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HIV drug resistance among adults and children in sub-Saharan Africa

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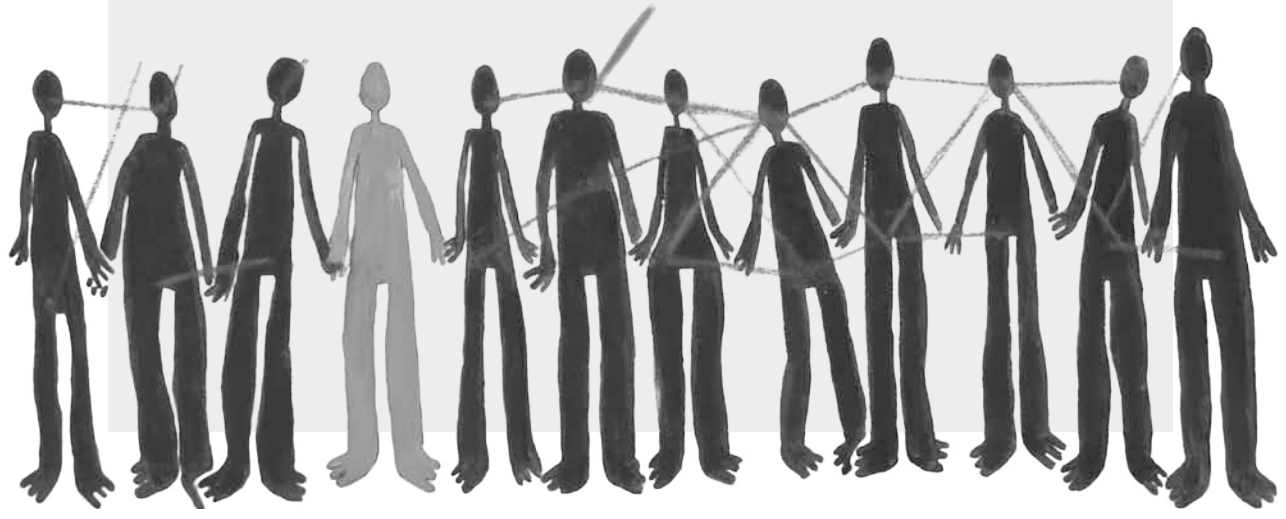
Chapter 14

General discussion



Since its introduction 15 years ago, combination antiretroviral therapy (ART) has saved millions of lives worldwide. In Africa, over five million HIV-infected people are currently estimated to receive ART, impacting nearly 50% of those in need.[1] Treatment programs in resource-limited countries have efficacy rates similar to those reported for developed countries.[2] However, from the moment ART was introduced and scaled-up in resource-limited countries, little attention has been paid to the emergence of HIV drug resistance and its potential consequences. Drug resistant HIV may severely restrict therapeutic options, and treatment costs will greatly increase when second and third-line ART regimens are needed. Therefore, a major challenge that will confront national HIV treatment programs is how to prevent and manage mounting drug resistance.

To address this challenge, the 'PharmAccess African Studies to Evaluate Resistance' or PASER program was established with the aim of developing regional capacity for the population-level assessment of acquired and transmitted HIV drug resistance. The PASER network consists of clinical sites, laboratories and research groups in Kenya, Nigeria, South Africa, Uganda, Zambia, and Zimbabwe. Within the PASER program, the PASER-Monitoring protocol assessed baseline and acquired resistance in adults receiving first- or second-line ART, and the PASER-Surveillance protocol assessed transmitted drug resistance in recently infected individuals. A spin-off project, the 'Monitoring Antiretroviral Resistance in Children' or MARCH pediatric cohort, was initiated in Uganda with similar objectives as the adult study. The studies included in this thesis were conducted as part of the PASER and MARCH programs.

PART 1: EPIDEMIOLOGY OF TRANSMITTED HIV DRUG RESISTANCE

Initial evidence of studies in resource-limited settings, using the World Health Organization (WHO) threshold survey methodology, suggested that in the first years of ART scale-up, the prevalence of transmitted drug resistance was limited, i.e. below 5%.[3] Studies performed in 2009-2010 as part of the PASER-Surveillance program have reported a rise in transmitted drug resistance in East Africa. Our study carried out among 70 recently diagnosed individuals in Kampala, Uganda, revealed drug resistance mutations in 8.6% (**chapter 2**). This study suggested increasing resistance levels compared with a previous survey in the same geographic region in which no drug resistance was found.[4]of this information or reproduction of this materialis strictly forbidden unless prior written permission is obtainedfrom Adobe Systems Incorporated.File: docxtoSangamML.xsltAuthor : Sachin Soni

In Mombasa, Kenya, we conducted a similar study using the WHO threshold survey methodology (**chapter 3**). Among 68 participants, the prevalence of transmitted drug

resistance was found to be as high as 13.2%. Although no prior surveys have been conducted in Mombasa, this level exceeds what has been previously reported in Kenya.

Reported transmitted drug resistance levels in these two urban African settings were highest for non-nucleoside reverse transcriptase inhibitors (NNRTIs), which is consistent with the widespread use of this drug class for first-line ART, the low genetic barrier of NNRTIs for resistance, and the relative fitness of NNRTI-resistant strains.[5, 6] The findings in Kampala and Mombasa corroborate other recent studies reporting an increase of transmitted drug resistance in parallel to the widespread distribution of ART in other African countries.[7-9] A Virology Laboratory CREMER/IMPM/IRD, Yaounde, Cameroon bCentral Hospital, Yaounde, Cameroon cUMI 233 TransVIHMI, Institut de Recherche pour le Developpement (IRD Indentifying recent infections is complicated and instead, HIV drug resistance can be measured among people at the time of ART initiation, although mutations may disappear due to reversion.[10] The PASER-Monitoring study assessed baseline (or pretreatment) resistance among chronically infected, antiretroviral-naïve persons and detected a drug resistance mutation in 5.6% before ART initiation.[11] The prevalence of resistance mutations appeared higher in Uganda (12%), where antiretroviral drugs were available ahead of other African countries. In this study, pretreatment resistance was estimated to increase with time since the start of the ART roll-out by 38% per year. In agreement, a recent meta-analysis also demonstrated an increasing prevalence of drug resistance since ART scale-up, in particular in East Africa.[12] Importantly, this study also found that the prevalence of mutations was not different between recently or chronically infected populations.

In 2010, the WHO recommended that the threshold for ART initiation be changed from 200 to 350 CD4 cells/mm^3 – substantially increasing the number of people eligible for ART.[13] In addition to the individual clinical benefits, earlier initiation of ART may reduce HIV incidence rates and the concept of treatment as prevention has attracted attention as a means to reduce the global HIV epidemic.[14, 15] We investigated the effect of the subsequent increased ART coverage on future transmitted drug resistance trends in East Africa using a mathematical model (**chapter 4**). This model was based on the rates of acquired and transmitted resistance rates as detected in the PASER-M and PASER-S studies, respectively, in Kampala and Mombasa. We estimated that in 10 years time, the prevalence of transmitted drug resistance will increase to 12% and 13% if ART is started at CD4 <350 cells/mm3 and to 18% and 19% if ART started at CD4 <500 cells/mm3 in Kampala and Mombasa, respectively. Our model confirmed that resistance will consist predominantly of NNRTI-associated mutations. This, however, can be prevented by providing wider access to virological monitoring and boosted PIs for second-line therapy. Importantly, we predict that the number of infections that will be averted by earlier ART initiation will far exceed the number of infections with a drug resistant virus.

PART 2: ACQUIRED HIV DRUG RESISTANCE IN PERSONS FAILING FIRST-LINE ANTIRETROVIRAL THERAPY

Increasing transmitted drug resistance is ultimately caused by increasing rates of virological failure and acquired resistance in persons receiving ART. A challenge for healthcare workers in resource-limited countries is how to identify patients who are experiencing therapy failure and to make timely decisions on switches to second-line therapy. The WHO public-health approach to ART administration recommends using clinical and/or immunological definitions of ART failure if viral load testing is not available.[13] The studies in part two of this thesis underscore the poor ability of these clinical and immunological criteria to diagnose ART failure. We demonstrated that among 250 patients who started second-line ART, first-line treatment failure had been diagnosed in 64 patients based on clinical and immunological criteria alone, in absence of viral load testing (**chapter 5**). In this group, an unnecessary switch occurred in 47%, as later determination of viral load revealed <1000 copies/ml. The use of targeted viral load testing by the treating clinician reduced the rate of unnecessary switches to 12%. In the same cross-sectional study, a high frequency (88%) of clinically significant mutations was detected among patients with clinical and/or immunological first-line failure and a viral load >1000 copies/ml. Extensive cross-resistance within the nucleoside reverse transcriptase inhibitor (NRTI) drug class was present in 48%, which is indicative of prolonged virological failure. In these patients, a switch to second-line ART would be expected to offer the benefit of the boosted protease inhibitor (PI), but limited or no additional effect of the NRTI backbone.

Drug resistance mutations associated with first-line regimens were investigated in more detail in our prospective study of 2588 patients with no prior exposure to antiretroviral medication (**chapter 6**). During a routine measurement after 12 months of first-line ART, 142 patients were evaluated for drug resistance after a viral load >1000 copies/ml was detected. Of these, 70% had at least one mutation and 49% had dual-class resistance. Compared to the study in chapter 5, in which most patients had prolonged treatment failure, extensive NRTI resistance was less common in this cohort where a routine viral load test was carried out after 12 months. For example, multiple thymidine analogue mutations, conferring broad NRTI cross-resistance, were found in 38% of patients in chapter 5 but only in 6% of patients in the prospective cohort in chapter 6. Hence, routine viral load measurement prevented prolonged virological failure and the accumulation of mutations. In chapter 6, the K65R mutation was frequently (12%) selected for after failure of stavudine or tenofovir containing regimens. This finding suggested that, after failure of a stavudine containing first-line regimen, zidovudine rather than tenofovir (as recommended by the current WHO guidelines)[13], may be the preferred second-line NRTI. The data reported in chapters 5 and 6 also indicated that the second-generation NNRTIs

etravirine or rilpivirine are unlikely to be effective as part of second-line ART, given the high frequencies of Y181C and G190A mutations.

We estimated the rate at which thymidine analogue mutations accumulate during continued ART failure in **chapter 7**. Among a small cohort comprising 43 South African patients, repeated resistance testing was performed in patients with persistent viremia while receiving first-line ART. Overall, thymidine analogue mutations were found to accumulate at a rate of 0.07 mutations per month, resulting in one new mutation for every 14.6 months of continued drug exposure. This was considerably faster than the rate of one thymidine analogue mutation in every 4.3 years described in a European study.[16] In patients who acquired at least 1 thymidine analogue mutation, the rate of accumulation was found to be 0.15 mutations per month, i.e. one mutation in 6.5 months. Again, we concluded that the degree and pattern of drug resistance detected after prolonged virological failure in this cohort are likely to negatively impact future regimens containing NRTI backbones.

The poor sensitivity and specificity of WHO clinical and immunological criteria to diagnose ART failure have been also pointed out by other studies in Africa.[17, 18] False-positive cases (clinical or immunological failure but suppressed viral load) cause unnecessary switching to second-line ART. In line with our observations, a Kenyan study estimated that up to 58% of participants would be misclassified as having ART failure and prematurely switched to second-line if only immunological criteria would be used.[19] We conclude that unnecessary regimens switches will exhaust drug options and augment costs. The use of targeted viral load testing to confirm suspected treatment failure and guide regimen switching is therefore likely to be cost-saving.[20] False-negative cases are more common as viral load elevation failure usually precedes clinical or immunological deterioration. This results in prolonged virological failure and subsequent accumulation of mutations, as was also clearly demonstrated by a study from Malawi confirming our work.[21] Overall, deferred treatment switch is common in resource-limited settings,[22, 23] and causes increased mortality.[24, 25] We conclude that prolonged failure could be prevented by routine viral load testing, for instance at 6-monthly or yearly intervals. When NRTI resistance, and especially the accumulation of thymidine analogue mutations, is less extensive, it might still be possible to construct an active NRTI backbone in second-line ART.

PART 3: OUTCOMES OF FIRST- AND SECOND-LINE ANTIRETROVIRAL THERAPY

In response to the rapid scale-up of ART in resource-limited settings, the WHO has formulated Early Warning Indicators, which constitute a key element of the organization's Global Strategy for HIV Drug Resistance Prevention and Assessment.[26] The indicators are ART site factors, such as prescribing practices, attrition rates and drug supply continuity, which may be associated with the emergence of HIV drug resistance and can be subsequently acted on to prevent or minimize resistance.[27] Within the PASER network, we assessed the Early Warning Indicators and identified vulnerable aspects of care (**chapter 8**). Of concern, nine out of thirteen sites failed to meet the target for at least one indicator. We studied the indicator "on-time antiretroviral drug pick-up" in detail among patients from two sites in Lusaka, Zambia. We found that this indicator was difficult to measure and had a low sensitivity (47%) for determining possible drug resistance after 12 months of first-line ART. Instead, the medication possession ratio (defined as the number of days that a person is in possession of drugs divided by the number of days between prescriptions) was better able to identify patients at risk for HIV drug resistance at 12 months, with a sensitivity of 67%. We therefore recommend including the medication possession ratio in the Early Warning Indicators.

Besides assessing the ability of ART programs to prevent HIV drug resistance emergence, part three of this thesis also evaluates the response to ART in patients with preexisting resistance. In **chapter 9**, the presence of pretreatment resistance was investigated among 2579 patients starting standard NNRTI-based first-line ART. We found that 123 (5%) patients harbored resistance to at least one prescribed drug and consequently received a suboptimal first-line regimen. In these patients, the risk of virological failure and further acquisition of resistance after the first year was more than double compared to patients without pretreatment resistance. The rate of virological failure was 10% of all patients who were still on treatment after 12 months. These findings were largely in agreement with results from a large collaborative analysis in Europe.[28] Our study emphasized the need for at least three fully-active antiretroviral drugs in first-line regimens to ensure a good virological response and to prevent the further acquisition of resistance. Independently of pretreatment resistance, we found that previous use of antiretroviral drugs and prolonged non-adherence below 95% were associated with virological failure and the further acquisition of resistance.

With more people receiving ART in resource-limited settings, treatment failure and the need to switch to second-line regimens is likely to increase. At present, less than 3% of patients are receiving second-line treatment, even though the proportion with virological failure on first-line ART is substantially higher.[1, 29] In **chapter 10**, we studied

the impact of resistance mutations selected for by the first-line regimen on the response to empirically prescribed second-line ART. Among 243 patients who started boosted PI-based regimens, 55% were predicted to receive partially active regimens because they harbored drug resistant virus with reduced susceptibility to at least one prescribed second-line drug. After 12 months, the virological failure rate among those still on treatment was 14% and was not increased in those patients receiving partially active regimens. The development of major PI mutations was very infrequent. Similar to our findings in the cohort on first-line ART, adherence levels below 95% increased the risk of second-line failure. We concluded that empirically prescribed PI-based second-line regimens can successfully re-suppress drug resistant HIV, even in absence of fully-active NRTI backbone. This corroborates the results of a Malawian study among patients with extensive NRTI resistance at the time of switch.[30] These findings bring up the question of whether boosted PI monotherapy could be used as second-line ART. This would have several potential advantages such as lower costs, fewer pills, fewer drug interactions and less NRTI-associated toxicity. Ongoing studies, such as the ACTG 5230 and EARNEST trials, will hopefully be able to answer this question in the coming years.[31]

PART 4: PEDIATRIC STUDIES

Pediatric HIV research and clinical care has always lagged behind progress for adults and has therefore been referred to as a neglected disease.[32, 33] Due to financial and technical constraints, monitoring HIV drug resistance in resource-limited settings is infrequently performed in adults and even less common in children. Most children with HIV infection live in resource-limited settings and unfortunately are at high risk of developing resistance to ART. In a systematic review, we searched the available literature reporting acquired drug resistance after failure of first-line pediatric regimens (**chapter 11**). We retrieved 30 eligible studies reporting outcomes of 3241 children. Viruses with resistance-associated mutations were isolated from 90% of children and most mutations were associated with the NNRTI (88%) and NRTI (80%) drug-classes. PI resistance was less common (54%) and found mostly in South-America and South-Africa where these drugs are used more frequently. A subgroup analysis pointed to higher resistance rates in regions with extensive antiretroviral exposure (i.e., Asia and Latin America).

As part of the MARCH pediatric study, we studied resistance patterns among children failing first-line ART at three clinical sites of the Joint Clinical Research Centre in Uganda (**chapter 12**). A total of 369 children were enrolled in the MARCH study, of which 319 initiated first-line ART regimens and 50 were switched to second-line after first-line ART virological failure. In the latter group, genotypic tests results were obtained for 44

children and 100% of these harbored drug resistant HIV. The observed rate of thymidine analogue mutations (any mutation in 64%, multiple mutations in 46%) was higher than reported by studies included in the systematic review in chapter 11. HIV susceptibility to NRTIs, with the exception of tenofovir, was reduced in ≥60% of children. Tenofovir, however, is only licensed for use in children above 12 years of age and therefore the selection of an active NRTI for second-line ART in young children is practically impossible. The extensive resistance patterns observed in this cohort could be due to clinicians' reluctance to switch to second-line ART, due to regimen costs and complexity. Both the systematic review and the MARCH study in Uganda underscore the fact that second-line drug access must be improved by increasing the availability of new-generation drugs with non-overlapping resistance profiles and tolerable formulations for children. The MARCH study is currently still ongoing in Uganda and will yield important prospective data regarding the efficacy of first- and second-line pediatric regimens.

In **chapter 13** we studied the barriers to initiation of pediatric HIV treatment in Uganda, drawing on both quantitative and qualitative data sources. The median age of children initiating first-line ART in the MARCH cohort was high (4.8 years) and 72% of children were diagnosed with a WHO clinical stage 3 or 4 event. This was surprising in light of the current guidelines which recommend initiation of ART in all HIV-infected children under the age of two, regardless of clinical symptoms or immunodeficiency.[34] We found that the main risk factors for late presentation were loss to follow-up between perinatal care and the pediatric HIV clinic, high transportation costs, caregivers' unawareness of HIV symptoms, fear and stigma. The problem of late presentation therefore requires a multifactorial approach, addressing both health system and individual-level factors. Over half of HIV-infected children without treatment will die before the age of two and therefore improving access to care is lifesaving in this vulnerable young age group.[35] Although HIV drug resistance was not studied in this chapter, those factors influencing health-seeking behavior might also influence retention in care and treatment adherence once ART is initiated. The challenge of optimizing service delivery so that it integrates HIV prevention, diagnosis, retention in care and lifelong treatment adherence is one of the key elements of the WHO Treatment 2.0 initiative.[1, 36] In the context of pediatric HIV care in Uganda, we demonstrated that these factors, along with the prevention of HIV drug resistance, are essential to achieve sustained and universal access.

FUTURE PERSPECTIVES

The aim of this thesis was to assess HIV drug resistance among adults and children in Africa. Data from PASER, MARCH as well as other studies now provide evidence that transmitted drug resistance in Africa is gradually increasing. Although HIV-infected persons thereby risk a suboptimum response to first-line regimens, this should not be an impediment to further scale-up of ART in the region. Universal access is yet to be attained, and the United Nations have expressed their commitment to do so by working toward the provision of ART to 15 million people worldwide by 2015.[37] HIV incidence rates are declining, but the yearly number of persons newly infected with HIV remains higher than the number of persons who start ART.[1] Although outside the scope of this thesis, it is a global priority to improve the prevention of HIV infection and to eliminate mother-to-child transmission. In addition, in order to curb the HIV epidemic, it is imperative that those who are infected with HIV at present have access to life-saving ART. Besides providing benefit to the individual patient, mounting scientific evidence suggests that increased access to ART is also contributing to a decline in the number of people acquiring HIV infection.[38]

HIV drug resistance poses a potential threat to the success of global HIV control and increased access to treatment should be accompanied by measures to protect the sustained effectiveness of ART regimens. First, African health systems must be prepared to provide care to more people, at an earlier stage of HIV infection and for a longer period of time. Procurement and supply management systems must be improved to prevent stock-outs and treatment interruptions, which are linked to the emergence of HIV drug resistance.[39, 40] Besides infrastructure, increased investments in human resources for health, including clinicians, counselors and laboratory staff, are critical. The WHO-defined Early Warning Indicators aim to identify specific health system deficiencies that are associated with HIV drug resistance development. Early Warning Indicator assessment is comparatively inexpensive and should become integrated into routine ART program monitoring and evaluation systems. To facilitate more widespread implementation, it is essential that the process of data abstraction, analysis, and reporting is as simple and straightforward as possible.

A second measure to prevent HIV drug resistance is the introduction of low-cost viral load measurement technologies. There is now compelling evidence from PASER and other studies that viral load testing is an important tool to preserve first-line regimens, guide regimen switching and prevent the accumulation of mutations. A recent model of HIV transmission predicted that resistance transmission in resource-limited settings will be reduced if routine viral load monitoring is introduced.[41] The scaling-up of viral-load

testing for individual patient management in Africa is possible since recent technological advances enable lower test cost, simplified sample storage and shipment using dried blood spots, as well as simpler real-time PCR machines that require less technical expertise.[42, 43] Dried blood spots are particularly suitable for use in children, as small blood volumes are required, which can be obtained from a finger- or heel prick. The Affordable Resistance Test for Africa (ART-A) project, coordinated by PharmAccess Foundation and the Amsterdam Institute for Global Health and Development, has developed a low-cost testing protocols for both viral load and genotypic resistance testing. Future research should focus on the implementation of the point-of-care qualitative viral load test under different field conditions. Furthermore, additional clinical studies should determine the optimal frequency of routine virological monitoring and examine the long-term benefits of early failure detection and timely switching.[44]

Third, in order to assess and respond to emerging HIV drug resistance, population-based resistance surveillance should be routinely integrated into national HIV treatment programs. Surveillance efforts should be directed at adults and children, the latter being an especially vulnerable population. Since observational data, meta-analyses and mathematical models have pointed to increasing levels of transmitted drug resistance, surveying resistance in populations prior to ART initiation should be prioritized. Given resource constraints, it seems most practical to sample chronically infected persons at the time of ART initiation, which will then be able to directly inform current ART guidelines. Although resistance among newly diagnosed persons most likely reflects true transmission of resistant virus, the challenges in identifying recent HIV infections result in labor-intensive and more expensive surveys. Standardized surveillance data from different ART programs and countries should be assembled in collaborative studies in order to increase the representativeness of research findings and have a stronger impact on policy makers. The Global HIV Drug Resistance Network (HIVResNet) coordinated by the WHO, has a leadership role in this respect and has published the first HIV Drug Resistance Global Report in 2012.[45]

As a fourth measure to respond to HIV drug resistance, ART guidelines should be adapted according to the findings of local or regional surveillance efforts. PASER results have demonstrated that pretreatment resistance will lead to higher rates of treatment failure and further resistance acquisition, and first-line ART regimens may have to be altered in specific populations in order to prevent this. Differentiation of therapy is already recommended by the pediatric guidelines: children below the age of two who were previously exposed to nevirapine should receive a boosted PI instead of NNRTI-based first-line regimens.[34] Similarly, PI-based first-line regimens should also be considered in adults who report previous use of (NNRTI-based) ART or PMTCT. Randomized clinical

studies, such as those that have been conducted in children,[46] are necessary to prove the benefits of first-line PI-based therapy in adults with previous antiretroviral exposure. For second-line ART, guideline recommendations may also have to be reconsidered. Studies in this thesis have shown that after failure of NNRTI-based regimens, a switch to second-line might offer the benefit of the boosted PI but only the toxicity of the NRTIs. Boosted PI monotherapy is an attractive option, but long-term efficacy still needs to be assessed.

As a final recommendation, it is essential to expand the available ART armamentarium for Africa. Guideline changes as mentioned above can only be implemented if PIs are made available as generic medicines or pressure is exerted on the pharmaceutical industry to reduce prices. Also, the introduction of viral load monitoring will only be of value if the cost of second-line (and hopefully, eventually, third line) regimens is sufficiently low to allow programs to adopt virologically determined switching. Especially for children, additional drugs that are licensed for use, well-tolerated, and have non-overlapping resistance profiles are urgently needed. Additional research is needed to examine the possible role of new drug classes (e.g. integrase inhibitors) in second and third-line therapy.

In conclusion, lifelong care is required for the effective and safe administration of ART to the millions of HIV-infected Africans who will eventually need it. At the beginning of the 20th century, high-level political commitment, international funding and pressure on pharmaceutical companies resulted in the large-scale introduction of ART in sub-Saharan Africa. At present, sustained investments are required to allow the further scale-up of treatment and optimize service delivery as emphasized by the Treatment 2.0 initiative.[1] The goal of making HIV treatment available to more people is currently under pressure due to declining resources and donors loosing political will. The findings in this thesis raise concern about a suboptimal response to the currently used antiretroviral drugs and call for the expansion of resistance surveillance efforts in Africa. Comprehensive HIV treatment must include strategies to monitor and prevent HIV drug resistance and provide options for the growing number of adults and children for whom standard first-line regimens will no longer be effective.

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