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

Gert U. van Zyl[a], Lisa M. Frenkel[b], Michael H. Chung[c], Wolfgang Preiser[a], John W. Mellors[f] and Jean B. Nachega[d,e,f,g]

*AIDS* 2014, **28**:2643–2648

**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.

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], 1 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

[a]Division of Medical Virology, Department of Pathology, Stellenbosch University and National Health Laboratory Service Tygerberg, Cape Town, South Africa, [b]Departments of Pediatrics, Laboratory Medicine and Global Health, University of Washington, and Seattle Children's Research Institute, [c]Departments of Global Health, Medicine, and Epidemiology, University of Washington, Seattle, Washington, USA, [d]Department of Medicine and Center for Infectious Diseases, Stellenbosch University Faculty of Medicine and Health Sciences, Cape Town, South Africa, [e]Departments of International Health and Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, [f]Division of Infectious Diseases, Department of Medicine, and [g]Department of Epidemiology, Pittsburgh School of Medicine and Graduate School of Public Health, Pittsburgh, Pennsylvania, USA.

Correspondence to Jean B. Nachega, MD, PhD, MPH, Associate Professor of Medicine, Infectious Diseases, Microbiology and Epidemiology, Pittsburgh Graduate School of Public Health, Infectious Diseases Epidemiology Research Program, 130 DeSoto Street, Parran Hall 503, Pittsburgh, PA 15261, USA.

Tel: +1 410 800 7803; fax: +1 410 502 6733; e-mail: jbn16@pitt.edu
Received: 20 April 2014; revised: 18 September 2014; accepted: 25 September 2014.

DOI:10.1097/QAD.0000000000000502

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

**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.

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.

## Acknowledgements

J.B.N. acknowledges grant support from the N.I.A.I.D./N.I.H., A.C.T.G. Stellenbosch University (S.U.) Clinical Trial Unit (C.T.U.) Award (2UM1AI069521–08); the US NIH-Fogarty International Center (F.I.C.)/Health Resources and Services Administration (H.R.S.A.)/US President Emergency Plan for AIDS Relief (P.E.P.F.A.R.) Grant Award (T84HA21652–01–00) for Medical Education Partnership Initiative (M.E.P.I.); and the Wellcome Trust Southern Africa Consortium for Research Excellence (S.A.C.O.R.E., WT087537MA).

### 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.

## References

1. Nachega JB, Uthman OA, Del Rio C, Mugavero MJ, Rees H, Mills EJ. **Addressing the Achilles' heel in the HIV care continuum for the success of a test-and-treat strategy to achieve an AIDS-free generation.** *Clin Infect Dis* 2014; **59 (Suppl 1)**:S21–S27.
2. Hosseinipour MC, Gupta RK, Van Zyl G, Eron JJ, Nachega JB. **Emergence of HIV drug resistance during first- and second-line antiretroviral therapy in resource-limited settings.** *J Infect Dis* 2013; **207 (Suppl 2)**:S49–S56.
3. Nachega JB, Marconi VC, van Zyl GU, Gardner EM, Preiser W, Hong SY, *et al*. **HIV treatment adherence, drug resistance, virologic failure: evolving concepts.** *Infect Disord Drug Targets* 2011; **11**:167–174.
4. Mills EJ, Nachega JB, Bangsberg DR, Singh S, Rachlis B, Wu P, *et al*. **Adherence to HAART: a systematic review of developed and developing nation patient-reported barriers and facilitators.** *PLoS Med* 2006; **3**:e438.
5. Orrell C, Harling G, Lawn SD, Kaplan R, McNally M, Bekker LG, *et al*. **Conservation of first-line antiretroviral treatment regimen where therapeutic options are limited.** *Antivir Ther* 2007; **12**:83–88.
6. Hoffmann CJ, Charalambous S, Sim J, Ledwaba J, Schwikkard G, Chaisson RE, *et al*. **Viremia, resuppression, and time to resistance in human immunodeficiency virus (HIV) subtype C during first-line antiretroviral therapy in South Africa.** *Clin Infect Dis* 2009; **49**:1928–1935.
7. World Health Organization. Monitoring response to ART and the diagnosis of treatment failure. In: *Consolidated guidelines on the use of antiretroviral drugs For treating and preventing HIV infection. Recommendations for a public health approach*. Geneva, Switzerland: World Health Organization; 2013. pp. 132–137.
8. Prosperi MC, Mackie N, Di Giambenedetto S, Zazzi M, Camacho R, Fanti I, *et al*. **Detection of drug resistance mutations at low plasma HIV-1 RNA load in a European multicentre cohort study.** *J Antimicrob Chemother* 2011; **66**:1886–1896.
9. Kantor R, Diero L, Delong A, Kamle L, Muyonga S, Mambo F, *et al*. **Misclassification of first-line antiretroviral treatment failure based on immunological monitoring of HIV infection in resource-limited settings.** *Clin Infect Dis* 2009; **49**:454–462.
10. Sawe FK, McIntyre JA. **Monitoring HIV antiretroviral therapy in resource-limited settings: time to avoid costly outcomes.** *Clin Infect Dis* 2009; **49**:463–465.
11. Badri M, Lawn SD, Wood R. **Utility of CD4 cell counts for early prediction of virological failure during antiretroviral therapy in a resource-limited setting.** *BMC Infect Dis* 2008; **8**:89.
12. Rowley CF. **Developments in CD4 and viral load monitoring in resource-limited settings.** *Clin Infect Dis* 2014; **58**:407–412.
13. Pannus P, Fajardo E, Metcalf C, Coulborn RM, Duran LT, Bygrave H, *et al*. **Pooled HIV-1 viral load testing using dried blood spots to reduce the cost of monitoring antiretroviral treatment in a resource-limited setting.** *J Acquir Immune Defic Syndr* 2013; **64**:134–137.
14. van Zyl GU, Preiser W, Potschka S, Lundershausen AT, Haubrich R, Smith D. **Pooling strategies to reduce the cost of HIV-1 RNA load monitoring in a resource-limited setting.** *Clin Infect Dis* 2011; **52**:264–270.
15. Hosseinipour MC, van Oosterhout JJ, Weigel R, Phiri S, Kamwendo D, Parkin N, *et al*. **The public health approach to identify antiretroviral therapy failure: high-level nucleoside reverse transcriptase inhibitor resistance among Malawians failing first-line antiretroviral therapy.** *AIDS* 2009; **23**:1127–1134.
16. Lapadula G, Calabresi A, Castelnuovo F, Costarelli S, Quiros-Roldan E, Paraninfo G, *et al*. **Prevalence and risk factors for etravirine resistance among patients failing on nonnucleoside reverse transcriptase inhibitors.** *Antivir Ther* 2008; **13**:601–605.
17. Taiwo B, Chaplin B, Penugonda S, Meloni S, Akanmu S, Gashau W, *et al*. **Suboptimal etravirine activity is common during failure of nevirapine-based combination antiretroviral therapy in a cohort infected with non-B subtype HIV-1.** *Curr HIV Res* 2010; **8**:194–198.
18. van Zyl GU, van der Merwe L, Claassen M, Zeier M, Preiser W. **Antiretroviral resistance patterns and factors associated with resistance in adult patients failing NNRTI-based regimens in the Western Cape, South Africa.** *J Med Virol* 2011; **83**:1764–1769.
19. Cordova E, Loiza E, Porteiro N, Mingrone H. **Predicted susceptibility of etravirine in HIV patients experiencing virological failure secondary to nonnucleoside reverse transcriptase inhibitor resistance in Argentina.** *Enferm Infecc Microbiol Clin* 2011; **29**:428–431.
20. Tang MW, Kanki PJ, Shafer RW. **A review of the virological efficacy of the 4 world health organization-recommended tenofovir-containing regimens for initial HIV therapy.** *Clin Infect Dis* 2012; **54**:862–875.
21. Van Zyl GU, Liu TF, Claassen M, Engelbrecht S, de Oliveira T, Preiser W, *et al*. **Trends in genotypic hiv-1 antiretroviral resistance between 2006 and 2012 in South African patients receiving first- and second-line antiretroviral treatment regimens.** *PLoS One* 2013; **8**:e67188.
22. Hill A, Sawyer W. **Effects of nucleoside reverse transcriptase inhibitor backbone on the efficacy of first-line boosted highly active antiretroviral therapy based on protease inhibitors: meta-regression analysis of 12 clinical trials in 5168 patients.** *HIV Med* 2009; **10**:527–535.
23. Sax PE, Tierney C, Collier AC, Daar ES, Mollan K, Budhathoki C, *et al*. **Abacavir/lamivudine versus tenofovir DF/emtricitabine as part of combination regimens for initial treatment of HIV: final results.** *J Infect Dis* 2011; **204**:1191–1201.
24. Walensky RP, Weinstein MC, Yazdanpanah Y, Losina E, Mercincavage LM, Toure S, *et al*. **HIV drug resistance surveillance for prioritizing treatment in resource-limited settings.** *AIDS* 2007; **21**:973–982.
25. Rosen S, Long L, Sanne I, Stevens WS, Fox MP. **The net cost of incorporating resistance testing into HIV/AIDS treatment in South Africa: a Markov model with primary data.** *J Int AIDS Soc* 2011; **14**:24.
26. Levison JH, Wood R, Scott CA, Ciaranello AL, Martinson NA, Rusu C, *et al*. **The clinical and economic impact of genotype testing at first-line antiretroviral therapy failure for HIV-infected patients in South Africa.** *Clin Infect Dis* 2013; **56**:587–597.
27. Wallis CL, Mellors JW, Venter WD, Sanne I, Stevens W. **Varied patterns of HIV-1 drug resistance on failing first-line antiretroviral therapy in South Africa.** *J Acquir Immune Defic Syndr* 2010; **53**:480–484.

28. Marconi VC, Sunpath H, Lu Z, Gordon M, Koranteng-Apeagyei K, Hampton J, *et al*. **Prevalence of HIV-1 drug resistance after failure of a first highly active antiretroviral therapy regimen in KwaZulu Natal, South Africa.** *Clin Infect Dis* 2008; **46**:1589–1597.
29. Sigaloff KC, Hamers RL, Wallis CL, Kityo C, Siwale M, Ive P, *et al*. **Second-line antiretroviral treatment successfully resuppresses drug-resistant HIV-1 after first-line failure: prospective cohort in Sub-Saharan Africa.** *J Infect Dis* 2012; **205**:1739–1744.
30. Margot NA, Enejosa J, Cheng AK, Miller MD, McColl DJ, Study T. **Development of HIV-1 drug resistance through 144 weeks in antiretroviral-naive subjects on emtricitabine, tenofovir disoproxil fumarate, and efavirenz compared with lamivudine/zidovudine and efavirenz in study GS-01-934.** *J Acquir Immune Defic Syndr* 2009; **52**:209–221.
31. Bulteel N, Bansi-Matharu L, Churchill D, Dunn D, Bibby D, Hill T, *et al*. **The emergence of drug resistant HIV variants at virological failure of HAART combinations containing efavirenz, tenofovir and lamivudine or emtricitabine within the UK Collaborative HIV Cohort.** *J Infect* 2014; **68**:77–84.
32. El-Khatib Z, Ekstrom AM, Ledwaba J, Mohapi L, Laher F, Karstaedt A, *et al*. **Viremia and drug resistance among HIV-1 patients on antiretroviral treatment: a cross-sectional study in Soweto, South Africa.** *AIDS* 2010; **24**:1679–1687.
33. Wallis CL, Mellors JW, Venter WD, Sanne I, Stevens W. **Protease inhibitor resistance is uncommon in HIV-1 subtype C infected patients on failing second-line lopinavir/r-containing antiretroviral therapy in South Africa.** *AIDS Res Treat* 2011; **2011**:769627.
34. Larrouy L, Chazallon C, Landman R, Capitant C, Peytavin G, Collin G, *et al*. **Gag mutations can impact virological response to dual-boosted protease inhibitor combinations in antiretroviral-naive HIV-infected patients.** *Antimicrob Agents Chemother* 2010; **54**:2910–2919.
35. Rabi SA, Laird GM, Durand CM, Laskey S, Shan L, Bailey JR, *et al*. **Multistep inhibition explains HIV-1 protease inhibitor pharmacodynamics and resistance.** *J Clin Invest* 2013; **123**:3848–3860.
36. Siliciano JD, Siliciano RF. **Recent trends in HIV-1 drug resistance.** *Curr Opin Virol* 2013; **3**:487–494.
37. van Zyl GU, van Mens TE, McIlleron H, Zeier M, Nachega JB, Decloedt E, *et al*. **Low lopinavir plasma or hair concentrations explain second-line protease inhibitor failures in a resource-limited setting.** *J Acquir Immune Defic Syndr* 2011; **56**:333–339.
38. Gandhi M, Ameli N, Bacchetti P, Anastos K, Gange SJ, Minkoff H, *et al*. **Atazanavir concentration in hair is the strongest predictor of outcomes on antiretroviral therapy.** *Clin Infect Dis* 2011; **52**:1267–1275.
39. Bisson GP, Gross R, Bellamy S, Chittams J, Hislop M, Regensberg L, *et al*. **Pharmacy refill adherence compared with CD4 count changes for monitoring HIV-infected adults on antiretroviral therapy.** *PLoS Med* 2008; **5**:e109.
40. Gandhi M, Ameli N, Bacchetti P, Gange SJ, Anastos K, Levine A, *et al*. **Protease inhibitor levels in hair strongly predict virologic response to treatment.** *AIDS* 2009; **23**:471–478.
41. Thompson MA, Mugavero MJ, Amico KR, Cargill VA, Chang LW, Gross R, *et al*. **Guidelines for improving entry into and retention in care and antiretroviral adherence for persons with HIV: evidence-based recommendations from an International Association of Physicians in AIDS Care panel.** *Ann Intern Med* 2012; **156**:817–833.
42. Gupta RK, Jordan MR, Sultan BJ, Hill A, Davis DH, Gregson J, *et al*. **Global trends in antiretroviral resistance in treatment-naive individuals with HIV after rollout of antiretroviral treatment in resource-limited settings: a global collaborative study and meta-regression analysis.** *Lancet* 2012; **380**:1250–1258.
43. Phillips AN, Pillay D, Garnett G, Bennett D, Vitoria M, Cambiano V, *et al*. **Effect on transmission of HIV-1 resistance of timing of implementation of viral load monitoring to determine switches from first to second-line antiretroviral regimens in resource-limited settings.** *AIDS* 2011; **25**:843–850.
44. Gandhi RT, Wurcel A, Rosenberg ES, Johnston MN, Hellmann N, Bates M, *et al*. **Progressive reversion of human immunodeficiency virus type 1 resistance mutations in vivo after transmission of a multiply drug-resistant virus.** *Clin Infect Dis* 2003; **37**:1693–1698.
45. Wainberg MA, Moisi D, Oliveira M, Toni TD, Brenner BG. **Transmission dynamics of the M184V drug resistance mutation in primary HIV infection.** *J Antimicrob Chemother* 2011; **66**:2346–2349.
46. Bennett DE, Myatt M, Bertagnolio S, Sutherland D, Gilks CF. **Recommendations for surveillance of transmitted HIV drug resistance in countries scaling up antiretroviral treatment.** *Antivir Ther* 2008; **13 (Suppl 2)**:25–36.
47. Hamers RL, Siwale M, Wallis CL, Labib M, van Hasselt R, Stevens WS, *et al*. **HIV-1 drug resistance mutations are present in six percentage of persons initiating antiretroviral therapy in Lusaka, Zambia.** *J Acquir Immune Defic Syndr* 2010; **55**:95–101.
48. Hamers RL, Wallis CL, Kityo C, Siwale M, Mandaliya K, Conradie F, *et al*. **HIV-1 drug resistance in antiretroviral-naive individuals in sub-Saharan Africa after rollout of antiretroviral therapy: a multicentre observational study.** *Lancet Infect Dis* 2011; **11**:750–759.
49. Bertagnolio S, Parkin N, Jordan M. **HIV drug resistance surveillance in low- and middle-income countries: 2004 to 2010.** *J Int AIDS Soc* 2012; **15 (Suppl 4)**:18083.
50. Stadeli KM, Richman DD. **Rates of emergence of HIV drug resistance in resource-limited settings: a systematic review.** *Antivir Ther* 2013; **18**:115–123.
51. Sigaloff KC, Mandaliya K, Hamers RL, Otieno F, Jao IM, Lyagoba F, *et al*. **Short communication: High prevalence of transmitted antiretroviral drug resistance among newly HIV type 1 diagnosed adults in Mombasa, Kenya.** *AIDS Res Hum Retroviruses* 2012; **28**:1033–1037.
52. Ndembi N, Hamers RL, Sigaloff KC, Lyagoba F, Magambo B, Nanteza B, *et al*. **Transmitted antiretroviral drug resistance among newly HIV-1 diagnosed young individuals in Kampala.** *AIDS* 2011; **25**:905–910.
53. Manasa J, Katzenstein D, Cassol S, Newell ML, de Oliveira T, Southern Africa T, *et al*. **Primary drug resistance in South Africa: data from 10 years of surveys.** *AIDS Res Hum Retroviruses* 2012; **28**:558–565.
54. Grant RM, Lama JR, Anderson PL, McMahan V, Liu AY, Vargas L, *et al*. **Preexposure chemoprophylaxis for HIV prevention in men who have sex with men.** *N Engl J Med* 2010; **363**:2587–2599.
55. Hurt CB, Eron JJ Jr, Cohen MS. **Preexposure prophylaxis and antiretroviral resistance: HIV prevention at a cost?** *Clin Infect Dis* 2011; **53**:1265–1270.
56. Baeten JM, Donnell D, Ndase P, Mugo NR, Campbell JD, Wangisi J, *et al*. **Antiretroviral prophylaxis for HIV prevention in heterosexual men and women.** *N Engl J Med* 2012; **367**:399–410.
57. Anderson PL, Glidden DV, Liu A, Buchbinder S, Lama JR, Guanira JV, *et al*. **Emtricitabine-tenofovir concentrations and preexposure prophylaxis efficacy in men who have sex with men.** *Sci Transl Med* 2012; **4**:151ra125.
58. Coutsinos D, Invernizzi CF, Xu H, Moisi D, Oliveira M, Brenner BG, *et al*. **Template usage is responsible for the preferential acquisition of the K65R reverse transcriptase mutation in subtype C variants of human immunodeficiency virus type 1.** *J Virol* 2009; **83**:2029–2033.
59. Coutsinos D, Invernizzi CF, Moisi D, Oliveira M, Martinez-Cajas JL, Brenner BG, *et al*. **A template-dependent dislocation mechanism potentiates K65R reverse transcriptase mutation development in subtype C variants of HIV-1.** *PLoS One* 2011; **6**:e20208.
60. Hoffmann CJ, Ledwaba J, Li JF, Johnston V, Hunt G, Fielding KL, *et al*. **Resistance to tenofovir-based regimens during treatment failure of subtype C HIV-1 in South Africa.** *Antivir Ther* 2013; **18**:915–920.
61. Sunpath H, Wu B, Gordon M, Hampton J, Johnson B, Moosa M-YS, *et al*. **High rate of K65R for antiretroviral therapy-naive patients with subtype C HIV infection failing a tenofovir-containing first-line regimen: South Africa.** *AIDS* 2012; **26**:1679–1684.
62. Invernizzi CF, Coutsinos D, Oliveira M, Schildknecht RS, Xu H, Gaseitsiwe S, *et al*. **The preferential selection of K65R in HIV-1 subtype C is attenuated by nucleotide polymorphisms at thymidine analogue mutation sites.** *J Antimicrob Chemother* 2013; **68**:2192–2196.
63. Abbas UL, Hood G, Wetzel AW, Mellors JW. **Factors influencing the emergence and spread of HIV drug resistance arising from rollout of antiretroviral preexposure prophylaxis (PrEP).** *PLoS One* 2011; **6**:e18165.

64. Gupta RK, Wainberg MA, Brun-Vezinet F, Gatell JM, Albert J, Sonnerborg A, *et al*. **Oral antiretroviral drugs as public health tools for HIV prevention: global implications for adherence, drug resistance, and the success of HIV treatment programs.** *J Infect Dis* 2013; **207 (Suppl 2)**:S101–S106.
65. Supervie V, Garcia-Lerma JG, Heneine W, Blower S. **HIV, transmitted drug resistance, and the paradox of preexposure prophylaxis.** *Proc Natl Acad Sci U S A* 2010; **107**:12381–12386.
66. van de Vijver DA, Nichols BE, Abbas UL, Boucher CA, Cambiano V, Eaton JW, *et al*. **Preexposure prophylaxis will have a limited impact on HIV-1 drug resistance in sub-Saharan Africa: a comparison of mathematical models.** *AIDS* 2013; **27**:2943–2951.
67. Abbas UL, Glaubius R, Mubayi A, Hood G, Mellors JW. **Antiretroviral therapy and preexposure prophylaxis: combined impact on HIV transmission and drug resistance in South Africa.** *J Infect Dis* 2013; **208**:224–234.
68. Abbas UL, Glaubius R, Hood G, Mellors JW. **Antiretroviral treatment, preexposure prophylaxis, and drug resistance in sub-Saharan Africa: a consensus among mathematical models.** *J Infect Dis* 2014; **209**:164–166.
69. Persaud D, Bedri A, Ziemniak C, Moorthy A, Gudetta B, Abashawl A, *et al*. **Slower clearance of nevirapine resistant virus in infants failing extended nevirapine prophylaxis for prevention of mother-to-child HIV transmission.** *AIDS Res Hum Retroviruses* 2011; **27**:823–829.
70. Kuhn L, Hunt G, Technau KG, Coovadia A, Ledwaba J, Pickerill S, *et al*. **Drug resistance among newly diagnosed HIV-infected children in the era of more efficacious antiretroviral prophylaxis.** *AIDS* 2013; **28**:1673–1678.
71. Ellis GM, Vlaskin TA, Koth A, Vaz LE, Dross SE, Beck IA, *et al*. **Simultaneous and sensitive detection of human immunodeficiency virus type 1 (HIV) drug resistant genotypes by multiplex oligonucleotide ligation assay.** *J Virol Methods* 2013; **192**:39–43.
72. Zhang G, Cai F, Zhou Z, Devos J, Wagar N, Diallo K, *et al*. **Simultaneous detection of major drug resistance mutations in the protease and reverse transcriptase genes for HIV-1 subtype C using a multiplex allele-specific (MAS) assay.** *J Clin Microbiol* 2013; **51**:3666–3674.
73. Jourdain G, Wagner TA, Ngo-Giang-Huong N, Sirirungsi W, Klinbuayaem V, Fregonese F, *et al*. **Association between detection of HIV-1 DNA resistance mutations by a sensitive assay at initiation of antiretroviral therapy and virologic failure.** *Clin Infect Dis* 2010; **50**:1397–1404.
74. Bronze M, Aitken SC, Wallis CL, Steegen K, Stuyver LJ, de Wit TF, *et al*. **Evaluation of an affordable HIV-1 virological failure assay and antiretroviral drug resistance genotyping protocol.** *J Virol Methods* 2013; **194**:300–307.
75. Tilghman MW, May S, Perez-Santiago J, Ignacio CC, Little SJ, Richman DD, *et al*. **A combined screening platform for HIV treatment failure and resistance.** *PLoS One* 2012; **7**:e35401.
76. Newman H, Breunig L, van Zyl G, Stich A, Preiser W. **A qualitative PCR minipool strategy to screen for virologic failure and antiretroviral drug resistance in South African patients on first-line antiretroviral therapy.** *J Clin Virol* 2014; **60**:387–391.
77. Dudley DM, Chin EN, Bimber BN, Sanabani SS, Tarosso LF, Costa PR, *et al*. **Low-cost ultra-wide genotyping using Roche/454 pyrosequencing for surveillance of HIV drug resistance.** *PLoS One* 2012; **7**:e36494.
78. Li JZ, Paredes R, Ribaudo HJ, Kozal MJ, Svarovskaia ES, Johnson JA, *et al*. **Impact of minority nonnucleoside reverse transcriptase inhibitor resistance mutations on resistance genotype after virologic failure.** *J Infect Dis* 2013; **207**:893–897.
79. Ji H, Li Y, Graham M, Liang BB, Pilon R, Tyson S, *et al*. **Next-generation sequencing of dried blood spot specimens: a novel approach to HIV drug-resistance surveillance.** *Antivir Ther* 2011; **16**:871–878.