARV therapy was selected on the basis of standard genotypic resistance testing but was

followed by assays for low-abundant variants not detectable by standard dideoxy-terminator Sanger sequencing (60-65).

Many studies have attempted to ascertain the effect of individual DRMs on the virological response to specific ARVs in a salvage therapy regimen. Most had too few patients relative to the large number of covariates associated with response to salvage therapy. Nonetheless, in a few large clinical trials the variability in patient characteristics and salvage therapy regimens was sufficiently controlled to detect a reliable association between a pre-therapy DRM and the risk of VF. Such studies have assessed the effects of thymidine analog mutations (TAMs) and of M184V and K65R on the virological response to an ABC- (66) or TDF- (67) containing regimen; PI DRMs on the response to an LPV/r- (68) or DRV/r (69)-containing regimen; NNRTI DRMs on the response to an etravirine (ETR)-containing regimen (70); and INI DRMs on the response to a dolutegravir (DTG)-containing regimen (71, 72).

DRM PREVALENCE IN DIFFERENT CLINICAL SCENARIOS

Before Starting Initial ARV Therapy

NNRTI and NRTI resistance are the most common forms of TDR (7, 14, 15). Table 4 shows the absolute and cumulative prevalence of the major NRTI and NNRTI DRMs in RT sequences from a recently published individual patient-level meta-analysis of more than 50,000 ARV-naïve patients in 287 published studies (16).

In the NRTI class, M184V was the most common transmitted major DRM, accounting for more than 50% of viruses with one or more major DRMs regardless of region or subtype. M184I, K65R, L74V/I, Y115F and the TAMs K70R and T215Y/F were the next most common transmitted major NRTI DRMs. The TAMs M41L, D67N/E/G and K219Q/E/N/R and the T215 revertant mutations were the most common non-major transmitted NRTI DRMs.

K103N, Y181C and G190A were the three most common NNRTI DRMs in all regions and subtypes, occurring in more than 80% of viruses with a major NNRTI DRM. V106M was the fourth most common NNRTI DRM in subtype C viruses. V106A, Y188L and G190S accounted for most of the

remaining transmitted major NNRTI DRMs. A98G and K101E were the most common non-major transmitted NNRTI DRMs.

VF on a 1st-Line NRTI/NNRTI-Containing Regimen

To identify sensitive and specific indicators of ADR in patients with VF on a 1st-line NRTI/NNRTI regimen, we analyzed published RT sequences from 4,926 patients with VF while receiving the most commonly used 1st-line therapy regimens in LMICs. Table S1 summarizes the number of patients according to 1st-line regimen and HIV-1 subtype. Fifty-five percent, 27%, 16% and 2% received a d4T-, AZT-, TDF- or ABC-containing regimen, respectively. Fifty-four percent received EFV and 46% received NVP. The most common subtypes were C (46%), circulating recombinant form (CRF) 01_AE (15%), B (11%), A (8%), G (8%) and CRF02_AG (7%). Seventy-three percent of patients had one or more major NRTI DRMs and one or more major NNRTI DRMs. Nine percent had a major NNRTI DRM but no major NRTI DRM; 2% percent had a major NRTI DRM but no major NNRTI DRM; and 16% had no major NRTI or NNRTI DRM.

Table 5 shows that in viruses with one or more major NRTI DRM the most common were M184V (91%) and M184I (4.3%), K65R (11%), and the TAMs K70R (14%), T215Y (10%) and T215F (8.6%). About one-half of the viruses with K65R did not have M184V, making K65R the second largest contributor to the cumulative proportion of viruses with a major NRTI DRM. K65R also occurred in 48% of 467 patients with VF on a 1st-line TDF-containing regimen (Table S2). The TAMs nearly always occurred in combination with M184V and contributed less to the cumulative proportion of viruses with a major NRTI DRM than did K65R. In patients with VF on a 3TC- or FTC-containing regimen, M184I often emerges before M184V. However, M184V outcompetes M184I within several weeks in most patients (73, 74).

The spectrum of DRMs in 712 children was similar to adults with the exception that L74V/I occurred more often in children because a higher proportion of children received an ABC-containing regimen (Tables S3 and S4). Indeed, among both adults and children receiving ABC, L74V/I were the

second most common major NRTI DRMs after M184V (34, 75), although L74V/I rarely occurred in the absence of M184V.

Table 5 shows that the most common NNRTI DRMs in viruses from the 3,899 patients with one or more major NNRTI DRMs were K103N (49%), Y181C (26%), G190A (20%) and V106M (17%). One or more of these four DRMs occurred in 89% of viruses with a major NNRTI DRM. V106M was the second-most common NNRTI DRM in subtype C viruses, occurring in 33% of patients with a major NNRTI DRM. The six next-most common NNRTI DRMs – V106A, Y181I/V, Y188L, and G190S/E – accounted for an additional 10% of viruses with one or more major NNRTI DRM.

Persistently Detectable Viremia on a 1st- or 2nd-Line PI-Containing Regimen

Table 6 shows the most common major LPV-associated DRMs in published protease sequences from 1,214 previously PI-naïve patients with VF on an LPV/r-containing regimen. Of these 1,214 patients, 203 (17%) had viruses with predicted intermediate or high-level LPV resistance. The most common major PI DRMs were V82A, I76V, I84V and L47A. One or more of these four DRMs occurred in 88% of viruses with intermediate or high-level LPV/r resistance. The next two most common major LPV DRMs – I50V and V82F – accounted for an additional 4% of viruses with predicted intermediate or high-level LPV resistance. The remaining 8% of viruses with predicted intermediate or high-level LPV resistance had a combination of two or more PI DRMs with lower mutation scores, including V32I, M46I, I54M/L/V, I47V, V82S/T/M and L90M. The most common subtypes of these 203 viruses were C (49%), CRF01_AE (14%), CRF01_AG (12%), B (8%), G (7%) and A (5%). Overall 170 (84%) of the 203 LPV-resistant viruses had predicted intermediate or high-level cross-resistance to ATV/r; 36 (18%) had predicted intermediate or high-level cross-resistance to DRV/r.

Few protease sequences are available from PI-naïve patients with VF on ATV/r- or DRV/r-containing regimens. Published reports of aggregated data indicate that I50L and N88S are the main DRMs developing in PI-naïve patients with VF on an ATV- or ATV/r-containing regimen (32, 76, 77).

These DRMs do not confer cross-resistance to LPV or DRV (51). In fact, I50L is associated with increased susceptibility to LPV, DRV and other PIs (78).

PROPOSED DRMS FOR POC TESTING

RTI DRMs can be used to identify TDR in ART-naïve patients starting ART, and ADR in patients receiving a 1st-line NRTI/NNRTI-containing regimen. PI DRMs can be used to identify acquired PI resistance in patients receiving a 1st- or 2nd-line PI-containing regimen. The proposed RTI DRMs should be particularly sensitive at detecting ADR on a 1st-line NRTI/NNRT-containing regimen because a false-negative test result in such patients could delay an appropriate treatment change. In contrast, failure to detect TDR would result in a patient receiving the standard-of-care, though possibly suboptimal, 1st-line regimen. For both RT and protease, different mutations at the same amino acid position (e.g., M184V and M184I) are treated as separate DRMs despite the fact that some POC assays may be able to detect more than one DRM at the same position.

Tier 1 RTI DRMs: K103N, V106M, Y181C and G190A (NNRTIs); K65R and M184V (NRTIs)

Proposed tier 1 DRMs include the four NNRTI and two NRTI DRMs with the highest cumulative sensitivity for detecting ADR on a 1st-line NRTI/NNRTI-containing regimen (Table 7). This set of six DRMs was 99% sensitive for detecting ADR on a 1st-line NRTI/NNRTI regimen and 82% sensitive for detecting TDR in ART-naïve patients. No significant differences in sensitivity were observed for the subset of LMIC patients with ADR or TDR, the subset of children with ADR on a 1st-line NRTI/NNRTI-containing regimen, or the subset of adult patients with ADR on a 1st-line TDF-containing regimen.

In an ART-naïve patient, the presence of each of the tier 1 DRMs except K65R may be considered an indication for starting an initial PI-containing regimen or closer virological monitoring based on cost-effectiveness or country policy. The presence of K65R would be an indication for using an AZT/3TC nucleoside backbone.

In patients with VF on a 1st-line NRTI/NNRTI-containing regimen, the presence of a Tier 1 DRM indicates that the regimen has reduced antiviral activity. Although the presence of a DRM in patients with VF on a 1st-line NRTI/NNRTI regimen does not preclude a virological response to continued therapy with improved adherence (79-81), continued therapy is expected to result in a higher rate of immunological and clinical deterioration than would occur if the patient is switched to a 2nd-line PI-based therapy.

Tier 2 RTI DRMs: Y188L and G190S (NNRTIs); L74V/I, Q151M, M184I, and T215Y/F (NRTIs)

Y188L and G190S were the most common major non-tier 1 NNRTI DRMs associated with ADR on a 1st-line NRTI/NNRTI-containing regimen and among the most common non-tier 1 NNRTI DRMs associated with TDR. L74V/I, Q151M, M184I, and T215Y/F were among the most common major non-tier 1 DRMs associated with ADR on a 1st-line NRTI/NNRTI-containing regimen and TDR.

Compared to an assay that just detected tier 1 RTI DRMs, an assay that detected both tier 1 and 2 RTI DRMs would have increased the sensitivity for detecting a major NRTI or NNRTI DRMs from 82% to 91% in patients with TDR. Such an assay would also have increased sensitivity for detecting both a major NRTI and a major NNRTI-associated DRM from 85% to 95% in the 3,475 patients with dual-class ADR on a 1st-line NRTI/NNRTI-containing regimen. However, an assay with tier 1 and 2 RTI DRMs would only marginally increase the sensitivity for detecting at least one major NRTI or NNRTI DRM in patients with ADR on a 1st-line NRTI/NNRTI-containing regimen from 98% to 99%.

Additional NNRTI DRMs that may eventually have a role in a POC genotypic test include L100I, K101P, Y181I/V and G190E – DRMs associated with high-level ETR and RPV resistance (29, 52, 70). Additional NRTI DRMs that may eventually have a role in a POC genotypic resistance test include K65N, K70E/G and Y115 – DRMs associated with reductions in ABC and TDF susceptibility (34, 49, 82-84).

Tier 1 PI DRMs: I47A, L76V, V82A and I84V

Patients with VF on an LPV/r-containing regimen who have one or more of these DRMs have evidence for reduced LPV susceptibility. The sensitivity of a POC assay for detecting intermediate or

high-level LPV resistance could be increased from 88% to 98%, if it also included the DRMs M46I and I54V. A test with these six mutations would require additional interpretation because, when they occur alone, M46I or I54V confer only low-level LPV resistance. The PI DRMs – I50L and N88S – are likely to be useful in regions where ATV/r is the most commonly used initial PI (32, 76, 77).

UNCERTAIN ISSUES AND FUTURE DIRECTIONS

There are several areas of uncertainty with the analyses and recommendations in this document including whether the analyzed datasets were sufficiently representative to identify the most common major DRMs associated with TDR and ADR, whether a POC assay for a limited number of DRMs can be a useful replacement for standard sequencing, and how changes in ARV-treatment strategies would influence the choice of POC DRMs.

Published Datasets

The sequences used to identify the most common major NRTI and NNRTI DRMs associated with TDR were obtained from a recently published meta-analysis of 287 studies including 151 studies from Sub-Saharan Africa and the LMICs of South/Southeast Asia (16). The predominance of four of the tier 1 DRMs in all regions and subtypes suggests that these DRMs are robust indicators of TDR.

Of the 3,282 LMIC patients with ADR with a major NRTI or NNRTI DRM, only 291 (9%) were receiving a TDF-containing regimen. Additionally, most of the virus sequences in LMICs were from patients whose virus levels were not being monitored and who may therefore have had prolonged VF. As routine virus load monitoring is introduced in more regions, VF will likely be associated with fewer DRMs. Ongoing surveillance remains necessary to track the most common NRTI and NNRTI DRMs that will arise in the increasing number of patients receiving a TDF-containing 1st-line regimen and/or undergoing virological monitoring.

Use of a POC Assay to Detect a Limited Number of DRMs

Although many allele-specific point mutation assays for HIV-1 drug resistance have been developed for research purposes, only a few have been developed and studied for their reliability and applicability in routine patient management (59, 85, 86). However, even a point mutation assay that reliably detected all tier 1 RTI DRMs would underestimate the extent of drug resistance in a virus sample. The finding of one or more DRMs by a point-mutation assay would have different implications from the finding of the same DRMs by Sanger sequencing. Therefore, clinical studies could be useful to determine how to optimally use a POC genotypic resistance test.

Several analyses of the cost-effectiveness of standard genotypic resistance testing for specific clinical indications have yielded different conclusions (87-91). One of the promises of POC HIV-1 drug resistance testing is that it is expected to be less expensive than standard genotypic resistance testing using DNA sequencing. However, developing a POC test will require surmounting technical and regulatory hurdles. Therefore, an economic analysis relevant to the costs of developing such an assay would need to consider its use over the range of potential clinical applications, including the selection of the most efficacious ART regimens for patients with TDR and for patients with 1st- and 2nd-line ADR.

Evolution of ART Strategies

The usefulness of a POC genotypic resistance test will depend on regional treatment options. The extent to which ATV/r will be used for 2nd-line therapy and the potential availability of DRV/r and the INIs are key areas of uncertainty. In contrast to LPV/r, ATV/r has not been studied for treating patients with VF on a 1st-line NRTI/NNRTI regimen. Although LPV/r and ATV/r-containing regimens are equally efficacious for initial ART (92, 93), ATV/r-containing regimens may be less efficacious for second-line therapy. ATV/r has a lower genetic barrier to resistance than LPV/r and ATV/r monotherapy has consistently been less effective than LPV/r for regimen simplification (94-96). These data suggest that ATV/r may be less effective than LPV/r in treating patients with NRTI resistance. Therefore, the extent of NRTI resistance following initial therapy will likely have greater implications for the use of ATV/r-containing than for LPV/r-containing 2nd-line regimens.

However, if ATV/r-containing 2nd-line regimens prove effective, their use would have favorable implications for both POC testing and 3rd-line treatment. I50L and N88S are the most commonly occurring major DRMs in PI-naïve patients receiving ATV/r. Identifying clinically relevant ATV resistance would therefore be simpler than identifying the more complex patterns of DRMs associated with LPV resistance. In addition, most patients with VF on a 2nd-line ATV/r-containing regimen are expected to have viruses that are fully susceptible to LPV and DRV making it possible to create a highly effective 3rd-line regimen using these PIs.

Although the NNRTI rilpivirine (RPV) has recently been approved in upper-income countries for use in a fixed-dose combination with TDF and FTC, further studies would be necessary before it could be considered a standard first-line treatment option in LMICs. In particular, RPV is approved only for patients with plasma HIV-1 RNA levels below 100,000 copies/ml whose viruses do not have the RT mutation E138A – a mutation, which occurs in about 5% of viruses belonging to subtypes A and C (97).

It is difficult to predict how the introduction of INIs will influence the development of POC genotypic resistance testing strategies because such strategies depend on which INIs will be introduced and on whether they will be used for 1st-, 2nd- or 3rd-line therapy. However, if INIs will be used beyond the first line of therapy and in combination with NRTIs, it may become important to identify the NRTI DRMs most likely to increase the risk of VF on an NRTI/INI-containing regimen.

EXECUTIVE SUMMARY

The increasing prevalence of acquired and transmitted HIV-1 drug resistance is an obstacle to successful antiretroviral therapy (ART) in the low- and middle-income countries (LMICs) hardest hit by the HIV-1 pandemic. Genotypic drug resistance testing could facilitate the choice of initial ART in areas with rising transmitted drug resistance (TDR) and enable care-providers to determine which patients with virological failure (VF) on a 1st- or 2nd-line ART regimen require a change in treatment. An inexpensive near point-of-care (POC) genotypic resistance test would be useful in settings where the resources,

capacity, and infrastructure to perform standard genotypic drug resistance testing are limited. Such a test would be particularly useful in conjunction with the POC HIV-1 viral load tests that are currently being introduced.

A POC genotypic resistance test is likely to involve the use of allele-specific point mutation assays for detecting drug-resistance mutations (DRMs). The inclusion of a DRM in a POC assay should be based on its sensitivity and specificity for identifying drug-resistant HIV-1 strains and its relevance to the success of ART. This document proposes a set of DRMs for POC genotypic resistance testing in LMIC settings and outlines how such an assay could be used to optimize ART. Considering the technical challenges associated with the inclusion of each additional DRM in a point-mutation assay, we organized DRMs into a core group of essential tier 1 DRMs and additional tiers of incremental clinical usefulness.

Nucleoside reverse transcriptase (RT) inhibitor (NRTI), nonnucleoside RT inhibitor (NNRTI), and protease inhibitor (PI)-associated DRMs were selected for inclusion based on their scores in the Stanford HIV Drug Resistance Database (HIVDB) genotypic resistance interpretation system and their prevalence in ART-naïve patients with TDR and ART-experienced patients with ADR. To identify the most common transmitted DRMs, we analyzed HIV-1 RT sequences described in a recent meta-analysis of 287 studies with more than 50,000 adult ART-naïve patients. To identify the most commonly acquired NRTI- and NNRTI-associated DRMs, we analyzed published HIV-1 RT sequences from nearly 5,000 adult and children with VF on a standard 1st-line NRTI/NNRTI-containing ART regimen. To identify the most common ritonavir-boosted lopinavir (LPV/r)-associated DRMs, we analyzed protease sequences from 1,214 previously PI-naïve patients with VF on an LPV/r-containing regimen.

One or more members of a set of six tier 1 RT DRMs – two major NRTI-associated DRMs (M184V and K65R) and four major NNRTI-associated DRMs (K103N, Y181C, G190A, and V106M) – were present in 82% of analyzed virus sequences from ART-naïve patients with TDR and 98% of analyzed virus sequences from patients with ADR on a 1st-line NRTI/NNRTI-containing regimen. The detection of one or more of these six RT DRMs in an ART-naïve patient or in a patient with VF on a 1st-line NRTI/NNRTI-containing regimen may be considered an indication for a PI-containing regimen or

closer virological monitoring based on cost-effectiveness or country policy. The six tier 1 RT DRMs were also highly sensitive for detecting ADR in the subsets of children receiving a 1st-line NRTI/NNRTI regimen and adults receiving a 1st-line TDF-containing NRTI/NNRTI regimen.

A set of Tier 2 RTI DRMs including the NNRTI DRMs Y188L and G190S and the NRTI DRMs L74V/I, Q151M, M184I, and T215F/Y increased the sensitivity for detecting TDR from 82% to 92% and for detecting dual class NRTI/NNRTI resistance in patients with VF on a 1st-line NRTI/NNRTI-containing regimen from 85% to 95%. However, considering the limited number of treatment options in many LMICs and the technical challenges associated with the inclusion of each additional DRM in a point mutation assay, the inclusion of the Tier 2 mutations in a POC assay is currently not a high priority.

Our analysis indicated that a set of four PI DRMs – I47A, L76V, V82A, and I84V – was 88% sensitive for detecting intermediate and high-level LPV resistance in patients receiving a 1st- or 2nd-line LPV/r-containing regimen. In published studies, the PI DRMs I50L and N88S are likely to be the most sensitive DRMs for detecting intermediate and high-level ATV resistance in patients receiving a 1st- or 2nd-line ATV/r-containing regimen. The inclusion of PI DRMs in a POC genotypic resistance test is likely to be useful primarily in settings in which third-line ART regimens are available.

Table 1. Prevalence of Nucleoside RT Inhibitor (NRTI) Drug-Resistance Mutations (DRMs) in Antiretroviral (ARV)-Naïve and -Treated Patients and Their Estimated Contributions to Reduced NRTI Susceptibility

DRM	HIVDB Score[a]	SDRM[b]	Prevalence (%)[c]		% Without Other Major DRMs[d]	Phenotypic Fold Resistance[e]			
			ARV-Naïve (n=54,728)	ARV-Treated (n=25,424)		3TC (n=1361)	ABC (n=1267)	AZT (n=1373)	TDF (n=1081)
M184V	60	✓	0.2	52	35	**>50**	**3**	0.3	0.5
K65R	60	✓	0.04	4	30	**5**	**3**	0.8	**2**
Q151M	60	✓	0	3	9	1.7	**4**	**5**	1.1
M184I	60	✓	0.03	2	28	**>50**	**1.7**	0.3	0.6
T215Y	45	✓	0.02	28	21	1.5	**1.8**	**6**	1.4
T215F	45	✓	0.01	10	7	1.5	**1.7**	**8**	**1.6**
Y115F	45	✓	0.01	2	1	1.4	**3**	**4**	1.7
T69i	45	✓	0	1	3	**3**	**5**	**18**	**4**
K70R	30	✓	0.07	18	12	1.3	1.3	**5**	**1.7**
L74V	30	✓	0.01	9	6	1	**1.5**	0.3	0.6
L74I	30	✓	0.02	4	2	0.8	1.2	0.8	0.8
D67d	30		0	0.09	0	NA	NA	NA	NA
M41L	15	✓	0.3	30	2	1.1	1.1	**2**	**1.5**
D67N	15	✓	0.04	28	1	1.2	1.2	**2**	1.2
L210W	15	✓	0.06	19	2	1.2	1.4	**4**	**1.6**
T215I	15	✓	0.03	1	6	1.8	**1.5**	**5**	**1.6**
T215S	15	✓	0.3	0.9	13	0.8	0.8	0.3	0.7
T215C	15	✓	0.09	0.8	20	0.9	1	1.2	0.8
T215D	15	✓	0.3	0.6	45	1.3	0.8	0.3	0.6
T215V	15	✓	0.01	0.6	4	1.1	1	1.7	1
K70E	15	✓	0.02	0.6	7	2	1.1	0.2	1
K70G	15		0	0.4	4	1.4	1.2	0.3	1.1
T69d	15		0	0.2	2	NA	NA	NA	NA
T215E	15	✓	0.1	0.2	39	2	1.1	1.2	1.3
K65N	15		0.03	0.1	20	NA	NA	NA	NA
K219Q	10	✓	0.09	11	2	1.1	1	0.9	1
T69D	10	✓	0.03	6	3	1.1	1	0.8	0.9
K219E	10	✓	0.03	6	2	1	0.9	0.4	0.8
V75M	10	✓	0.03	3	1	0.9	1.3	1.4	1.1
K219N	10	✓	0.04	3	3	1.2	1.1	1.1	-1
K219R	10	✓	0.07	3	2	1.8	**1.6**	**3**	**1.5**
D67G	10	✓	0.05	2	5	1.1	1.1	**2**	1.2
F116Y	10	✓	0.01	2	0	1.1	1	**3**	1.2
F77L	10	✓	0.1	2	2	1	0.9	**4**	1.4
V75T	10	✓	0	1	6	1.8	**1.6**	0.8	0.9
D67E	10	✓	0.01	0.6	1	1.2	**1.5**	1	**1.5**
K70T	10		0.03	0.3	8	0.1	0.9	**3**	**1.8**
K70N	10		0.03	0.3	6	1	1.3	1.6	1.4
K70Q	10		0.02	0.2	3	NA	NA	NA	NA
K65E	10		0.03	0.1	22	NA	NA	NA	NA
A62V	5		0.9	4	3	0.9	1.0	1.2	1.2
V75I	5		0.04	3	4	1.5	1.1	1.8	0.9

[a]HIVDB Score: Highest penalty score according to the Stanford HIV Drug Resistance Database (HIVDB) genotypic resistance interpretation program (version 7.0) for lamivudine (3TC), abacavir (ABC), zidovudine (AZT), and tenofovir (TDF). Scores of 15 to 29, 30 to 59, and ≥60 indicate low, intermediate, and high-level resistance. Emtricitabine (FTC) and 3TC scores are identical. [b]Surveillance Drug Resistance Mutation (SDRM): In ARV-naïve patients, these DRMs are indicators of transmitted drug resistance (TDR) (30). [c]DRM prevalence in samples from patients with known ARV

treatment history in HIVDB. The ARV-Naïve category excludes viruses with ≥2 SDRMs considered to be consistent with TDR rather than natural variation. [d]Proportion of patient samples having the DRM and no other major NRTI DRM (score ≥30) / all patient samples with the DRM. [e]Estimated contribution to fold-reduced susceptibility based on linear regression analysis of PhenoSense susceptibility test results (50) (http://hivdb.stanford.edu/pages/genopheno.dataset.html). 'NA': fewer than three phenotypes with the DRM. Fold-resistance levels in bold (≥1.5 for ABC and 3TC, ≥2 for AZT, and ≥3 for 3TC) indicate a statistically and probable clinically significant increase above 1.0 compared with wildtype.

Table 2. Prevalence of Non-Nucleoside RT Inhibitor (NNRTI) Drug-Resistance Mutations (DRMs) in Antiretroviral (ARV)-Naïve and -Treated Patients and Their Estimated Contributions to Reduced NNRTI Susceptibility

DRM	HIVDB Score[a]	SDRM[b]	Prevalence (%)[c]		Without / With other Major DRMs[d]	Phenotypic Fold Resistance[e]			
			ARV-Naïve (n=54,728)	ARV-Treated (n=25,424)		NVP (n=1694)	EFV (n=1687)	ETR (n=484)	RPV (n=183)
K103N	60	✓	1.0	36	37	**24**	**21**	1.3	2
Y181C	60	✓	0.1	20	29	**16**	**2**	**8**	**3**
G190A	60	✓	0.2	15	12	**11**	**11**	0.9	1.3
V106M	60	✓	0.01	5	15	**18**	**32**	0.6	NA
L100I	60	✓	0.01	4	1	**3**	**14**	**6**	**7**
Y188L	60	✓	0.04	4	55	**>50**	**>50**	**3**	**10**
G190S	60	✓	0.01	3	26	**35**	**>50**	0.9	NA
M230L	60	✓	0.02	2	5	**6**	**7**	**4**	**5**
V106A	60	✓	0.01	2	13	**>50**	**7**	0.4	NA
K103S	60	✓	0.04	2	5	**11**	**7**	1.5	1.7
K101P	60	✓	0	1	5	**18**	**25**	**22**	**>50**
Y188C	60	✓	0.01	0.9	19	**>50**	**35**	NA	NA
Y181I	60	✓	0.01	0.9	49	**>50**	1.4	**30**	**24**
Y181V	60	✓	0	0.6	56	**>50**	2	**>50**	NA
G190E	60	✓	0.02	0.5	67	**>50**	**>50**	**>50**	**27**
Y188H	60	✓	0.03	0.5	7	**5**	**9**	NA	NA
G190Q	60		0	0.3	70	**>50**	**>50**	NA	NA
K101E	30	✓	0.2	8	4	**2**	**3**	1.5	**2**
A98G	30		0.2	6	13	**2**	**2**	1.4	**3**
P225H	30	✓	0.02	4	2	**2**	**3**	1.2	NA
F227L	30		0.04	3	5	1.4	**2**	2	NA
K238T	30		0.04	2	3	**3**	**2**	1.4	NA
Y318F	30		0.1	2	4	NA	NA	NA	NA
E138K	30		0.1	0.4	18	-0.6	1	2	**1.6**
F227C	30		0	0.04	13	NA	NA	NA	NA
N348I	15		0.09	14	17	NA	NA	NA	NA
V108I	15		0.5	9	5	**2**	**3**	1	0.9
E138A	15		3	3	32	1.5	1.6	2	**1.8**
K101H	15		0	1	3	**3**	**3**	1.3	1
E138Q	15		0.03	1	3	1.4	1	NA	NA
E138G	15		0.3	0.7	18	**2**	1.4	**3**	**1.7**
V179F	15	✓	0	0.3	0	0.9	**3**	**3**	0.4
V179D	10		2	3	18	**3**	**5**	**3**	**1.8**

[a]HIVDB Score: The highest mutation penalty score according to the Stanford HIV Drug Resistance Database (HIVDB) genotypic resistance interpretation program (version 7.0) for nevirapine (NVP), efavirenz (EFV), etravirine (ETR), and rilpivirine (RPV). Total scores of 15 to 29, 30 to 59, and ≥60 indicates low-level, intermediate, and high-level resistance. [b]Surveillance Drug Resistance Mutation (SDRM): When present in ARV-naïve patients, these DRMs are considered specific indicators of transmitted drug resistance (TDR) (30). [c]Prevalence of DRM in samples from patients with known ARV treatment history in HIVDB. The ARV-Naïve category excludes viruses containing ≥2 SDRMs as these were considered to be consistent with TDR rather than natural variation. Nonetheless, the 1.0% prevalence of K103N in ARV-naïve patients reflects its common occurrence in patients with TDR. [d]Proportion of patient samples having the DRM and no other major NNRTI DRM (score ≥60) / all patient samples with the DRM. [e]Estimated contribution to fold-reduced susceptibility based on linear regression analysis of PhenoSense susceptibility test results (50) (http://hivdb.stanford.edu/pages/genopheno.dataset.html). 'NA': fewer than three phenotypes with the DRM. Fold-resistance levels in bold (≥2 for NVP, EFV, and NVP and ≥3 for ETR) indicate a statistically and probable clinically significant increase above 1.0 compared with wildtype.

Table 3. Prevalence of Protease Inhibitor (PI) Drug-Resistance Mutations (DRMs) PI-Naïve and -Treated Patients and Their Estimated Contributions to Reduced PI Susceptibility

DRM	HIVDB Score[a]	SDRM[b]	Prevalence (%)[c]		Without / With other Major DRMs[d]	Phenotypic Fold Resistance[e]		
			PI-Naïve (n=60,537)	PI-Treated (n=13,660)		ATV (n=1100)	DRV (n=590)	LPV (n=1389)
I84V	60	✓	0.02	15	7	**4**	**3**	**3**
N88S	60	✓	0.03	2	70	**7**	0.9	1.1
I47A	60	✓	0	0.5	8	0.9	2	**43**
I50L	60	✓	0.01	0.5	48	**6**	0.4	0.3
I84A	60	✓	0	0.2	54	**47**	NA	**6**
I84C	60	✓	0	0.2	64	**5**	NA	1.9
V82A	30	✓	0.04	24	27	1.5	0.8	**3**
G48V	30	✓	0	4	3	**4**	0.8	2
L76V	30	✓	0.01	4	9	0.4	2	**4**
I50V	30	✓	0.02	2	11	0.9	**4**	**4**
V82F	30	✓	0	2	22	**2**	**4**	**7**
G48M	30	✓	0	0.5	0	**2**	1.1	1.8
L90M	25	✓	0.3	33	23	**3**	1.2	1.9
V82T	25	✓	0	3	18	2	0.6	**3**
V82S	25	✓	0	1	25	**4**	NA	**6**
V82M	25	✓	0	0.3	22	1	1.2	0.9
I54L	20	✓	0.01	3	4	**2**	**3**	1.9
I54M	20	✓	0	3	3	**2**	**5**	**3**
I54V	15	✓	0	27	1	**3**	1.4	**4**
M46I	15	✓	0.3	23	3	1.2	1.2	1.6
V32I	15	✓	0.01	5	2	**3**	**3**	1.3
I47V	15	✓	0.03	5	0.6	0.9	1.3	**4**
I54A	15	✓	0	1	0.5	**12**	NA	11
I54T	15	✓	0.01	0.9	2	**9**	**6**	**9**
I54S	15	✓	0	0.7	2	**10**	2	**11**
M46L	10	✓	0.3	10	4	1.5	1.3	1.6
G73S	10	✓	0.03	9	3	**2**	1.2	1.5
D30N	10	✓	0.02	6	28	**3**	-0.9	1.1
L24I	10	✓	0.02	6	3	**2**	1	1.8
F53L	10	✓	0.04	6	1	1.7	1.1	1.3
K20T	10		0.1	5	7	**2**	1.1	1.9
G73T	10	✓	0	3	1	**2**	1.6	1.5
T74P	10		0.04	2	6	**2**	1.5	1.4
G73C	10	✓	0	1	0.6	1.7	1.1	1.6
N83D	10	✓	0.02	0.8	12	**3**	0.9	1.3
V82C	10	✓	0	0.6	4	1.3	1.3	**3**
V82L	10	✓	0.02	0.3	30	**2**	1.5	1.2
L33F	5		0.4	13	2	1.2	2	1.8
L10F	5		0.2	10	3	1.4	1.5	2
L89V	5		0.06	4	4	1.2	**3**	1.4

[a]HIVDB Score: The highest mutation penalty score according to the Stanford HIV Drug Resistance Database (HIVDB) genotypic resistance interpretation program (version 7.0) for atazanavir (ATV), darunavir (DRV), and lopinavir (LPV). Total scores of 15 to 29, 30 to 59, and ≥60 indicates low-level, intermediate, and high-level resistance. [b]Surveillance Drug Resistance Mutation (SDRM): When present in ARV-naïve patients, these DRMs are considered specific indicators of transmitted drug resistance (TDR) (30). [c]Prevalence of DRM in samples from patients with known ARV treatment history in HIVDB. The ARV-Naïve category excludes viruses containing ≥2 SDRMs as these were considered to be consistent with TDR rather than natural variation. [d]Proportion of patient samples having the DRM and no other major NNRTI DRM (score ≥30) / all patient samples with the DRM. [e]Estimated contribution to fold-reduced susceptibility based on linear regression analysis of PhenoSense susceptibility test results (50) (http://hivdb.stanford.edu/pages/genopheno.dataset.html). 'NA': fewer than three phenotypes with the DRM. Fold-resistance levels in bold (≥2 for ATV and ≥3 for LPV and DRV) indicate a statistically and probable clinically significant increase compared with wildtype.

Table 4. Absolute and Cumulative Prevalence of Each Major Nucleoside (NRTI) and Nonnucleoside RT Inhibitor (NNRTI) Drug-Resistance Mutation (DRM) In Patients With Transmitted Drug Resistance and ≥1 Major NRTI or NNRTI DRM in a Meta-Analysis of 287 Studies Published Between 2000 and 2013

LMICs[a] (n=24,173 individuals)			Upper-income countries[a] (n=24,898 individuals)		
Prevalence of Each Major NRTI DRM[b] (n=285 viruses with ≥1 major NRTI DRM)			Prevalence of Each Major NRTI DRM[b] (n=782 viruses with ≥1 major NRTI DRM)		
DRM	Absolute %[d]	Cumulative %[e]	DRM	Absolute %[d]	Cumulative %[e]
184V	63.5	63.5	184V	52.1	52.1
70R	19.9	73.9	215Y	31.4	72.1
184I	7.5	80.1	70R	24.4	87.5
65R	6.6	85.1	65R	5.3	91.2
215Y	19.5	90	215F	10.8	94.1
74I	5.8	94.2	74V	6.8	95.8
115F	2.9	97.1	184I	3.5	97.4
215F	10.8	99.2	151M	3.3	98.7
74V	4.6	100	74I	4.8	99.8
151M	1.7	100	115F	2.9	100

Prevalence of Each Major NNRTI DRM[c] (n=732 viruses with ≥1 major NNRTI DRM)			Prevalence of Each Major NNRTI DRM[c] (n=1,089 viruses with ≥1 major NNRTI DRM)		
DRM	Absolute %[d]	Cumulative %[e]	DRM	Absolute %[d]	Cumulative %[e]
103N	55.2	55.2	103N	67	67
181C	23.7	75.5	181C	14.7	78
190A	16.4	86.1	190A	11.7	86.4
188L	3.1	89	188L	5.1	90.5
103S	4.3	90.8	103S	3.4	92.2
106M	2	92.2	188H	1.6	93.6
190S	1.8	93.7	188C	1.4	94.9
190E	1.4	95.1	106A	1.6	96
100I	1.8	96.3	106M	1.1	97
106A	1	97.1	190S	1.3	97.8
188C	1.2	98	190E	0.8	98.7
230L	2	98.8	230L	0.8	99.2
181I	0.6	99.4	181I	1	99.6
188H	0.6	99.8	100I	2.4	99.9
181V	0.6	100	101P	1.1	100
101P	0.6	100	181V	0.1	100

[a]LMICs: Low- and Middle-Income Countries of Sub-Saharan Africa, South / Southeast Asia, and Latin America and Caribbean; Upper-Income Countries: Upper-Income Countries of North America, Europe, and Southeast Asia. [b]NRTI DRM with an HIVDB score ≥30. There were no insertions or deletions between codons 67 and 70. [c]NNRTI DRMs with an HIVDB score ≥60. [d]Absolute %: number of individuals with DRM / number of individuals with a major DRM of the same drug class (NRTI or NNRTI). [e]Cumulative %: number of individuals with one or more of the preceding DRMs in the list / number of individuals with a major DRM of the same drug class (NRTI or NNRTI).

Table 5. Absolute and Cumulative Prevalence of Each Major Nucleoside (NRTI) and Nonnucleoside RT Inhibitor (NNRTI) Drug-Resistance Mutation (DRM) in 4,926 Patients with Virological Failure and Acquired Drug Resistance while Receiving a 1st-Line NRTI/NNRTI-Containing Regimen[a]

LMICs[b] (n=3,981 individuals)			Upper-income countries[b] (n=945 individuals)		
Prevalence of Each Major NRTI DRM[c] (n=3,110 viruses with ≥1 major NRTI DRM)			Prevalence of Each Major NRTI DRM[c] (n=514 viruses with ≥1 major NRTI DRM)		
DRM	Absolute %[e]	Cumulative %[f]	DRM	Absolute %[e]	Cumulative %[f]
184V	91.7	91.7	184V	87.4	87.4
65R	9.8	96.7	65R	16.7	94.2
184I	3.7	98.8	184I	8	97.9
215Y	11.1	99.3	115F	4.9	98.4
70R	14.7	99.6	215Y	5.6	99
215F	9.4	99.8	70R	8.9	99.4
151M	3.6	99.9	74I	3.7	99.6
115F	3	99.9	151M	0.4	99.8
74I	2.3	100	74V	5.4	100
74V	4.1	100	215F	3.9	100

Prevalence of Each Major NNRTI DRM[d] (n=3,291 viruses with ≥1 major NNRTI DRM)			Prevalence of Each Major NNRTI DRM[d] (n=608 viruses with ≥1 major NNRTI DRM)		
DRM	Absolute %[e]	Cumulative %[f]	DRM	Absolute %[e]	Cumulative %[f]
103N	45.7	45.7	103N	63.8	63.8
181C	27.2	66.9	181C	19.1	78.3
106M	18.8	79.9	190A	13.5	83.6
190A	21.2	89.3	190S	6.6	88.3
188L	5.7	92.8	188L	5.3	92.3
190S	2.9	95	106M	4.6	94.6
106A	1.7	96.2	190E	1.6	96.2
181V	1.2	97.1	106A	2	97.5
190E	0.9	97.9	188C	1.8	98
181I	0.8	98.6	188H	1.3	98.5
190Q	0.6	99.1	190Q	0.5	98.8
188C	2	99.6	230L	3	99.2
103S	2.8	99.8	181V	0.5	99.5
230L	5	99.9	181I	0.5	99.8
188H	1.2	100	100I	6.4	100
100I	2.2	100	101P	0.7	100

[a]Regimens include four AZT/d4T-containing regimens – AZT/d4T+3TC+EFV/NVP (n=4,020), four TDF-containing regimens – TDF+3TC/FTC+EFV/NVP (n=772), and two ABC-containing regimens – ABC+3TC+NVP/EFV (n=134). [b]LMICs: Low- and Middle-Income Countries of Sub-Saharan Africa, South / Southeast Asia, and Latin America and Caribbean; Upper-Income Countries: Upper-Income Countries of North America, Europe, and Southeast Asia. [c]NRTI DRM with an HIVDB score ≥30. There were no insertions or deletions between codons 67 and 70. [d]NNRTI DRMs with an HIVDB score ≥60. [e]Absolute %: number of individuals with DRM / number of individuals with a major DRM of the same drug class (NRTI or NNRTI). [f]Cumulative %: number of individuals with one or more of the preceding DRMs in the list / number of individuals with a major DRM of the same drug class (NRTI or NNRTI).

Table 6. Absolute and Cumulative Prevalence of Major Lopinavir-Associated Mutations in 203 Lopinavir (LPV)-Resistant Viruses From 1,214 Previously PI-Naïve Patients with Virological Failure on a Ritonavir-Boosted LPV (LPV/r)-Containing Regimen[a]

DRM	Prevalence of Major LPV/r DRMs (n=203 Viruses with Intermediate or High-Level LPV Resistance)	
	Absolute %[b]	Cumulative %[e]
V82A	59.6	59.6
L76V	32.5	74.9
I84V	15.3	82.8
I47A	8.4	88.2
V82F	2.5	90.1
I50V	4.9	91.6
Other[d]	8.4	100

[a]Resistance was defined as the presence of a cumulative HIVDB LPV mutation penalty score ≥30. [b]Absolute %: number of individuals with DRM / number of individuals with a major LPV/r DRM. [c]Cumulative %: number of individuals with one or more of the preceding major LPV/r DRMs in the list / number of individuals with a major LPV/r DRM. [d]Other included viruses having intermediate or high-level resistance arising from an accumulation of mutations with an HIVDB penalty score <30 including: M46I/I54V/V82S (n=4), I54V/V82M (n=3), I54V/L90M (n=1), V32I/M46I/I47V/I54M/L90M (n=1), I54V/V82T/L90M (n=1), M46I/I54V/V82T (n=1), I54V/V82T (n=1), I54V/V82S/V82T (n=1), L90M (n=1), M46I/L90M (n=1), M46I/I47V/I54V/V82S (n=1).

Table 7. Cumulative Prevalence or Sensitivity of the Six Tier 1 RT Inhibitor (RTI) Drug-Resistance Mutations (DRMs) for Detecting Transmitted or Acquired Drug Resistance in Viruses from Patients with one or more Major NRTI or NNRTI DRM[a]

RTI-DRM	TDR	1st-Line ADR		
All Regions, All Subtypes				
	Total (n=1761)	Total (n=3,996)	Children (n=734)	TDF (n=558)
K103N	50	47.3	49	47.3
M184V	65.6	91.3	94.8	79.2
Y181C	74.7	95	98	87.5
G190A	80.2	96.1	98.4	88.9
V106M	81	97.6	98.9	93
K65R	81.9	98.5	99.3	97
LMICs, All Subtypes				
	Total (n=573)	Total (n=3,282)	Children (n=725)	TDF (n=291)
K103N	46.8	45	49.4	40.5
M184V	62.7	92	95.2	79
Y181C	76.1	95.6	97.9	87.6
G190A	81.7	96.7	98.3	89
V106M	82.9	98.4	98.9	95.9
K65R	83.9	99	99.3	99
All Regions, Subtype C				
	Total (n=157)	Total (n=1,909)	Children (n=473)	TDF (n=242)
K103N	45.9	51.1	56.7	38.8
M184V	56.7	92	96.2	75.6
Y181C	71.3	94.6	97.5	83.5
G190A	80.3	95.5	97.9	85.1
V106M	83.4	98.5	98.7	94.2
K65R	84.7	99.4	99.4	98.8

[a]TDR and ADR were defined as having one or more major DRMs. Major NRTI-associated DRMs (HIVDB score ≥30) included K65R, D67 deletion, T69 insertion, K70R, L74V/I, Y115F, Q151M, M184I/V, and T215F/Y. Major NNRTI-associated DRMs (HIVDB score ≥60) included: L100I, K101P, K103N/S, V106A/M, Y181C/I/V, Y188L/H/C, G190A/S/E/Q, and M230L.

Table S1. Summary of Sequences from Patients Receiving NRTI/NNRTI First-Line Regimens

Regimen	No. Pts	No. Refs	Subtype %									
			A	B	C	01	02	D	G	F	Others
AZT/3TC/EFV	644	53	14.8	21.9	32.6	8.7	6.5	3.6	9.9	1.2	0.8
D4T/3TC/EFV	1318	44	2.1	6	86.7	2.4	0.9	0.3	0.6	0.3	0.6
TDF/3TC/EFV	251	16	2	15.9	72.9	0.4	4	0	4.4	0.4	0
TDF/FTC/EFV	349	11	6	39.3	20.9	8.6	5.2	2	15.5	1.4	1.1
ABC/3TC/EFV	113	9	5.3	13.3	75.2	0	2.7	0	3.5	0	0
AZT/3TC/NVP	673	54	17.8	8.9	25	10.1	13.7	9.1	11.9	0.7	2.8
D4T/3TC/NVP	1385	50	6.1	2.7	25.1	38.7	11.5	2.4	8.7	0.8	4
TDF/3TC/NVP	85	12	2.4	11.8	45.9	3.5	18.8	2.4	15.3	0	0
TDF/FTC/NVP	87	9	9.2	31	16.1	6.9	8	3.4	25.3	0	0
ABC/3TC/NVP	21	7	19	33.3	4.8	4.8	19	0	19	0	0
Total	4926	95	7.6	11.2	46	14.9	7.4	2.7	7.7	0.7	1.8

Abbreviations: AZT (zidovudine), 3TC (lamivudine), EFV (efavirenz), D4T (stavudine), TDF (tenofovir), FTC (emtricitabine), ABC (abacavir), NVP (nevirapine).

Table S2. Absolute and Cumulative Percent of Each Major Nucleoside (NRTI) Drug-Resistance Mutation (DRM) in 467 Patients with Virological Failure and Acquired NRTI Drug Resistance while Receiving a 1st-Line TDF Containing Regimen[a]

DRM	Absolute %[b]	Cumulative %[c]
M184V	72.6	72.6
K65R	48	93.4
M184I	13.7	98.7
Y115F	11.6	99.6
Q151M	0.9	99.8
T215Y	1.9	100
L74I	6.2	100
L74V	4.9	100
K70R	4.7	100
T215F	1.7	100

[a] NRTI DRM with an HIVDB score ≥30.

[b] Absolute. %: number of individuals with DRM / number of individuals with a major NRTI DRM.

[c] Cumulative. %: number of individuals with one or more of the preceding DRMs in the list / number of individuals with a major NRTI DRM .

Table S3. Absolute and Cumulative Percent of Each Major Nucleoside (NRTI) Drug-Resistance Mutation (DRM) [†] **in 712 Children with Virological Failure and Acquired NRTI Drug Resistance while Receiving a 1st-Line NRTI/NNRTI Regimen**[a]

DRM	Absolute %[b]	Cumulative %[c]
M184V	94.8	94.8
K65R	5.6	97.9
M184I	2.7	99.6
K70R	10	99.9
T215Y	6.7	100
T215F	8	100
L74V	8	100
Y115F	3.9	100
Q151M	3.5	100
L74I	2	100

[a]NRTI DRM with an HIVDB score ≥30.
[b]Absolute. %: number of individuals with DRM / number of individuals with a major NRTI DRM.
[c]Cumulative. %: number of individuals with one or more of the preceding DRMs in the list / number of individuals with a major NRTI DRM .

Table S4. Absolute and Cumulative Percent of Each Major Nonnucleoside (NNRTI) Drug-Resistance Mutation (DRM) in 721 Children with Virological Failure and Acquired NNRTI Drug Resistance while Receiving a 1st-Line NRTI/NNRTI Regimen[a]

DRM	Absolute %[b]	Cumulative %[c]
K103N	49.9	49.9
V106M	28.3	69.6
Y181C	21.5	84.9
G190A	17.6	90.8
Y188L	6.8	94.7
G190S	2.1	96.4
G190Q	1.2	97.6
G190E	1	98.5
Y181V	0.7	99
M230L	6.1	99.4
V106A	0.6	99.7
L100I	3.9	99.9
Y188C	2.2	100
K103S	3.3	100
K101P	2.5	100
Y188H	1.1	100

[a]NNRTI DRM with an HIVDB score ≥60.
[b]Absolute. %: number of individuals with DRM / number of individuals with a major NNRTI DRM.
[c]Cumulative. %: number of individuals with one or more of the preceding DRMs in the list / number of individuals with a major NNRTI DRM .

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