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Emerging antiretroviral drug resistance in sub-Saharan Africa: novel affordable technologies are needed to provide resistance testing for individual and public health benefits

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Emerging antiretroviral drug resistance in sub-Saharan Africa: novel affordable technologies are needed to provide resistance testing for individual and public health benefits

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Keywords

Africa; antiretroviral therapy; HIV/AIDS; resistance testing

In industrialized countries, viral load monitoring and genotypic antiretroviral drug resistance testing (GART) play an important role in the selection of initial and subsequent combination antiretroviral therapy (cART) regimens. In contrast, resource constraints in Africa limit access to assays that could detect virologic failure, transmitted drug resistance (TDR) and acquired drug resistance to cART. This has adverse consequences for both individual and public health. Although the further roll-out of antiretrovirals for prevention, including preexposure prophylaxis (PrEP) and universal test and treat (UTT) strategies, could reduce HIV-1 incidence, these strategies may increase TDR [1,2]. Here, we present arguments that the scale up of antiretrovirals use should be accompanied by cost-effective assays for early detection of virologic failure, surveillance of TDR and GART for individual patient management.

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Conflicts of interest

J.W.M. serves on the Scientific Advisory Board of Gilead Sciences and holds a US Patent Filing No. 12/599951 with Azido Purine Nucleosides for Treatment of Viral Infections as well as shared options with RFS Pharmaceuticals. The other authors declare no conflicts of interest.

It is theoretically possible to remain on the same cART regimen for life, when an individual is infected with an antiretroviral susceptible strain, with adequate adherence, retention, optimal drug bioavailability and the absence of structural barriers (e.g. cART stock outs, etc.) [3,4]. The desired outcome of cART is achieving and maintaining suppression of HIV replication with viral load below the detection limit of standard HIV-1 RNA assays (<50 copies/ml). Adherence monitoring is required to detect lapses in adherence leading to virologic rebound which could benefit from adherence counseling intensification before emergence of drug resistance [5,6], l or switching patients to second line, who have sustained viral load more than 1000 copies/ml despite documented optimal adherence, according to WHO criteria [7]. The threshold of 1000 copies/ml is based on commercial GART sensitivity, but with 'homebrew' methods on plasma samples, drug resistance is often detected at lower viral load [8], whereas a higher threshold would apply for dried blood spots. Unfortunately, few settings in sub-Saharan Africa have access to routine viral load testing because of the cost, whereas clinical and immunological monitoring are only moderately sensitive and specific measures of virologic failure, resulting in either delayed or unnecessary cART switches [9–11]. New low-cost point-of-care viral load testing could increase access and enhance the cascade of care through immediately available results [12], whereas centralized testing could reduce costs by economy of scale or pooled testing [13,14], but would require sample transport infrastructure and ideally the use of information systems with confidential/coded automated mobile health text messaging of results to patient and providers.

When HIV replicates under conditions that favour selection of spontaneously generated, mutant variants, (e.g. monotherapy or dual therapy or inadequate drug concentrations), these drug-resistant variants would predominate. Their emergence and persistence are influenced by the genetic barrier to resistance (number of mutations required for resistance), pharmacokinetic properties including antiretroviral half-lives, relative fitness of resistant HIV variants compared with wild type, and interactions between mutations that may increase or decrease susceptibility to other antiretrovirals.

The particular regimen, chosen, impacts on the risk of resistance: thymidine analogue mutations (TAMs) accumulate in patients with prolonged virologic failure on stavudine (D4T) or zidovudine [15]; nevirapine(NVP) is associated with a higher risk of TAMs than efavirenz (EFV) and etravirine (ETV) [16–19]; NVP/tenofovir (TDF)/lamivudine (3TC) is associated with higher rates of virologic failure and K65R compared with EFV/TDF/emtricitabine (FTC) or 3TC [20,21]; abacavir (ABC)/3TC compared with TDF/FTC combined with a protease inhibitor has greater rates of virologic failure and accompanied resistance [22,23]. Similarly, ABC/3TC/EFV has greater virologic failure compared with TDF/FTC/EFV in patients with high baseline viral loads [23], possibly from cross-resistance between 3TC and ABC resulting in a lower regimen genetic barrier.

Models dispute the cost-effectiveness of GART [24–26]. The underlying assumptions vary: the cost of GART (~$300 for commercial assays compared with ~$150 for homebrew tests); predictive value of bulk sequencing; and rates of poor adherence with first-line virologic failure, which impacts rates of detectable drug resistance mutations [18,25,27,28]. Although initial response rates to LPV/r regimens in second-line therapy is good irrespective of

preexisting nucleos(t)ide reverse transcriptase inhibitor (NRTI) resistance [29], GART may help determine the most durable NRTI backbone for a second-line regimen, or to detect mutations that would be relevant for future third-line or salvage regimens, which may later no longer be detectable by bulk sequencing. When patients fail a first-line regimen of TDF/3TC or FTC/non-nucleoside reverse transcriptase inhibitor (NNRTI), the resistance pattern is predictable, although the duration of virologic failure influences the number of mutations i.e. only NNRTI and/or 3TC/FTC resistance; or, even later, the addition of TDF resistance [30,31]. Virologic failure during second-line protease inhibitor regimens in resource-limited settings (RLS) is associated with less than 10% protease inhibitor resistance because most patients were protease inhibitor naive and lopinavir (LPV)/r provides a high genetic barrier to selection of antiretroviral-resistant variants [18,32,33]. Polymorphisms in the gag cleavage site [34] or in envelope [35,36] have been associated with protease inhibitor resistance. However, these mutations are not detected by routine GART and their contribution to protease inhibitor failure is understudied. Nevertheless, current data support that inadequate drug exposure from poor adherence is the major contributor to virologic failure [37,38].

When the pretest probability of resistance is low, such as with virologic failure during second-line cART, blanket resistance testing would not be cost-effective. ART adherence assessed by objective tools such as pharmacy refill data [39] or hair concentrations [40] predictive of poor adherence [37] could identify individuals requiring adherence interventions [41]. Reserving GART for patients for whom virologic failure is unexplained by very poor adherence would optimize resource allocation [37]. In a context of documented virologic failure, therapy and adherence history and by GART testing, when available and affordable, will be critical to identify patients requiring third line and to construct regimens, which include darunavir and raltegravir.

TDR from primary or ‘super-infection’ is associated with increased cART coverage [42] and inadequate management of persons with virologic failure [43]. The WHO suggests surveillance of TDR in young persons, more likely to have recent infection and less likely to have TDR mutations overgrown by wild-type virus [44,45], and defines a prevalence of less than 5% as ‘low’, 5–15% as ‘intermediate’ and more than 15% as ‘high’ [46]. However, the largest studies of antiretroviral-naive patients assessed GART at cART initiation [42,47,48]. Currently, the WHO is considering adding this practical surveillance strategy. Recent data suggest increased TDR in RLS [48–52]. In contrast, data from South Africa suggest that TDR may have peaked [53], perhaps due to viral load monitoring [43].

New biologic approaches to HIV prevention, including PrEP and UTT strategies, are gaining momentum. In studies of PrEP, the major risk for resistance appears to be initiation of mono- or dual-drug PrEP during undiagnosed primary HIV infection [54–57]. As UTT and PrEP often rely on TDF as a regimen component, the possible more rapid selection of K65R in HIV-1 subtype C [58–61], although abrogated by other mutation interactions[62], is of concern for regions of sub-Saharan Africa where this subtype predominates. In clinical trials of PrEP, HIV testing to detect incident HIV infection occurred monthly, but with expansion to RLS this is likely to occur less frequently, increasing the risk for selection of drug-resistant variants which may emerge if failure of PrEP occurs unnoticed (e.g. due to

suboptimal adherence), and as a consequence, the HIV infection being exposed to suboptimal dual therapy (FTC/TDF) instead of full cART [63,64]. Therefore, monitoring of PrEP adherence as well as implementation of targeted adherence interventions, when necessary, will be critical for the optimization of clinical and public health benefit of PrEP [41]. Although models suggest variable effects of PrEP on resistance [65–67], the effects of cART failure on resistance prevalence are much greater [66–68].

Drug resistance testing at the time of HIV-1 diagnosis may not be efficient as TDR levels are less than 10%. Children infected, despite current highly effective PMTCT regimens, may have an increased risk of resistance and need prioritization [69,70]. Increased access to GART would be facilitated by new technologies (Table 1). Given that relatively few antiretroviral agents are available in Africa, testing for resistance with point mutation assays [71,72] may be worthwhile before first-line or second-line cART regimens or after single-dose NVP exposure [73]. In-house GART methods and collective bargaining with suppliers – as the case of the Southern African Treatment and Resistance Network –can make testing more affordable, when performed by laboratories participating in international external quality-assurance programmes. Sequencing reverse transcriptase amino acid positions 41–230 is sufficient for patients with virologic failure during first-line ART, and could reduce costs [74], as could approaches that combine screening for virologic failure with sequencing for reverse transcriptase mutations using pooled specimens [75,76] or next generation sequencing (NGS) [77]. Deep sequencing with NGS platforms to detect minor variant NNRTI probably adds clinical value [78] but is costly. An alternative use of NGS coverage is the pooling of many individually ‘primer barcoded’ samples, potentially making this an affordable alternative to GART by bulk sequencing in a centralized high-throughput laboratory service [79].

In summary, the recent and dramatic increase in cART coverage in Africa is associated with an increase in HIV drug resistance. The possible widespread use of UTT and/or introduction of PrEP may escalate TDR and acquired resistance, emphasizing the need for TDR surveillance and new affordable technologies to manage cART. Optimally, low-cost assays are needed that can be performed while patients wait in the clinic. An ideal assay would detect both virologic failure and important resistance mutations on a single platform at the point of care.

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Table 1

The major strengths and challenges of current technologies for genotypic antiretroviral resistance testing.

Method	Strengths	Challenges
Dye terminator ‘consensus’ or ‘population’ sequencing	Mutations correlated with reference to clinical outcomes	Limited potential for automation
	Cost savings can be incurred by the use of in-house methods, shorter fragment sequencing and the use of qualitative polymerase chain reaction (PCR) for screening of patients with possible failure without performing viral load testing [75]	Not suited for parallel[a] testing
		Cannot detect variants that occur at a low frequency
Point mutation assays (e.g. allele-specific PCR, oligonucleotide ligation assays and multiplex allele-specific assays)	High sensitivity for minor variants	No commercial assays available
	Relatively economical	Multiplexing allows for detection of a set of common mutations only
Next generation sequencing (various platforms including: 454, Illumina and Ion torrent sequencers)	Parallel testing: ability to pool multiple labelled specimens is cost-saving	Complex workflow is labour intensive
	High sensitivity for minor variants (ultradeep sequencing)	Requirement for specialised facilities
		Long turn-around times PCR errors can lead to overestimating resistance Possible read problems dependent on template composition[b]

[a] Parallel testing refers to the ability to test multiple HIV templates in a viral population. In addition, many patients can be tested at once through the use of ‘barcoding’ or ‘indexing’ the sequences. Although a single next generation sequencing reaction is costly this may allow for pooled testing of multiple samples. Allele-specific assays are more affordable than dye terminator sequencing or next generation sequencing, and can suffice if the requirement is to look for a few specific mutations; however, when required to detect more mutations, this approach become more costly.

[b] Assays that are dependent on pyrosequencing (e.g. 454 and Iontorrent) may be inaccurate in determining the sequence in regions with homopolymers, whereas sequencing by synthesis methods (e.g. Illumina) may be prone to unequal sequencing coverage depending to the CG : AT composition of the genome.