Emergence of HIV Drug Resistance During First- and Second-Line Antiretroviral Therapy in Resource-Limited Settings

Mina C. Hosseinipour,[1,2] Ravindra K Gupta,[3] Gert Van Zyl,[4] Joseph J. Eron,[2] and Jean B. Nachega[5,6,7]

[1]University of North Carolina Project, Lilongwe, Malawi; [2]University of North Carolina School of Medicine, Department of Medicine, Chapel Hill; [3]Division of infection and Immunity, University College London Medical School, United Kingdom; [4]Division of Medical Virology, Department of Pathology, Stellenbosch University and National Health Laboratory Service, Cape Town, South Africa, [5]Department of Medicine and Center for Infectious Diseases, Stellenbosch University Faculty of Medicine and Health Sciences, Cape Town, South Africa; [6]Departments of International Health and Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland; and [7]Department of Epidemiology, Pittsburgh Graduate School of Public Health, Pennsylvania

Introduction. Antiretroviral therapy (ART) in resource-limited settings has expanded in the last decade, reaching >8 million individuals and reducing AIDS mortality and morbidity. Continued success of ART programs will require understanding the emergence of HIV drug resistance patterns among individuals in whom treatment has failed and managing ART from both an individual and public health perspective. We review data on the emergence of HIV drug resistance among individuals in whom first-line therapy has failed and clinical and resistance outcomes of those receiving second-line therapy in resource-limited settings.

Results. Resistance surveys among patients initiating first-line nonnucleoside reverse-transcriptase inhibitor (NNRTI)–based therapy suggest that 76%–90% of living patients achieve HIV RNA suppression by 12 months after ART initiation. Among patients with detectable HIV RNA at 12 months, HIV drug resistance, primarily due to M184V and NNRTI mutations, has been identified in 60%–72%, although the antiretroviral activity of proposed second-line regimens has been preserved. Complex mutation patterns, including thymidine-analog mutations, K65R, and multinucleoside mutations, are prevalent among cases of treatment failure identified by clinical or immunologic methods. Approximately 22% of patients receiving second-line therapy do not achieve HIV RNA suppression by 6 months, with poor adherence, rather than HIV drug resistance, driving most failures. Major protease inhibitor resistance at the time of second-line failure ranges from 0% to 50%, but studies are limited.

Conclusions. Resistance of HIV to first-line therapy is predictable at 12 months when evaluated by means of HIV RNA monitoring and, when detected, largely preserves second-line therapy options. Optimizing adherence, performing resistance surveillance, and improving treatment monitoring are critical for long-term prevention of drug resistance.

Keywords. antiretroviral drug resistance; resource-limited settings; second-line therapy.

In the past decade, the marked expansion of human immunodeficiency virus (HIV) treatment programs in resource-limited settings has resulted in the receipt of life-saving therapy by >8 million individuals [1, 2]. In line with World Health Organization (WHO)

guidelines, most countries have adopted similar approaches for initial antiretroviral treatment, with the overwhelming majority of programs choosing first-line regimens based on nonnucleoside reverse transcriptase inhibitors (NNRTIs) and 2 nucleoside reverse-transcriptase inhibitors (NRTIs). Countries are moving to phase out stavudine (d4T), used initially because of its low cost and twice-daily fixed-dose formulation with lamivudine (3TC) and nevirapine (NVP) [3], and are adopting tenofovir (TDF)–based therapy because of its more favorable long-term safety profile and once-daily fixed-dose combination with either 3TC or emtricitabine (FTC) plus efavirenz (EFV) or with zidovudine

(ZDV)–based therapy [4]. Second-line therapy has consisted of a protease inhibitor (PI) with 2 NRTIs; most programs have not yet adopted third-line regimens.

As a component of antiretroviral therapy (ART) roll out, surveillance for HIV drug resistance has been a critical to WHO recommendations and includes monitoring for early indicators of poor program performance, surveillance of transmitted drug resistance, and evaluation of drug resistance acquired during therapy [5]. Alongside WHO activities, treatment programs and research groups have evaluated the emergence of drug resistance among treated populations to better inform new treatment guidelines. To date, emergence of drugs resistance varies according to initial regimen choice, treatment monitoring strategies, and individual patient adherence and program factors.

For this scientific overview, we review WHO publications, systematic reviews, and relevant abstracts and manuscripts on the topic of drug resistance and summarize presentations and expert discussions conducted at the Collaborative HIV and Drug Resistance Network workshop in Geneva, Switzerland, on 10 and 11 October 2012. Specifically, we summarize the emergence of first-line drug resistance mutations after 12 months of ART and, on the basis of monitoring strategies, describe the outcomes to second-line therapy and summarize HIV resistance among patients in whom second-line therapies have failed.

OVERVIEW

First-Line Treatment Failure and HIV Drug Resistance
The prevalence of HIV drug resistance at the time of first-line ART failure was systematically reviewed in 2010 [6], as well as in a recent report, which emphasized 12-month resistance outcomes [1]. For the vast majority of resistance surveillance work conducted to date in resource-limited settings, the threshold HIV RNA load for genotyping has been >1000 copies/mL. Additional resistance may be identified by using lower thresholds [7].

In the systematic review by Barth et al [6], all studies that reported HIV RNA results at least 3 months after ART initiation were eligible for analysis. A total of 89 studies with 13 288 patients were included, and the median duration of treatment was 10 months. The prevalence of HIV suppression was 78% after 6 months of treatment, 76% after 12 months, and 67% after 24 months. Among the subset of studies reporting treatment failure with HIV-specific resistance mutation data (27 studies with 734 patients), the most common mutations were the M184V mutation, found in 65% of patients, and the K103N mutation, found in 52% of patients. Thymidine-analog mutations (TAMs; M41L, D67N, K70R, L210W, T215Y/F, and K219Q/E) were less common, ranging from 5%–20% of patients, and the K65R mutation was found in 5% of patients. In a single study, only a small proportion of patients received PI therapy, which was almost exclusively saquinavir/ritonavir (RTV) [8]. Among these, the most common mutations were

the L90M and V82A/F/T/S mutation, found in 16% of patients. The design of the studies was heterogeneous, and most had limited follow-up time.

In a more recent review of published studies that reported genotype resistance outcomes at 12 months, 9 studies from 8 countries were included [1]. Among the 4248 patients evaluated, treatment in 573 (13.5%) failed by 12 months. Among these cases of treatment failure, 60% had documented HIV resistance, and 40% had wild-type virus. Similar to the previous review, the most common mutations were NRTI mutations (primarily M184V), detected in 55% of subjects, and NNRTI mutations, detected in 46%.

As part of WHO drug resistance surveillance activities [5], multiple surveys of drug resistance at baseline and 12 months after ART initiation have been conducted [1, 9–13]. Among 36 surveys across 12 countries in which WHO drug resistance surveillance was conducted, the overall resistance among patients about to start first-line ART ranged from 4.8% (in 2007) to 6.8% (in 2010), and this increase was driven by NNRTI-associated mutations. Particularly in East and Southern Africa, an increasing prevalence of HIV drug resistance has been noted [14]. Whether the presence of resistant HIV before therapy initiation represents transmitted drug resistance or previous drug exposure is uncertain.

The WHO surveillance methods for assessing acquired resistance at 12 months classify patients into the following groups: resistance prevention (HIV RNA load of <1000 copies/mL), possible resistance (HIV RNA load of >1000 copies/mL but no resistance detected; patients have been lost to follow-up or stopped therapy), or documented resistance (HIV RNA load of >1000 copies/mL with resistance mutations identified) [1, 5]. Among patients from the 29 surveys that evaluated resistance at 12 months, 76.1% were in the resistance prevention group, 5.1% were in the documented resistance group, and 18.8% were in the possible resistance group. After restriction of the evaluation to living patients receiving treatment, approximately 90% had HIV RNA suppression, and 9.4% had treatment failure [1]. Among patients with detectable HIV RNA, resistance was confirmed in 72.1%, and the remainder had wild-type virus. Like other surveys, the predominant resistance mutations consisted of NNRTI mutations (69.5% of cases), NRTI mutations (62.5%), and both (59.9%). Similarly, the K103N and M184V mutations were the most commonly identified NNRTI and NRTI mutations, respectively, with TAMs occurring less frequently. The majority of resistance (85% of cases) was acquired during therapy, and only 15% of failures were explained by baseline resistance.

PharmAccess African Studies to Evaluate Resistance (PASER) is a multicountry, 13-site cohort study that provides additional data on drug resistance at baseline and 12 months after treatment initiation [15, 16]. The PASER cohort, which overlaps with some WHO surveillance sites, includes a larger

population of individuals receiving TDF-based first-line therapy, thereby serving as a useful resource to programs that are shifting first-line regimens away from d4T. Specifically, 37% of the cohort received ZDV, 27% received d4T, and 33.5% received TDF. From an intent-to-treat perspective, 70% of patients achieved HIV RNA suppression, and, like the WHO survey, 90% of those alive and receiving treatment had HIV RNA suppression. Among those in whom treatment was failing at 12 months, 71% had virus with resistance mutations identified, and 29% had wild-type virus. Nearly all resistance (96% of cases) was acquired during therapy in this cohort. The predominant mutations again were K103N (28.9% of cases) and M184V (53.5%), but the additional NRTI mutations varied according the NRTI backbone. TAMs occurred in 12.7% of patients receiving ZDV, in 5% receiving d4T, and in 4.3% receiving TDF. However, the K65R mutation was more frequent among patients in whom TDF-based therapy failed (27.7%), compared with those in whom d4T-based treatment (15%) or ZDV-based treatment (0%) failed.

However, higher frequencies of the K65R mutation have been reported after failure of TDF-based first-line therapy, and resistance may vary according to the NNRTI medication. In a study from Durban, South Africa, 585 patients initiated TDF + 3TC + NNRTI as a first-line regimen. Thirty-five patients (6.0%) had virologic failure (HIV RNA load, >1000 copies/mL) after 5 months of treatment. Twenty-three of 33 patients (69.7%) with virologic failure had virus with the K65R mutation. There were few additional NRTI-associated mutations. The authors concluded that this high rate may reflect faster in vivo selection, longer use of a failing regimen, or transmitted drug resistance [17]. In a recent review of the 4 WHO-recommended regimens, TDF/3TC/NVP was associated with an increased risk of failure, compared with ZDV/3TC/NVP, TDF/3TC/EFV, and TDF/FTC/EFV. K65R was also more prevalent among patients for whom TDF/3TC/NVP therapy had failed [18].

On the basis of survey monitoring data obtained 12 months after treatment initiation [1, 15], the predicted activity of various NRTIs can support recommendations for next lines of therapy. Overall, without regard for the first-line NRTI chosen at time of first-line failure, it is predicted that ZDV would be fully active in 90% of patients and that TDF would be active in 73%–85%. Abacavir and didanosine would have less activity (in about 60% of recipients), largely because of decreased activity in the setting of the M184V mutation. The predicted activity of either NVP or EFV is low (10%–30% of recipients), and etravirine (ETR) activity may also be at least partially compromised, with activity predicted in 41% of recipients. When considering first-line backbones among patients in whom treatment was failing at 12 months, full activity of TDF is expected after ZDV failure, as significant TAMs have not yet developed. Likewise, ZDV should remain fully active after initial TDF failure.

HIV resistance becomes more complex when virologic monitoring is not used to identify treatment failure [19–21], and accumulation of additional resistance mutations when continuing a failing regimen has been well documented [22–28]. In the Nevirapine OR Abacavir (NORA) substudy of Development of Antiretroviral Therapy in Africa (DART), which retrospectively performed resistance testing on virus, an average of 2.89 TAMs accumulated between weeks 48 and 96 among clients with non-suppressed HIV RNA who were receiving ZDV/3TC and NVP [24]. In some South African sites where routine virologic monitoring is available, the prevalence of TAMs are much lower [6, 26, 29, 30] than that in settings that rely on clinical and immunologic monitoring. Similarly, NNRTI mutations accumulate when there is prolonged failure [27], and the use of NVP, rather than EFV, could contribute to cross-resistance to the second-line NNRTI ETR [26]. In the case of d4T failure, mutations seem unpredictable, with selection of both TAMs and the K65R mutation when identified from clinical failure scenarios [19]. For example, in Malawi, among patients in whom d4T-based therapy failed, the most common mutation pattern was the M184V mutation plus NNRTI mutations with ≥1 TAMs, which occurred in 56% of patients. However, virus in 23% of subjects developed either K65R or K70E, despite no exposure to TDF [19]. The emergence of K65R during d4T-based therapy may be influenced by viral subtype, with some in vitro evidence [31, 32], clinical observations, and a meta-analysis [33] suggesting that K65R may emerge more commonly in subtype C virus. Meta-analysis evaluating the evolution of resistance during receipt of d4T-containing regimens also suggests that cotreatment with NVP-containing regimens is associated with an increased risk of TAMs, K65R, and Q151M, although duration of therapy does not influence the emergence of K65R [33]. However, this does not entirely explain why in some regions, such as Malawi, where virtually all viruses are subtype C, prolonged failure during d4T-based therapy sometimes results in selection of TAMs and sometimes results in selection of K65R. In addition, the pattern of TAMs may also impact the relative activity of TDF in second-line therapy, with the TAM 1 pathway (41L, 215Y) having more of impact on TDF susceptibility [34]. Additionally, we have little information on the evolution of resistance during multiple treatment substitutions that occur for toxicity management. More-complex mutation patterns may be observed if substitutions occur during unrecognized virologic failure.

Second-Line Treatment Failure and HIV Drug Resistance

Despite the successful roll out of first-line therapy across resource-limited settings, <3% of all patients undergoing treatment—a small proportion of the total number in whom treatment is failing—are receiving second-line therapy [2]. As mentioned, clinical and immunologic monitoring detect failure late, after the accumulation of more-complex mutations that

render standard second-line NRTI backbones less effective. Thus, understanding outcomes of second-line treatment among patients with resistant virus identified at the time of first-line treatment failure is critical.

The outcomes of second-line therapy have been satisfactory and largely consistent across different settings. Among 19 studies reporting outcomes of second-line treatment in low-income and middle-income countries, the proportion of patients for whom therapy had failed was 21.8% at 6 months, 23.1% at 12 months, 26.7% at 24 months, and 38% at 36 months [35]. Death was relatively uncommon across most studies, although some programs that used clinical monitoring to detect treatment failure reported high early mortality rates (10%) at the time of initiation of second-line treatment [36]. Among 5 second-line treatment studies reporting associations between adherence and treatment outcomes, all reported that poor adherence was associated with failure. Use of hair sampling to detect lopinavir (LPV) level as a measure of adherence found that those with treatment failure had the lowest levels of drug exposure [37].

Only small studies have evaluated PI resistance at the time of second-line failure. PI resistance mutations are present in approximately 18% of genotyped viruses [35], ranging from 0% to 50%, with some variation according to region (Table 1). The PI most commonly used for these studies was RTV-boosted LPV (LPV/r). The prevalence of PI resistance at the time of failure was highest in the study that included patients primarily taking RTV-boosted indinavir and RTV-boosted atazanavir (ATV/r) combinations. Interestingly, up to 67% of patients in whom second-line therapies have failed have wild-type virus, underscoring nonadherence as the etiology of treatment failure. Emergence of PI resistance at the time of virologic failure is very uncommon in PI-naive patients in the developed world who experience virologic failure during receipt of their first PI regimen, and this may also be true in resource-limited settings. However, we acknowledge that we do not fully understand the nature of PI failure. Although, the *gag* gene is not sequenced during standard genotyping, mutations in *gag* have been known to contribute to viral fitness in the presence of PI mutations. Recent publications have identified mutations in Gag protein cleavage sites and other sites that could per se contribute to PI resistance [38–41]. It is not known whether mutations in *gag* only contribute to a sizable proportion of patients with unexplained PI failure [42], especially in settings where non–subtype B HIV type 1 is prevalent [43].

Evaluation of second-line treatment outcomes according to resistance mutations detected at the time of first-line failure is limited because of small sample sizes in published studies. In Malawi, treatment responses for those with the highest levels of NRTI resistance appeared to be better than responses for those with less extensive resistance and those with no resistance (97% vs 92% and 71%, respectively), although the differences were not statistically significant, likely because of the small sample size for each resistance category [36]. This again may reflect adherence, because patients with failure of first-line treatment and limited or no resistance likely had poor adherence to first-line therapy, whereas those with extensive resistance mutations at the time of virologic failure of first-line treatment may actually be more likely to adhere to second-line therapy. In a larger multicenter cohort study, response rates were similar between patients receiving a fully active second-line regimen versus those receiving a partially active regimen, with adherence again playing a more significant role in treatment failure [44]. Multicenter studies evaluating resistance data will likely be required to determine the role of resistance in the response to second-line treatment.

However, given the potency of boosted PIs, treatment responses may be adequate even in patients with virus exhibiting high-level NRTI resistance. Boosted PIs have relatively high rates of virologic suppression when used as monotherapy in PI-naive individuals [45], although virologic suppression is slightly higher when NRTI backbones are concurrently used [46]. Recently, LPV/r monotherapy was evaluated as second-line treatment, with excellent results. In an open-label, single-arm study, 87% of subjects had an HIV RNA load of <400 copies/mL at 24 weeks during monotherapy, and 96% of subjects had viral suppression after treatment was intensified with TDF/FTC [47]. In a 2-arm trial comparing LPV/r monotherapy to LPV/r plus NRTIs, responses were excellent in both arms, but a greater proportion of patients receiving LPV/r plus NRTIs achieved an HIV RNA load of <50 copies/mL. [48] However, both trials have limited follow-up durations, and PI resistance may emerge more quickly with such strategies, particularly in the absence of virologic monitoring.

CONCLUSIONS

Our understanding of HIV resistance has yielded several important themes that can inform the development of strategies for preventing drug resistance. Drug resistance patterns detected at the time of first-line failure of 2 NRTI and NNRTI combinations are largely predictable and have a low risk of compromising second-line treatment options if these patterns are identified early after virologic failure or ≤12 months after initiating ART. Specifically, the most likely mutations will include NNRTI mutations and the M184V mutation, with the K65R mutation likely developing with TDF-containing combinations. Thereafter, if failure is not detected, accumulated drug resistance can be expected with ZDV-containing combinations, which result in TAMs, and with d4T regimens, which select for K65R, TAMs, or Q151M on the basis of viral clade or NNRTI companion drug. Hence, use of HIV RNA monitoring to detect virologic failure ≤12 months after treatment initiation will prevent complex drug resistance and should be preferred over

Table 1. Summary of Studies Describing Human Immunodeficiency Virus (HIV) Drug Resistance to Second-Line Therapy From Resource-Limited Settings

Variable	Wallis et al [54]	El-Khatib et al [55]	Saravanan et al [56]	Levison et al [57]	van Zyl et al [37]	Ferradini et al [58]	Maiga et al [59]	Reynolds et al [60]	Hosseinipour et al (unpublished data)
Country	South Africa	South Africa	India	South Africa	South Africa	Cambodia	Mali	Uganda	Malawi
PI(s)	LPV/r	LPV/r for 114 patients, ATZ/r for 1	ATZ/r for 47.6% of patients, IND/r for 44.8%, LPV/r for 7.5%	LPV/r	LPV/r	LPV/r	LPV/r	LPV/r	LPV/r
HIV sequenced, % of patients	75	35	45	33	33	4	93	6	12
Duration of second-line therapy, mo	16		>6	10		≥24	≥48		12
Wild-type virus, patients, %	39	NR	NR	67	NR	25	20	NR	58
Major PI mutation								0	
Patients, %	7	6	≥49	0	6	50	28	0	16
Mutations	M46I, L76V, V82A	Q58E, M46L, L90M, N88S	M46I, I54V/A, V82A, L90M	. . .	NR	NR	12L76V,[a] M46I/L, I47V/A, I54M/L, Q58E, V82A/F/T/S, I84V, L90M	. . .	NR

Abbreviations: ATV/r, ritonavir-boosted atazanavir; IND/r, ritonavir-boosted indinavir; LPV/r, ritonavir-boosted lopinavir; NR, not reported; PI, protease inhibitor.

[a] Detected in 12% of patients.

clinical or immunologic monitoring. Furthermore, because d4T has a less predictable pattern of resistance, movement away from this agent in first-line therapy will also improve prediction of the emergence of drug resistance mutations if treatment failure is identified late.

Optimizing treatment adherence and retention at all components of care is critical to the prevention of resistance. By use of the WHO approaches to evaluate acquired resistance, high rates of possible resistance were identified because of loss to follow-up from clinics. Tracing patients who are no longer adherent to therapy can reduce loss to follow-up and allow resuppression of HIV RNA in 61% of those who resume first-line therapy [49], but it may not be a feasible public health approach in countries with underresourced health systems. For both first-line and second-line therapies, a significant proportion of individuals have wild-type virus at treatment failure, suggesting nonadherence as the etiology of treatment failure. Opportunities exist to proactively monitor adherence and, when necessary, intervene with cost-effective interventions, including adherence counseling [50], or other emerging adherence interventions, such as mobile phone technology that delivers weekly text messages [51], to maintain or improve adherence, to prevent virologic failure and drug resistance, and, ultimately, to preserve treatment options [52]. Given the high proportion of patients in whom second-line therapy has failed and who do not have virus with PI mutations, consideration is being given to the role of resistance testing prior to switching to third-line regimens [53].

Currently, we have gaps in knowledge of the influence of first-line resistance patterns on second-line outcomes. Multiple cohorts and multicenter trials will need to be combined to have sufficient power to evaluate the effect of resistance on treatment outcomes. Also, emergence of resistance during second-line treatment remains poorly described. To date, studies have been small, and the vast majority have used LPV/r as second-line therapy. However, a new fixed-dose combination of ATV/r is expected, and many countries are expected to shift to ATV/r second-line regimens, such that additional data on treatment outcomes and emergence of drug resistance will be required. Additionally, we also need to consider the influence of alternative first-line therapies, such as PIs (which are increasingly being used among pregnant women for prevention of mother-to-child transmission), on drug resistance, because initial resistance profiles are likely to differ [61].

In summary, increasing access to and use of virologic monitoring over other strategies to identify treatment failure will prevent the accumulation of resistance mutations. Continued surveillance of resistance at the time of treatment failure will allow the optimal choice of antiretroviral therapy, preserving treatment options for life-long therapy in areas where access to the full range of antiretroviral drug classes is limited.

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