UNIVERSITY OF AMSTERDAM

UvA-DARE (Digital Academic Repository)

Drug-resistant HIV-1 in sub-Saharan Africa: clinical and public health studies

Hamers, R.L.

Publication date
2013

Link to publication

Citation for published version (APA):
Hamers, R. L. (2013). *Drug-resistant HIV-1 in sub-Saharan Africa: clinical and public health studies*. [Thesis, fully internal, Universiteit van Amsterdam].

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: https://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, P.O. Box 19185, 1000 GD Amsterdam, The Netherlands. You will be contacted as soon as possible.

UvA-DARE is a service provided by the library of the University of Amsterdam (https://dare.uva.nl)

Download date:13 Nov 2026

Chapter 1

General introduction

HIV GLOBAL EPIDEMIC

The first cases of a novel immunodeficiency syndrome were reported in San Francisco, US, in 1981 [1]. In 1983, the causative agent was discovered to be a novel retrovirus, which was named Human Immunodeficiency Virus (HIV) [2]. The syndrome was named Acquired Immunodeficiency Syndrome (AIDS). Since the 1980s, the HIV/AIDS epidemic has had a detrimental impact worldwide. To date, HIV/AIDS has claimed the lives of more than 25 million people, and more than 30 million people are estimated to be living with HIV/AIDS worldwide [3]. Sub-Saharan Africa is the region most heavily affected by HIV/AIDS, accounting for 68% of all people living with HIV/AIDS, 70% of all new HIV infections in 2010, and 72% of all AIDS-related deaths [3]. The epidemic is most severe in southern Africa. Recently, the largest epidemics in the region —in Ethiopia, Nigeria, South Africa, Zambia, and Zimbabwe— have either stabilized or are showing signs of decline [3]. The number of annual AIDS-related deaths is steadily decreasing, reflecting the introduction of combination antiretroviral therapy (ART) as well as a decreasing incidence [3].

HIV is an enveloped retrovirus that belongs to the genus of lentiviridae, which is subdivided in two types: HIV-1, most common globally, and HIV-2, a less pathogenic variant concentrated in west Africa. HIV-1 has been divided into four distinct genetic groups: M, N, O and P [4-6]. Group M (major) is responsible for over 90% of HIV-1 infections globally. Natural genetic variation has led to the sub-classification of HIV-1 group M into nine subtypes (A-D, F-H, J, and K) and numerous circulating recombinant forms (CRFs) [7]. Subtype B is most prevalent in Europe, North America and Australia [8]. In sub-Saharan Africa, HIV-1 subtype C is responsible for 56% of infections, mainly in southern and east Africa, whereas smaller proportions of infections are caused by subtypes A, D, G, CRF_AG and other CRFs [8].

ANTIRETROVIRAL TREATMENT

HIV-1 can be transmitted sexually, through parenteral exposure to blood, or from mother to child during pregnancy, birth or breast-feeding. HIV-1 primarily targets CD4+ T-lymphocytes and macrophages. The acute infection is characterized by a burst of viral replication and immune activation [9, 10] and followed by a symptom-free interval of on average eight to ten years. During chronic infection, the number of CD4+ T-lymphycytes gradually declines. Without ART, cell-mediated immunity will eventually be lost and the immunodeficient HIV-infected individual becomes susceptible to opportunistic infections and neoplasms. The final stage, AIDS, will lead to death, if untreated.

ART reduces HIV-related morbidity and mortality by lowering of the viral load to minimum levels, thereby allowing the immune system to recover and preventing opportunistic infections [11, 12]. Notably, with currently available treatment modalities, HIV-1 cannot be eliminated from infected persons. Since ART became available in 1996, HIV no longer inevitably leads to AIDS and death. As a result of social mobilization, high-level political commitment and substantial international funding during the past decade, access to ART in resource-limited countries has been rapidly scaled-up. By the end of 2010, more than five million people in sub-Saharan Africa were receiving ART, reaching nearly 50% of those in immediate need [3]. HIV-related morbidity and mortality have significantly decreased for individuals receiving ART in the region [11, 13]. Despite these impressive gains, access is still not universal, and the United Nations General Assembly has recently committed to a target of treating 15 million people by 2015 worldwide [14].

To allow the scale-up of ART in resource-limited countries, a public health approach, developed by the World Health Organization (WHO) has been critical [15]. This approach is based on simplified ART protocols, including standard first-line and second-line ART regimens, limited laboratory monitoring, and a decentralized service delivery. Standard first-line regimens consist of a single non-nucleoside reverse transcriptase inhibitor (NNRTI) and a dual nucleoside/nucleotide reverse transcriptase inhibitor (NRTI) backbone, often available as generic fixed-dose combinations [16, 17]. Recommended second-line regimens combine a ritonavir-boosted protease inhibitor (bPI) with two previously unused and/or recycled NRTIs [16, 17], although availability of second-line regimens is still restricted in many settings. Because of resource constraints, plasma viral load testing is not generally available to monitor therapy effectiveness and detect therapy failure. Instead, WHO-defined HIV clinical staging and –if available– CD4 cell counts are commonly used to guide decision-making about regimen switching [16, 17].

HIV DRUG RESISTANCE

HIV-1 infection is characterized by high genetic diversity of the virus. This is the result of high levels of viral replication [18, 19] coupled with a high mutation frequency in the HIV genome, which is generated by error-prone reverse transcription during the HIV replication cycle [20, 21]. Consequently, a large pool of genetically related but distinct genetic virus variants, called quasispecies, are present within the infected individual [22]. Distinct quasispecies may have either deleterious mutations, mutations that reduce their fitness, or mutations that provide a fitness advantage in a particular environment, such as in the presence of antiretroviral drugs. Mutations (i.e. point mutations, insertions or deletions) may result in changes in the amino acid coding of the HIV proteins,

potentially altering the structure and/or function of these proteins and affecting the fitness of the viral strain. Mutations can cause drug resistance by structural alteration of the target molecule that prevents or reduces inhibitor binding (in the case of PIs, NRTIs, NNRTIs, and entry inhibitors), or by directly affecting the mechanism of action of the reverse-transcriptase enzyme (in the case of NRTIs).

ART applies a combination of antiretroviral agents from different drug classes to minimize the risk of drug resistance development. An effective ART regimen will suppress the replication of most quasispecies. However, quasispecies containing one or more resistance-associated mutations may continue to replicate at a very low rate. In the presence of the selective pressure of ART, viruses with reduced susceptibility to one or more of the drugs in the regimen will out-compete the wild-type (i.e. drug-susceptible) virus, and eventually become predominant in the quasispecies population. If the selective pressure of ART is removed, the virus variants harbouring resistance may be replaced by more efficiently replicating wild-type virus [23]. The drug-resistant variants persist as minority quasispecies, archived in the proviral DNA, and may re-emerge if selective pressure is re-applied by restarting the antiretroviral drug that selected for them. Overall, mutation and selection is a dynamic process determined by the potency of the ART regimen, drug concentrations, cross-resistance, and the effects of resistance on viral fitness.

Drug-resistant HIV-1 variants that are selected by ART during residual viral replication, called acquired drug resistance, constitute a reservoir for onward transmission to newly infected individuals, called primary or transmitted drug resistance (TDR) [24, 25]. Individuals who are newly infected with a drug-resistant variant can further contribute to the spread of drug-resistant HIV-1 [26, 27]. Although TDR variants may persist in untreated individuals [28], they may revert to wild-type virus over time or diminish to levels below detection by population-based genotyping [29]. Some of the challenges in studying the epidemiology of TDR include differences between studies in the definition and interpretation of resistance test results, duration of HIV-1 infection, time period, geographic region, subpopulation, and the possibility of undisclosed previous exposure to antiretroviral drugs.

SCALE-UP OF ANTIRETROVIRAL TREATMENT IN RESOURCE-LIMITED SETTINGS

In resource-limited countries, concern has been raised about the potential emergence and spread of HIV-1 drug resistance and its public health implications after the scale-up of antiretroviral drugs. In Europe and North America, the wider use of ART has been as-

sociated with an increase in levels of TDR [30-32], peaking in some settings at over 20% before levelling off at 9-15% in the era of (highly active) ART [24, 25, 33, 34]. Notably, its evolution has occurred in the context of (non-potent) sequential mono and dual therapies of NRTIs before 1996 [30-32]. By contrast, in resource-limited countries, the history and conditions of HIV treatment have been very different. The rapid scale-up of ART since 2003-2004 has been rightfully given priority to save the lives of millions of HIV-1 infected Africans, using potent, triple combination therapy from the onset. Relatively little attention has been paid to the development and spread of drug-resistant HIV-1 as a potential consequence of the widespread distribution of ART.

Factors contributing to HIV-1 drug resistance in resource-limited countries can be broadly grouped into four categories: regimen- and drug-specific, virus-related, patient-specific, and programmatic. A recognized limitation of NNRTI-based regimens is their relatively lower genetic barrier to resistance when compared to bPI regimens. Suboptimal regimens, such as the peripartum use of single-dose nevirapine to prevent mother-to-child HIV transmission (PMTCT), drug-drug interactions, inappropriate prescribing practices, use of non-quality assured drugs can further increase the risk of acquiring drug resistance [35]. For example, concomitant use of rifampicin in tuberculosis co-infected patients has been shown to reduce levels of nevirapine [36]. Poor adherence to ART is a predictor of virological failure [37-41], drug resistance, disease progression [42-44] and death [45]. Programme-level factors, such as limited human resources, inadequate infrastructure and weak supply management systems, can also negatively affect treatment adherence, retention in care and ultimately facilitate the emergence of population-level HIV drug resistance. Fragile drug procurement and supply management systems can result in drug stock-outs [46]. The absence of routine viral load monitoring, which is a more sensitive indicator of treatment failure than clinical-immunological parameters, may lead some patients to experience prolonged periods of virological failure prior to change of regimen [47, 48]. Moreover, although current evidence is limited, it has been suggested that the propensity to develop drug resistance and the spectrum of mutations that are acquired during ART, may differ across the various HIV-1 subtypes and CRFs [49].

The threat of increased TDR after the ART scale-up in sub-Saharan Africa has the potential to compromise the effectiveness of first-line ART regimens. Therefore, a new challenge that may confront national HIV treatment programs is how to manage emerging drug-resistant HIV-1. However, few data exist to adequately inform policy.

RESEARCH SETTING: THE PASER NETWORK

In 2006, a collaborative bi-regional program was established in sub-Saharan Africa and Asia, (*Linking African and Asian Societies for an Enhanced Response to HIV/AIDS*, denoted LAASER) with the primary aim of developing the regional capacities for the population-based assessment of acquired and transmitted HIV-1 drug resistance, thereby advancing the epidemiological, clinical and laboratory knowledge of the management of drug resistance in the regions. LAASER received financial support from The Netherlands Ministry of Foreign Affairs in partnership with Stichting AidsFonds (2006-2011).

As part of LAASER, the *PharmAccess African Studies to Evaluate Resistance* (PASER) network was established as a collaborative partnership of clinical sites, laboratories and research groups in Kenya, Nigeria, South Africa, Uganda, Zambia, and Zimbabwe. Table 1 summarizes some relevant country characteristics. PASER has implemented two laboratory-based study protocols: prospective cohorts to assess pre-therapy and acquired resistance in patients receiving first- or second-line ART (Monitoring, PASER-M), and cross-sectional surveys to assess TDR in recently HIV-1 infected populations (Surveillance, PASER-S). PASER contributes to fulfilling the goals of the Global HIV Drug Resistance Network (HIVResNet), developed by the WHO (http://www.who.int/hiv/topics/drugresistance/hivresnet). The implementation of HIV-1 drug resistance surveys in resource-limited countries is challenged by the high cost and complexity of genotypic resistance testing. To address this issue, PASER has initiated a public–private consortium, called *Affordable Resistance Test for Africa* (ART-A), which aims to develop a more affordable test algorithm for HIV-1 drug resistance. The studies included in this thesis were conducted as part of the PASER and ART-A programs.

Table 1. HIV/AIDS characteristics of PASER countries (source: WHO/UNAIDS)

Country	Number of people infected with HIV (millions) (2009)	National adult HIV prevalence (2009)	Year of introduction of national HIV treatment program	Number of people receiving ART (2010)	National ART coverage (2010)
Kenya	1.5	6.3%	2003	432,621	61%
Nigeria	3.3	3.6%	2002	359,181	26%
South Africa	5.6	17.8%	2004	1,389,865	55%
Uganda	1.2	6.5%	2000	248,222	47%
Zambia	1.0	13.5%	2003	344,407	72%
Zimbabwe	1.2	14.3%	2004	326,241	59%

RESEARCH OBJECTIVES

The aim of this thesis was to study the extent of HIV-1 drug resistance and its potential public health implications after the scale-up of ART in sub-Saharan Africa.

The research objectives of the thesis were:

- To define the epidemiology of TDR in HIV-1 infected populations after the scale-up of ART.
- To assess the effects of pre-therapy HIV-1 drug resistance on the response to first-line or second-line ART in routine ART programs.
- To assess patterns of HIV-1 drug resistance mutations and their clinical impact in patients experiencing failure of standard first-line or second-line ART in routine ART programs.
- To explore the implications of emerging HIV-1 drug resistance for public health policy in resource-limited countries.

OUTLINE OF THESIS

Background

As an introduction to the thesis, the first three chapters include a review of data on HIV-1 drug resistance in sub-Saharan Africa that were available before the start of the PhD research (**Chapter 2**), including an illustrative patient case study (**Chapter 3**), and a profile of the PASER-M cohort (**Chapter 4**).

The core of the thesis comprises three parts that include studies on epidemiological (**Part I**), clinical (**Part II**) and public health (**Part III**) aspects of drug-resistant HIV-1 in sub-Saharan Africa.

Transmitted HIV-1 drug resistance (Part I)

The first part focuses on the epidemiology of TDR in various countries and populations after the scale-up of ART, based on data from the PASER-M and PASER-S studies. The first study compares the prevalence and patterns of pre-therapy resistance between antiretroviral-naïve and antiretroviral-exposed individuals in Lusaka, Zambia, who are about to start standard first-line ART (**Chapter 5**). Subsequently, we assess TDR prevalence in antiretroviral-naive adults from 11 regions in Kenya, Nigeria, South Africa, Uganda, Zambia, and Zimbabwe, and examine if wider use of ART in sub-Saharan Africa is associated with rising prevalence of TDR (**Chapter 6**). Finally, we assess TDR in a recently HIV-1 infected population in Kampala, Uganda (**Chapter 7**).

Antiretroviral treatment and acquired resistance (Part II)

The second part includes clinical studies on therapy response and drug resistance patterns in patients receiving first-line ART (**Chapters 8 and 9**), switching to second-line ART (**Chapter 10**), or receiving second-line ART (**Chapter 11**), who are enrolled in 13 regular ART programs in six African countries. All studies are based on data from the PASER-M cohort. The first study prospectively assesses the effect of pre-therapy resistance on the immunological, virological and resistance outcomes of first-line ART (**Chapter 8**). Subsequently, the patterns of drug resistance mutations in patients experiencing virological failure after 12 months of first-line ART are described, including the implications for second-line therapy strategies (**Chapter 9**). In **Chapter 10**, we investigate patients at time of switch to a second-line regimen who experienced prolonged first-line failure, in the absence of plasma viral load monitoring. Particularly, we assess the diagnostic accuracy of clinico-immunological failure criteria –i.e. the proportion of patients who are misdiagnosed with virological failure and switched unnecessarily–, and the patterns of HIV-1 drug resistance mutations that are present at time of switch after prolonged failure. Finally, we prospectively assess the response to empiric second-line ART, including the effect of first-line resistance, and the patterns of drug resistance mutations in patients failing second-line ART (**Chapter 11**).

Public health policy (Part III)

The third part expands on the implications of emerging drug-resistant HIV-1 for clinical practice and public health policy in resource-limited countries. First, we discuss the operational experiences, achievements and challenges in establishing the PASER network (**Chapter 12**), and report the results of a site-level assessment of WHO-recommended early-warning indicators of HIV-1 drug resistance in all clinical sites collaborating in the PASER network (**Chapter 13**). In **Chapter 14**, we report a model-based analysis on the costs, life-expectancy and cost-effectiveness associated with laboratory-based diagnostic monitoring of patients receiving ART in sub-Saharan Africa using either CD4 cell counts or plasma viral loads only, as compared to clinical monitoring. In **Chapter 15**, a systematic review was undertaken of the usefulness and limitations of dried fluid spots as a practical, affordable specimen matrix to measure HIV-1 viral load and genotypic resistance in resource-limited countries. Finally, we expound recommendations and priorities for public health policy in view of rising drug-resistant HIV-1 in sub-Saharan Africa (**Chapter 16**).

Discussion

The final chapter (**Chapter 17**) is a summary and general discussion of the main research findings of this thesis, followed by some concluding remarks.

REFERENCES

1. Gottlieb MS, Schroff R, Schanker HM, Weisman JD, Fan PT, Wolf RA, Saxon A. Pneumocystis carinii pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. N Engl J Med 1981;305:1425-1431.
2. Barre-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, Chamaret S, Gruest J, et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science 1983;220:868-871.
3. World Health Organization and Joint United Nations Programme on HIV/AIDS. Global HIV/AIDS Response: Epidemic update and health sector progress towards Universal Access: progress report 2011. Available at: http://www.who.int/hiv/pub/progress_report2011/hiv_full_report_2011.pdf.
4. Fonjungo PN, Dash BC, Mpoudi EN, Torimiro JN, Alemnji GA, Eno LT, et al. Molecular screening for HIV-1 group N and simian immunodeficiency virus cpz-like virus infections in Cameroon. AIDS 2000;14:750-752.
5. Jaffe HW, Schochetman G. Group O human immunodeficiency virus-1 infections. Infect.Dis.Clin. North Am. 1998;12:39-46.
6. Plantier JC, Leoz M, Dickerson JE, De Oliveira F, Cordonnier F, Lemee V, et al. A new human immunodeficiency virus derived from gorillas. Nat Med 2009;15:871-872.
7. Robertson DL, Anderson JP, Bradac JA, Carr JK, Foley B, Funkhouser RK, et al. HIV-1 nomenclature proposal. Science 2000;288:55-56.
8. Hemelaar J, Gouws E, Ghys PD, Osmanov S. Global trends in molecular epidemiology of HIV-1 during 2000-2007. Aids 2011,25:679-689.
9. Daar ES, Moudgil T, Meyer RD, Ho DD. Transient high levels of viremia in patients with primary human immunodeficiency virus type 1 infection. N Engl J Med 1991;324:961-964.
10. Koup RA, Safrit JT, Cao Y, Andrews CA, McLeod G, Borkowsky W, et al. Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. J Virol 1994;68:4650-4655.
11. Jahn A, Floyd S, Crampin AC, Mwaungulu F, Mvula H, Munthali F, et al. Population-level effect of HIV on adult mortality and early evidence of reversal after introduction of antiretroviral therapy in Malawi. Lancet 2008;371:1603-1611.
12. Williams BG, Granich R, De Cock KM, Glaziou P, Sharma A, Dye C. Antiretroviral therapy for tuberculosis control in nine African countries. Proc Natl Acad Sci U S A 2010;107:19485-19489.
13. Braitstein P, Brinkhof MW, Dabis F, Schechter M, Boulle A, Miotti P, et al. Mortality of HIV-1-infected patients in the first year of antiretroviral therapy: comparison between low-income and high-income countries. Lancet 2006;367:817-824.
14. UN General Assembly. Political Declaration on HIV/AIDS: Intensifying our Efforts to Eliminate HIV/AIDS. 10 June 2011. Available at: http://www.unaids.org/en/media/unaids/contentassets/documents/document/2011/06/20110610_UN_A-RES-65-277_en.pdf.
15. Gilks CF, Crowley S, Ekpini R, Gove S, Perriens J, Souteyrand Y, et al. The WHO public-health approach to antiretroviral treatment against HIV in resource-limited settings. Lancet 2006;368: 505-510.
16. World Health Organization. Antiretroviral therapy for HIV infection in adults and adolescents: Recommendations for a public health approach, 2006 revision. Available at: http://www.who.int/hiv/pub/guidelines/artadultguidelines.pdf. Geneva, 2006.

17. World Health Organization. Antiretroviral therapy for HIV infection in adults and adolescents: Recommendations for a public health approach, 2010 revision. Available at: http://whqlibdoc.who.int/publications/2010/9789241599764_eng.pdf. Geneva, 2010.
18. Ho DD, Neumann AU, Perelson AS, Chen W, Leonard JM, Markowitz M. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. Nature 1995;373:123-126.
19. Wei X, Ghosh SK, Taylor ME, Johnson VA, Emini EA, Deutsch P, et al. Viral dynamics in human immunodeficiency virus type 1 infection. Nature 1995,373:117-122.
20. Preston BD, Poiesz BJ, Loeb LA. Fidelity of HIV-1 reverse transcriptase. Science 1988;242:1168-1171.
21. Roberts JD, Bebenek K, Kunkel TA. The accuracy of reverse transcriptase from HIV-1. Science 1988; 242:1171-1173.
22. Coffin JM. HIV population dynamics in vivo: implications for genetic variation, pathogenesis, and therapy. Science 1995;267:483-489.
23. Halfon P, Durant J, Clevenbergh P, Carsenti H, Celis L, Khiri H, et al. Kinetics of disappearance of resistance mutations and reappearance of wild-type during structured treatment interruptions. Aids 2003;17:1351-1361.
24. Vercauteren J, Wensing AM, van de Vijver DA, Albert J, Balotta C, Hamouda O, et al. Transmission of drug-resistant HIV-1 is stabilizing in Europe. J Infect Dis 2009;200:1503-1508.
25. Wheeler WH, Ziebell RA, Zabina H, Pieniazek D, Prejean J, Bodnar UR, et al. Prevalence of transmitted drug resistance associated mutations and HIV-1 subtypes in new HIV-1 diagnoses, U.S.-2006. Aids 2010;24:1203-1212.
26. Yerly S, Junier T, Gayet-Ageron A, Amari EB, von Wyl V, Gunthard HF, et al. The impact of transmission clusters on primary drug resistance in newly diagnosed HIV-1 infection. Aids 2009,23: 1415-1423.
27. Brenner BG, Roger M, Routy JP, Moisi D, Ntemgwa M, Matte C, et al. High rates of forward transmission events after acute/early HIV-1 infection. J Infect Dis 2007;195:951-959.
28. Little SJ, Frost SD, Wong JK, Smith DM, Pond SL, Ignacio CC, et al. Persistence of transmitted drug resistance among subjects with primary human immunodeficiency virus infection. J Virol 2008,82:5510-5518.
29. Johnson JA, Li JF, Wei X, Lipscomb J, Irlbeck D, Craig C, et al. Minority HIV-1 drug resistance mutations are present in antiretroviral treatment-naive populations and associate with reduced treatment efficacy. PLoS Med 2008;5:e158.
30. Grant RM, Hecht FM, Warmerdam M, Liu L, Liegler T, Petropoulos CJ, et al. Time trends in primary HIV-1 drug resistance among recently infected persons. JAMA 2002;288:181-188.
31. Little SJ, Holte S, Routy JP, Daar ES, Markowitz M, Collier AC, et al. Antiretroviral-drug resistance among patients recently infected with HIV. N.Engl.J.Med. 2002;347:385-394.
32. Wensing AM, van de Vijver DA, Angarano G, Asjo B, Balotta C, Boeri E, et al. Prevalence of drug-resistant HIV-1 variants in untreated individuals in Europe: implications for clinical management. J.Infect.Dis. 2005;192:958-966.
33. Cane P, Chrystie I, Dunn D, Evans B, Geretti AM, Green H, et al. Time trends in primary resistance to HIV drugs in the United Kingdom: multicentre observational study. BMJ 2005;331:1368.
34. Rahim S, Fredrick LM, da Silva BA, Bernstein B, King MS. Geographic and temporal trends of transmitted HIV-1 drug resistance among antiretroviral-naive subjects screening for two clinical trials in North America and Western Europe. HIV Clin Trials 2009;10:94-103.
35. Cockburn R, Newton PN, Agyarko EK, Akunyili D, White NJ. The global threat of counterfeit drugs: why industry and governments must communicate the dangers. PLoS Med 2005;2:e100.

36. Boulle A, Van Cutsem G, Cohen K, Hilderbrand K, Mathee S, Abrahams M, et al. Outcomes of nevirapine- and efavirenz-based antiretroviral therapy when coadministered with rifampicin-based antitubercular therapy. Jama 2008;300:530-539.
37. Lucas GM, Chaisson RE, Moore RD. Highly active antiretroviral therapy in a large urban clinic: risk factors for virologic failure and adverse drug reactions. Annals of internal medicine 1999;131: 81-87.
38. Nachega JB, Hislop M, Dowdy DW, Chaisson RE, Regensberg L, Maartens G. Adherence to non-nucleoside reverse transcriptase inhibitor-based HIV therapy and virologic outcomes. Annals of internal medicine 2007;146:564-573.
39. Arnsten JH, Demas PA, Farzadegan H, Grant RW, Gourevitch MN, Chang CJ, et al. Antiretroviral therapy adherence and viral suppression in HIV-infected drug users: comparison of self-report and electronic monitoring. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America 2001;33:1417-1423.
40. Shuter J, Sarlo JA, Kanmaz TJ, Rode RA, Zingman BS. HIV-infected patients receiving lopinavir/ritonavir-based antiretroviral therapy achieve high rates of virologic suppression despite adherence rates less than 95%. Journal of acquired immune deficiency syndromes 2007;45:4-8.
41. Martin M, Del Cacho E, Codina C, Tuset M, De Lazzari E, Mallolas J, et al. Relationship between adherence level, type of the antiretroviral regimen, and plasma HIV type 1 RNA viral load: a prospective cohort study. AIDS research and human retroviruses 2008;24:1263-1268.
42. Bangsberg DR, Acosta EP, Gupta R, Guzman D, Riley ED, Harrigan PR, et al. Adherence-resistance relationships for protease and non-nucleoside reverse transcriptase inhibitors explained by virological fitness. AIDS 2006,20:223-231.
43. Harrigan PR, Hogg RS, Dong WW, Yip B, Wynhoven B, Woodward J, et al. Predictors of HIV drug-resistance mutations in a large antiretroviral-naive cohort initiating triple antiretroviral therapy. The Journal of infectious diseases 2005;191:339-347.
44. Bangsberg DR, Perry S, Charlebois ED, Clark RA, Roberston M, Zolopa AR, Moss A. Non-adherence to highly active antiretroviral therapy predicts progression to AIDS. AIDS 2001;15:1181-1183.
45. Sethi AK, Celentano DD, Gange SJ, Moore RD, Gallant JE. Association between adherence to antiretroviral therapy and human immunodeficiency virus drug resistance. Clin.Infect.Dis. 2003; 37:1112-1118.
46. Oyugi JH, Byakika-Tusiime J, Ragland K, Laeyendecker O, Mugerwa R, Kityo C, et al. Treatment interruptions predict resistance in HIV-positive individuals purchasing fixed-dose combination antiretroviral therapy in Kampala, Uganda. Aids 2007;21:965-971.
47. 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.
48. Sigaloff KC, Hamers RL, Wallis CL, Kityo C, Siwale M, Ive P, et al. Unnecessary antiretroviral treatment switches and accumulation of HIV resistance mutations; two arguments for viral load monitoring in Africa. J Acquir Immune Defic Syndr 2011;58(1):23-31.
49. Wainberg MA, Zaharatos GJ, Brenner BG. Development of antiretroviral drug resistance. N Engl J Med 2011;365:637-646.