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SUPPLEMENT ARTICLE

Key Factors Influencing the Emergence of Human Immunodeficiency Virus Drug Resistance in Low- and Middle-Income Countries

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The emergence and spread of human immunodeficiency virus (HIV) drug resistance from antiretroviral roll-out programs remain a threat to long-term control of the HIV-AIDS epidemic in low- and middle-income countries (LMICs). The patterns of drug resistance and factors driving emergence of resistance are complex and multifactorial. The key drivers of drug resistance in LMICs are reviewed here, and recommendations are made to limit their influence on antiretroviral therapy efficacy.

Keywords. HIV-1 drug resistance; HIV subtype; antiretroviral therapy failure.

The last 20 years has seen a rapid expansion of antiretroviral (ARV) access, with approximately 18 million people receiving antiretroviral therapy (ART) as of 2016 [1]. In low- and middle-income countries (LMICs), first-line treatment regimens generally include 1 nonnucleoside reverse transcriptase inhibitor (NNRTI) and 2 nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), with second-line regimens substituting a boosted protease inhibitor (PI) for the NNRTI and alternate NRTIs [2]. Factors related to specific ART regimens, prior drug exposure, the timing of ART initiation, and viral subtypes in LMICs are important for the development of resistance to commonly used drugs. This article reviews these factors with special emphasis on viral subtypes.

PRETREATMENT HUMAN IMMUNODEFICIENCY VIRUS DRUG RESISTANCE

In LMICs, pretreatment drug resistance is rising and ranges 1%–12.3% in different regions [3]. A meta-analysis of sequences from 50 870 individuals initiating therapy found that pretreatment, especially NNRTI, resistance is increasing in sub-Saharan Africa [4]. There were 4 NNRTI mutations—K101E, K103N, Y181C, and G190A—that accounted for >80% of the pretreatment resistance across all HIV subtypes. In addition, 16 NRTI mutations accounted for >69% of the pretreatment resistance, but pretreatment resistance linked to PIs was low [3]. Similarly, in the Pan African Studies to Evaluate Resistance cohort, 5% of individuals starting standard first-line treatment had pretreatment resistance [5]. Importantly, individuals with pretreatment resistance to the regimen prescribed were more likely to fail therapy compared with those with no resistance (odds ratio [OR], 2.13; 95% CI, 1.44–3.14; $P < .0001$). Single-dose nevirapine (sdNVP) for prevention of mother-to-child transmission of HIV may result in pretreatment drug resistance. The Optimal Combination Therapy after Nevirapine Exposure study examined outcomes following first-line treatment in women with or without sdNVP exposure before starting a nevirapine (NVP)–based first-line regimen [6, 7]. The frequency of NNRTI resistance prior to treatment initiation was 45% in the sdNVP-exposed group and 18% in the group that had no prior sdNVP exposure, and ART failure was more likely in the women with NNRTI resistance and prior sdNVP exposure. Similarly, prior exposure to RTV or indinavir increases PI cross-resistance and probability of failure and drug resistance from lopinavir/ritonavir (LPV/r) in both children and adults [8], and prior exposure to integrase strand transfer inhibitor exposure increases the chance of failure and drug resistance to dolutegravir [9]. Pretreatment resistance can fade over time and form minority variants, which can have an impact on outcome (covered in separate articles in this supplement). These findings highlight the need for cost-effective strategies to assess drug resistance prior to treatment initiation [10] or to change the initial treatment strategy [11].

TIMING OF TREATMENT INITIATION AND TREATMENT MONITORING

Individuals who initiate treatment with CD4 cell counts >500 cells/mm^3 have better first-line outcomes compared with those with CD4 cell counts <350 cells/mm^3 [12]. Several lines of evidence support the notion that delayed therapy is associated with higher viral diversity and increased viral failure [13, 14]. Cohen et al [14, 15] found that there was an increased chance

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of experiencing viral failure on rilpivirine (RPV) and efavirenz (EFV) when starting treatment with a viral load >100 000 RNA copies/mL. A combined analysis of the TMC278 against HIV, in a once-daily regimen versusefavirenz and efficacy comparison in treatment-naive, HIV-infected subjects of TMC278 and efavirenz studies found that there was a 4.4% difference in response rate in individuals with a starting viral load of 100 000 RNA copies/mL versus 500 000 RNA copies/mL and an 8% increase when the starting viral load was >500 000 RNA copies/mL [16]. The World Health Organization (WHO) has recommended treatment for all HIV-infected individuals, but uptake of this recommendation is incomplete, resulting in delayed initiation of ART [17].

A major driver of the development of HIV drug resistance is the way treatment is monitored. Plasma HIV RNA (viral load) monitoring scale-up is incomplete (reviewed in this issue), and clinical and immunological indicators are often used to determine treatment success. Multiple studies show that the longer an individual is left on a failing regimen the more complex the resistance profile [18], with 1 study showing accumulation of drug-resistance mutations at a rate of 1.45 per year after first virologic failure, resulting in declining drug susceptibility after continued failure [19]. Table 1 shows the progression of resistance as viral load monitoring thresholds are made less stringent or not used at all and the time to switching increases.

ANTIRETROVIRAL THERAPY ADHERENCE

Adherence to ART is a key contributor to treatment success, and the "partially" adherent individual is the most vulnerable to developing resistance [20]. Inadequate medication exposure leads to selection pressure, which increases the chance of developing resistance. Intermittent adherence of ARVs with different half-lives results in periods of monotherapy and consequent development of resistance. Suboptimal dosing in growing children and poor tolerance of specific ARVs are important factors in inadequate exposure [21, 22]. There is no consensus on the optimal way to measure adherence and ARV exposure. Self-reported and clinic-based pill counts are poor indicators of adherence compared with electronic drug monitoring, therapeutic drug monitoring, and pharmacy-based calculations [23]. Measures to improve adherence have focused on both patient and ARV factors. Sex and age have been closely linked to treatment success, with females and those of increased age having better adherence and less resistance. Studies of specific adherence support measures have yielded conflicting results, and studies are ongoing to test a variety of electronic interventions [24, 25]. In studies of second-line ART regimens, participants entering with a high level of resistance to first-line treatment had better outcome compared with those with no resistance, possibly indicating that better adherence to first-line treatment was a marker of second-line treatment adherence and success [26–28]. Fixed-dose, single tablet regimens have been shown to improve adherence over multipill regimens and also reduce the development of resistance [29], and long-acting formulations may provide an added advantage in the future. Developing tools that monitor and aid adherence is an active area of research, clearly needed to control HIV drug resistance levels in LMICs.

ANTIRETROVIRAL THERAPY REGIMEN

Certain medication combinations rather than their individual components may also increase the risk of failure and drug resistance. Tang et al [30] provided interesting evidence that the risk sum of a regimen is more than its parts. Specifically, K65R emerged frequently with tenofovir (TDF)/lamivudine (3TC)/

Table 1. Comparison of Large Resistance Datasets Showing Higher Resistance Mutation Frequency and Complex Resistance Mutations Profiles as Individuals Are Left on a Failing Regimen for Longer Time

Site	Malawi Lilongwe [60]	South Africa Cape Town [61]	South Africa Johannesburg [31]	South Africa Durban [62]	South Africa CIPRA-SA [63]
Sample size	96	110	226	115	67
Clinical sites	1	1	2	2	2
Switch criteria	Clinical or immunological	HIV RNA >5000 copies/mL	HIV RNA >5000 or 1000 copies/mL	HIV RNA >1000 copies/mL	HIV RNA >1000 copies/mL
Frequency of monitoring	Not applicable	6 monthly HIV RNA and CD4+ T cell	6 monthly HIV RNA and CD4+ T cell	6 monthly HIV RNA and CD4+ T cell	3 monthly HIV RNA and CD4+ T cell
% with failure and resistance	95%	85%	83%	83.5%	82%
M184V/I	81%	78%	72%	64.3%	67.2%
NNRTI	93%	86%	78%	Unknown	75%
K103N	28%	55%	38%	51%	50%
V106M	7%	31%	17%	19%	14%
>3 TAMS	44%	23%	11%	32.2%	1.5%
K65R	19%	9%	4.5%	2.6%	3%
Q151M	19%	Not reported	2.5%	0.9%	0%
NRTI+NNRTI	91%	83%	73%	64.3%	63%

Abbreviations: 3TC, lamivudine; AZT, zidovudine; d4T, stavudine; EFV, efavirenz; FTC, emtricitabine; NNRTI, nonnucleoside reverse transcriptase inhibitor; NVP, nevirapine; TAMs, thymidine analog mutations; TDF, tenofovir.

NVP, less frequently with TDF/emtricitabine (FTC)/NVP or TDF/3TC/EFV, and rarely with TDF/FTC/EFV. Thus, all first-line regimens, despite consisting of ARVs from the same drug classes, are not equal, and important drug–drug and mutational interactions that drive success or failure need to be better understood. Monitoring the virologic outcomes following roll-out of related but different regimens should be performed at the population level to identify subtler differences in regimen efficacy that may be missed in smaller clinical trials.

HUMAN IMMUNODEFICIENCY VIRUS SUBTYPE

During first-line ART, the mutational signature that develops is linked both to the type of ART prescribed and the HIV subtype (Figure 1). Each of these influences is described herein.

Stavudine and Tenofovir

In HIV subtype C, an increase in frequency of K65R after stavudine (d4T) exposure [31] or TDF exposure [32] has been observed. Unlike subtype B, for which there was no K65R observed when individuals were exposed to d4T, in subtype C, 4.5% of individuals experiencing viral failure after first-line treatment in South Africa had the K65R mutation [31]. When d4T was replaced by TDF, very high levels of K65R (69%) were observed in this subtype. More frequent development of the K65R mutation may be a result of subtype C nucleotide sequence difference, and/or a delay in treatment switch, and/or combination of d4T and TDF treatment [33]. Analysis of sequences from the SELECT study, made up of predominantly subtypes C, D, and A1, found that K65R occurred in 22% (n = 107) of participants, and when divided by NRTI was 2% (n = 5) in those treated with zidovudine (AZT)/d4T; 70% (n = 63) in those treated with TDF; and 38% (n = 39) in those treated with both TDF and AZT/d4T [34]. Association of mutation by subtype in the SELECT study was confounded by small non-subtype C numbers; however, other studies have found K65R to occur significantly more in subtype C than in other subtypes [35, 36].

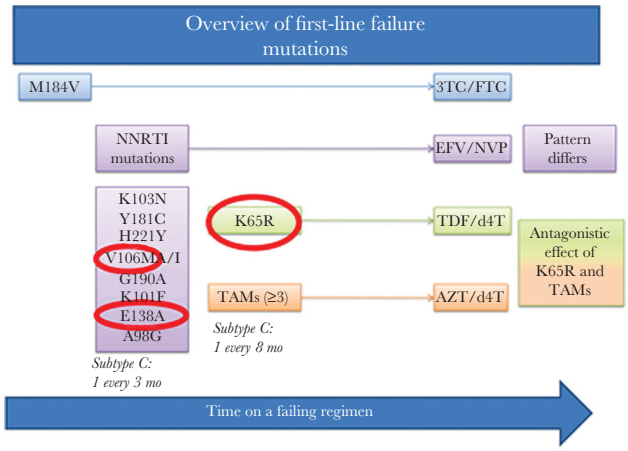

Figure 1. Overview of mutations linked to first-line failure. Generally, the first mutation to develop is the M184V mutation linked to 3TC/FTC exposure, followed concurrently by nonnucleoside reverse transcriptase inhibitor mutations at a rate of an additional 1 mutation for every 3 months on a failing regimen and nucleoside/nucleotide reverse transcriptase inhibitor (NRTI) mutations. The NRTI mutation patterns depends on the NRTI used. Mutations circled in red have been associated with subtype C. Abbreviations: 3TC, lamivudine; AZT, zidovudine; d4T, stavudine; EFV, efavirenz; FTC, emtricitabine; NNRTI, nonnucleoside reverse transcriptase inhibitor; NVP, nevirapine; TAMs, thymidine analog mutations; TDF, tenofovir.

Nevirapine versus Efavirenz

The mutational signatures of NVP and EFV differ even though there is a considerable amount of cross-resistance between the 2 ARVs. Efavirenz selects a wider range of mutations compared with NVP, and the mutations selected by both NNRTIs are influenced by subtype [31, 37]. For example, the Y181C mutation is more commonly selected by NVP compared with EFV, and the subtype C–specific V106M mutation [38, 39] is more often selected by EFV (34%) than NVP (2%) [31]. K103N occurs at a greater frequency and in higher levels in women with subtypes C and D rather than subtype A [40]. Sluis-Cremer et al [41] reported that E138A naturally occurs in subtype C compared with subtype B and that E138K or E138Q are more common in treatment-experienced subtype C sequences (1.0% and 1.1%, respectively) than in subtype B sequences (0.3% and 0.6%, respectively). Phenotyping showed that E138A/K/Q in subtype C decreased RPV susceptibility 2.9-, 5.8-, and 5.4-fold, respectively. These observations suggest that E138A could impact treatment or prevention strategies that contain NNRTIs in geographic areas where subtype C infection is prevalent; further investigation is required. The different mutation profiles caused by either NVP or EFV usage and subtype may impact the success of second-generation NNRTIs (eg, etravirine) in future third-line or salvage regimens.

Phenotypic Analysis

Two studies have examined the impact of HIV-1 subtype C on phenotypic resistance and genotype interpretation algorithms that were developed based on subtype B datasets [42–44]. These results showed that although the phenotypic scores were concordant between subtype B and C for NVP, EFV, and 3TC, there were differences in TDF, RPV, and ETR, and resistance was misclassified in 17%, 30%, and 30%, respectively, of isolates that showed phenotypic susceptibility despite resistance predicted by algorithms. This discrepancy may result from the presence of compensatory and/or epistatic mutations in reverse transcriptase that maintain or increase susceptibility to ETR, RPV, and TDF.

Second-line antiretroviral therapy regimen

WHO guidelines recommend either LPV/r or atazanavir/ritonavir (ATV/r) with 2 NRTIs as second-line therapy. Resistance testing after first-line failure has been found to be cost effective [45, 46], supporting concerns about unnecessary switches to more expensive regimens. Cost effectiveness was achieved at a test cost of <$100 and was dependent on the prevalence of wild-type virus and timely response to test results. Three large

treatment studies (SELECT, SECOND-LINE, and EARNEST [26, 27, 47]) in both high-income countries and LMICs found that using 1 or 2 new ARV classes (2 NRTI + PI vs PI + integrase inhibitor) had equivalent outcomes. More important, NRTI mutations at start of treatment initiation were strongly associated with better outcome versus not having NRTI mutations [26, 27, 47]. Tailoring NRTI selection against baseline resistance data did not improve outcome [28]. Possible explanations include better adherence in individuals failing first-line treatment with resistance. Alternatively, the current phenotyping and genotyping algorithms may not appropriately account for residual activity of NRTIs despite resistance mutations or for combination therapy and therefore overestimate resistance to NRTIs when used in combination with protease inhibitors.

The EARNEST study also demonstrated inferior virologic suppression rates for LPV/r monotherapy after 12 weeks of induction therapy with LPV/r and raltegravir, accompanied by high rates of LPV/r resistance [28]. Four small studies of dolutegravir (DTG) monotherapy, used for regimen simplification after virologic suppression, have demonstrated high rates of DTG resistance after virologic failure [48]. These studies, as well as others yielding similar data after darunavir/ritonavir (DRV/r) monotherapy, discourage the use of monotherapy for second-line ART and for regimen simplification [49, 50].

Studies show that the level of protease resistance that develops is low after LPV/r treatment failure [51, 52]; however, the level of drug resistance is increasing (22%) as second-line regimens are rolled out on a larger scale [53, 54]. There are minimal data on ATV/r resistance in LMICs, but it is known that mutations develop more easily during ATV exposure compared with LPV [55]. Depending on subtype (subtype B, C, or CRF02_AG) and PI (nelfinavir, LPV, ATV), the effect of a polymorphism at codon 36 of protease has been found to have a differential effect on both drug susceptibility and the viral replication capacity [56]. There are multiple other protease polymorphisms across different subtypes that may increase the likelihood of additional protease mutations and thus result in higher levels of resistance [56]. In an LPV/r monotherapy study, it was found that subtype AG and G were linked to lower PI susceptibility and subsequent response to treatment [57]. Overall, resistance to PIs is complex, and ongoing work is elucidating mutations in both the *gag* [58] and *env* gene [59] that may be associated with treatment failure and subtype.

CONCLUSIONS

The development of resistance is a major hindrance to successful treatment programs in LMICs. Multiple factors can affect the emergence of resistance during therapy, including the presence of pretreatment resistance; the timing of treatment initiation; HIV subtype; ARVs used in first-and second-line ART; whether viral load monitoring is performed, along with the schedules for monitoring; and medication adherence. Several unanswered questions still exist, however. How will mutations selected by new ARVs (DTG, capsid inhibitors, and others) alter resistance and cross-resistance patterns? Will new ARVs select mutations that affect outcomes differently across subtypes? Can genotypic results accurately predict virologic outcomes in all subtypes, or will different subtype-specific mutations require region-specific interpretation and guidelines? What are the consequences of mutations that develop outside the gene targeted by an ARV; for example, mutations in *gag* and *gp41* that may affect resistance to protease inhibitors? These unanswered questions highlight the need for ongoing research in the field of HIV drug resistance.

Notes

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