Rational use of antiretroviral therapy in low-income and middle-income countries: optimizing regimen sequencing and switching

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Introduction

During the 4 years to the end of 2007, the number of people in low-income and middle-income countries (LMICs) receiving antiretroviral therapy (ART) increased from 400 000 to 3 million [1,2]. Although early mortality [3] and retention in care [4] remain significant challenges, the majority of reports from LMICs have shown encouraging immunological, virological and survival outcomes [5–12]. Reported rates of switching to second-line ART regimens have been lower than expected [13–15], in part due to actual rates of treatment success, but mainly because of limited access to both virological monitoring [16] and second-line drugs [14]. Clinicians have also been reluctant to switch therapy [15] due to regimen cost, complexity, inconvenience and lack of subsequent treatment options. As cohorts mature and expand and access to virological monitoring and second-line regimens increase, however, rates of diagnosed treatment failure and switch to second-line regimens will increase [17]. As the cost of second-line regimens are currently three to 20 times more expensive than that of first-line regimens [18], these increases will challenge the cost-effectiveness [19,20] and sustainability [21] of HIV-treatment programmes.

An effective response to the challenges of HIV treatment failure in LMICs must include reductions in the cost of second-line agents [22], but changes to commercial regulations, particularly in India, suggest the scale of price reductions seen with first-line agents are unlikely to occur with second-line agents. Strategies to maximize the effectiveness of first-line and second-line regimens and optimize the timing of regimen switching are required to

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fully utilize the survival benefit of available treatment options, maintain programme cost-effectiveness and enable achievement of universal access to HIV treatment. A comprehensive strategy must be evidence based and focused on the rational long-term use of ART at a population level. The objective of this review is to support the development of these strategies by providing an overview of available evidence with an emphasis on regimen sequencing and switching.

How can we prolong effective use of first-line antiretroviral therapy?

Drug resistance prior to initiation of combination antiretroviral therapy

Primary (transmitted) resistance to nonnucleoside reverse transcriptase inhibitors (NNRTIs) increases the risk of NNRTI-based regimen failure [23], whereas the equivalent relationship remains to be defined for ritonavir-boosted protease inhibitor-based regimens. Although the use of mono-therapy and bi-therapy has been uncommon in LMICs, previous experience of sub-optimal ART is a significant contributor to virological failure in some settings [24,25]. The use of nevirapine monotherapy for the prevention of perinatal transmission is more common and is associated with development of resistance to NNRTIs [26] and reduced virological response to subsequent nevirapine-based therapy [27]. Virological responses can be improved by delaying therapy after delivery [28]. Alternatively, resistance can be limited with the use of alternative or additional [29] antiretroviral agents.

Recent analyses suggest primary NNRTI resistance is unlikely to have a significant population-level impact on the effective use of first-line NNRTI regimens in most LMICs in the near future [30,31]. Nevertheless, adequately resourced HIV drug resistance surveillance programmes [32] have the potential to inform treatment strategies and determine the utility of drug resistance testing for treatment of naive patients.

Timing of treatment initiation

The timing of initiation of ART has important implications for optimizing clinical benefit of first-line regimens. Low CD4 cell count at the time of initiation of ART is common in LMICs [3,5–11] due to constraints on HIV testing and treatment and limited access to CD4 cell counting to guide treatment initiation. This results in increased mortality [33,34], virological failure [35] and drug resistance [36], whereas use of ART is associated with reduced mortality even at high CD4 cell counts [37]. These relationships favour initiation of therapy when the CD4 cell count falls below 350 cells/μl or earlier, but this strategy depends on early diagnosis and referral and the ability of health systems to manage larger cohorts. Given treatment interruption is not currently recommended [37–40], this strategy would also lead to longer duration of drug exposure, with attendant costs and risks of virological failure, drug resistance and subsequent clinical progression in settings with limited treatment options [41]. The long-term risks and benefits of alternative initiation strategies in LMICs remain unknown, but data from treatment interruption studies suggest earlier treatment initiation may be associated with similar or greater reduction in clinical events compared with the use of this strategy in high-income countries [38,42].

Selection of first-line regimen

First-line antiretroviral regimens are highly effective in suppressing viral replication. Clinical studies of two nucleoside reverse transcriptase inhibitors (NRTIs) combined with either a NNRTI or a boosted protease inhibitor published since 2003 demonstrate a probability of virological success (HIV RNA $<$50 copies/ml at 48 weeks) between 59 and 83% by intent-to-treat analysis [43–53]. Efavirenz and nevirapine are both potent agents from the NNRTI class, but in a randomized comparison, equivalence could not be demonstrated [43], and some observational data suggest superior virological outcomes with efavirenz [54–57]. Moreover, mutations associated with reduced activity of etravirine, such as Y181C, are more commonly selected in patients failing nevirapine than in those failing efavirenz [57]. Randomized studies of efavirenz versus a boosted protease inhibitor, lopinavir/ritonavir, have shown better virological suppression in the efavirenz arm, but greater CD4 cell count increase in the lopinavir/ritonavir arm [59]. Ritonavir-boosted protease inhibitors have a high genetic barrier to resistance and are associated with fewer resistance mutations at the time of virological failure compared with NNRTI-based regimens [52,59]. Boosted protease inhibitors are an option when NNRTIs are contra-indicated: infection with HIV-2, intolerance of both nevirapine and efavirenz and in pregnant women requiring treatment for tuberculosis during first trimester. These advantages are, however, counterbalanced by their toxicity, drug interactions, greater potential for sub-optimal adherence [60], storage requirements of some agents and, despite recent price reductions, continuing high cost. A combination of two NRTIs and a NNRTI therefore continues to be recommended by WHO as standard first-line treatment [61]. These assessments may need to be revisited if further price reductions or formulation changes occur.

NRTIs are used in combinations due to increased potency and genetic barrier to resistance. Randomized comparative studies have shown significantly greater virological response with tenofovir + emtricitabine as compared to zidovudine + lamivudine [49] and equivalence of virological response when abacavir + lamivudine was compared to zidovudine + lamivudine [50] and stavudine + lamivudine was compared to tenofovir + lamivudine [46]. In all these studies, the NRTI backbones were

combined with efavirenz. Comparative data on NRTI combinations administered with nevirapine or boosted protease inhibitor are limited.

The selection of an NRTI agent for use in combination with lamivudine/emtricitabine and a NNRTI is driven primarily by cost and toxicity in most LMICs. Despite its significant long-term toxicity, stavudine remains more widely used than zidovudine [14] owing to its lower cost and limited short-term toxicity [62]. Some studies suggest that the risk of zidovudine-associated anaemia can be reduced by starting with a stavudine-containing regimen and routinely substituting stavudine with zidovudine after 6 months of therapy [63,64]. Tenofovir is associated with less toxicity in randomized trials than are thymidine analogues [46,49] and emerging data from populations in LMICs with lower body weight are reassuring [65]. Although there are concerns regarding the prevalence of renal disease in Africa [66] and the feasibility of renal monitoring in many LMICs, the main barrier to more widespread use of tenofovir in first-line regimens is cost: currently a median three-fold increase in total regimen cost in LMICs compared to current first-line regimens [62].

An alternative to two class regimens is a nucleoside-only regimen. Randomized studies in high-income countries have shown that triple NRTI regimens lacking a thymidine analogue fail rapidly [67,68] and zidovudine + abacavir + lamivudine is inferior to standard regimens. Combining tenofovir and zidovudine has potential benefit due to antagonistic resistance pathways. A pilot randomized study comparing zidovudine + lamivudine + efavirenz and abacavir + lamivudine + zidovudine + tenofovir showed equivalent virological suppression [69] and in a large randomized trial ongoing in Uganda and Zimbabwe, the use of zidovudine + lamivudine + tenofovir was associated with HIV RNA more than 1000 copies/ml in 24% at 48 weeks [70], which is comparable to outcomes seen with the use of dual class initial regimens. Within the latter trial, a randomized comparison has shown superior virological suppression, but borderline increase in new WHO stage 3/4 events or death when zidovudine + lamivudine was combined with nevirapine compared to combination with abacavir [71]. The mechanism for this finding is not clear and further comparative studies are warranted.

Agents from two new classes have been trialled in treatment-naive subjects. When compared with efavirenz in subjects with pretreatment CD4 cell count above 100 cells/μl, the integrase inhibitor raltegravir demonstrated comparable activity and less toxicity in combination with tenofovir and lamivudine [72]. The potential role of this drug class in LMICs within second-line or even first-line therapy is significant and will be shaped by cost and accrual of additional efficacy, toxicity and drug interaction data. Maraviroc, a CCR-5 antagonist, showed similar, but slightly inferior efficacy when compared with efavirenz [73]. The restriction of efficacy of this agent to patients with R5-tropic only virus and the need for pretreatment tropism assays suggests the role of this class will be limited in most LMICs. Early reports suggested subtype C viruses rarely utilize CXCR-4, but more recent reports from Zimbabwe [74] and South Africa [75] have shown substantial use of this co-receptor. From the NNRTI class, rilpivirine is an investigational agent with potent in-vitro antiretroviral activity and high genetic barrier to resistance [76]. Its low dosage, lack of potential interactions and the possibility of daily dosing suggest this agent may play an important role in LMICs.

Adherence to antiretroviral therapy

Adherence to ART is a major determinant of the longevity of first-line regimens [77]. A recent meta-analysis of adherence to ART in Africa and North America found a pooled estimate of adherence of 77% [95% confidence interval (CI) 68–85] for African studies compared to 55% (95% CI 49–62) for North American studies [78]. The African data were largely drawn from small cohorts (median $n = 100$) and additional data from large treatment programmes are needed. Understanding the determinants of adherence behaviour across populations and over time remains critical to ensuring the success of first-line therapy, yet relevant data are currently extremely limited [79]. The main barriers to ART adherence in LMICs identified to date are financial constraints, transportation or disruption to supply, forgetting or disrupted routine, fear of disclosure and lack of understanding of treatment benefits. Despite the widespread implementation of adherence support programmes in LMICs, a recent meta-analysis of randomized trials of adherence interventions did not identify any studies conducted in these settings [80].

Programme and structural factors are also likely to be important determinates of treatment outcomes, although these relationships remain poorly documented. Important factors may include the quality of drug production and distribution, the quality of clinical management, accessibility of ART centres [81], treatment preparedness and community support [82], and stigma and discrimination in healthcare settings and in broader society.

How can we accurately detect first-line failure and optimize switch to second-line therapy?

Monitoring and switch strategies

The rational use of ART in LMICs is critically dependent on the accurate detection of treatment failure and optimizing the timing of switch to alternative regimens. Monitoring and switch strategies aim to balance the risks of HIV drug resistance and compromised efficacy of second-line therapy, immunological and clinical progression and inappropriate early switching, taking into

Table 1. Performance characteristics of WHO clinico-immunological criteria for the diagnosis of virological failure.

Report	Setting	*N*	Gold standard	Criteria	Period	Sens	Spec	PPV	NPV
An *et al.* [82]	Cambodia	399	VL >50 copies/ml	WHO 2003 criteria	>M6 (median 12.8 months)	0.30	0.89	0.20	0.93
Meya *et al.*[a] [83]	Uganda	496	VL >400 copies/ml	WHO 2006 criteria	Not reported	0.24	0.87	0.17	0.91
Van Griensven *et al.* [81]	Rwanda	863	VL >40 copies/ml	WHO 2006 immunological criteria	>M12	0.27	0.82	0.17	0.89

M, month of antiretroviral treatment; NPV, negative predictive value; PPV, positive predictive value; Sens, sensitivity; Spec, specificity; VL, viral load. [a]Adapted from data presented in publication.

account variable factors such as the monitoring tools that are available, the risks and benefits of alternative regimens and current and future drug availability. Three broad approaches can be described.

Clinico-immunological monitoring

Table 1 shows available data regarding the performance characteristics of current WHO clinico-immunological definition of treatment failure [61] for detection of virological failure [83–85]. Although these data are limited, they suggest these criteria detect approximately a quarter of patients with virological failure and that the majority of patients fulfilling these criteria are not failing virologically. Some studies suggest a CD4 cell count increase of less than 50 cells/μl by month 6 of therapy may more rapidly identify individuals with potential virological failure than current criteria, although significant inaccuracy remains due to discordant immunological and virological responses to initial ART [86–89]. One study suggested use of CD4 cell count decrease of more than 30% between months 12 and 24 was associated with an improved positive predictive value of 54%, but similar sensitivity [90] and cross-sectional studies have identified some improvements in sensitivity, but positive predictive values have generally remained below 25% [84,85,91,92].

Targeted HIV viral load testing

HIV viral load testing can be restricted to patients with an increased risk of virological failure using a high-sensitivity/low-specificity clinico-immunological algorithm [84,86, 87,90]. For example, CD4 cell count decrease or increase of less than 10–20% or 150–200 cells/μl over 6–12 months is 70–80% sensitive for virological failure [84,86,90]. Alternatively, HIV viral load testing can be targeted to patients fulfilling current clinico-immunological algorithms [93,94], preventing inappropriate early switching and minimizing testing volumes, but with low sensitivity for virological failure. No prospective studies of targeted testing strategies have been reported.

Universal HIV viral load testing

Data from high-income countries suggest that more frequent HIV RNA viral load monitoring improves virological outcomes [95] and universal testing every 3–4 months has been the standard of care in high-income countries for many years [40,96,97]. Data largely from African cohorts, however, have shown no evidence that access to HIV viral load reduces mortality during early ART [3] and a recently published mathematical model suggested little survival benefit from addition of CD4 or viral load monitoring to clinical monitoring [98]. A randomized trial of different monitoring strategies conducted in Uganda found in an adjusted analysis over a median 3 years of follow-up that adding CD4 cell counting to clinical monitoring was associated with a reduced risk of new AIDS-defining event or death, but no morbidity or mortality benefit was seen with the further addition of HIV viral load monitoring [99]. Other randomized trials of treatment monitoring are ongoing in Thailand [100] and Uganda and Zimbabwe [101].

The advantages of universal testing are high sensitivity and specificity for treatment failure and collection of robust individual patient outcome data. Although feasibility is currently limited in many settings [61], this is likely to improve as technologies evolve [102]. Inexpensive qualitative or semi-quantitative assays detecting loss of viral suppression are an urgent need [103]. The cost-effectiveness of viral load testing will be dependent on morbidity, survival and resistance benefits, reduction of inappropriate switching, costs associated with increased use of second-line regimens and costs of viral load testing itself. One study suggested universal HIV viral load testing was cost-effective in South Africa [104], whereas another model suggested poor cost-effectiveness [98]. Additional data are needed to support cost-effective analyses in a range of settings. No comparisons of universal and targeted testing strategies have been performed, and their relative cost benefits are unknown.

HIV viral load testing has also been used as an indicator of ART adherence [15,103]. Under this strategy, individuals with virological failure receive an adherence intervention and continue without regimen change if viral re-suppression occurs. Viral re-suppression of up to 70% has been documented [15], but the durability of virological suppression and rate of accumulation of NRTI resistance is not known. Longer term prospective studies with assessment of viral suppression and drug resistance are needed.

HIV viral load test frequency and switch threshold

Two key questions for HIV viral load testing strategies are the frequency of virological monitoring required to minimize clinical, immunological and virological pro-

gression and accumulation of drug resistance mutations and the appropriate viral load threshold for regimen switching.

A number of cohort studies of patients receiving predominantly unboosted protease inhibitor-based therapy in high-income countries have shown that immunological progression is unlikely if the HIV viral load is below 10–20 000 copies/ml [105–108] or suppressed approximately 1.5 log copies/ml or more below pretherapy baseline [107,109]. Moreover, the risk of disease progression or death has been shown to be lower if the HIV viral load is below 10–20 000 copies/ml [109–112]. Unfortunately, the only randomized trial to examine this question, which randomized patients to immediate versus delayed switch, did not recruit fully and was not able to demonstrate noninferiority [113].

In contrast, it is clear that resistance mutants accumulate even at low concentrations of plasma HIV RNA [114] and cohort studies of patients taking unchanged failing predominantly unboosted protease inhibitor-based regimens have largely shown that the rate of accumulation of drug resistance mutations is independent of HIV viral load [115–119], although one study found a higher rate of resistance accumulation for patients with moderate HIV viraemia (3–4 log copies/ml) or higher HIV viral load slope [120]. Overall, the observed rates of drug resistance mutation accumulation was 39–77% of patients over a median 6–14 months [115,116,118–121] with an incidence of new resistance-associated mutations of 0.93 [115] and 1.96 [119] over 6 months and 1.61 mutations per year [120].

These data may not, however, be generalizable to patients taking NNRTI-based regimens in LMICs, despite the use of similar NRTI backbones to the cohorts described above. In an analysis of patients with triple class failure and stable viral load published by the PLATO collaboration multivariate linear regression demonstrated a negative CD4 cell count slope with use of NNRTI-based therapy (−23.0 cells/μl per year; 95% CI −35.0 to −11.0) compared with a positive slope with protease inhibitor-based therapy (+18.0 cells/μl per year; 95% CI +7.0 to +28.0) [107]. Furthermore, NNRTI resistance may be more closely associated with increased mortality [58,122] and may have less impact on viral fitness [123,124] when compared with protease inhibitor resistance. Indeed, the ART-LINC collaboration reported that patients taking protease inhibitor-sparing regimens (mostly NNRTI based) were less likely to experience a positive immunological response in the setting of persistent HIV viraemia during early ART [88]. Other studies from LMICs suggest rapid accumulation of drug resistance mutations following virological failure of NNRTI-based therapy [125] and an association between resistance mutations and subsequent immunological failure [126]. Whether HIV viral load is associated with accumulation of resistance mutations in these settings remains unclear [125,126].

In the absence of more extensive data, the optimal frequency of viral load testing cannot be determined. Although this frequency may be dependent on factors such as time on therapy, duration of prior viral suppression and adherence, it is likely that in general the optimal frequency lies somewhere between 3 and 12 monthly. Similarly, in the absence of more definitive data, current WHO guidelines have provisionally suggested a HIV RNA level of 10 000 copies/ml [61] as a threshold for first-line regimen switching in LMICs.

Drug resistance genotyping

Resistance testing at time of treatment failure results in improved short-term virological response in treatment-experienced patients [127,128] and is cost-effective in high-income countries when based on HIV viral genotyping [129–131]. Expert advice increases its benefit [128], although free, open-interpretation systems may reduce the need for expert advice for many patients [132,133]. Cost-effectiveness is likely to be different in LMICs and related to the treatment, monitoring and switch strategies employed. A substantial proportion of patients with virological failure of first-line therapy lack resistance mutations [134–138], suggesting that resistance testing may reduce costs by triaging patients to adherence support rather than to second-line therapy. Although resistance testing is currently expensive (US$50–300 per test), this cost could be lowered by use of technologies targeting commonly selected mutations in order to demonstrate that failure is caused by resistance and not only by nonadherence [139].

Monitoring and switch strategies are heavily influenced by the agents employed in first-line and second-line regimens. Even with current treatment regimens, however, scarcity of data limits the development of rational strategies. It can be assumed that mutations associated with resistance to NNRTIs and lamivudine/emtricitabine will be rapidly selected in patients failing therapy. The key question is, therefore, which monitoring and switch strategies most cost-effectively minimize inappropriate early switching, medium-term and long-term immunological and clinical progression and the development of reverse transcriptase cross-resistance (engendered by mutations such as thymidine analogue mutations (TAMs), K65R, Q151M and T69 insertions and mutations associated with impaired efficacy of second-generation NNRTIs). The heterogeneity of LMICs makes a single answer unlikely. Setting-specific approaches are necessary and dependent on robust data from diverse settings.

Which second-line regimen should be used?

The efficacy of second-line regimens will be a function of the first-line regimen used, the duration of viral replication in the presence of these drugs and the range

of drugs available to construct a second-line regimen. Subtype polymorphism at drug-related resistance positions may influence the selection of resistance, although this has not been found to be a major factor in treatment outcomes to date [140,141].

There is currently limited evidence to guide the selection of second-line regimens in LMICs after failure of first-line NNRTI-based therapy [142]. Clinical data suggest continued benefit from NRTI despite the presence of confirmed resistance [143,144], possibly due to reduced fitness of mutated virus [145]. NRTIs should therefore be continued as a component of second-line therapy even in the setting of resistance if other potent combination regimens are not available. Ritonavir-boosted protease inhibitors have shown impressive results when used in treatment-experienced patients in high-income countries [146,147]. High plasma concentrations and a broad genetic barrier to resistance appear to reduce the need for support from other fully active drugs in the regimen. Based on these characteristics and the tolerability, simplicity, costs and availability of certain fixed-dose combinations, WHO guidelines for a public health approach currently recommend a boosted protease inhibitor with two NRTIs.

Choice of nucleoside reverse transcriptase inhibitors

The choice of NRTIs used within a second-line regimen depends on those used in the first-line regimen (Fig. 1). Failure of zidovudine-containing or stavudine-containing first-line regimens select for TAMs,

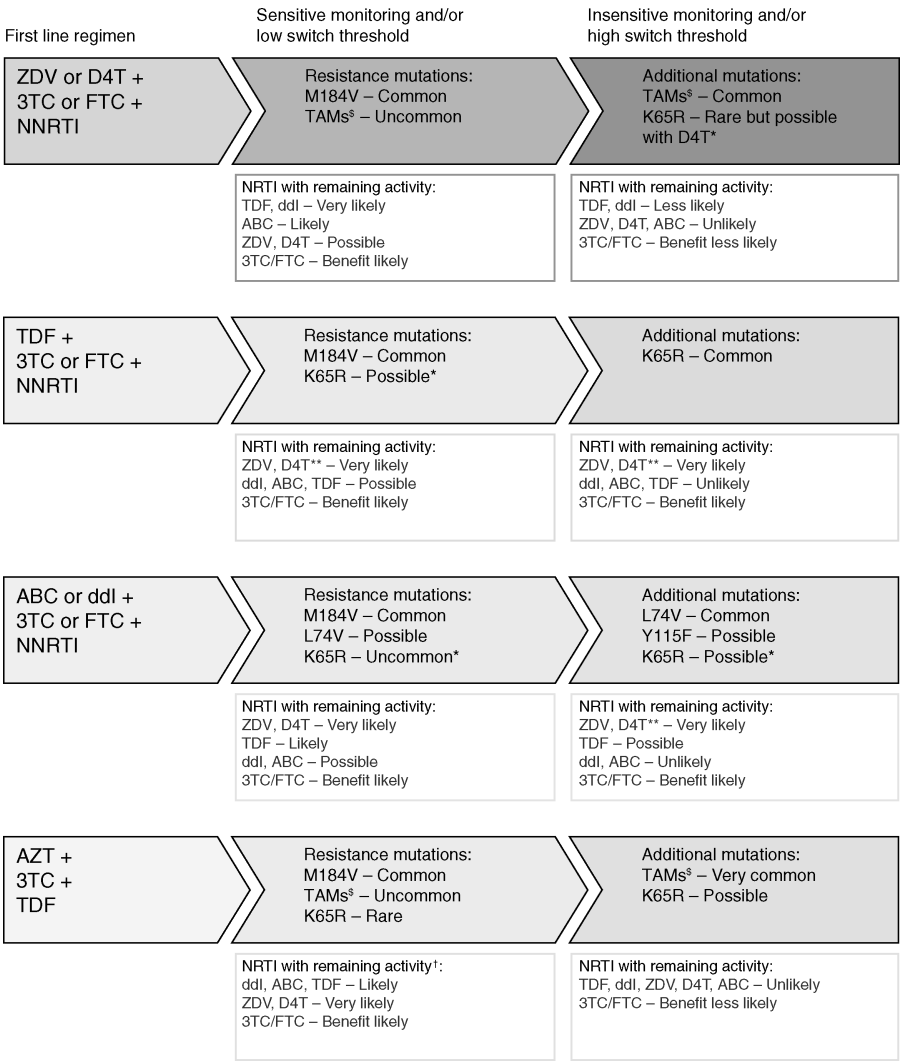

Fig. 1. Influence of first-line regimen and monitoring/switch strategies on activity of nucleoside reverse transcriptase inhibitor (NRTI) agents in second-line antiretroviral therapy. ABC, abacavir; ddI, didanosine; D4T, stavudine; FTC, emtricitabine; TDF, tenofovir; ZDV, zidovudine; 3TC, lamuvidine. $TAM1 mutations (M41L, L210W, T215Y) have a greater impact on NRTI activity than TAM2 mutations (D67N, K70R, T215F, K219Q/E). *Potential increased risk of K65R with subtype C virus. **Fewer data on D4T activity in presence of K65R. †Few data on early failure with this regimen.

commonly along one of two pathways: TAM 1 (M41L, L210W and T215Y) or TAM 2 (D67N, K70R, T215F and K219Q/E). Stavudine combined with drugs with a low genetic barrier has been associated with a higher probability of type 1 TAMs [148,149]. Accumulation of two or more TAM 1 mutations has been most tightly correlated with broad NRTI resistance and inability to construct a potent second-line NRTI backbone [149,150]. Combinations with lamivudine or emtricitabine that select for the M184V mutation or high genetic barrier triple NRTI combinations such as zidovudine + lamivudine/emtricitabine + tenofovir may reduce or delay TAM selection [151]. Nevertheless, multiple TAMs and broad NRTI resistance will ultimately develop with all thymidine analogue containing regimens if viral replication continues unchecked [70,152].

The K65R HIV-1 reverse transcriptase mutation is a multidrug resistance mutation associated most commonly with tenofovir, but also with didanosine, abacavir and rarely stavudine [153,154]. The prevalence of this mutation among viruses from subtype B-infected individuals receiving frequent virological monitoring has remained below 5% in clinical trials with tenofovir [46]. TAMs and the L74V mutation occur rarely in the presence of K65R [155] and in one recent study of patients with K65R followed over 18 months, no patient developed additional multinucleoside resistance (Q151M or T69 insertions) [156]. In-vitro data suggest that the combination of the K65R and M184V mutations, as seen at time of failure of tenofovir + lamivudine/emtricitabine, is associated with reduced viral replication capacity, although clinical correlates of this parameter are lacking [157]. Susceptibility to zidovudine is increased in the presence of K65R and/or M184V [158], and selection of resistance will require multiple zidovudine mutations if these mutations are maintained. This supports the use of zidovudine following failure of tenofovir + lamividine/emtricitabine. Although laboratory data on stavudine are less clear, some clinical data suggest it has similar attributes. This provides support for the use of a thymidine analogue following tenofovir failure. One caveat is that preliminary in-vitro data suggest K65R may be selected more commonly in subtype C than B [159] and a higher frequency of K65R has been observed in subtype C patients failing didanosine-based or stavudine-based therapies [160].

Based on the above data, first-line regimens containing zidovudine and lamivudine/emtricitabine may therefore be associated with greater residual efficacy of NRTIs in second-line regimens than stavudine-containing regimens and tenofovir is likely to be an effective second-line agent in this situation, depending on the prevalence of broad NRTI resistance and K65R. Alternatively, there are some resistance data to support a sequencing strategy that employs tenofovir followed by a thymidine analogue. Lamivudine/emtricitabine provides a useful option for a second-line NRTI, as some degree of efficacy can be assured regardless of the availability of resistance testing and cost and toxicity are minimal. Continuation of both thymidine analogue and tenofovir may increase the probability of regimen efficacy in the absence of resistance testing and may reduce the incidence of additional mutations. Didanosine and abacavir are alternatives to tenofovir, but the toxicity of the former and cost of the latter do not compare favourably. In addition, the M184 mutation increases tenofovir susceptibility but increases resistance to abacavir and, to a lesser degree, resistance to didanosine.

Choice of boosted protease inhibitor

Ritonavir-boosted protease inhibitors have shown good utility in drug-experienced patients [161,162] and use in early salvage therapy from high-income countries shows favourable efficacy for many of these agents [121]. Comparative data are complicated by the use of different doses, study populations and outcome measures [163]. Head-to-head comparisons of boosted protease inhibitors for second-line treatment in LMICs are not available. Early data from cohorts without universal access to HIV viral load testing have shown good short-term outcomes, suggesting that the boosted protease inhibitor component is providing the majority of treatment efficacy [13,25]. The choice of protease inhibitor is mainly dictated by cost and logistic requirements and to date lopinavir/ritonavir is the only boosted protease inhibitor that does not require refrigeration. Once ritonavir in booster dose becomes available in heat-stable formulation, other convenient combinations will be feasible in LMICs. For example, atazanavir/ritonavir has been shown to be noninferior to lopinavir/ritonavir in this setting, has a more favourable lipid profile, and is dosed once daily [146]. Darunavir/ritonavir has shown improved efficacy and similar tolerability in treatment-experienced patients when compared with control protease inhibitor/ritonavir [164] or lopinavir/ritonavir [147]. WHO has prioritized lopinavir/ritonavir and atazanavir/ritonavir as the main protease inhibitors to be used in the setting of a second-line regimen in LMICs [165].

Alternative approaches

Monotherapy with a boosted protease inhibitor is an alternative approach to treatment of patients with failure of initial NNRTI-based therapy. This approach is theoretically attractive for LMICs, as efficacy is independent of cross-resistance to first-line agents, NRTI toxicity is eliminated, and drug costs are minimized. Data available to date, however, suggest moderately reduced efficacy of this strategy compared with standard protease inhibitor/ritonavir-based therapy [166–168], and non-B subtype virus has been associated with lopinavir/ritonavir monotherapy failure [169]. Similarly, the use of dual boosted protease inhibitors alone is a strategy that would be free of concerns regarding cross-resistance to first-line agents. A number of small studies have examined this approach with encouraging pharmacokinetic and early clinical results, but significant toxicity [170–172]. Available data do not,

therefore, support the use of a protease inhibitor only strategy for second-line regimens at this time.

Dose reduction is another strategy that holds potential for cost minimization in LMICs. Dose ranging studies and post-licensing clinical studies have shown adequate virological responses for doses lower than those currently licensed for indinavir/ritonavir [173,174], lopinavir/ritonavir [175] and atazanavir [176]. Although this strategy can reduce toxicity as well as cost, greater levels of adherence may be required and the risks of treatment failure and resistance need to be evaluated carefully in adequately powered safety and efficacy trials.

Interruption of treatment at fixed or CD4-guided intervals has been investigated in a number of studies reviewed elsewhere [177]. Although some studies in which therapy was reinitiated at relatively high CD4 cell counts suggested a role for treatment interruption [42,178], the Trivacan and DART studies showed increased severe morbidity in patients undergoing CD4-guided treatment interruptions and the SMART study demonstrated that CD4-guided treatment interruption was associated with an increase in mortality, opportunistic disease and major cardiovascular, renal or hepatic disease [37–39]. Therefore, this approach cannot be recommended at this time and additional clinical endpoint studies are unlikely.

Recent studies have shown the promise of integrase inhibitors [179,180] and CCR5 antagonists [181–183] and new nucleoside [184] and nonnucleoside [185] reverse transcriptase inhibitors to dramatically improve the management of treatment-experienced patients in high-income countries. Although there is unlikely to be a need for large-scale access to 'third-line' treatment regimens in LMICs for many years [186], these data suggest the possibility of constructing effective second-line therapy from two classes not used in first-line therapy, reducing the impact of delayed diagnosis of first-line failure and NRTI and NNRTI cross-resistance. Adequately powered efficacy trials of this strategy in nonsubtype B infected patients in LMICs are a priority.

Conclusion

The beginning of global ART scale-up characterized by the '3 by 5' target has transitioned to a post-emergency phase defined by the goal of universal access [2,187]. Larger treatment goals and longer horizons have led to an increased focus on programme sustainability, which is challenged by the prospect of significant increases in direct drug expenditure as larger proportions of patients switch to second-line agents. Although reduction in the price of protease inhibitors in low-income and middle-income countries [22,188] is an essential step toward programme sustainability, these price reductions are likely to be insufficient.

A comprehensive approach to the long-term use of ART is needed to maximize therapeutic effectiveness and minimize costs over the long term. The aims of a comprehensive strategy should be explicit and address short-term treatment outcomes, accumulation and transmission of drug resistance, long-term morbidity and mortality and programme costs. Much is already known (Table 2), but many important evidence gaps exist and require urgent investigation (Table 3). As our knowledge improves, new drugs become available and monitoring and drug costs continue to fall, strategies for

Table 2. Key knowledge for rational use of antiretroviral therapy in low-income and middle-income countries.

Domain	Key knowledge
How can we prolong effective use of first-line ART?	
Sub-optimal ART	Use of monotherapy or bi-therapy should be avoided
Impact of PMTCT	Use of nevirapine monotherapy should be minimized
Primary drug resistance	Drug resistance genotyping is not required for treatment-naïve patients
Timing of treatment initiation	At a minimum ART should be initiated for patients in WHO Clinical Stage 1 or 2 when CD4 cell count is less than 200 cells/μl
Selection of first-line regimen	Two NRTIs plus either a NNRTI or a boosted protease inhibitor is highly effective No modification based on subtype is necessary
ART adherence	Adherence is a major determinant of the longevity of first-line regimens
How can we accurately detect first line failure and optimize switch to second-line therapy?	
Clinico-immunological monitoring	Currently recommended clinico-immunological criteria are insensitive to virological failure
Drug resistance genotyping	Drug resistance genotyping for patients failing initial therapy is cost-effective in high-income countries
Which second-line regimen should be used?	
Choice of NRTIs	If a thymidine analogue and lamivudine/emtricitabine have been used in a first-line regimen, tenofovir, didanosine or abacavir should be used and lamivudine/emtricitabine continued If tenofovir has been used in a first-line regimen, a thymidine analogue should be used Cross-resistance will reduce the efficacy of all NRTIs in late failure
Choice of boosted protease inhibitor	Boosted protease inhibitor-based regimens are effective in the treatment of patients with first-regimen failure
Alternative approaches	Single class boosted protease inhibitor regimens, dose reduction or interrupted therapy are not recommended strategies at present

ART, antiretroviral therapy; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PMTCT, prevention of mother-to-child transmission.

Table 3. Key knowledge gaps for rational use of antiretroviral therapy in low-income and middle-income countries.

Domain	Key knowledge gaps
How can we prolong effective use of first-line ART?	
Sub-optimal ART	What are the most effective methods to reduce sub-optimal use of ART in settings where unregulated private sector access to ART is significant?
	What is the optimal regimen for patients with known previous sub-optimal use of ART?
Impact of PMTCT	What is the most effective, safe and feasible ART strategy for PMTCT?
Primary drug resistance	How rapidly will any increase in the prevalence of primary resistance in LMIC occur?
Timing of treatment initiation	Should ART be initiated earlier in patients in WHO Clinical Stage 1 or 2 (CD4 cell count <350 cells/μl)?
Selection of first-line regimen	At what price difference does the improved toxicity profile of tenofovir-containing regimens make these regimens more cost-effective than thymidine analogue-containing regimens?
	How does the safety and efficacy of thymidine analogue-containing nucleoside-only regimens compare to standard first-line therapy in LMIC?
	Will improvements in cost, toxicity and formulation combined with favourable resistance effects make protease inhibitor-based regimens a feasible alternative to NNRTI-based regimens in LMIC?
	Is there a role for new drug classes in first-line therapy in LMIC?
	Are there undocumented influences of sub-type on selection of resistance to some drugs?
ART adherence	What is the medium-term level of adherence in large treatment programmes in LMIC?
	Are there differences in adherence between regimens?
	What are the barriers to adherence in LMIC?
	What are the most effective ways to support ART adherence in LMIC?
How can we accurately detect first line failure and optimize switch to second-line therapy?	
Clinico-immunological monitoring	What are the best clinico-immunological criteria for use in settings without access to viral load monitoring?
Targeted HIV viral load testing	What are the long-term outcomes associated with use of targeted HIV viral load testing?
Universal HIV viral load testing	What are the medium-term resistance outcomes of continuing NNRTI-based first-line therapy in patients who re-suppress following adherence interventions?
	How do the efficacy and cost-effectiveness of targeted and universal HIV viral load testing strategies compare?
	Can novel technologies play a role in improving the feasibility and cost-effectiveness of universal HIV viral load testing?
HIV viral load test frequency and switch threshold	What is the most cost-effective frequency of HIV viral load testing and threshold for switching therapy?
Drug resistance genotyping	What is the role of drug resistance genotyping in LMIC?
	What is the clinical utility and cost-effectiveness of inexpensive assays for detecting specific resistance mutations in LMIC?
Which second-line regimen should be used?	
Choice of NRTIs	What is the incidence of K65R in nonsubtype B viruses?
	What is the comparative efficacy, toxicity and cost of using tenofovir versus abacavir versus didanosine in second-line regimens?
	Should a thymidine analogue be continued?
	What are the relative costs and benefits of sequencing a thymidine analogue prior to versus subsequent to tenofovir?
	What is the impact of multidrug NRTI mutations on the medium to long-term efficacy of ritonavir-boosted protease inhibitor-based second-line regimens?
Choice of boosted protease inhibitor	What is the optimal protease inhibitor for LMIC with access to heat stable booster dose ritonavir?
Alternative approaches	What is the efficacy of second-line therapy constructed from two classes not used in first-line therapy?

ART, antiretroviral therapy; LMIC, low-income and middle-income countries; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PMTCT, prevention of mother-to-child transmission.

the use of ART in LMICs will continue to evolve. The challenge is profound, but so are the benefits of sound investment in rational and comprehensive strategies.

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References

1. *Progress on global access to HIV antiretroviral therapy: a report on ''3 by 5'' and beyond* Geneva: World Health Organization; 2006.
2. *Towards universal access: scaling up priority HIV/AIDS interventions in the health sector: progress report.* Geneva: WHO, UNAIDS, UNICEF; 2008.
3. 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.
4. Rosen S, Fox MP, Gill CJ. **Patient retention in antiretroviral therapy programs in sub-Saharan Africa: a systematic review.** *PLoS Med* 2007; **4**:e298.
5. Weidle PJ, Malamba S, Mwebaze R, Sozi C, Rukundo G, Downing R, *et al*. **Assessment of a pilot antiretroviral drug therapy programme in Uganda: patients' response, survival, and drug resistance.** *Lancet* 2002; **360**:34–40.
6. Ferradini L, Jeannin A, Pinoges L, Izopet J, Odhiambo D, Mankhambo L, *et al*. **Scaling up of highly active antiretroviral therapy in a rural district of Malawi: an effectiveness assessment.** *Lancet* 2006; **367**:1335–1342.
7. Laurent C, Diakhate N, Gueye NF, Toure MA, Sow PS, Faye MA, *et al*. **The Senegalese government's highly active antiretroviral therapy initiative: an 18-month follow-up study.** *AIDS* 2002; **16**:1363–1370.
8. Tassie JM, Szumilin E, Calmy A, Goemaere E. **Highly active antiretroviral therapy in resource-poor settings: the experience of Medecins Sans Frontieres.** *AIDS* 2003; **17**:1995–1997.
9. Coetzee D, Hildebrand K, Boulle A, Maartens G, Louis F, Labatala V, *et al*. **Outcomes after two years of providing antiretroviral treatment in Khayelitsha, South Africa.** *AIDS* 2004; **18**:887–895.
10. Seyler C, Anglaret X, Dakoury-Dogbo N, Messou E, Toure S, Danel C, *et al*. **Medium-term survival, morbidity and immunovirological evolution in HIV-infected adults receiving antiretroviral therapy, Abidjan, Cote d'Ivoire.** *Antivir Ther* 2003; **8**:385–393.
11. Kumarasamy N, Solomon S, Chaguturu SK, Mahajan AP, Flanigan TP, Balakrishnan P, Mayer KH. **The safety, tolerability and effectiveness of generic antiretroviral drug regimens for HIV-infected patients in south India.** *AIDS* 2003; **17**:2267–2269.
12. Ivers LC, Kendrick D, Doucette K. **Efficacy of antiretroviral therapy programs in resource-poor settings: a meta-analysis of the published literature.** *Clin Infect Dis* 2005; **41**:217–224.
13. Pujades M, Calmy A, O'Brien D, Humblet P, Group MHAw. **Outcomes of adults receiving second-line ART in M,decins Sans Frontières-supported projects in resources-limited countries.** *Proceedings of the 14th Conference on Retroviruses and Opportunistic Infections*; 25–28 February 2007; Los Angeles.
14. Renaud-Thery F, Nguimfack BD, Vitoria M, Lee E, Graaff P, Samb B, Perriens J. **Use of antiretroviral therapy in resource-limited countries in 2006: distribution and uptake of first- and second-line regimens.** *AIDS* 2007; **21 (Suppl 4)**:S89–S95.
15. Orrell C, Harling G, Lawn SD, Kaplan R, McNally M, Bekker LG, Wood R. **Conservation of first-line antiretroviral treatment regimen where therapeutic options are limited.** *Antivir Ther* 2007; **12**:83–88.
16. *Consultation on technical and operational recommendations for scale-up of laboratory services and monitoring HIV antiretroviral therapy in resource-limited settings*. Geneva: World Health Organization; 2006.
17. Galarraga O, O'Brien ME, Gutierrez JP, Renaud-Thery F, Nguimfack BD, Beusenberg M, *et al*. **Forecast of demand for antiretroviral drugs in low and middle-income countries: 2007–2008.** *AIDS* 2007; **21 (Suppl 4)**:S97–S103.
18. WHO. Global price reporting mechanism. http://www.who.int/hiv/amds/price/hdd/index.aspx. [Accessed 16 May 2008]
19. Freedberg KA, Kumarasamy N, Losina E, Cecelia AJ, Scott CA, Divi N, *et al*. **Clinical impact and cost-effectiveness of antiretroviral therapy in India: starting criteria and second-line therapy.** *AIDS* 2007; **21 (Suppl 4)**:S117–S128.
20. Over M, Revenga A, Masaki E, Peerapatanapokin W, Gold J, Tangcharoensathien V, Thanprasertsuk S. **The economics of effective AIDS treatment in Thailand.** *AIDS* 2007; **21 (Suppl 4)**:S105–S116.
21. Greco DB, Simao M. **Brazilian policy of universal access to AIDS treatment: sustainability challenges and perspectives.** *AIDS* 2007; **21 (Suppl 4)**:S37–S45.
22. Ford N, Wilson D, Costa CG, Lotrowska M, Kijtiwatchakul K. **Sustaining access to antiretroviral therapy in the less-developed world: lessons from Brazil and Thailand.** *AIDS* 2007; **21 (Suppl 4)**:S21–S29.
23. Kuritzkes DR, Lalama C, Ribaudo H, Marcial M, Meyer W, Shikuma CM, *et al*. **Baseline resistance to NNRTI as a predictor of virological failure in treatment-naïve subject receiving efavirenz-based regimens in ACTG A5095.** *Proceedings of the 14th Conference on Retroviruses and Opportunistic Infections*; 25–28 February 2007; Los Angeles.
24. Pujades M, Calmy A, O'Brien D, Humblet P, MSF HIV/AIDS working group. **Outcomes of adults receiving second-line ART in Medecins Sans Frontieres-supported projects in resources-limited countries.** *Proceedings of the 14th Conference on Retroviruses and Opportunistic Infections*; 25–28 February 2007; Los Angeles.
25. Ferradini L, Segeral O, Nouhin J, Leakhena S, Vara O, Dulioust A, *et al*. **Efficacy of Kaletra-based second-line ART in Cambodia.** *Proceedings of the 14th Conference on Retroviruses and Opportunistic Infections*; 25–28 February 2007; Los Angeles.
26. Flys T, Nissley DV, Claasen CW, Jones D, Shi C, Guay LA, *et al*. **Sensitive drug-resistance assays reveal long-term persistence of HIV-1 variants with the K103N nevirapine (NVP) resistance mutation in some women and infants after the administration of single-dose NVP: HIVNET 012.** *J Infect Dis* 2005; **192**:24–29.
27. Jourdain G, Ngo-Giang-Huong N, Le Coeur S, Bowonwatanuwong C, Kantipong P, Leechanachai P, *et al*. **Intrapartum exposure to nevirapine and subsequent maternal responses to nevirapine-based antiretroviral therapy.** *N Engl J Med* 2004; **351**:229–240.
28. Lockman S, Shapiro RL, Smeaton LM, Wester C, Thior I, Stevens L, *et al*. **Response to antiretroviral therapy after a single, peripartum dose of nevirapine.** *N Engl J Med* 2007; **356**:135–147.

29. Chi BH, Sinkala M, Mbewe F, Cantrell RA, Kruse G, Chintu N, *et al*. **Single-dose tenofovir and emtricitabine for reduction of viral resistance to nonnucleoside reverse transcriptase inhibitor drugs in women given intrapartum nevirapine for perinatal HIV prevention: an open-label randomised trial.** *Lancet* 2007; **370**:1698–1705.
30. Vardavas R, Blower S. **The emergence of HIV transmitted resistance in Botswana: "when will the WHO detection threshold be exceeded?".** *PLoS ONE* 2007; **2**:e152.
31. 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.
32. WHO. The global HIV drug resistance surveillance network. www.who.int/drugresistance/hivaids/network/en/index.html. [Accessed 16 May 2008]
33. Egger M, May M, Chene G, Phillips AN, Ledergerber B, Dabis F, *et al*. **Prognosis of HIV-1-infected patients starting highly active antiretroviral therapy: a collaborative analysis of prospective studies.** *Lancet* 2002; **360**:119–129.
34. Hogg RS, Yip B, Chan KJ, Wood E, Craib KJ, O'Shaughnessy MV, Montaner JS. **Rates of disease progression by baseline CD4 cell count and viral load after initiating triple-drug therapy.** *JAMA* 2001; **286**:2568–2577.
35. Deeks SG. **Determinants of virological response to antiretroviral therapy: implications for long-term strategies.** *Clin Infect Dis* 2000; **30 (Suppl 2)**:S177–S184.
36. Uy J, Armon C, Buchacz K, Brooks J, and the HOPS Investigators. **Initiation of HAART at CD4 cell counts over 350 cells/mm^3 is associated with a lower prevalence of antiretroviral resistance mutations at virologic failure.** *Proceedings of the 4th IAS Conference on HIV Pathogenesis, Treatment and Prevention;* 22–25 July 2007; Sydney.
37. El Sadr WM, Lundgren JD, Neaton JD, Gordin F, Abrams D, Arduino RC, *et al*. **CD4+ count-guided interruption of antiretroviral treatment.** *N Engl J Med* 2006; **355**:2283–2296.
38. Danel C, Moh R, Minga A, Anzian A, Ba-Gomis O, Kanga C, *et al*. **CD4-guided structured antiretroviral treatment interruption strategy in HIV-infected adults in west Africa (Trivacan ANRS 1269 trial): a randomised trial.** *Lancet* 2006; **367**:1981–1989.
39. DART Trial Team. **Fixed duration interruptions are inferior to continuous treatment in African adults starting therapy with CD4 cell counts <200 cells/microl.** *AIDS* 2008; **22**:237–247.
40. US Department of Health and Human Services. *Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents*. Washington: US Department of Health and Human Services; 2008.
41. Phillips A, Lepri A, Lampe F, Johnson M, Sabin C. **When should antiretroviral therapy be started for HIV infection? Interpreting the evidence from observational studies.** *AIDS* 2003; **17**:1863–1869.
42. Ananworanich J, Gayet-Ageron A, Le Braz M, Prasithsirikul W, Chetchotisakd P, Kiertiburanakul S, *et al*. **CD4-guided scheduled treatment interruptions compared with continuous therapy for patients infected with HIV-1: results of the Staccato randomised trial.** *Lancet* 2006; **368**:459–465.
43. van Leth F, Phanuphak P, Ruxrungtham K, Baraldi E, Miller S, Gazzard B, *et al*. **Comparison of first-line antiretroviral therapy with regimens including nevirapine, efavirenz, or both drugs, plus stavudine and lamivudine: a randomised open-label trial, the 2NN Study.** *Lancet* 2004; **363**:1253–1263.
44. Gulick RM, Ribaudo HJ, Shikuma CM, Lustgarten S, Squires KE, Meyer WA III, *et al*. **Triple-nucleoside regimens versus efavirenz-containing regimens for the initial treatment of HIV-1 infection.** *N Engl J Med* 2004; **350**:1850–1861.
45. Saag MS, Cahn P, Raffi F, Wolff M, Pearce D, Molina JM, *et al*. **Efficacy and safety of emtricitabine vs stavudine in combination therapy in antiretroviral-naive patients: a randomized trial.** *JAMA* 2004; **292**:180–189.
46. Gallant JE, Staszewski S, Pozniak AL, DeJesus E, Suleiman JM, Miller MD, *et al*. **Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naive patients: a 3-year randomized trial.** *JAMA* 2004; **292**:191–201.
47. Eron J Jr, Yeni P, Gathe J Jr, Estrada V, DeJesus E, Staszewski S, *et al*. **The KLEAN study of fosamprenavir-ritonavir versus lopinavir-ritonavir, each in combination with abacavir-lamivudine, for initial treatment of HIV infection over 48 weeks: a randomised noninferiority trial.** *Lancet* 2006; **368**:476–482.
48. Johnson MA, Gathe JC Jr, Podzamczer D, Molina JM, Naylor CT, Chiu YL, *et al*. **A once-daily lopinavir/ritonavir-based regimen provides noninferior antiviral activity compared with a twice-daily regimen.** *J Acquir Immune Defic Syndr* 2006; **43**:153–160.
49. Gallant JE, DeJesus E, Arribas JR, Pozniak AL, Gazzard B, Campo RE, *et al*. **Tenofovir DF, emtricitabine, and efavirenz vs. zidovudine, lamivudine, and efavirenz for HIV.** *N Engl J Med* 2006; **354**:251–260.
50. DeJesus E, Herrera G, Teofilo E, Gerstoft J, Buendia CB, Brand JD, *et al*. **Abacavir versus zidovudine combined with lamivudine and efavirenz, for the treatment of antiretroviral-naive HIV-infected adults.** *Clin Infect Dis* 2004; **39**:1038–1046.
51. DeJesus E, McCarty D, Farthing CF, Shortino DD, Grinsztejn B, Thomas DA, *et al*. **Once-daily versus twice-daily lamivudine, in combination with zidovudine and efavirenz, for the treatment of antiretroviral-naive adults with HIV infection: a randomized equivalence trial.** *Clin Infect Dis* 2004; **39**:411–418.
52. Bartlett JA, Johnson J, Herrera G, Sosa N, Rodriguez A, Liao Q, *et al*. **Long-term results of initial therapy with abacavir and lamivudine combined with efavirenz, amprenavir/ritonavir, or stavudine.** *J Acquir Immune Defic Syndr* 2006; **43**:284–292.
53. Moyle GJ, DeJesus E, Cahn P, Castillo SA, Zhao H, Gordon DN, *et al*. **Abacavir once or twice daily combined with once-daily lamivudine and efavirenz for the treatment of antiretroviral-naive HIV-infected adults: results of the Ziagen Once Daily in Antiretroviral Combination Study.** *J Acquir Immune Defic Syndr* 2005; **38**:417–425.
54. Matthews GV, Sabin CA, Mandalia S, Lampe F, Phillips AN, Nelson MR, *et al*. **Virological suppression at 6 months is related to choice of initial regimen in antiretroviral-naive patients: a cohort study.** *AIDS* 2002; **16**:53–61.
55. Phillips AN, Pradier C, Lazzarin A, Clotet B, Goebel FD, Hermans P, *et al*. **Viral load outcome of nonnucleoside reverse transcriptase inhibitor regimens for 2203 mainly antiretroviral-experienced patients.** *AIDS* 2001; **15**:2385–2395.
56. Kamya MR, Mayanja-Kizza H, Kambugu A, Bakeera-Kitaka S, Semitala F, Mwebaze-Songa P, *et al*. **Predictors of long-term viral failure among Ugandan children and adults treated with antiretroviral therapy.** *J Acquir Immune Defic Syndr* 2007; **46**:187–193.
57. Bannister WP, Ruiz L, Cozzi-Lepri A, Mocroft A, Kirk O, Staszewski S, *et al*. **Comparison of genotypic resistance profiles and virological response between patients starting nevirapine and efavirenz in EuroSIDA.** *AIDS* 2008; **22**:367–376.
58. MacArthur RD, Novak RM, Peng G, Chen L, Xiang Y, Hullsiek KH, *et al*. **A comparison of three highly active antiretroviral treatment strategies consisting of nonnucleoside reverse transcriptase inhibitors, protease inhibitors, or both in the presence of nucleoside reverse transcriptase inhibitors as initial therapy (CPCRA 058 FIRST Study): a long-term randomised trial.** *Lancet* 2006; **368**:2125–2135.
59. Riddler SA, Haubrich R, DiRienzo AG, Peeples L, Powderly WG, Klingman KL, *et al*. **Class-sparing regimens for initial treatment of HIV-1 infection.** *N Engl J Med* 2008; **358**:2095–2106.
60. Sow PS, Otieno LF, Bissagnene E, Kityo C, Bennink R, Clevenbergh P, *et al*. **Implementation of an antiretroviral access program for HIV-1-infected individuals in resource-limited settings: clinical results from 4 African countries.** *J Acquir Immune Defic Syndr* 2007; **44**:262–267.
61. *Antiretroviral therapy for HIV infection in adults and adolescents in resource-limited settings: towards universal access. Recommendations for a public health approach*. Geneva: World Health Organization; 2006.
62. WHO. *Global price reporting mechanism (GPRM)*. Geneva: WHO; 2007.
63. Huffam SE, Srasuebkul P, Zhou J, Calmy A, Saphonn V, Kaldor JM, Ditangco R. **Prior antiretroviral therapy experience protects against zidovudine-related anaemia.** *HIV Med* 2007; **8**:465–471.

64. Toeung PD, Pouv S, Chel S, Huffam S, Khol V, Bun V, *et al.* **Routine switch after 6 months from d4t to AZT containing antiretroviral therapy, at an outpatient HIV clinic in Phnom Penh, Cambodia.** *Proceedings of the 4th IAS Conference on HIV Pathogenesis, Treatment and Prevention*; 22–25 July 2007; Sydney.
65. Gayet-Ageron A, Ananworanich J, Jupimai T, Chetchotisakd P, Prasithsirikul W, Ubolyam S, *et al.* **No change in calculated creatinine clearance after tenofovir initiation among Thai patients.** *J Antimicrob Chemother* 2007; **59**:1034–1037.
66. Peters P, Moore D, Mermin J, Brooks J, Downing R, Were W, *et al.* **Renal function improves among Ugandans on NNRTI-based HAART: 24-Month follow-up from the home-based AIDS care program in rural Uganda.** *Proceedings of the 14th Conference on Retrovirsuses and Opportunistic Infections*; 25–28 February 2007; Los Angeles.
67. Landman R, Descamps D, Peytavin G, Trylesinski A, Katlama C, Girard PM, *et al.* **Early virologic failure and rescue therapy of tenofovir, abacavir, and lamivudine for initial treatment of HIV-1 infection: TONUS study.** *HIV Clin Trials* 2005; **6**:291–301.
68. Khanlou H, Yeh V, Guyer B, Farthing C. **Early virologic failure in a pilot study evaluating the efficacy of therapy containing once-daily abacavir, lamivudine, and tenofovir DF in treatment-naive HIV-infected patients.** *AIDS Patient Care STDS* 2005; **19**:135–140.
69. Moyle G, Higgs C, Teague A, Mandalia S, Nelson M, Johnson M, *et al.* **An open-label, randomized comparative pilot study of a single-class quadruple therapy regimen versus a 2-class triple therapy regimen for individuals initiating antiretroviral therapy.** *Antivir Ther* 2006; **11**:73–78.
70. DART. **Virological response to a triple nucleoside/nucleotide analogue regimen over 48 weeks in HIV-1-infected adults in Africa.** *AIDS* 2006; **20**:1391–1399.
71. Munderi P, Walker S, Kityo C, Kallebu P, Ssali F, Lyagoba F, *et al.* **Discordance between virological/immunological and clinical outcomes at 48 weeks, in a randomised comparison of ZDV/3TC/NVP and ZDV/3TC/ABC in 600 patients with low CD4 counts in Africa.** *Proceedings of the 4th IAS Conference on HIV Pathogenesis, Treatment and Prevention*; 22–25 July 2007; Sydney.
72. Markowitz M, Nguyen BY, Gotuzzo E, Mendo F, Ratanasuwan W, Kovacs C, *et al.* **Rapid and durable antiretroviral effect of the HIV-1 integrase inhibitor raltegravir as part of combination therapy in treatment-naive patients with HIV-1 infection: results of a 48-week controlled study.** *J Acquir Immune Defic Syndr* 2007; **46**:125–133.
73. Saag M, Ive P, Heera J, Tawadrous M, DeJesus E, Clumeck N, *et al.* **A multicenter, randomized, double-blind, comparative trial of a novel CCR5 antagonist, maraviroc versus efavirenz, both in combination with Combivir (zidovudine [ZDV]/lamivudine [3TC]), for the treatment of antiretroviral naive patients infected with R5 HIV 1: week 48 results of the MERIT study.** *Proceedings of the 4th IAS Conference on HIV Pathogenesis, Treatment and Prevention*; 22–25 July 2007; Sydney.
74. Johnston ER, Zijenah LS, Mutetwa S, Kantor R, Kittinunvorakoon C, Katzenstein DA. **High frequency of syncytium-inducing and CXCR4-tropic viruses among human immunodeficiency virus type 1 subtype C-infected patients receiving antiretroviral treatment.** *J Virol* 2003; **77**:7682–7688.
75. Connell B, Michler K, Capovilla A, Venter W, Stevens W, Papathanasopoulos M. **Emergence of X4 usage among HIV-1 subtype C: evidence for an evolving epidemic in South Africa.** *AIDS* 2008; **22**:896–899.
76. Goebel F, Yakovlev A, Pozniak AL, Vinogradova E, Boogaerts G, Hoetelmans R, *et al.* **Short-term antiviral activity of TMC278: a novel NNRTI – in treatment-naive HIV-1-infected subjects.** *AIDS* 2006; **20**:1721–1726.
77. Nieuwkerk PT, Oort FJ. **Self-reported adherence to antiretroviral therapy for HIV-1 infection and virologic treatment response: a meta-analysis.** *J Acquir Immune Defic Syndr* 2005; **38**:445–448.
78. Mills EJ, Nachega JB, Buchan I, Orbinski J, Attaran A, Singh S, *et al.* **Adherence to antiretroviral therapy in sub-Saharan Africa and North America: a meta-analysis.** *JAMA* 2006; **296**:679–690.
79. 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.
80. Simoni JM, Pearson CR, Pantalone DW, Marks G, Crepaz N. **Efficacy of interventions in improving highly active antiretroviral therapy adherence and HIV-1 RNA viral load. A meta-analytic review of randomized controlled trials.** *J Acquir Immune Defic Syndr* 2006; **43 (Suppl 1)**:S23–S35.
81. Louis C, Ivers LC, Smith Fawzi MC, Freedberg KA, Castro A. **Late presentation for HIV care in central Haiti: factors limiting access to care.** *AIDS Care* 2007; **19**:487–491.
82. Zachariah R. **Comment on: Community support is associated with better antiretroviral treatment outcomes in a resource-limited rural district in Malawi.** *Trans R Soc Trop Med Hyg* 2007; **101**:627–628.
83. Van Griensven J, Corthouts A, Att E, Alonso J. **Validity of WHO-recommended immunological criteria for treatment failure.** *The 2007 HIV/AIDS Implementers' Meeting*; 16–19 June 2007; Kigali.
84. An S, Koole O, Haverkamp M, Sculier D, Thai S, Lynen L. **Predictors of virological failure in a Cambodian setting: findings from a cross-sectional study at Sihanouk Hospital Centre of HOPE, Phnom Penh.** *Proceedings of the 4th IAS Conference on HIV Pathogenesis, Treatment and Prevention*; 22–25 July 2007; Sydney, Australia.
85. Meya D, Tibenderana H, John L, Spacek L, Namugga I, Magero S, *et al.* **Evaluation of clinical and laboratory parameters to predict viral response to ART in Uganda.** *Proceedings of the 14th Conference on Retroviruses and Opportunistic Infections*; 25–28 February 2007; Los Angeles.
86. Bisson GP, Gross R, Strom JB, Rollins C, Bellamy S, Weinstein R, *et al.* **Diagnostic accuracy of CD4 cell count increase for virologic response after initiating highly active antiretroviral therapy.** *AIDS* 2006; **20**:1613–1619.
87. Moore DM, Mermin J, Awor A, Yip B, Hogg RS, Montaner JS. **Performance of immunologic responses in predicting viral load suppression: implications for monitoring patients in resource-limited settings.** *J Acquir Immune Defic Syndr* 2006; **43**:436–439.
88. Grabar S, Le MV, Goujard C, Leport C, Kazatchkine MD, Costagliola D, Weiss L. **Clinical outcome of patients with HIV-1 infection according to immunologic and virologic response after 6 months of highly active antiretroviral therapy.** *Ann Intern Med* 2000; **133**:401–410.
89. Tuboi SH, Brinkhof MW, Egger M, Stone RA, Braitstein P, Nash D, *et al.* **Discordant responses to potent antiretroviral treatment in previously naive HIV-1-infected adults initiating treatment in resource-constrained countries: The Antiretroviral Therapy in Low-Income Countries (ART-LINC) Collaboration.** *J Acquir Immune Defic Syndr* 2007; **45**:52–59.
90. Ferradini L. **Clinico-immunological criteria for the detection of virological failures. Implications for the use of viral load in Cambodia.** *Proceedings of the 1st Phnom Penh Symposium on HIV Medicine*; 14–15 September 2006; Phnom Penh.
91. Chaiwarith R, Wachirakaphan C, Kotarathititum W, Praparatanaphan J, Sirisanthana T, Supparatpinyo K. **Sensitivity and specificity of using CD4+ measurement and clinical evaluation to determine antiretroviral treatment failure in Thailand.** *Int J Infect Dis* 2007; **11**:413–416.
92. Badri M, Lawn SD, Wood R. **Usefulness of CD4 cell count changes in predicting virological failure in patients receiving HAART in a resource-poor setting.** *Proceedings of the 14th Conference on Retroviruses and Opportunistic Infections*; 25–28 February 2007; Los Angeles.
93. Hosseinipour M, van Oosterhout J, Weigel R, Mzigangira D, Saukila N, Mhango B, *et al.* **Validating clinical and immunological definitions of antiretroviral treatment failure in Malawi.** *Proceedings of the 4th IAS Conference on HIV Pathogenesis, Treatment and Prevention*; 25–28 February 2007; Sydney.
94. Cardiello P, Pertraro P, Chalamilla G, Tharakan J. **Does use of viral load result enhance accuracy in defining first line ARV failure?** *Proceedings of the HIV/AIDS Implementers Meeting*; 12–15 June 2006; Durban.

95. Haubrich RH, Currier JS, Forthal DN, Beall G, Kemper CA, Johnson D, *et al*. **A randomized study of the utility of human immunodeficiency virus RNA measurement for the management of antiretroviral therapy.** *Clin Infect Dis* 2001; **33**:1060–1068.
96. Gazzard B, Bernard AJ, Boffito M, Churchill D, Edwards S, Fisher N, *et al*. **British HIV Association (BHIVA) guidelines for the treatment of HIV-infected adults with antiretroviral therapy (2006).** *HIV Med* 2006; **7**:487–503.
97. Hammer SM, Saag MS, Schechter M, Montaner JS, Schooley RT, Jacobsen DM, *et al*. **Treatment for adult HIV infection: 2006 recommendations of the International AIDS Society–USA panel.** *JAMA* 2006; **296**:827–843.
98. Phillips AN, Pillay D, Miners AH, Bennett DE, Gilks CF, Lundgren JD. **Outcomes from monitoring of patients on antiretroviral therapy in resource-limited settings with viral load, CD4 cell count, or clinical observation alone: a computer simulation model.** *Lancet* 2008; **371**:1443–1451.
99. Coutinho A, Mermin J, Ekwaru J, Were W, Bunnell R, Kaharuza F, *et al*. **Utility of routine viral load, CD4 cell count, and clinical monitoring among HIV-infected adults in Uganda: a randomized trial.** *Proceedings of the 15th Conference on Retroviruses and Opportunistic Infections*; 3–6 February 2008; Los Angeles.
100. Monitoring highly active antiretroviral therapy in HIV-infected parents in Thailand. http://clinicaltrials.gov/ct2/show/NCT00162682. [Accessed 16 May 2008]
101. Development of AntiRetroviral Therapy in Africa. A randomised trial of monitoring practice and structured treatment interruptions in the management of antiretroviral therapy in adults with HIV infection in Africa. http://www.controlled-trials.com/ISRCTN13968779/. [Accessed 16 May 2008]
102. Fiscus SA, Cheng B, Crowe SM, Demeter L, Jennings C, Miller V, *et al*. **HIV-1 viral load assays for resource-limited settings.** *PLoS Med* 2006; **3**:e417.
103. Calmy A, Ford N, Hirschel B, Reynolds SJ, Lynen L, Goemaere E, *et al*. **HIV viral load monitoring in resource-limited regions: optional or necessary?** *Clin Infect Dis* 2007; **44**:128–134.
104. Vijayaraghavan A, Efrusy MB, Mazonson PD, Ebrahim O, Sanne IM, Santas CC. **Cost-effectiveness of alternative strategies for initiating and monitoring highly active antiretroviral therapy in the developing world.** *J Acquir Immune Defic Syndr* 2007; 46:91–100.
105. Le MV, Thiebaut R, Chene G, Leport C, Cailleton V, Michelet C, *et al*. **Predictors of long-term increase in CD4+ cell counts in human immunodeficiency virus-infected patients receiving a protease inhibitor-containing antiretroviral regimen.** *J Infect Dis* 2002; **185**:471–480.
106. Tenorio AR, Smith KY, Kuritzkes DR, Sha BE, Donoval B, Young R, *et al*. **HIV-1-infected antiretroviral-treated patients with prolonged partial viral suppression: clinical, virologic, and immunologic course.** *J Acquir Immune Defic Syndr* 2003; **34**:491–496.
107. Ledergerber B, Lundgren JD, Walker AS, Sabin C, Justice A, Reiss P, *et al*. **Predictors of trend in CD4-positive T-cell count and mortality among HIV-1-infected individuals with virological failure to all three antiretroviral-drug classes.** *Lancet* 2004; **364**:51–62.
108. Raffanti SP, Fusco JS, Sherrill BH, Hansen NI, Justice AC, D'Aquila R, *et al*. **Effect of persistent moderate viremia on disease progression during HIV therapy.** *J Acquir Immune Defic Syndr* 2004; **37**:1147–1154.
109. Deeks SG, Barbour JD, Grant RM, Martin JN. **Duration and predictors of CD4 T-cell gains in patients who continue combination therapy despite detectable plasma viremia.** *AIDS* 2002; **16**:201–207.
110. Murri R, Lepri AC, Cicconi P, Poggio A, Arlotti M, Tositti G, *et al*. **Is moderate HIV viremia associated with a higher risk of clinical progression in HIV-infected people treated with highly active antiretroviral therapy: evidence from the Italian cohort of antiretroviral-naive patients study.** *J Acquir Immune Defic Syndr* 2006; **41**:23–30.
111. Duncombe C, Kerr SJ, Ruxrungtham K, Dore GJ, Law MG, Emery S, *et al*. **HIV disease progression in a patient cohort treated via a clinical research network in a resource limited setting.** *AIDS* 2005; **19**:169–178.
112. Grabar S, Le MV, Goujard C, Egger M, Leport C, Kazatchkine MD, *et al*. **Response to highly active antiretroviral therapy at 6 months and long-term disease progression in HIV-1 infection.** *J Acquir Immune Defic Syndr* 2005; **39**:284–292.
113. Riddler SA, Jiang H, Tenorio A, Huang H, Kuritzkes DR, Acosta EP, *et al*. **A randomized study of antiviral medication switch at lower- versus higher-switch thresholds: AIDS Clinical Trials Group Study A5115.** *Antivir Ther* 2007; **12**:531–541.
114. Aleman S, Soderbarg K, Visco-Comandini U, Sitbon G, Sonnerborg A. **Drug resistance at low viraemia in HIV-1-infected patients with antiretroviral combination therapy.** *AIDS* 2002; **16**:1039–1044.
115. Bangsberg DR, Charlebois ED, Grant RM, Holodniy M, Deeks SG, Perry S, *et al*. **High levels of adherence do not prevent accumulation of HIV drug resistance mutations.** *AIDS* 2003; **17**:1925–1932.
116. Kantor R, Shafer RW, Follansbee S, Taylor J, Shilane D, Hurley L, *et al*. **Evolution of resistance to drugs in HIV-1-infected patients failing antiretroviral therapy.** *AIDS* 2004; **18**:1503–1511.
117. Kristiansen TB, Pedersen AG, Eugen-Olsen J, Katzenstein TL, Lundgren JD. **Genetic evolution of HIV in patients remaining on a stable HAART regimen despite insufficient viral suppression.** *Scand J Infect Dis* 2005; **37**:890–901.
118. Hatano H, Hunt P, Weidler J, Coakley E, Hoh R, Liegler T, *et al*. **Rate of viral evolution and risk of losing future drug options in heavily pretreated, HIV-infected patients who continue to receive a stable, partially suppressive treatment regimen.** *Clin Infect Dis* 2006; **43**:1329–1336.
119. Cozzi-Lepri A, Phillips AN, Ruiz L, Clotet B, Loveday C, Kjaer J, *et al*. **Evolution of drug resistance in HIV-infected patients remaining on a virologically failing combination antiretroviral therapy regimen.** *AIDS* 2007; **21**:721–732.
120. Napravnik S, Edwards D, Stewart P, Stalzer B, Matteson E, Eron JJ Jr. **HIV-1 drug resistance evolution among patients on potent combination antiretroviral therapy with detectable viremia.** *J Acquir Immune Defic Syndr* 2005; **40**:34–40.
121. Youle M. **Overview of boosted protease inhibitors in treatment-experienced HIV-infected patients.** *J Antimicrob Chemother* 2007; **60**:1195–1205.
122. Hogg RS, Bangsberg DR, Lima VD, Alexander C, Bonner S, Yip B, *et al*. **Emergence of drug resistance is associated with an increased risk of death among patients first starting HAART.** *PLoS Med* 2006; **3**:e356.
123. Joly V, Descamps D, Peytavin G, Touati F, Mentre F, Duval X, *et al*. **Evolution of human immunodeficiency virus type 1 (HIV-1) resistance mutations in nonnucleoside reverse transcriptase inhibitors (NNRTIs) in HIV-1-infected patients switched to antiretroviral therapy without NNRTIs.** *Antimicrob Agents Chemother* 2004; **48**:172–175.
124. Koval CE, Dykes C, Wang J, Demeter LM. **Relative replication fitness of efavirenz-resistant mutants of HIV-1: correlation with frequency during clinical therapy and evidence of compensation for the reduced fitness of K103N + L100I by the nucleoside resistance mutation L74V.** *Virology* 2006; **353**:184–192.
125. Sungkanuparph S, Manosuthi W, Kiertiburanakul S, Piyavong B, Chumpathat N, Chantratita W. **Options for a second-line antiretroviral regimen for HIV type 1-infected patients whose initial regimen of a fixed-dose combination of stavudine, lamivudine, and nevirapine fails.** *Clin Infect Dis* 2007; **44**:447–452.
126. Seyler C, Adje-Toure C, Messou E, Dakoury-Dogbo N, Rouet F, Gabillard D, *et al*. **Impact of genotypic drug resistance mutations on clinical and immunological outcomes in HIV-infected adults on HAART in West Africa.** *AIDS* 2007; **21**:1157–1164.
127. Durant J, Clevenbergh P, Halfon P, Delgiudice P, Porsin S, Simonet P, *et al*. **Drug-resistance genotyping in HIV-1 therapy: the VIRADAPT randomised controlled trial.** *Lancet* 1999; **353**:2195–2199.
128. Tural C, Ruiz L, Holtzer C, Schapiro J, Viciana P, Gonzalez J, *et al*. **Clinical utility of HIV-1 genotyping and expert advice: the Havana trial.** *AIDS* 2002; **16**:209–218.
129. Sax PE, Islam R, Walensky RP, Losina E, Weinstein MC, Goldie SJ, *et al*. **Should resistance testing be performed for treatment-naive HIV-infected patients? A cost-effectiveness analysis.** *Clin Infect Dis* 2005; **41**:1316–1323.

130. Weinstein MC, Goldie SJ, Losina E, Cohen CJ, Baxter JD, Zhang H, *et al*. **Use of genotypic resistance testing to guide HIV therapy: clinical impact and cost-effectiveness.** *Ann Intern Med* 2001; **134**:440–450.
131. Sendi P, Gunthard HF, Simcock M, Ledergerber B, Schupbach J, Battegay M. **Cost-effectiveness of genotypic antiretroviral resistance testing in HIV-infected patients with treatment failure.** *PLoS ONE* 2007; **2**:e173.
132. Shafer RW. **Rationale and uses of a public HIV drug-resistance database.** *J Infect Dis* 2006; **194 (Suppl 1)**:S51–S58.
133. Fox ZV, Geretti AM, Kjaer J, Dragsted UB, Phillips AN, Gerstoft J, *et al*. **The ability of four genotypic interpretation systems to predict virological response to ritonavir-boosted protease inhibitors.** *AIDS* 2007; **21**:2033–2042.
134. Chaix ML, Rouet F, Tony TD, Danel C, Moh R, Gabillard D, *et al*. **Low rate of resistance to ARV drugs 6 months after HAART initiation in HIV-1-infected sub-Saharan African: The ANRS 1269 TRIVACAN trial.** *Proceedings of the 14th Conference on Retroviruses and Opportunistic Infections*; 25–28 February 2007; Los Angeles.
135. Spacek LA, Shihab HM, Kamya MR, Mwesigire D, Ronald A, Mayanja H, *et al*. **Response to antiretroviral therapy in HIV-infected patients attending a public, urban clinic in Kampala, Uganda.** *Clin Infect Dis* 2006; **42**:252–259.
136. Fischer A, Karasi JC, Kibibi D, Omes C, Lambert C, Uwayitu A, *et al*. **Antiviral efficacy and resistance in patients on antiretroviral therapy in Kigali, Rwanda: the real-life situation in 2002.** *HIV Med* 2006; **7**:64–66.
137. Ramadhani HO, Thielman NM, Landman KZ, Ndosi EM, Gao F, Kirchherr JL, *et al*. **Predictors of incomplete adherence, virologic failure, and antiviral drug resistance among HIV-infected adults receiving antiretroviral therapy in Tanzania.** *Clin Infect Dis* 2007; **45**:1492–1498.
138. Ferradini L, Laureillard D, Prak N, Ngeth C, Fernandez M, Pinoges L, *et al*. **Positive outcomes of HAART at 24 months in HIV-infected patients in Cambodia.** *AIDS* 2007; **21**:2293–2301.
139. Wallis CL, Mahomed I, Morris L, Chidarikire T, Stevens G, Rekhviashvili N, Stevens W. **Evaluation of an oligonucleotide ligation assay for detection of mutations in HIV-1 subtype C individuals who have high level resistance to nucleoside reverse transcriptase inhibitors and nonnucleoside reverse transcriptase inhibitors.** *J Virol Methods* 2005; **125**:99–109.
140. Grossman Z, Paxinos EE, Averbuch D, Maayan S, Parkin NT, Engelhard D, *et al*. **Mutation D30N is not preferentially selected by human immunodeficiency virus type 1 subtype C in the development of resistance to nelfinavir.** *Antimicrob Agents Chemother* 2004; **48**:2159–2165.
141. Pillay D, Walker AS, Gibb DM, De Rossi A, Kaye S, Ait-Khaled M, *et al*. **Impact of human immunodeficiency virus type 1 subtypes on virologic response and emergence of drug resistance among children in the Paediatric European Network for Treatment of AIDS (PENTA) 5 trial.** *J Infect Dis* 2002; **186**:617–625.
142. Humphreys EH, Hernandez LB, Rutherford GW. Antiretroviral regimens for patients with HIV who fail first-line antiretroviral therapy. Cochrane Database Syst Rev 2007:CD006517.
143. Deeks SG, Hoh R, Neilands TB, Liegler T, Aweeka F, Petropoulos CJ, *et al*. **Interruption of treatment with individual therapeutic drug classes in adults with multidrug-resistant HIV-1 infection.** *J Infect Dis* 2005; **192**:1537–1544.
144. Castagna A, Danise A, Menzo S, Galli L, Gianotti N, Carini E, *et al*. **Lamivudine monotherapy in HIV-1-infected patients harbouring a lamivudine-resistant virus: a randomized pilot study (E-184V study).** *AIDS* 2006; **20**:795–803.
145. Wei X, Liang C, Gotte M, Wainberg MA. **Negative effect of the M184V mutation in HIV-1 reverse transcriptase on initiation of viral DNA synthesis.** *Virology* 2003; **311**:202–212.
146. Johnson M, Grinsztejn B, Rodriguez C, Coco J, DeJesus E, Lazzarin A, *et al*. **96-week comparison of once-daily atazanavir/ritonavir and twice-daily lopinavir/ritonavir in patients with multiple virologic failures.** *AIDS* 2006; **20**:711–718.
147. Madruga JV, Berger D, McMurchie M, Suter F, Banhegyi D, Ruxrungtham K, *et al*. **Efficacy and safety of darunavir-ritonavir compared with that of lopinavir-ritonavir at 48 weeks in treatment-experienced, HIV-infected patients in TITAN: a randomised controlled phase III trial.** *Lancet* 2007; **370**: 49–58.
148. De Luca A, Di Giambenedetto S, Romano L, Gonnelli A, Corsi P, Baldari M, *et al*. **Frequency and treatment-related predictors of thymidine-analogue mutation patterns in HIV-1 isolates after unsuccessful antiretroviral therapy.** *J Infect Dis* 2006; **193**:1219–1222.
149. Cozzi-Lepri A, Ruiz L, Loveday C, Phillips AN, Clotet B, Reiss P, *et al*. **Thymidine analogue mutation profiles: factors associated with acquiring specific profiles and their impact on the virological response to therapy.** *Antivir Ther* 2005; **10**:791–802.
150. Flandre P, Descamps D, Joly V, Meiffredy V, Tamalet C, Izopet J, *et al*. **Predictive factors and selection of thymidine analogue mutations by nucleoside reverse transcriptase inhibitors according to initial regimen received.** *Antivir Ther* 2003; **8**:65–72.
151. Turner D, Brenner BG, Routy JP, Petrella M, Wainberg MA. **Rationale for maintenance of the M184v resistance mutation in human immunodeficiency virus type 1 reverse transcriptase in treatment experienced patients.** *New Microbiol* 2004; **27**:31–39.
152. Rey D, Krebs M, Partisani M, Hess G, Cheneau C, Priester M, *et al*. **Virologic response of zidovudine, lamivudine, and tenofovir disoproxil fumarate combination in antiretroviral-naive HIV-1-infected patients.** *J Acquir Immune Defic Syndr* 2006; **43**:530–534.
153. Garcia-Lerma JG, MacInnes H, Bennett D, Reid P, Nidtha S, Weinstock H, *et al*. **A novel genetic pathway of human immunodeficiency virus type 1 resistance to stavudine mediated by the K65R mutation.** *J Virol* 2003; **77**:5685–5693.
154. Margot NA, Lu B, Cheng A, Miller MD. **Resistance development over 144 weeks in treatment-naive patients receiving tenofovir disoproxil fumarate or stavudine with lamivudine and efavirenz in Study 903.** *HIV Med* 2006; **7**:442–450.
155. Gonzales MJ, Wu TD, Taylor J, Belitskaya I, Kantor R, Israelski D, *et al*. **Extended spectrum of HIV-1 reverse transcriptase mutations in patients receiving multiple nucleoside analog inhibitors.** *AIDS* 2003; **17**:791–799.
156. Chappell BJ, Margot NA, Miller MD. **Long-term follow-up of patients taking tenofovir DF with low-level HIV-1 viremia and the K65R substitution in HIV-1 RT.** *AIDS* 2007; **21**:761–763.
157. Frankel FA, Invernizzi CF, Oliveira M, Wainberg MA. **Diminished efficiency of HIV-1 reverse transcriptase containing the K65R and M184V drug resistance mutations.** *AIDS* 2007; **21**:665–675.
158. White KL, Chen JM, Feng JY, Margot NA, Ly JK, Ray AS, *et al*. **The K65R reverse transcriptase mutation in HIV-1 reverses the excision phenotype of zidovudine resistance mutations.** *Antivir Ther* 2006; **11**:155–163.
159. Brenner BG, Oliveira M, Doualla-Bell F, Moisi DD, Ntemgwa M, Frankel F, *et al*. **HIV-1 subtype C viruses rapidly develop K65R resistance to tenofovir in cell culture.** *AIDS* 2006; **20**:F9–F13.
160. Wallis CL, Bell C, Boulme R, Sanne I, Venter F, Papathanasopoulos M, Stevens W. **Emerging ART drug resistance in subtype C: experience from the 2 clinics in Johannesburg, South Africa.** *Proceedings of the 14th Conference on Retroviruses and Opportunistic Infections*; 25–28 February 2007; Los Angeles.
161. Hall CS, Raines CP, Barnett SH, Moore RD, Gallant JE. **Efficacy of salvage therapy containing ritonavir and saquinavir after failure of single protease inhibitor-containing regimens.** *AIDS* 1999; **13**:1207–1212.
162. Tebas P, Patick AK, Kane EM, Klebert MK, Simpson JH, Erice A, *et al*. **Virologic responses to a ritonavir: saquinavir-containing regimen in patients who had previously failed nelfinavir.** *AIDS* 1999; **13**:F23–F28.
163. de Mendoza C, Valer L, Ribera E, Barreiro P, Martin-Carbonero L, Ramirez G, Soriano V. **Performance of six different ritonavir-boosted protease inhibitor-based regimens in heavily antiretroviral-experienced HIV-infected patients.** *HIV Clin Trials* 2006; **7**:163–171.
164. Clotet B, Bellos N, Molina JM, Cooper D, Goffard JC, Lazzarin A, *et al*. **Efficacy and safety of darunavir-ritonavir at week 48 in treatment-experienced patients with HIV-1 infection in POWER 1 and 2: a pooled subgroup analysis of data from two randomised trials.** *Lancet* 2007; **369**:1169–1178.

165. *Prioritizing second-line antiretroviral drugs for adults and adolescents: a public health approach. Report of a WHO Working Group Meeting*. Geneva: World Health Organization; 2007.
166. Arribas JR, Pulido F, Delgado R, Lorenzo A, Miralles P, Arranz A, *et al*. **Lopinavir/ritonavir as single-drug therapy for maintenance of HIV-1 viral suppression: 48-week results of a randomized, controlled, open-label, proof-of-concept pilot clinical trial (OK Study).** *J Acquir Immune Defic Syndr* 2005; **40**:280–287.
167. Swindells S, DiRienzo AG, Wilkin T, Fletcher CV, Margolis DM, Thal GD, *et al*. **Regimen simplification to atazanavir-ritonavir alone as maintenance antiretroviral therapy after sustained virologic suppression.** *JAMA* 2006; **296**:806–814.
168. Delfraissy JF, Flandre P, Delaugerre C, Ghosn J, Horban A, Girard PM, *et al*. **Lopinavir/ritonavir monotherapy or plus zidovudine and lamivudine in antiretroviral-naive HIV-infected patients.** *AIDS* 2008; **22**:385–393.
169. Flandre P, Delaugerre C, Ghosn J, Chaix ML, Horban A, Girard PM, *et al*. **Prognostic factors of virological success in antiretroviral-naive patients receiving LPV/r monotherapy in the MONARK trial.** *Proceedings of the 11th European AIDS Conference*; 24–27 October 2007; Madrid.
170. Hellinger J, Cohen C, Morris A, Sheble-Hall S, Gordon D, Foy C, *et al*. **Pilot study of saquinavir and lopinavir/ritonavir twice daily in protease inhibitor-naive HIV-positive patients.** *HIV Clin Trials* 2005; **6**:107–117.
171. Staszewski S, Babacan E, Stephan C, Haberl A, Carlebach A, Gute P, *et al*. **The LOPSAQ study: 48 week analysis of a boosted double protease inhibitor regimen containing lopinavir/ritonavir plus saquinavir without additional antiretroviral therapy.** *J Antimicrob Chemother* 2006; **58**:1024–1030.
172. Ribera E, Azuaje C, Lopez RM, Diaz M, Feijoo M, Pou L, *et al*. **Atazanavir and lopinavir/ritonavir: pharmacokinetics, safety and efficacy of a promising double-boosted protease inhibitor regimen.** *AIDS* 2006; **20**:1131–1139.
173. Boyd M, Mootsikapun P, Burger D, Chuenyam T, Ubolyam S, Mahanontharit A, *et al*. **Pharmacokinetics of reduced-dose indinavir/ritonavir 400/100 mg twice daily in HIV-1-infected Thai patients.** *Antivir Ther* 2005; **10**:301–307.
174. Konopnicki D, De Wit S, Poll B, Crommentuyn K, Huitema A, Clumeck N. **Indinavir/ritonavir-based therapy in HIV-1-infected antiretroviral therapy-naive patients: comparison of 800/100 mg and 400/100 mg twice daily.** *HIV Med* 2005; **6**:1–6.
175. Murphy RL, Brun S, Hicks C, Eron JJ, Gulick R, King M, *et al*. **ABT-378/ritonavir plus stavudine and lamivudine for the treatment of antiretroviral-naive adults with HIV-1 infection: 48-week results.** *AIDS* 2001; **15**:F1–F9.
176. Sanne I, Piliero P, Squires K, Thiry A, Schnittman S. **Results of a phase 2 clinical trial at 48 weeks (AI424-007): a dose-ranging, safety, and efficacy comparative trial of atazanavir at three doses in combination with didanosine and stavudine in antiretroviral-naive subjects.** *J Acquir Immune Defic Syndr* 2003; **32**:18–29.
177. Ananworanich J, Hirschel B. **Intermittent therapy for the treatment of chronic HIV infection.** *AIDS* 2007; **21**:123–134.
178. Maggiolo F, Ripamonti D, Gregis G, Quinzan G, Callegaro A, Suter F. **Effect of prolonged discontinuation of successful antiretroviral therapy on CD4 T cells: a controlled, prospective trial.** *AIDS* 2004; **18**:439–446.
179. Cooper D, Gatell J, Rockstroh J, Katlama C, Yeni P, Lazzarin A, *et al*. **Results of BENCHMRK-1, a phase III study evaluating the efficacy and safety of MK-0518, a novel HIV-1 integrase inhibitor, in patients with triple-class resistant virus.** *Proceedings of the 14th Conference on Retrovirsuses and Opportunistic Infections*; 25–28 February 2007; Los Angeles.
180. Zolopa A, Mullen M, Berger D, Ruane P, Hawkins T, Zhong L, *et al*. **The HIV integrase inhibitor GS-9137 demonstrates potent antiretroviral activity in treatment-experienced patients.** *Proceedings of the 14th Conference on Retrovirsuses and Opportunistic Infections*; 25–28 February 2007; Los Angeles.
181. Gulick RM, Van der Ryst E, Lampiris H, Fatkenheuer G, Raffi F, Lalezari J, *et al*. **Efficacy and safety of once-daily (QD) compared with twice-daily (BID) maraviroc plus optimized background therapy (OBT) in treatment-experienced patients infected with CCR5-tropic-HIV-1: 24-week combined analysis of the MOTIVATE 1 and 2 studies.** *Proceedings of the 4th IAS Conference on HIV Pathogenesis, Treatment and Prevention*; 22–25 July 2007; Sydney.
182. Van der Ryst E, Cooper D, Konourina I, Saag M, Goodrich J, Tawadrous M, *et al*. **Efficacy of Maraviroc in combination with at least one other potent new antiretroviral drug: 24-week combined analysis of the MOTIVATE 1 and 2 studies.** *Proceedings of the 4th IAS Conference on HIV Pathogenesis, Treatment and Prevention*; 22–25 July 2007; Sydney.
183. Gulick RM, Su Z, Flexner C, Hughes MD, Skolnik PR, Wilkin TJ, *et al*. **Phase 2 study of the safety and efficacy of vicriviroc, a CCR5 inhibitor, in HIV-1-infected, treatment-experienced patients: AIDS clinical trials group 5211.** *J Infect Dis* 2007; **196**:304–312.
184. Cahn P, Schuermann D, Reuss F, Wit F, Boehm E, Lange J. **A phase-II study of 14 days monotherapy with the nucleoside-analogue Fosalvudine Tidoxil in treatment-naive HIV-1 infected adults.** *Proceedings of the 4th IAS Conference on HIV Pathogenesis, Treatment and Prevention*; 22–25 July 2007; Sydney.
185. Madruga JV, Cahn P, Grinsztejn B, Haubrich R, Lalezari J, Mills A, *et al*. **Efficacy and safety of TMC125 (etravirine) in treatment-experienced HIV-1-infected patients in DUET-1: 24-week results from a randomised, double-blind, placebo-controlled trial.** *Lancet* 2007; **370**:29–38.
186. Phillips AN, Leen C, Wilson A, Anderson J, Dunn D, Schwenk A, *et al*. **Risk of extensive virological failure to the three original antiretroviral drug classes over long-term follow-up from the start of therapy in patients with HIV infection: an observational cohort study.** *Lancet* 2007; **370**:1923–1928.
187. *Treating 3 million by 2005: making it happen: The WHO Strategy*. Geneva: World Health Organization; 2003.
188. Nunn AS, Fonseca EM, Bastos FI, Gruskin S, Salomon JA. **Evolution of antiretroviral drug costs in Brazil in the context of free and universal access to AIDS treatment.** *PLoS Med* 2007; **4**:e305.