An Evidence-Based Public Health Approach To Establish An Antiretroviral Treatment Program In A Resource-Limited Setting

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Chapter 1

Introduction and outline of thesis

Since the description of the first case in 1981 of what came to be known as an HIV infection, the HIV/AIDS pandemic has grown into one of the most devastating public health threats of our time. As of 2011, 33 million individuals worldwide are infected and in sub-Saharan Africa every day 5000 new infections occur (1.8 mill in 2010)[1].

HIV has become one of the most studied infectious diseases having broadened our knowledge in basic sciences including immunology, viral pathogenesis, pharmacology and in behavioural sciences, but as of now no cure nor effective preventative public health method has been found.

Molecular biology of HIV

Origin and classification

HIV belongs to the genus lentivirus of the family retroviridae[2]. Retroviruses derive their name from the Latin word 'backwards' because they transcribe their genomic RNA into DNA, establishing a provirus in the chromosome of the host cell, thus reversing the usual flow of genetic information. Retroviruses are classified into 7 genera, subfamilies[3], and are found in a wide range of animals[4]. Four retrovirus species, two belonging to the subfamily of lentiviruses (HIV -1[5, 6, 7], HIV-2[8, 9]) and two to the subfamily of Gamma retroviruses (HTLV1[10] and HTLV2[11, 12]), also infect humans. Non-human primate lentiviruses, SIVs, have been isolated from many African monkey species where they generally cause a non-pathogenic infection of the immune system of natural host species[13]. HIV is phylogenetically closely related to the lentivirus that infects chimpanzees (SIVchz)[14]. This genetic relationship combined with epidemiological evidence suggests that HIV entered human populations through multiple zoonotic infections from SIVchz infected chimpanzees[15]. The Asian Macaque monkey is the only known monkey which develops AIDS on SIV infection[16], a fact that has been used for investigating AIDS pathogenesis and prevention.

HIV particle

HIV is a RNA virus. The HIV virion is surrounded by an outer lipid membrane of host cellular origin in which multiple viral glycoprotein complexes, composed of external GP120 and transmembrane gp41, are inserted. Closely associated with the outer membrane is the viral matrix protein into which the gp41 molecules are anchored.

In the centre of the viral particle is a core shell, formed by the capsid protein, that holds two strands of genomic RNA as well as the reverse transcriptase (RT), protease (PR) and integrase (IN) enzymes. The genome contains three structural genes, the env gene encoding for the envelop glycoproteins, the pol gene encoding the main enzymes and the gag gene encoding the matrix and capsid proteins. The viral genome also contains six accessory genes which are essential to the viral lifecycle; another important genomic region essential for integration and viral replication is the long terminal repeat (LTR) situated on both ends of the viral genome. The LTR has promoter and enhancer sequences which are responsive to many cellular and viral (Transcriptional transactivator, Tat) signaling proteins.

HIV Lifecycle

Cell tropism of HIV is determined by the affinity of its envelope gp120 for the CD4 molecule found on T helper cells, macrophages, and microglia of CNS and by the presence of one of the two corecpetors, namely CCR5, mainly expressed on activated and effector/memory T cells or CXCR4 on naïve T cells. Gp41 is crucial for the fusion of the host and viral cell membranes. Upon cell entry the capsid is released into the host cell cytplasma and the viral RNA is converted into a double-stranded viral DNA, called provirus, catalyzed by viral enzyme RT using cellular building blocks. A pre-integration complex (PIC) is formed from viral DNA, integrase and other viral and host proteins and is transported into the nucleus where integrase catalyses the transfer of viral DNA into the host chromosome.

Changes in the cellular activation state and/or external stimuli can induce transcription of the HIV provirus resulting in the synthesis of new viral particles. Full-length and multiply spliced RNA transcripts are transported to the cytoplasma where they are translated into viral proteins using the host cell machinery. At the host cell plasma membrane the viral particles are assembled and are released through budding from the cell. Final maturation into an infective virus is completed through rearrangements of the capsid protein in the budded particle catalysed by the protease enzyme.

Interestingly the host cell has the ability to block viral reverse transcription in the target cell through the host cell APOBEC enzyme which is incorporated to the budding virus. This 'intracellular immunity' is counteracted by the viral accessory vif protein which blocks the packaging of APOBEC and thus protects viral infectivity[17]. Another cellular restriction factor, TRIM5α, is able to block the PIC and thus to prevent transfer of the viral DNA into the nucleus. TRIM5α belongs to a large family of proteins which are involved in various cellular processes including interference with virus replication[18]. TRIM5α of African (old world) monkeys inhibit monkey cells from getting infected with HIV, while human TRIM5α is less effective in suppressing HIV infection[19]. A current research focus is to fully elucidate the functions of this protein family as they might be involved in innate immunity[20]. Currently licenced antiretroviral (ARV) drugs act by blocking the viral life cycle at specific steps namely viral cell entry, reverse transcription, proviral integration and particle maturation.

Viral diversity, distribution and transmission

HIV is characterized by a high mutagenic potential[21] as a result of the error-prone functioning of the RT enzyme[22], and the ability of the virus to build recombinant forms as both RNA strands are used for reverse transcription[23]. The high mutagenesis in combination with a high replication rate (generation time 2 days)[24, 25] have lead on a global level to the establishment of distinct HIV subtypes which are unevenly distributed worldwide. HIV-1 subtype C is the major subtype representing greater than 50% of all HIV-1 infections worldwide and more than 90% of all HIV infections in Southern Africa[26, 27]. On an individual level HIV exists as quasispecies[28, 29] i.e. genetically closely related swarms of viruses, rather than genetically identical copies of a distinct virus, enabling HIV to adapt to a range of local environments e.g. evading the control of the immune system or escaping the pressure of ARV drugs causing ARV drug resistance [30].

HIV is mainly transmitted through infected blood, breast milk and/or genital secretions. Worldwide and especially in sub-Saharan Africa (SSA) the majority of new HIV infections are caused by heterosexual transmission and mother-to-child transmission (MTCT) but important subepidemics exist in risk populations such as among men having sex with men (MSM) and intravenous (IV) drug abusers.

Course of HIV infection

Early events

HIV is present in sexual secretions as free and/or as cell- associated viruses[31]. The process from viral exposure at the sexual mucosa to the infection of the draining lymph node (LN) is a complex interplay between innate immune responses[32, 33, 34] and adaptive immunity [35,36]. In general, HIV infection of healthy sexual mucosal membrane is effective in only 1:100 to 1:1000[37]; however infection risk is much increased when the mucosa integrity is impaired through trauma, ulcers or inflammation.

Following an initial burst of viral replication in susceptible CD4 T cell targets within the local LN, the virus is disseminated via lymph ducts to the whole body including the gut, where especially in the lamina propria of the gut mucosa it causes a massive infection and loss of the CD4 T cells i.e. effector memory CCR5 subsets of CD4 T cells[38, 39, 40, 41, 42]. In healthy individuals the gut is the site where most CD4 T cells reside. Within a few weeks of HIV infection half of all body CD4 T memory cells are destroyed[43, 41], an event that is not significantly reflected in peripheral blood CD4 T cell count. Death occurs both by direct infection/killing of the infected cells and by bystander killing of uninfected cells through the mechanism of apoptosis[44]. While the relevance of this massive loss for immune function is still unclear, the associated damage of the intestinal epithelial barrier has consequences for integrity of the mucosa[45, 46], notably permitting the translocation of microbial products that contribute to systemic immune activation in chronic disease[47].

The early extensive viral replication in gut-associated and other lymphatic tissue leads to plasma viremia which peaks at 3-4 weeks up to 10 mill copies/ml[48] and then declines over several months before reaching a steady state. The initial viral decline is associated with the occurrence of cytotoxic HIV-specific CD8 T cells which are able to eliminate HIV infected CD4 T cells through MHC class I restricted cytolysis and are thought to significantly contribute to initial viral control establishing a viremic setpoint[49]. The effectiveness of the CD8 T cell response depends on the presence of HIV specific CD4 T cells[50, 51], CD4 T cells that, as part of their biological function, have become committed by interaction with peptide antigens (HIV epitopes)

bound to MHC-II molecules on antigen presenting cells. Antibodies (AB) against HIV are detectable within the first weeks of infection[52] and are used for diagnostic purposes, but generally early ABs are not able to neutralize circulating viral strains. Neutralizing ABs targeted against circulating HIV variants emerge within the first months[53, 54], stimulating viral diversification. The polyclonal AB response resulting from ongoing AB production and viral escape is typical for HIV infection. Of note, in a fraction of patients maturation of certain AB over time (months to years) become able to neutralize HIV variants[55, 56, 57]. Such broad neutralizing ABs are of enormous interest for vaccine research[58, 59].

Recent investigations suggest that initially only a single[60] or a few viruses are transmitted (genetic bottleneck of transmission)[61]. With the emergence of the cellular and humoral immune response HIV rapidly evolves and selects for viral variants which are able to escape from ABs[54] and CD8 T cell responses[62]. Viral diversification continues throughout the course of HIV infection enabling newly formed viruses to evade the pressure exerted by the immune system and/or antiretroviral drugs[63].

Established infection

Established HIV infection is characterized by chronic immune activation[64] and constant viral diversification providing the potential to escape from immune control.

Chronic immune activation

Systemic immune activation[65] is a hallmark of chronic HIV-infection. While its etiology is still unclear, recent research findings strongly suggest that in addition to the high HIV-specific antigen burden, events associated with the initial gut mucosa injury play a key role[38]. The compromised intestinal mucosal barrier allows microbial products such as lipopolysaccharides (LPS) from the cell wall of gram-negative bacteria to enter the circulation (microbial translocation) where they stimulate macrophages and monocytes[66, 46]. Soluble CD14, a protein produced by LPS-stimulated macrophages and monocytes, has been shown to be an independent predictor of HIV disease progression[67].

Also, recent studies have shown that CD4 T cells producing IL-17 (Th17 cells) are preferentially depleted from the gastrointestinal tract of chronically HIV-infected individuals. Th17 cells are thought to play a central role in antibacterial immunity, particularly at mucosal surfaces[68, 46, 69 70, 71].

Signs of chronic immune activation include the increased frequency of activated immune cells[72, 73], the increased turnover of lymphocytes, i. e. T cell proliferation and death (BrU and Ki67 proliferation assays)[74, 75, 76], the polyclonal B cell activation[77], and the increased levels of inflammation markers[78]. These markers of immune activation are found to be a better predictor of the course of infection than viral load or CD4 T cell levels[79, 80]. Interestingly apathogenic SIV infection in the natural monkey host is characterized by the absence of chronic immune activation[81].

Chronic inflammation induced by HIV infection also leads to inflammatory changes in other tissues such as bones, kidneys and the vascular epithelium[82]. Mounting evidence from epidemiological and laboratory studies suggests that HIV infection possibly directly contributes to certain non-AIDS related illnesses such as osteoporosis, artherosclerosis, and kidney and liver dysfunction which develop in the course of HIV infection[83].

Immune deficiency/CD4 decline

The vast majority of HIV-infected individuals experience a progressive decline of CD4 T cells and increased immunodeficiency that ultimately leads to opportunistic infections (OIs) and death. The mechanism that drives the CD4 decline is not yet fully understood.

Recent advances in immunophenotyping of T lymphocytes[84] make it possible to classify lymphocytes into different subsets characterized by maturity/ development stage, senescence, activation stage, and function according to the presence of immune markers on their surface. While the understanding of the immune cell biology is evolving, there is, however, broad consensus to discriminate the following subtypes[85, 86, 87]. Naïve T lymphocytes, after emigration from the thymus, mainly reside in secondary lymphoid tissues (LNs) and on stimulation by their cogent antigen (AG) generate effector and memory cells. A broad distinction divides

memory T lymphocytes into central memory (Tcm) and effector memory (Tem) cells. Tcm lymphocytes traffic through the secondary lymph nodes, are long- lived and, similar to naïve T lymphocytes, have regenerative potential i.e. are capable of homeostatic proliferation. (Tem) lymphocytes are more prevalent in peripheral tissue and provide immediate effector function i.e. they can rapidly produce cytokines or cytolytic enzymes. (Tem) cells highly express the CCR5 co-receptor and thus are primary target for HIV infection.

Chronic immune activation is associated with an increased T cell turnover, particularly of the activated (Tem) cell subset. The heightened proliferation of susceptible target cells provides the virus with a fertile substrate for replication and in a vicious circle maintains immune activation[88]. Ultimately, the pool of naïve T lymphocytes and (Tcm) cells, which replenishes activated cells, is drained and in conjunction with reduced thymic output leads to regenerative failure of naïve CD4 T cells[89, 90, 91, 92]. Of note only a small proportion of CD4 T cells are productively infected in chronic HIV infection[93] and apoptotic cell death of un-infected bystander CD4 T cells plays an essential role.

Additional mechanisms contribute to the overall loss of immune competence. Particularly the quality of the CD8 T cell function gets progressively impaired, a process that is aggravated by the decline of robust HIV-specific CD4 T cell help[94]. CD8 exhaustion is reflected by the appearance of inhibitory molecules such as PD-1[95,96], and CTLA-4 and is associated with disease[97,98].

Maintenance of an HIV reservoir

A key strategy of HIV for persistence in the host and evasion of the immune system is the establishment of a viral reservoir in lymphoid tissue and in resting T cells. The network of the follicular dendritic cells (FDC) in the follicles of lymphoid tissue have the ability to trap replication-competent viruses on their surface without infecting the cell[99, 100]. Massive quantities of viruses are retained in the lymphoid tissue for months and years, forming an archive of genetically diverse viruses that have been created over time.

A second, cellular reservoir is formed in latently infected cells i.e. resting CD4 cells (memory T cells) that carry un-integrated or integrated HIV DNA that can give rise to infectious virus upon cellular stimulation[101]. Mathematical modeling of HIV dynamics has suggested that it will take up to 73 years to eliminate HIV in these reservoirs[102, 103]. Recent research focuses on approaches to purge these latent reservoirs in order to cure HIV infection[104, 105].

Natural protection

There are exceptions to the natural course of HIV infection as indicated by HIV-infected persons who control HIV infection over many years without signs of immune dysfunction. About 5-15% of HIV-infected individuals are thought to fall in the category of long-term non- progressors[106, 107, 108, 109] i.e. being infected for over 10 years with a stable high CD4 T count maintaining a low plasma viral load, while 1% (elite controllers) have undetectable plasma viral load. Also a number of individuals, mainly commercial sex workers (CSW) or partners in discordant couples, have been described who stay HIV-negative despite repeated, unprotected exposure (highly exposed persistently seronegatives, HEPS)[110].

Natural HIV resistance or delayed disease progression have been associated with certain genetic traits in the host and/or viral factors. Well documented among these features are polymorphisms/ deletions of the CCR5 co- receptor[111, 112], certain alleles within the HLA[113, 114] and the killer immunoglobulin-like receptor (KIR) genes[115], superior capacity of antiviral CTL immunity[116, 117], and attenuated viruses such as nef-deficient HIV strains[118].

Care and Prevention

Antiretroviral therapy

The introduction of drugs that are able to block viral replication at different stages of the viral lifecycle have revolutionized HIV management and have reversed the fatal outcome of HIV infection[119, 120]. As of 2011 four classes of ARV drugs (over 25 agents) are licenced which act by either inhibiting viral entry (fusion inhibitors and Co-receptor inhibitors), inhibiting the RT enzyme (NRTI, NNRTI), blocking integration of the virus into the host chromosome (INI), or interfering with viral assembly (PIs)[121, 122].

The current regimens used in first-line therapy combine antiretroviral drugs from two synergistic classes. The treatment aims, as stated by the US Department of Health and Human Services[123], are the following, namely to drive and maintain plasma HIV RNA viremia levels below limits of detection of most sensitive clinical viral load assays available (less than 50 copies/ml) in naïve and experienced persons; to preserve and restore immune function and prevent/delay clinical disease progression; to improve and maintain quality of life with potent, simple and well-tolerated once-daily regimens. Many newer combination antiretroviral regimens can achieve most of these aims.

Combination antiretroviral therapy (cART), however, is unable to eradicate the virus nor able to fully reconstitute the immune system. Importantly, even after longstanding suppressive cART there is evidence of persisting low-level viremia which could be caused by ongoing rounds of replication in intermittently activated latently infected cells or by episodic production of HIV by long-lived cells[104]. Also, whether a complete immunological recovery can be reached especially when cART is started in late disease is still doubtful. Areas of concern are the findings of accelerated senescence and the poor recovery of the gut-associated lymphoid tissue that was damaged in early disease. On the other hand, peripheral CD4 cell gain can continue up to 10 years and even in patients who started cART in late disease have reached normal T cell levels[124]. The choice of ARV combinations depends on cost/ availability, tolerability, convenience and genotypic pattern. In resource-limited settings drug costs play an important role in the selection of an ARV regimen[125].

Prevention of HIV infection

A vaccine is still regarded as the most desirable public health intervention but despite enormous research efforts and resource allocation an effective vaccine does not seem to be realistic in the next decade. Avoidance of contact with infected fluids/secretions (blood, breast milk, genital secretions) remains the most effective prevention method. Prevention of infection through behavior change and condom use have been advocated since more than a decade; however no convincing results have been reported up to date.

In recent years a number of public health interventions have been shown to potentially be effective to mitigate the epidemic. The enormous success of PMTCT[126, 127], the effect of ART to protect discordant couples[128], the efficacy of male circumcision to protect HIV-negative males[129] and the efficacy of pericoital use of microbicides by HIV un-infected women[130] or the oral use TDF in men[131] all give hope to be able to produce a prevention package tailored to the needs of individual persons.

Public Health Challenge

The HIV epidemic in Botswana

Botswana, a country in Southern Africa approximately the size of France with a population of 1.7 million people, with stable political leadership and a growing economy, with revenues coming largely from its diamond industry, had establish a countrywide health care infrastructure with impressive health statistics in the years since the country's independence in 1966. In the late 1990s these achievements were dramatically reversed with the explosive spread of the HIV/AIDS epidemic. By 2000 Botswana had the highest HIV-1 prevalence in the world, with an overall documented prevalence of 38% among pregnant women and 17.1% in the general population[132], life expectancy had decreased from 64 to 32 years between 1980 and 2000[133], incident tuberculosis rates doubled, and mortality among 25-54 year olds increased fourfold between 1990 and the early 2000s[134], resulting in devastating consequences for the nation's economy.

Efforts to stem the spread of HIV early in the epidemic through an extensive HIV information, education, and communication (IEC) campaign since 1988, national HIV sentinel surveillance since 1992, provision of voluntary HIV-1 counseling and testing since 1994, and implementation of a prevention of mother-to-child transmission program since 1999 did little to control the epidemic.

Response of the international community

The initial hesitation to introduce in Africa cART, which since 1995 had been proven to significantly reduce morbidity and mortality in the US and Europe, was based

not only on financial considerations but also on scientific concerns regarding the potential benefits of widespread ART in resource-limited settings. Specifically, existing knowledge about HIV-1 at that time was based on research among HIV-1 subtype B-infected persons, and information on the susceptibility of ARV medications to other HIV-1 subtypes was lacking; also most African communities lacked the degree of health care infrastructure that was thought to be needed for establishment of antiretroviral treatment (ART) programs; furthermore profound doubts were expressed about whether patients initiated on ART in rural or urban African settings would be able to maintain the high level of medication adherence that was required for successful viral suppression. These doubts were coupled with the fear of creating new ARV drug resistant epidemics[135, 136].

Throughout all sub-Saharan Africa, the humanitarian crisis that evolved from the HIV-1 pandemic is unparalleled for any infectious disease in modern times. Thus, in the early 2000 an impressive concerted response began to form, led by international agencies and the corporate world, in their commitment to assist nations in their fight against HIV/AIDS. Early steps in raising awareness and the formation of coalitions were initiated by concerned physicians and academicians who urged for access to HAART in resource-limited settings of the world[137, 138].

Botswana's response to the HIV epidemic

In 1996, in the absence of its own medical school, Botswana initiated a public-private partnership with the Harvard School of Public Health (HSPH), the initial goal being to better understand and to characterize HIV-1C infection, building research capacity and informing national and regional public policy. These activities significantly contributed to the establishment of the national PMTCT and the national ART program, two programs that have since proven highly successful and are amongst the most advanced in Africa. Botswana was the first country in sub-Saharan Africa to launch a national public ART program. Beginning in January 2002 from the first national ART site at the Infectious Disease Care Clinic (IDCC) in the capital city of Gaborone the daunting challenge of building a 'truly' national ART program[139] was responded to by the rapid national scale-up of ART facilities. By December 2010, approximately 130,000 persons were receiving public ART at more

than 150 designated sites across the country, corresponding to a coverage of over 80% among HIV-infected individuals qualifying for therapy.

Important components of the initial Botswana national ARV treatment guidelines[140] included the adult ART eligibility criteria of (i) the presence of an AIDS defining illness and/or (ii) having a CD4 cell count of less than 200 cells/mm^3, as well as the decision to regularly monitor plasma HIV-1 RNA levels, which differed substantially from other neighboring countries where this was not feasible due to laboratory and financial constraints. When Botswana announced its intent to start a countrywide cART program it was obvious that the choice of ARV regimens had to be made from a population rather than an individual patient's perspective. This public health approach is based on the assumption that the predominant virus circulating in the population is highly susceptible to the selected cART regimen, called the 'first-line' ART regimen. The first-line cART regimen consisted of two nucleoside reverse transcriptase inhibitors (NRTI's), namely zidovudine (ZDV) plus lamivudine (3TC) being given with one non-nucleoside reverse transcriptase inhibitor (NNRTI), namely efavirenz (for all males, and females without reproductive potential) or nevirapine (for all women having reproductive potential). Botswana also offered an alternate, second-line PI-based regimen for those failing their initially prescribed first-line regimen. Of note, this switch was to be performed without the use of genotypic resistance testing. Changes in the national 2008 HIV treatment guidelines[141] included the raise of CD4 count to qualify for cART to 250 cells /mm^3 and to introduce tenofovir as N(t)RTI component of the new first-line regimen The establishment and monitoring of these programs have been closely linked to past and ongoing basic scientific and clinical research, which will be described more extensively here.

Thesis objective

The aim of the thesis is to describe the evidence–based approach in guiding public health policy to control HIV infection in a generalized epidemic by using selected research findings that informed the implementation of a national ART program in Botswana.

Outline of the thesis:

Precursory studies

Important background information preceding the introduction of cART concerned knowledge of the reference level of clinical/immunological characteristics in the general population and the genotypic description of the prevalent circulating virus. The CD4-T lymphocyte count plays a crucial role in the monitoring and management of HIV infection. Variability in the normal values of white blood cells and of the CD4 cell subsets had been reported among different populations in Africa. In **Chapter 2** of the thesis we assess the T lymphocytes reference values of HIV un-infected adults in Botswana. The development of HIV drug resistance has been recognized as one of the major threats of successful introduction of ART in resource-limited setting. Knowledge of HIV-1 antiretroviral (ARV) drug resistance to HIV-1 subtype C (HIV-1C), the most prevalent subtype in sub-Saharan Africa (SSA), was sparse when Botswana considered to publically offer ART. **Chapter 3** presents the evaluation of population-level HIV-1C genotypes and their potential for ARV drug resistance before Botswana's public ARV treatment program began. In industrialized countries, it is recommended that HIV-infected adults undergo baseline screening for pathogens that might cause latent or active infections, such as syphilis, hepatitis B, hepatitis C, infection due to Toxoplasma gondii, and cytomegalovirus infection. A paucity of data exists from sub-Saharan Africa describing the prevalence of these pathogens. In **Chapter 4** we report sero-prevalence data for these pathogens among HIV-1–infected adults referred for initiation of ART in Botswana.

Program Implementation

The provision of ART through the public health care system necessitated multiple adaptation of the existing health care infrastructure. **Chapter 5** describes the challenges and lessons learnt from establishing the first public ART clinic in Botswana. The efficacy, tolerability and optimal adherence strategies of ARV medication for populations in SSA, particularly those infected with HIV-1C were largely unknown when ART was introduced in Botswana. The "Tshepo" Study was the first large scale, randomized, clinical trial that addressed these important issues among HIV-1 subtype C–infected ARV treatment–naive adults in southern Africa.

Chapter 6 provides the results of this randomized trial. The majority of HIV-infected women in Botswana and SSA in need of highly effective antiretroviral therapy are of reproductive age. Pregnancy rates are usually high in developing countries, and efavirenz (EFV) use in women of childbearing age is of concern because of its potential teratogenicity. **Chapter 7** studies the pregnancy outcome of women on EFV containing cART in the 'Tshepo' clinical trial. Numerous ART initiatives have now been established in many SSA countries showing early benefits. **Chapter 8** reviews the outcome of the first ART initiatives.

Program Outcomes

A key component to successfully manage an ART program is the monitoring and evaluation of program goals and indicators. **Chapter 9** provides a model for affordable patient-level ART monitoring which can be used for program evaluation that was piloted at Princess Marina Hospital in Gaborone, Botswana's first public ART outpatient clinic. While ART initiatives in many SSA countries show early benefits, to date, few results are available concerning long-term clinical outcomes in these treatment programs. **Chapter 10** analyzes the long-term treatment outcome of the first patients who initiated public ART in Botswana. Development of transmitted (primary) and/or acquired ARV drug resistance is a major obstacle for the successful delivery of ARV therapy. WHO has designed a survey to monitor the emergence of transmitted drug resistance in resource-limited settings. **Chapter 11** assesses the level of transmitted drug resistance in Botswana five years into the national ART program and provides a critical review of the proposed methodology.

Chapter 2

Low CD4+ T-lymphocyte values in Human Immunodeficiency Virus-negative adults in Botswana

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Abstract:

CD4+ lymphocyte counts (LCs) play a crucial role in the management and monitoring of HIV infection. Variability in CD4+ LCs has been reported to occur as a result of measurement techniques and/or biological variation. We report the CD4+ LCs of healthy human immunodeficiency virus (HIV)-seronegative adults in Botswana. Samples were obtained from HIV-seronegative blood donors. The median CD4+ LC was 726 cells/mm^3 (for females, 782 cells/mm^3 ; for males 698 cells/mm^3). The median CD8+ LC was 488 cells/mm^3 (for females, 494 cells/mm^3; for males 485 cells/mm^3). The median CD4+ to CD8 ratio was 1.57 (for females 1.66; for males 1.51). Our findings of low CD4+ LCs among HIV-negative adults in Botswana are significant and have important implications for the management of HIV disease in this sub-Saharan African population.

Key words: Africa, Botswana, CD4, HIV/AIDS, reference ranges

Introduction

CD4+ lymphocyte counts (LCs) are recognized as the most important measurement of overall human immunodeficiency virus (HIV)- induced immune impairment[28]. CD4+ LCs are an established predictor of disease-free survival and serve as an important guide in the decision to begin prophylactic interventions[27]. In addition, CD4+ LCs help to determine when to start combination antiretroviral therapy in routine clinical practice[8, 13]. CD4+ LCs also serve to monitor immune recovery in patients on antiretroviral therapy [2].

Variability in CD4+ LCs among healthy persons has been widely reported, and has been attributed both to biological influences and to differences in methodology used for T cell enumeration. Biological factors that influence CD4+ LCs include gender[22,32], age[16, 39], exercise and diurnal variation[19, 23, 30], pregnancy[37, 41] and co-morbid medical conditions. Variation in the distribution of white blood cell counts and, specifically, CD4+ cell counts among ethnic groups, has also been reported. Published reference ranges for CD4+ LCs in HIV-negative populations from Africa and Asia vary widely[1, 10, 20, 28, 29, 39, 40, 43, 44, 45]. Importantly, some of the reported values are significantly lower than the values established for North American and European cohorts, the population in which the kinetics of CD4+ LC decline in HIV disease are best documented[14, 29, 39].

Flow cytometry, the present reference method used to count the absolute numbers of CD4+ T cells, is a rapidly evolving diagnostic approach utilizing various permutations in the techniques used. These variations highlight the need for a standardized methodology to ensure that precise and reproducible CD4+ LCs are obtained. Significant inter-laboratory CD4+ LC variability has been reported by use of the conventional two-step procedure, which couples the percentage of CD4+ cells obtained by flow cytometry with the absolute lymphocyte counts obtained with a hematology analyzer (dual-platform technology)[3, 7, 11]. Recent recommendations from the Division of AIDS, United States National Institutes of Health, favor the use of single-platform methods which directly count the absolute CD4+ LCs values from a single tube[4, 6, 12, 34, 35].

Botswana, where HIV prevalence is one of the highest in the world, began a national antiretroviral treatment program in the public sector in January 2002. The Botswana Ministry of Health initially chose to offer highly active antiretroviral therapy (HAART) to all symptomatic HIV-infected citizens (those with an AIDS-defining illness) and/or those with a CD4+ T lymphocyte value of less than 200 cells/mm^3 [5]. International consensus guidelines for adults recommend that physicians consider initiating HAART when CD4+ LCs are higher (less than 350 cells/mm^3), especially in patients with plasma HIV-1 RNA levels greater than 50,000-100 000 copies/ml[9], or with rapid decline in CD4+ LCs. Botswana's decision to initiate HAART when CD4+ LCs are lower was influenced largely by logistical and financial considerations, with HAART initially offered to those who clinically needed therapy the most. The normal ranges of CD4+ LCs in HIV-negative individuals have not yet been described in this region of Africa. Differences in normal cell count ranges from this region of the world may lead to a local reevaluation of treatment guidelines that have been based on findings of studies with non-African cohorts.

The goal of this study is to describe CD4+ LC reference ranges among HIV-negative adults in Botswana by evaluating blood specimens obtained from two distinct populations: (i) blood donors and (ii) participants of the Botswana 2001 HIV Sentinel (Seroprevalence) Surveillance. Sentinel surveillance has been carried out annually in Botswana since 1992 to monitor the course of the HIV epidemic.

Materials and Methods

Populations. (i) Blood Donors: From August to October 2001, whole blood samples were collected from adult blood donors at the National Blood Transfusion Center at Princess Marina Hospital in Botswana's capital city, Gaborone. All blood donors were screened for the following conditions: weight loss, lung disease, tuberculosis, abdominal disease, heart disease, low or high blood pressure, kidney disease, epilepsy, diabetes mellitus, rheumatic fever, cerebrovascular accident, circulatory problems, venereal disease, allergies or asthma, goiter, jaundice, liver disease, and malaria. Potential donors exhibiting any of the above conditions were disqualified from donation. Additionally, a history of recent or current injections, vaccinations, medicines, or major surgery, or a recent illness could exclude potential donors.

(ii) The 2001 Sentinel Surveillance Participants: Women and men presenting at 11 representative health districts from July to September 2001 were included in this study. The women were presenting for the first time during their current pregnancy for antenatal care (ANC) and the men were presenting for symptoms suggestive of sexually transmitted infections (STI) .

This study was reviewed and approved by the Health Research Development Unit (HRDU) (ethical review board) of the Botswana Ministry of Health.

Sample collection and processing: Unlysed whole-blood samples from both populations were collected in tubes containing EDTA (Becton Dickinson) to prevent clotting. Blood donor samples were collected in the afternoon and were stored at ambient temperature (10-20° Celsius) until testing, which was completed within 36 hours of blood collection. The blood samples from Sentinel Surveillance participants were collected at participating sites each morning and were transported to the Botswana-Harvard HIV Reference Laboratory at ambient temperature. These samples were labeled with a serialized code number that could not be linked back to individual participants, and that corresponded to numbers on abbreviated demographic questionnaires. To be included in the analysis, samples had to (i) reach the laboratory within 24 hours of collection (ii) be properly labeled, and (iii) exhibit adequate sample integrity (i.e. no visible clotting and/or hemolysis). All samples were processed within 12 hours of arrival at the Botswana-Harvard HIV Reference Laboratory, which is within the allowable time limit stipulated by the instructions of the manufacturer.

Sample testing and quality assurance: Screening of all samples for HIV type 1 (HIV-1) was performed by parallel testing by enzyme-linked immunosorbent assay (ELISA) with the Murex HIV (version 1.2.0) assay (Abbott Pharmaceuticals, Inc.) and the HIV-1/HIV-2 AB-Capture ELISA Test System (Ortho-Clinical Diagnostics, Inc) to detect the presence of HIV-1 and HIV-2 antibodies.

A FACSCount fluorescence-activated cell sorter (FACS) system (Becton Dickinson) was used to enumerate absolute values for CD4 (helper/ inducer T lymphocytes) and CD8 (suppressor/ cytotoxic T lymphocytes) as well as CD4+ to CD8+ ratios for

each sample. FACSCount instrument performance was ensured by the following: (i) testing of numerous specimens for the concordance of the results by using a separate FACSCount instrument at an independent laboratory, (ii) regular servicing and maintenance of the FACSCount machine by Becton Dickinson and (iii) internal validation of the FACSCount machine against the Botswana-Harvard HIV Reference Laboratory's FACS Calibur flow cytometer. The performance of the Botswana-Harvard HIV Reference Laboratory is monitored regularly, as it participates in the quality assurance program of the United Kingdom National External Quality Assessment Scheme (UK-NEQAS) for Leucocyte Immunophenotyping.

Demographic data: Each Sentinel Surveillance participant was asked to complete an anonymous questionnaire that provided demographic information (including age, gender, and the location of the participating health facility) for the purposes of this study. Demographic data (age and gender) for the blood donors were retrieved from blood donor registration records.

Statistical analysis: Medians, means, ranges, 2.5^{th} to 97.5^{th} percentiles, standard deviations, and 95% confidence intervals for the mean were calculated for each immunohematological parameter. The non-parametric Wilcoxon rank-sum test was used to compare the distributions of immunohematological parameters by gender and population sampled.

Results

A total of 547 blood donor samples were collected, and of these, 437 (80%) tested HIV-negative on dual ELISA. Of the HIV-negative blood donors, 143 were female and 294 were male.

A total of 589 samples were collected from Sentinel Surveillance participants. Of these, 499 were suitable for T-lymphocyte subset enumeration, 251 (50%) of which were HIV-negative. 207 sentinel surveillance participants were female and 44 were male. Sentinel Surveillance samples were representative of both urban (61%) and rural (39%) populations from 11 health districts located throughout Botswana. The median age for HIV-negative female donors was 27 years (inter-quartile range, 19 to

35 years) and that for HIV-negative males was 29 years (inter-quartile range, 23 to 36 years). The median age of HIV-negative female antenatal clinic attendees was 23 years (inter-quartile range, 20 to 28 years) and that for HIV-negative male STI clinic attendees was 26 years (inter-quartile range, 21 to 28years).

Summary statistics and ranges for absolute CD4+ LC, CD8+ LC and CD4+ to CD8+ ratios are reported in Tables 1, 2, and 3 respectively. Among the HIV-negative blood donors, the median absolute CD4+ LC was 726 cells/mm^3, the median absolute CD8+ LC was 488 cells/mm^3, and the median CD4+ to CD8+ ratio was 1.57. Among the blood donors differences were seen by gender for CD4+ LCs ($p<0.001$) and CD4+ to CD8+ ratio ($p=0.016$) but not CD8+ LCs ($p=0.36$); women had higher median CD4+ LCs and CD4+ to CD8+ ratios.

Table 1. CD4+ lymphocyte counts (cells/mm^3) of HIV-negative adults in Botswana

Group	Sex	n	Median	Range	2.5th-97.5th Percentile	Mean	S t d Dev	95% CI for mean
Blood Donor	Female	143	786	344 - 1558	438 – 1328	827	245	(787, 868)
	Male	294	698	171 - 1652	366 – 1252	725	238	(698, 753)
	Both	437	726	171 - 1652	366 – 1318	759	245	(736, 782)
Sentinel Surveillenace	Female	207	612	152 - 1245	276 – 1062	626	200	(599, 653)
	Male	44	591	208 - 1282	233 – 1276	626	246	(551, 701)
	Both	251	599	152 - 1282	275 – 1114	626	208	(600, 652)

Table 2. CD8+ lymphocyte counts (cells/mm^3) of HIV-negative adults in Botswana

Group	Sex	n	Median	Range	2.5th-97.5th Percentile	Mean	Std Dev	95% CI for mean
Blood Donor	Female	143	494	155 - 1198	228 - 1062	523	203	(490, 557)
	Male	294	485	90 - 1573	178 - 994	502	205	(479, 526)
	Both	437	488	90 - 1573	190 - 1014	509	205	(490, 528)
Sentinel Surveillance	Female	207	428	138 - 1457	178 - 791	450	171	(427, 474)
	Male	44	491	228 - 1325	232 - 1299	577	294	(488, 666)
	Both	251	434	138 - 1457	179 - 985	473	203	(447, 498)

Table 3. CD4+ to CD8+ ratio of HIV-negative adults in Botswana*

Group	Sex	n	Median	Range	2.5th-97.5th Percentile	Mean	S t d Dev	95% CI for mean
Blood Donor	Female	143	1 66	0 59 - 3 86	0 89 - 3 18	1 72	0 57	(1 6 2 , 1 81)
	Male	294	1 51	0 45 - 5.83	0 76 - 2 82	1 58	0 60	(1 5 2 , 1 65)
	Both	437	1 57	0 45 - 5 83	0.76 - 2 88	1 63	0 60	(1 5 7 , 1 68)
Sentinel Surveillance	Female	207	1 44	0 19 - 3 44	0 79 - 2 75	1 50	0 53	(1 4 3 , 1 57)
	Male	44	1 18	0 25 - 2 63	0 26 - 2 51	1 29	0 57	(1 1 1 , 1 46)
	Both	251	1 40	0.19 - 3 44	0 63 - 2 71	1 46	0 54	(1 . 4 0 , 1 53)

* CD8+ LC values >2000 cell/mm³ were set to 2000 cell/mm³ when calculating the CD4+ to CD8+ ratio

Among the Sentinel Surveillance population, the median CD4+ LC was 599 cells/ mm³, the median absolute CD8+ LC was 434 cells/mm³, and the CD4+ to CD8+ ratio was 1.40. No significant differences were detected when median CD4+ LCs were compared by gender (p=0.58). Males had significantly higher median CD8+ LCs (p=0.029) and lower CD4+ to CD8+ ratios (p=0.009).

The CD4+ LCs and CD4+ to CD8+ ratios were significantly higher for the male and female participants in the blood donor population (Table 4) that those for male and female participants in the Sentinel Surveillance population.

Table 4. Comparison of lymphocyte subsets for study populations by gender

Population/Group Characteristic	Subgroup Median		Wilcoxon p-value
Females:	Blood Donor	ANC	
CD4+ LC (cells/mm³)	786	612	<0.001
CD8+ LC (cells/ mm³)	494	428	0.001
CD4+/CD8+	1.66	1.44	<0.001
Males:	Blood Donor	STI	
CD4+ LC (cells/mm³)	698	591	0.005
CD8+ LC (cells/ mm³)	485	491	0.40
CD4+/CD8+	1.51	1.18	0.001

Discussion

The primary objective of this study was to characterize CD4+ LCs among representative populations of HIV-negative adults in Botswana. The median CD4+ LCs for our study populations were lower than the reference values from Tanzania, Uganda, Cameroon, the Central African Republic, and Ethiopia. The median CD4+ LCs for samples collected in Botswana are also lower than those for the European control group evaluated in the Ethiopian study[20, 28, 39, 40, 46] (Table 5).

Table 5: Comparison of reported CD4 reference values from populations and technology used

Population	Description	N	CD4+ lymphocyte counts (cells/mm^3)				Technology used
			Mean	SD	Median	2 5-97 5 percentile range/ range†	
Botswana (present study)	ANC and STI Sentinel Survey participants	251	626	208	599	275 - 1114	FACSCount
Botswana (present study)	Blood donors	437	759	245	726	366 - 1318	FACSCount
Tanzania [17]	Local population near hospital	147	980 (*)	310	968	372 - 1588	FACSCount
Cameroon [36]	Healthy visitors/ guardians at hospital	203	980 *			350 - 1610	FACSCount
Uganda[20]	Visitors to AIDS Info center	183	1256 *			559 - 2333	FACScan
Ethiopia [18]	Factory workers	142	775 *	225	761	366 - 1235	FACScan
Dutch [18]		1356	993 (*)	319	950	509 - 1761	FACScan
Central African Republic [24]		Male 68 Female 82	927 940	349 291	851 912	380 - 1617 386-1454	FACS Calibur

*Women reported to have higher values than men

(*) gender comparison not reported

† 95 percentile ranges are presented for all but the Tanzanian and Cameroonian populations For the Tanzanian study we estimated the 2 5-97 5 percentile using mean±1 96*SD The 2 5-97 5 percentile range was not available for Cameroon so the range is presented

Previous studies show that population differences in CD4+ LC reference ranges are influenced by a variety of factors including genetics and environmental characteristics. Howard and Fasano [14] found significantly different CD+ LCs among healthy Asian and Non-Asian populations living in the United States. In two other

studies[29, 39], Dutch control groups had significantly higher CD4+ LC values than the investigated Ethiopian population.

International working groups have recently addressed the concern of inter-laboratory variability in absolute CD4+ LC measurement[25, 33] especially when various T-lymphocytes immunophenotyping methods are used. The absolute CD4+ LCs in the present study were measured with the FACSCount system, a single platform technology which is regarded as a reliable and robust method for the enumeration of CD4+ lymphocytes[36] and with excellent performance in terms of inter-laboratory variability[3, 6, 21]. Two other studies that used the same technology and that were conducted with different African populations (Cameroonians and Tanzanians) reported higher CD4+ LCs (Table 5). These results indicate that absolute CD4+ LC vary among different populations and classify our HIV–1 negative Botswana populations as having comparably low absolute CD4+ LCs. The median CD4+ LC values among potentially representative study populations within Botswana itself vary considerably: 726 cells/mm^3 for blood donors and 599 cells/mm^3 for Sentinel Surveillance participants.

These differences may be due to a multitude of factors including host factors like gender and pregnancy, varied specimen collection times, and the level of screening for possible confounding co-morbid medical conditions. Recent studies show that HIV-negative females have higher average CD4+ LCs as compared to males[22, 32,38], a finding supported by the results of our studies with both populations, particularly blood donors. Similar studies also report that CD4+ LCs decline during early pregnancy in women without HIV infection, [18, 37]. The findings from a more recent study characterizing the effects of pregnancy on CD4+ LCs (conducted with HIV-infected pregnant women) are less conclusive[41]. The effect of pregnancy may have decreased absolute CD4+ LCs in the Sentinel Surveillance population, thus decreasing the differences between males and females, and accentuating the differences in CD4+ LCs when compared to the blood donor population.

CD4+ LCs are influenced by the presence of co-morbid medical conditions such as Mycobacterium tuberculosis infections[42]. Data derived from the Sentinel

Surveillance population primarily comprise those for women presenting for ANC in early pregnancy and men presenting with symptoms referable to underlying sexually transmitted infections (who have a very high risk for HIV infection). Apart from these indicated conditions and the known negative HIV-1 test, nothing is known about the underlying health status of these participants. Given the high incident rates of tuberculosis in Botswana (an estimated 620 new cases per 100,000 inhabitants per year) [31], it is possible that individuals with active tuberculosis and even an acute retroviral infection (pre-seroconversion) may have been included in this analysis.

Large diurnal variation in CD4+ LCs has been reported[10, 23, 30] and these variations may have influenced the results obtained in the present study, as sample collection times were different for the two populations: blood was drawn from Sentinel Surveillance participants in the early morning, while blood was collected from blood donor participants in the afternoon.
Because of these potential limitations, we believe that the blood donor population is a more representative reference population. The CD4+ LCs for this group are somewhat higher than those for the Sentinel Surveillance participants, and are more consistent with findings from previous studies.

The absolute CD8+ LCs for our study population were comparable to the absolute CD8+ LCs reported from European blood donors [39]. The CD8+ LCs for adults in Botswana, however, are lower than those reported in other African populations. The median Botswana CD4+ to CD8+ ratio (1.18 – 1.66) therefore lies between those found in populations with high CD4+ LCs and low CD8+ LCs (Europe) and those with low CD4+ LCs and high CD8+ LCs (Ethiopia).

Limitations: There were several limitations to this study. All the information on the form used to screen blood donors was filled out by blood donors and was not independently verified. It is therefore possible that some donors did not remember recent infections or use of medications, especially herbal and over-the-counter products. In addition, recent seroconversion would not have been detected by ELISA and would have been included in the HIV-negative analysis. Our method of

T-Lymphocyte subset counting (with a FACSCount system) does not include the CD4+ percentage, a parameter that has been shown to be less variable in some situations. The clinical utility of CD4+ lymphocytes as a percentage of total lymphocytes (especially for adult populations) is less well defined, yet guidelines recommend that they be reported in clinical trials [34].

Implications: Our finding of low CD4+ LCs among HIV-negative adults in Botswana adds to emerging data supporting the presence of significant population differences in reference CD4+ LCs. The pivotal role of CD4+ LCs in making decisions on initiating and monitoring of HAART in Botswana and other developing countries underscores the importance of establishing CD4+ LC reference ranges for local population.

Validation of the low CD4+ LCs observed in these populations will also be important, especially as predictors of the extent and rate of HIV disease progression. Internationally established CD4+ LC cut-off values for the management of HIV disease are derived from comparably higher baseline CD4+ LCs, yet the functional significance of smaller pools of CD4+ cells at the outset of HIV disease remains unclear. A cohort of individuals in the early stages of HIV infection (who are not yet receiving antiretroviral treatment) should be followed longitudinally, an approach which is currently in the early stages of implementation in Botswana.

The increasing reliance on CD4+ LCs as a means to govern the initiation and monitoring of HAART among HIV-infected individuals in resource-limited settings makes it imperative that standardized, precise, and affordable methodologies for CD4+ LC determination be used [16, 17]. In addition, implementation of uniform quality control procedures for routine clinical assays will be important [3, 24] to ensure inter- and intra-laboratory comparabilities of baseline and longitudinal CD4+ LC measurements.

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47. Summary
48. Current HIV-1 antiretroviral (ARV) drug resistance knowledge is limited to HIV-1 subtype B (HIV-1B). We addressed whether unique genetic and phenotypic properties of HIV-1 subtype C (HIV-1C), southern Africa's most prevalent subtype, may foment earlier and/or distinct resistance mutations. Population-level HIV-1C genotypes were evaluated

with respect to drug resistance prevalence before Botswana's public ARV treatment program began.

49. Viruses were genotyped from 11 representative districts of northern and southern Botswana, and consensus sequence from 71 individuals and 51 previously reported sequences from HIV-positive blood donors were constructed.
50. Phylogenetic analysis classified all 71 sequences but one, which exhibited pol gene mosaicism, as HIV-1C. The protease and reverse transcriptase coding region had no detectable known primary mutations associated with HIV-1B protease inhibitor (PI) drug resistance. Secondary mutations associated with PI drug resistance were found in all sequences Several HIV-1C–specific polymorphic sites were found across the pol gene Northern and southern Botswana viral sequences showed no significant differences from each other.

Chapter 3

HIV-1 subtype C drug-resistance background among ARV-naïve adults in Botswana

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Summary

Current HIV-1 antiretroviral (ARV) drug resistance knowledge is limited to HIV-1 subtype B (HIV-1B). We addressed whether unique genetic and phenotypic properties of HIV-1 subtype C (HIV-1C), southern Africa's most prevalent subtype, may foment earlier and/or distinct resistance mutations. Population-level HIV-1C genotypes were evaluated with respect to drug resistance prevalence before Botswana's public ARV treatment program began.

Viruses were genotyped from 11 representative districts of northern and southern Botswana, and consensus sequence from 71 individuals and 51 previously reported sequences from HIV-positive blood donors were constructed.

Phylogenetic analysis classified all 71 sequences but one, which exhibited pol gene mosaicism, as HIV-1C. The protease and reverse transcriptase coding region had no detectable known primary mutations associated with HIV-1B protease inhibitor (PI) drug resistance. Secondary mutations associated with PI drug resistance were found in all sequences. Several HIV-1C–specific polymorphic sites were found across the pol gene. Northern and southern Botswana viral sequences showed no significant differences from each other.

Population genotyping shows that without countrywide ARV treatment, HIV-1C–infected Batswana harbor virtually no primary mutations known to confer resistance to the three major HIV-1B ARV drug classes. Some secondary PI mutations and polymorphic sites in the protease enzyme necessitate continuous population monitoring, particularly after introduction of countrywide ARV treatment in Botswana. Although its PI resistance development rate and kinetics are not known, our data may suggest increased susceptibility and readiness of HIV-1C to develop resistance under drug pressure when the PI class of drugs are used.

Key words: ARV drug resistance; sub–Saharan Africa (Botswana); HIV-1 subtype C; genotype polymorphisms

Introduction

Transmission of drug-resistant virus in the AIDS epidemic has been increasingly documented from different regions in the world (Geretti *et al.*, 2001; Geretti *et al.*, 2002; Boden *et al.*, 1999; Salomon *et al.*, 2000; Frater *et al.*, 2001). An increase in ARV drug resistance, from 8% to 22.7% among recently infected persons in the USA, has been reported from 1995 to 2000 (Little *et al.*, 2002). Drug-resistance rates of up to 29% among seroconverters or treatment naïve, chronically infected persons have been estimated in cohorts from the UK (UK Collaborative Group, 2001), Spain (Briones *et al.*, 2001) and Belgium (Van Vaerenbergh *et al.*, 2001). Significantly lower rates were reported from other cohorts (Descamps *et al.*, 2001),(Perno *et al.*, 2002), (Yerly *et al.*, 1999), (Bennett *et al.*, 2003), (Jayaraman *et al.*, 2003) (Chaix *et al.*, 2003), including a large European multi-country cohort (Wensing *et al.*, 2003). Little information is available on baseline drug resistance in non-industrialized countries, and especially from regions of the world where HIV-1C infection predominates (Vergne *et al.*, 2003; Adje-Toure *et al.*, 2003; Gordon *et al.*, 2003; Shafer *et al.*, 1997). Specific biological characteristics of HIV-1C, including high genetic diversity, may potentiate the emergence and spread of ARV drug-resistant HIV strains (Novitsky *et al.*, 1999; Montano *et al.*, 1997; Montano *et al.*, 1998; Montano *et al.*, 2000), which warrants further studies.

Botswana has one of the highest rates of HIV-1 infection prevalence in the world, with HIV-1C being the most prevalent subtype. The 2003 Botswana Sentinel Surveillance documented an HIV prevalence of 37.4% among pregnant women presenting for routine antenatal care (NACA, 2003). The 2003 estimate of HIV seroprevalence in Botswana is consistent with those obtained during the previous 4 years (35.4% to 38.5%).

In response to the epidemic, the Botswana government began comprehensive educational and primary prevention activities in the early 1990s, and in January 2002, it initiated its national ARV treatment program, providing public highly active antiretroviral therapy (HAART) to all qualifying citizens. It was estimated that approximately 110,000 HIV-infected individuals in Botswana urgently need HAART. The national ARV treatment program began at Princess Marina Hospital,

in the capital Gaborone, and has expanded to 21 sites; as of September 2004, approximately 21,000 persons were receiving publicly funded HAART. Adults eligible for HAART must have symptomatic HIV disease (AIDS-defining conditions) and/or a CD4+ cell count of less than 200 cells/mm^3. Current first-line treatment consists of zidovudine plus lamivudine (given together as Combivir™) with either efavirenz (male) or nevirapine (females with reproductive potential). Adults who fail first-line treatment are presently offered didanosine, stavudine and nelfinavir in combination.

The purpose of this study was (i) to analyze the baseline ARV drug-resistance patterns in Botswana before widespread use of ARVs, (ii) to determine potential regional differences in drug resistance, and (iii) to further characterize the biologic and virologic properties of the HIV-1C virus. We analyzed 2001 countrywide HIV Sentinel Surveillance samples for baseline drug resistance of HIV-1C by genotyping a representative number of samples from the northern and southern parts of the country.

Materials and Methods

Study subjects

From July to September of 2001, the 2001 Botswana HIV Sentinel Surveillance enrolled women attending antenatal clinics (ANC) and men with symptoms of sexually transmitted infections from 22 health districts of Botswana. A total of 71 samples from documented HIV-1 infected adults were randomly selected from 11 representative health districts, as shown in Fig. 1.

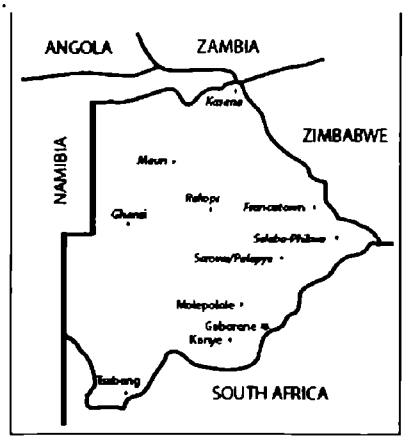


Fig 1: Map of Botswana depicting the 11 national health districts used in this study. Kasane, Francistown, and Gaborone are major international border posts

Although the status of ARV use was not documented among 2001 Sentinel Surveillance participants, it is highly unlikely that participants knew their HIV status or were receiving HAART at or before the time of blood collection. The availability of HAART was very limited in Botswana prior to the official opening of the national ARV program in January of 2002. Sample collection for the purposes of this analysis was completed by September 2001.

Therefore, the probability that analyzed samples represented circulating ARV-naïve HIV-1 strains is relatively high. The Institutional Review Board of the Ministry of Health, Botswana, approved the study. Screening for antibodies to HIV-1 was performed by double EIA testing using Murex HIV 1.2.0 (Abbott Pharmaceuticals, Abbott Park, Il, USA) and Ortho HIV-1/HIV-2 AB-Capture ELISA Test System (Ortho-Clinical Diagnostics, Rochester, NY, USA) assays.

Resistance testing

Plasma virus was analyzed for the presence of drug-resistant mutations. Viral genotyping was performed by using ViroSeq HIV-1 Genotyping System (Celera Diagnostics, formerly Applied Biosystems, Alameda, CA, USA), according to the manufacturer's instructions. Briefly, HIV-1 RNA was extracted from viral particles pelleted from plasma by isopropanol/ethanol precipitation, converted to cDNA and amplified in PCR. The amplicon represented the HIV-1 pol region spanning the entire protease (PR) and 335 codons of the reverse transcriptase (RT). The PCR product was then sequenced (both strands) using Big Dye chemistry on an ABI 3100 Genetic Analyzer.

Consensus sequences

HIV-1C RT and PR extended consensus sequences (Novitsky *et al.*, 2002) were built for the following subsets of sequences: northern Botswana 2001 (BW North); southern Botswana 2001 (BW South); a total Botswana consensus sequence (BW) constructed from 121 sequences, including 70 sentinel sequences plus 51 nearly full-length genome sequences from ARV treatment-naïve blood donors (Novitsky *et al.*, 2002); and South African, KwaZulu-Natal sequences (ZA) (Gordon *et al.*, 2003).

Analysis

Comparison of amino acid residue frequencies at polymorphic sites on different sequences was performed using SAS statistical software with Fisher's Exact test for R x C tables. All tests used a significance level of 0.10 percent level (p=0.001). No adjustments were made for multiple comparisons. We used Monte-Carlo methods to estimate p-values using StatXact software (Cytel Software) for amino acid residue at PR position 63 and RT positions 135, 207 and 334. The HIV-1 subtype B and C reference sequences from HIV-1 infected treatment-naïve patients (HIV-1B ref and HIV-1C ref, respectively) were retrieved from the Stanford HIV RT and Protease Sequence Database (http://hivdb.stanford.edu/hiv/).

Phylogenetic analysis was performed to determine the HIV-1 subtype and to characterize the phylogenetic relationship between sequences. The HIV-1 subtyping was performed using a set of reference sequences obtained from the HIV Sequence Database(http://www.hiv.lanl.gov/content/hiv-db/SUBTYPE_REF/align.html).

We also compared the pol regions of the 71 HIV-1C 2001 Botswana sequences to isolates from South Africa, Ethiopia, India, Brazil and HIV-1 subtype references. The multiple alignment of RT and PR sequences was performed by ClustalX (version 1.81) (Thompson *et al.*, 1997), accompanied by manual editing using BioEdit (Novitsky *et al.*, 2000). Determination of the pairwise distances of nucleotide alignment was performed by DNADist with the Kimura two-parameter model. Pairwise distances between translated amino acid alignments were computed by PROTDist with the PAM model. Both DNADist and PROTDist are components of the PHYLIP package (phylogeny inference package, versions 3.52c and 3.572c; University of Washington, Seattle).

The neighbor-joining method was employed for tree-building. For the recombinant analysis, we employed the neighbor-joining bootscan analysis with a sliding window of 300 bp incremented by 10 bp with reference sequences of HIV-1 subtypes A1, A2, B, C, D, F1, F2, G, H, J, K and known recombinants, using SimPlot (Lole *et al.*, 1999). The possibility of unidentified recombinant viruses cannot be excluded with certainty because only the pol region was analyzed in this study.

Accession numbers

The 71 new HIV-1C nucleotide sequences from Botswana were deposited in GenBank under accessions numbers AY829268 to AY829338.

Results

HIV-1 subtyping

Of the 71 samples, 70 (36 from northern Botswana and 34 from southern Botswana) were identified as HIV-1 subtype C strains based on their phylogenetic relationship (Fig. 2). One sample (01BW2413) from the southern part of Botswana did not cluster within HIV-1C. We performed neighbor-joining bootscan analysis for this sample within the available 1,302 bp that spanned the entire protease and 5'-portion of reverse transcriptase (data not shown). The bootscan analysis revealed that sample 01BW2413 could be classified as a complex A/J recombinant virus similar to a viral isolate described elsewhere (Novitsky *et al.*, 2000). Within the new set of 70 HIV-1C from Botswana, no subcluster(s) of the geographically different sequences (BW North and BW South) was found (Fig. 2). According to our results, 70 (98.6%) of 71 new sequences belong to HIV-1C, which confirms our previous finding (Novitsky *et al.*, 2002) that HIV-1C is the predominant circulating viral strain in Botswana.

Analysis of genetic distances

The pairwise nucleotide and translated amino acid distances for the Botswana and South Africa HIV-1C PR and RT are shown in Table 1. We compared genetic distances within the entire set of 196 HIV-1C sequences (121 from Botswana and 75 from South Africa) (Gordon *et al.*, 2003). The Botswana sequences included subsets of 34 new sequences from the south of Botswana, 36 sequences from the north of Botswana, and 51 previously described isolates (Novitsky *et al.*, 1999; Novitsky *et al.*, 2002; Ndung'u *et al.*, 2000). The mean value of genetic distances within PR and RT was about 5% on both nucleotide and amino acid levels, with minimal variation between the subsets (Table 1). Interestingly, genetic distances were significantly greater in Botswana samples than in South African samples ($p<0.001$). Distances among the southern Botswana subset were greater than those of the northern Botswana subset ($p<0.001$).

Table 1. Nucleotide (Nt) and translated amino acid (Prot) distances within the subsets of HIV-1C protease and reverse transcriptase (RT) sequences, mean values (%).

	Distances	BW* n=121	ZA** n=75	HIV-1C† n=196		BW South n=34	BW North n=36
Protease	Nt	5.6		4.9	5.4	5.9	5.0
	Prot	5.8		5.6	5.8	5.8	4.8
RT	Nt	5.6		5.0	5.5	5.6	5.0
	Prot	4.8		4.5	4.7	4.6	4.3

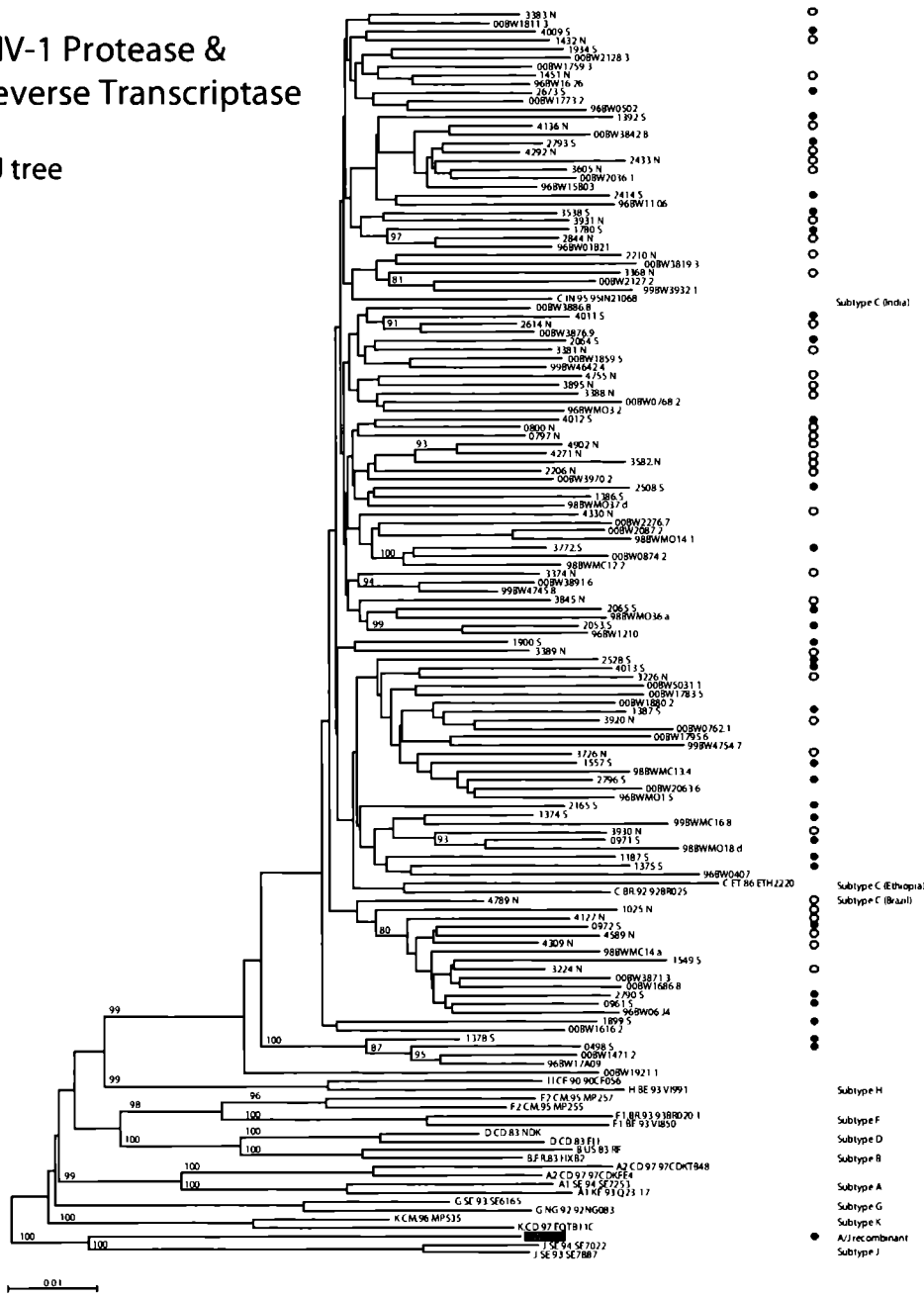
* BW, Botswana. ** ZA, South Africa † Combined total for BW and ZA.
Comparison of nucleotide and translated amino acid distances between the subsets of HIV-1C protease and reverse transcriptase sequences revealed significantly higher diversity in Botswana than in South Africa, and in the Botswana South as compared with the Botswana North subset ($p<0.01$ for all comparisons).
The pairwise distances of nucleotide alignment was performed by DNADist with the Kimura two-parameter model. Pairwise distances between translated amino acid alignments were computed by PROTDist with the PAM model.

Presence of mutations known to cause drug-resistance in HIV-1 subtype B

No primary drug-resistance mutations (mutations that reduce drug susceptibility by themselves (Shafer, 2002)) to the PIs were detected in any of 70 BW North and BW South samples. Secondary mutations (mutations that are associated with drug resistance in isolates containing other mutations) were found at the following positions in the PR enzyme: L10I, K20R, M36I, L63P/A/Q/S/H/C/T/I, V77I and I93L. Each sequence had at least one known secondary PI drug-resistance mutation. Thirty-one sequences (44.3%) had two secondary resistance mutations, of which 26 had the combination of M36I + I93L.

Thirty sequences had three or four secondary mutations. Two mutations (D30N and V82A) were found within the previously reported sequences from Botswana (Novitsky *et al.*, 1999; Novitsky *et al.*, 2002; Ndung'u *et al.*, 2000) that have been primarily reported among HIV-1B-infected individuals treated with the protease inhibitors nelfinavir (D30N), or indinavir, ritonavir, or saquinavir (Shafer, 2002). No primary and/or secondary nucleoside RT inhibitor (NRTI) drug-resistance mutations were found in the RT coding region in any of the 70 BW North or BW South samples. At known HIV-1B resistance mutation sites in RT, amino acid residues not specifically associated with non-NRTI (NNRTI) drug resistance were found, namely one A98S (BW North), one V179T (BW South), and one G333E (BW South). One mutation (G190E) that confers resistance to efavirenz (NNRTI) was found in the set of 51 blood donors.

Fig. 2: Phylogenetic analysis of the pol region of HIV-1 sequences from 71 Botswana 2001 Sentinel Surveillance sequences.



Treetopology was inferred by neighbor-joining method and was based on the alignment of 1302 bp that spanned the entire protease and 5´-portion of the reverse transcriptase. Sequences from northern Botswana are designated by open circles; sequences from southern Botswana are designated by shaded circles.

Polymorphism in the PR and RT of Botswana sequences

The Botswana consensus sequence was built using 121 HIV-1C translated amino acid sequences. The PR coding region displayed multiple amino acid residues, in low frequency, which were unique when compared with other subtype C sequences (Fig. 3). The PR coding region also displayed a significant number (high frequency) of HIV-1C amino acid substitutions at the following PR positions: 12S (77%), 15V (91%), 19I (82%), 41K (93%), 69K (99%) and 89M (89%) (Table 2).

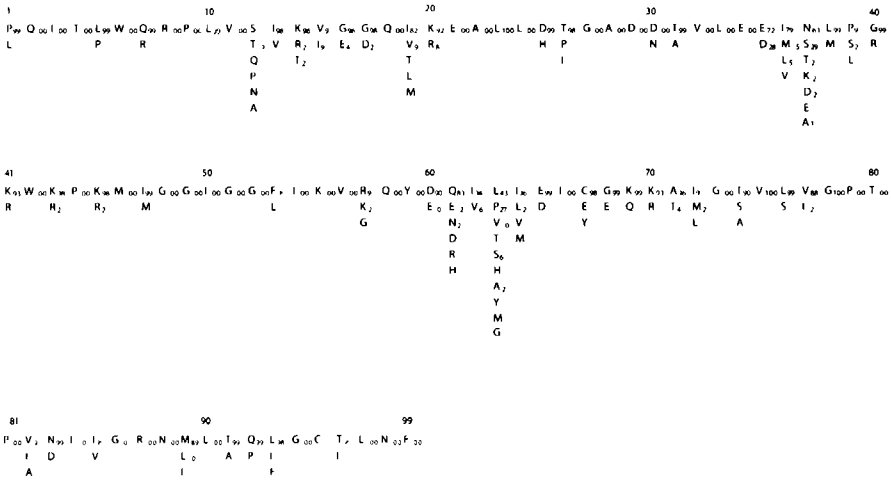
Similarly, within the RT coding region we found multiple low frequency polymorphic sites (Fig. 4) containing amino acid residues unique to subtype C sequences. In addition, significant numbers (high frequency) of HIV-1C-specific amino acid substitutions were found at the following RT positions: 35T (93%), 36A (81%), 39E (92%), 48T (93%), 122E (87%), 123G (31%), 173A (71%), 177E (78%), 200A (94%), 207E (48%), 211K (70%), 245Q (75%), 272P (77%), 277R (61%), 286A (59%), 291D (96%), 292I (94%) and 293V (89%) (Table 3).

Comparison of polymorphic sites in different sets of sequences

We compared sequences from the north (BW North) to those from the south (BW South) of Botswana. No significant differences were observed at any position within the PR region. However, there was a significant difference between the north and south at amino acid position 27 within the RT region ($p=0.02$), where a substitution from threonine (T) to serine (S) was found in 17% of northern sequences. This substitution was found in none of the southern Botswana sequences, and in only 3% of the ZA sequences (Gordon *et al.*, 2003).

We also compared HIV-1C reference sequences to the BW and ZA sequences. Within the PR coding region, amino acid residues at positions 19, 37, 41, 61, 63 ($P<0.0001$),12 ($P=0.0008$), 14, 77 ($P=0.003$), 93 ($P=0.005$) and 36 ($P=0.011$) of the BW sequences were significantly different than HIV-1C reference consensus sequences of the Stanford Database ($p<0.001$). BW sequences also differed significantly ($p<0.01$) from ZA sequences at positions 19 P=O.0049), 37 (P=0.0071, 14 (P=0.011) and 61 (P=0.019. See Table 2.

Fig. 3: Frequency and type of amino acid substitutions in protease gene from the Botswana consensus sequences built from 121 HIV-1 subtype C sequences.



Within the RT coding region, amino acid residues at positions 39, 60, 135, 173, 200, 207, 250, 334 (all p-values<0.0001) and 286 (p=0.0004), 48 (P=0.0012), 211 (P=0.0014), 248 (P=0.004),174 (P=0.014), 36 (P=0.024)and 122 (P=0.027) of the BW sequences were significantly different than HIV-1C reference consensus sequences of the Stanford Database (Table 3). BW sequences also differed significantly at amino acid positions 60 (p<0.0001) and 174, 250, 286 and 311 (all p-values<0.0001) compared to ZA sequences.

Discussion

We confirmed that the predominant HIV-1 virus in the Botswana epidemic is HIV-1C. Based on the geography of Botswana and the resulting trans-national migratory patterns of populations, dividing the country into a northern zone with active border crossings to and from Zambia and Zimbabwe, and a southern zone with population influx mainly from the Republic of South Africa, one might expect to see distinct differences in the HIV-1C epidemic within Botswana itself. Results obtained from phylogenetic analysis of the pol region in this study, however, do not suggest any regional clustering of viruses within Botswana. No phylogenetic distinctions within HIV-1C pol were found in samples from northern Botswana, southern Botswana,

or viruses reported from neighboring countries. Therefore, our data suggest that the AIDS epidemic in southern Africa is caused by a heterogeneous swarm of HIV-1C. Interestingly, one sequence showed a pol gene mosaicism that resembled the structure of a previously described HIV-1 A/J recombinant found in a patient with advanced AIDS in Botswana in 1998 (Novitsky *et al.*, 2000).

We studied the intra-subtype diversity of HIV-1C isolates from Botswana and South Africa (KwaZulu-Natal). Sequences from southern Botswana had increased sequence variability when compared to those from northern Botswana. Overall sequence diversity of samples from Botswana was higher when compared to South African isolates. These differences may suggest a more advanced and established HIV-1 epidemic in southern Botswana.

We also compared sequences obtained from ARV-naïve HIV-1C-infected persons with the geographically distinct sequences from Botswana and South Africa (Gordon *et al.*, 2003). Several significant polymorphisms in the PR and RT encoding region were found. This may suggest the evolution of geographically distinct strains or the influence of selective pressure from factors other than ARV treatment.

Our sequence data, obtained before Botswana initiated its public national ARV treatment program, document that there is virtually no known primary drug resistance to the three major classes of ARV drugs. Review of HIV-1 subtype C isolates obtained from ARV-naïve persons in Israel (Grossman et al., 2001), Ethiopia (Loemba *et al.*, 2002), Zambia (Handema *et al.*, 2003), Zimbabwe (Kantor *et al.*, 2002) and South Africa (Gordon *et al.*, 2003) corroborate our findings.

Table 2: Comparison of polymorphic codon sites (greater than 10%) in protease encoding region from treatment-naïve HIV-1B ref with HIV-1C consensus sequences from Botswana (BW), and KwaZulu-Natal South Africa (ZA), and a set of subtype C ref sequences.

PR Position	HIV-1B ref n=(1715)-(1947)	HIV-1C ref n=(341)-(362)	BW n=121	ZA n=75	Significance†
12	T_{88} S_4 A_3 P_2 I_2 N_1	S_{59} T_{36} P_3 A_2	S_{77} T_{19} Q_1 P_1 N_1 A1	S_{73} T_{19} P_4 A_4	§ p=0.0008 * NS
14	K_{89} R_{11}	K_{90} R_{10}	K_{96} R_2 T_2	K_{83} R_{13} T_3 S_1	§ p=0.0028 * p=0.011
15	I_{84} V_{16}	V_{84} I_{16}	V_{91} I_9	V_{93} I_7	§ NS * NS
19	L_{91} I_7 Q_1 V_1	I_{55} L_{28} V_9 T_7 Q_1	I_{82} V_9 T_7 L_1 M_1	I_{59} V_{23} T_{12} L_4 E1 A_1	§ p<0.0001 * p=0.0049
36	M_{87} I_{13}	I_{77} M_{11} V_8 T_2 L_2	I_{78} M_{16} L_5 V_1	I_{85} M_9 L_4 V_1 T_1	§ p=0.011 * NS
37	N_{65} S_{18} D_9 T_3 H_2 E_2 C_1 K_0	N_{60} K_{27} S_9 D_3 A_1	N_{63} S_{29} T_2 K_2 D_2 E_1 A_1	N_{78} S_{11} D_5 A_4 T_1 E_1	§ p<0.0001 * p=0.0071
41	R_{77} K_{23}	K_{66} N_{26} R_8	K_{93} R_7	K_{91} R_9	§ p<0.0001 * NS
61	Q_{98} E_2	Q_{96} E_4	Q_{83} E_{12} N_2 D_1 R_1 H_1	Q_{98} H_1 E_1	§ p<0.0001 * p=0.019
63	P_{53} L_{33} S_5 A_3 H_2 T_2 Q_1 C_1	L_{54} P_{28} V_6 T_5 S_4 A_1 H_1 I_1	L_{43} P_{27} V_{10} T_7 S_6 H_2 A_2 Y_1 M_1 G_1	L_{61} P_{22} V_7 T_5 S_1 Q_1 I_1 H_1 D_1	§ p<0.0001 * NS
69	H_{94} K_1 Q_3 Y_2	K_{98} Q_1 H_1	K_{99} Q_1	K_{98} Q_1 Y_1	§ NS * NS
77	V_{76} I_{24}	V_{95} I_5	V_{87} I_{13}	V_{92} I_8	§ p=0.003 * NS
89	L_{99} M_1	M_{85} L_{13} I_2	M_{89} L_{10} I_1	M_{88} L_{11} I_1	§ NS * NS
93	I_{76} L_{24}	L_{94} I_6	L_{98} I_1 F_1	L_{97} I_3	§ p=0.005 * NS

§ significant difference between HIV-1C ref and BW.
* significant difference between BW and ZA.
† NS, not significant.
Shaded: codon positions carrying mutations known to confer secondary drug resistance. P-values computed between frequencies of overall distributions of amino acid residues. Note: position 35 and 60 were polymorphic in both HIV-1B and HIV-1C consensuses.

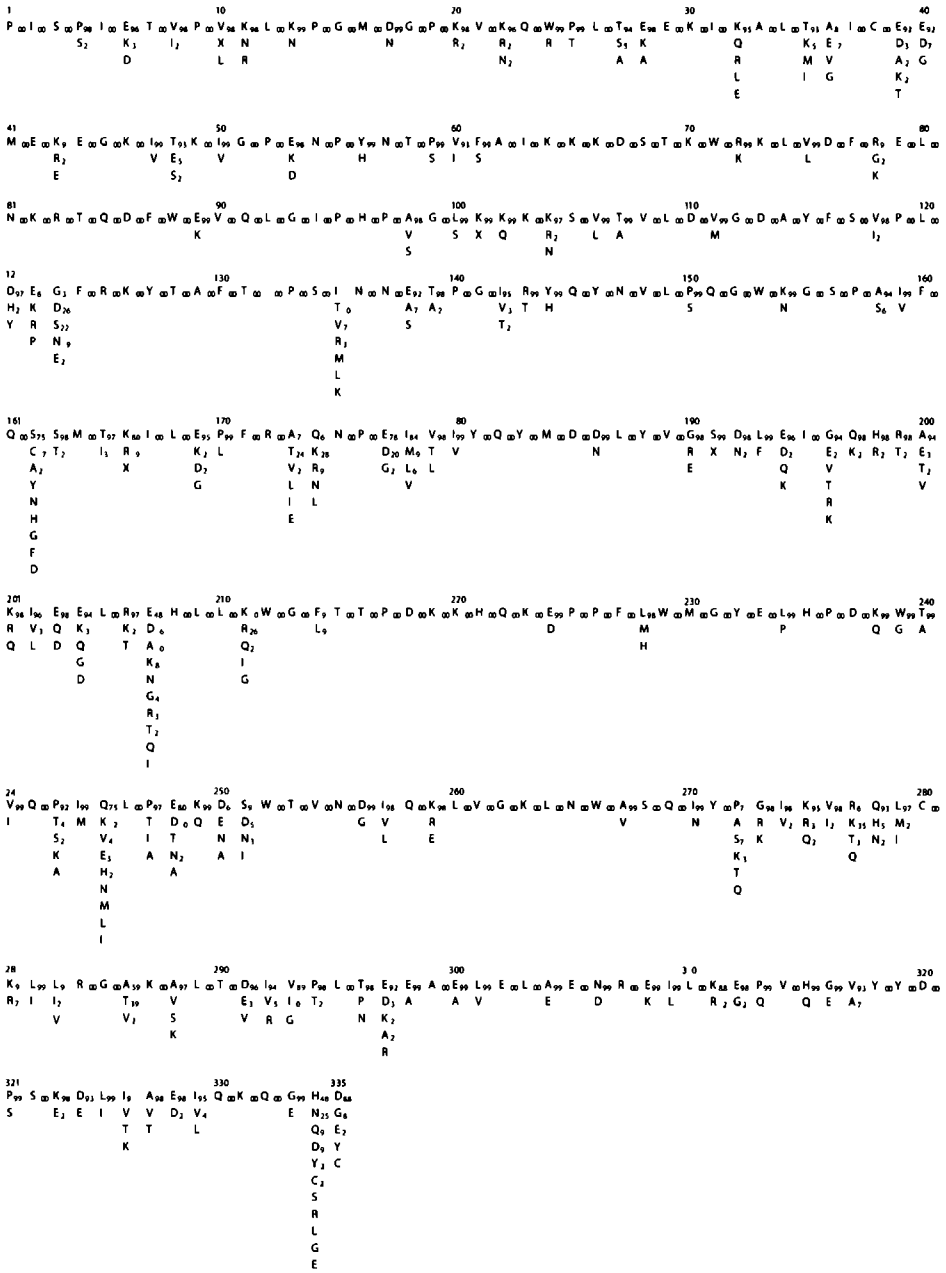
None of the mutations known to cause primary resistance to zidovudine, lamivudine, didanosine, stavudine or abacavir among HIV-1B-infected persons were found in the RT coding region of Botswana sequences. A few amino acid substitutions known to facilitate resistance were found at positions 211, 214 and 333. The F214L mutation in combination with R211K has been described to facilitate zidovudine and lamuvidine resistance (Shafer, 2002), and it was fairly common in our Botswana HIV-1C sequences (5 [7.1%] of 70 in our samples) (Loemba *et al.*, 2002). The G333E

mutation, reported in 6% of ARV-treatment-naïve HIV-1B-infected persons, facilitates zidovudine resistance in the presence of nucleotide excision mutations (Shafer, 2002). Additionally, none of the mutations known to cause primary resistance to nevirapine and efavirenz (mutations located between codons 98–108 and 179–190) were found in the RT coding region of our sequences. Only one sequence showed a substitution at position 190 (G190E)—a mutation that has been associated with reduced NNRTI susceptibility (Bacheler et al., 2001).

Two mutations associated with primary resistance to PIs (one D30N and one V82A) were found in the PR gene. However, a significant number of sequences harbored multiple secondary drug-resistance mutations in the PR coding region, namely L10I, K20R, M36I, L63P/A/Q/S/H/C/T/I, V77I and I93L (Shafer *et al.*, 1997; Grossman *et al.*, 2001). Drug resistance to PIs typically develops in a gradual fashion from the additive accumulation of primary and/or secondary mutations. Although the rate and kinetics of PI resistance are not known for HIV-1C, our findings may suggest an increased susceptibility and readiness of HIV-1C to develop resistance under selective drug pressure.

While the effects of naturally occurring substitutions in HIV-1C (mean of 10 PR and 14 RT substitutions per sequence in this study) on the kinetics and patterns of drug-resistance development are largely unknown, several studies have attempted to address their in vitro and biochemical significance. Velazquez-Campoy et al. suggested that the effect of drug-resistance mutations within PR may be enhanced by subtype-specific background polymorphisms (2002). Brenner *et al.* have shown that a common HIV-1C polymorphism at RT codon position 106 facilitates high-level multi-NNRTI resistance mutations (V106M) among patients under selective drug pressure, specifically those patients being treated with efavirenz (2003). A London cohort, evaluating HIV-1 non-subtype B- infected, ARV-naïve African patients, showed that the presence of specific baseline polymorphisms did not negatively influence future response to PI-containing HAART, but this is still preliminary as subjects have only had one year of follow-up (Frater *et al.*, 2001). Additional research to elucidate the impact of HIV-1C variability on the development of ARV drug resistance is needed.

Fig. 4: Frequency and type of amino acid substitutions in reverse transcriptase gene from the Botswana consensus sequences built from 121 HIV-1 subtype C sequences



In summary, our primary objective was to establish the background ARV drug-resistance profile for Botswana prior to the initiation of its large-scale national public ART program. Our results provide a rationale for offering PI-sparing (dual NRTI, single NNRTI) HAART as a first-line treatment in Botswana. Our data also emphasize the importance of monitoring ARV drug-resistance patterns over time, especially as the public ARV program is rapidly scaling up. Although genotypic resistance testing may become affordable in some settings, its large-scale use in sub-Saharan Africa for individual patient management is not at present economically feasible, thereby stressing the need for longitudinal population-based surveillance efforts guiding first- and second-line HAART choices. However it will also be important to characterize trends in the emergence of drug resistance among ARV-naïve and ARV-experienced individuals (Havlir et al., 2002; Wainberg & Friedland, 1998), as well as monitor drug-resistance trends among recently infected persons in this region of the world. Our data serve as an important benchmark for future regional population-based ARV resistance studies.

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Chapter 4

Serological evidence of HIV-associated infection among HIV-1 infected adults in Botswana

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Abstract

In industrialize countries, it is recommended that adults with human immunodeficiency virus type 1 (HIV-) infection undergo baseline screening for pathogens that might cause latent or active infections, such as syphilis, hepatitis B, hepatitis C, infection due to Toxoplasma gondii and cytomegalovirus infection. A paucity of data exist from sub-Saharan Africa describing the prevalence of these pathogens. We report data for HIV-1 infected adults referred for initiation of highly active antiretroviral therapy in Botswana.

Key words: Africa, HIV / AIDS, Botswana, Hepatitis B and C, Syphilis, Toxoplasma gondii, and Cytomegalovirus

Introduction

Adult patients with HIV infection and/or AIDS are at risk for co-infection with other sexually transmitted pathogens such as hepatitis B virus (HBV), hepatitis C virus (HBC), *Treponema pallidum*, and *herpes simplex virus* depending on their risk factors for HIV-1 infection. HBV is the leading cause of liver disease worldwide [1-2] and HIV-1 infection is associated with an increased risk for the development of chronic hepatitis B after HBV exposure. Numerous studies have documented a high rate of HCV infection (50-90%) among HIV-1 infected injection-drug users and persons with hemophilia [3-5]. Other possible modes of HCV transmission include mother-to-infant, needle-stick, or sexual transmission [5-10]. Documentation and close monitoring of persons who are co-infected with HIV-1 and HCV is needed because long-term studies now predict that between 2-20% of patients with chronic HCV infection develop cirrhosis within 20 years [11], and this rate of progression increases with older age, alcoholism, and HIV-1 infection [11-13].

HIV-1 infection also appears to alter the diagnosis, natural history, management, and outcome of *Treponema pallidum* infection [14-16]. In addition, HIV-1 infected adults with more advanced disease (i.e. those with CD4+ cell counts of < 100 cells/ mm^3) are at risk for illness due to re-activation of various viral and protozoan pathogens including Cytomegalovirus (CMV; which causes retinitis, colitis, and encephalitis) and *Toxoplasma gondii* (which causes encephalitis).

Very limited data are available on the serologic prevalence of syphilis, HBV infection, HCV infection, *Toxoplasmosis gondii* infection, and CMV infection among HIV-1 infected adults in sub-Saharan Africa, especially among HIV-1 individuals who are presenting for HAART initiation, and therefore have evidence of very advanced HIV-1 disease. The majority of existing literature is from West or East Africa and reports the serologic prevalence of these important disease pathogens among at-risk adult populations (i.e. women, blood donors, prisoners) with unknown HIV-1-infection status [17-25].

Methods

A total of 160 adult patients presenting for longitudinal care between May 2001 and January 2002 at the Infectious Disease Care Clinic for outpatient HIV care at Princess Marina Hospital in Gaborone, Botswana, were consecutively evaluated for the presence of syphilis, HBV infection, HCV infection, toxoplasmosis, and CMV infection. Baseline HIV-1 antibody levels, CD4+ cell counts and plasma HIV-1 RNA levels were also obtained. Not all adults were tested for HCV, T gondii and CMV antibodies because once a consistent test result trend was found (i.e. ≥ 40 patients with test results negative for the particular pathogen), serologic testing for that pathogen was discontinued.

Non-treponemal tests for syphilis (rapid plasma regain [RPR] and Veneral Disease Research Laboratory tests) were performed using RPR test (Randox Laboratories) according to the manufacturer's instructions. Tests for HBV and HCV were performed using Murex test kits (version 3.0 for Hepatitis B surface antigen and anti-HCV version 4.0 for HCV) according to the manufacturer's instructions.

Descriptive univariate analyses included mean values, median values, and SD for normally distributed continuous data and percentages for categorical data. To evaluate for potential differences by gender, age, and CD4+ cell count, analyses were performed with the Statistical Product and Service Solutions software, version 14.0 (SPSS Inc., Chicago, Illinois, USA) using the Kruskal-Wallis 1-way analysis of variance test. Unpaired Student's *t*-test was used to compare differences between the groups. *P*-values less than 0.05 were considered to be statistically significant

This study was approved by the Health Research and Development Committee of the Botswana Ministry of Health and the Harvard School of Public Health Human Subjects Committee.

Results

Our patients were all citizens of Botswana and were referred by senior medical officers practicing in the outpatient medical department at Princess Marina Hospital. Of the 163 patients referred, 3 were found to be HIV-1 negative and were excluded from analysis. The remaining 160 adults were antiretroviral treatment-naïve. The median age of these patients was 35.8 years, and 63.6% being female. Median CD4+

cell count was 104 cells/mm^3 (Interquartile range (IQR) 25th-75th percentile equals 37– 195) and median plasma HIV-1 RNA was 325,000 copies/mL (IQR 25-75 equals 116,026 - > 750,000). A total of 66 (47.6%) of the screened patients had CD4+ cell counts < 100 cells/mm^3 (table 1).

TABLE 1: Serological test results indicating prevalence of syphilis, hepatitis B, hepatitis C, Toxoplasmosis infection, and Cytomegalovirus infection among consecutively screened adults with HIV-1 infection in Botswana in 2001.

Pathogen	Serologic Test	Sample Size	Percentage (%) Testing Positive
Treponema pallidum	VDRL	143	13 3%
Hepatitis B	Hepatitis B surface antigen	141	10.6%
Hepatitis B	Hepatitis B e antigen	15 (Hepatitis B surface antigen positive patients)	40%
Hepatitis B	Hepatitis B core IgG antibody	141	58.2%
Hepatitis B	Hepatitis B surface antibody	141	37.1%
Hepatitis C	Hepatitis C antibody	50	0%
Toxoplasma gondii	IgG antibody	46	6.5%
Toxoplasma gondii	IgM antibody	46	0%
Cytomegalovirus	IgG antibody	43	95.3%
Cytomegalovirus	IgM antibody	43	0%

Serologic data evaluating evidence of prior syphilis were available for 143 patients in the initial cohort; 19 (13.3%) had positive non-treponemal (RPR) test results. One patient had a maculopapular truncal rash that involved the palms and soles, consistent with secondary syphilis. Analyzing by age, we found that 6 (10.3%) of 58 of adults < 35 years of age had positive RPR and 9 (14.3%) of 63 of adults ≥ to 35 years of age had positive RPR (P = 0.51). Analyzing by baseline CD4+ cell count, we found that 11 (16.7%) of 66 patients with baseline CD4+ cell count values < 100 cells/ mm^3 had positive RPR results and 8 (11.8%) of 68 with baseline CD4+ cell counts of ≥ 100 cells/mm^3 were RPR positive (P-value = 0.42). To assess test performance, we performed treponemal hemagglutination (TPHA) syphilis test for all screened adult patients, and found that 5 (3.5%) of 143 had positive RPR test results and negative TPHA test results; these patients were defined as having false positive RPR

test result. Of the positive RPR test results, 5 (26.3%) of 19 were not confirmed by the TPHA. Conversely, 8 (5.6%) of 143 patients had negative RPR test results and positive TPHA test results; these patients were defined as having no active syphilis disease and they most likely had experienced previous syphilis infection or received past treatment for syphilis and were without evidence of active disease. Fifteen (10.6%) of 141 patients tested had test results positive for Hepatitis B surface antigen; hepatitis B e antigen was detected in 6 (40%) of the 15 hepatitis B surface antigen positive adults. Of 140 evaluated patients 82 (58.2%) had results positive for core IgG antibody and 52 (37.1%) had results positive for surface antibody. HCV antibody was not detected in any of the 50 patients screened.

Anti-toxoplasmal IgG antibody was found in 3 (6.5%) of 46 patients screened. None of the 46 patients screened had IgM antibody against *T. gondii*. CMV IgG antibody levels were positive in 41 (95.3%) of 43 patients. None of the 41 screened had anti-CMV IgM antibody.

Discussion

Evidence of prior syphilis infection was common among the first group of HIV-1 infected patients to initiate highly active antiretroviral therapy in the Botswana National ARV Treatment program (found in 13.3% of patients). Recent Botswana syphilis prevalence data (from 2002) among adults with unknown HIV-1 status documented positivity rates ranging from 1.7-5.1% [26] among adults presenting with symptoms of sexually transmitted disease and attending family planning clinics; the rate was 6.6-9.9% among pregnant women presenting for antenatal care as part of the 2003 National Sentinel Surveillance [27]. Both syphilis and HIV-1 share a common epidemiological mode of transmission;therefore, a higher prevalence of co-infection among patients with known advanced HIV-1 disease is not surprising.
By performing both non-treponemal (RPR) and treponemal (TPHA) tests for all patients, we were able to document that ~ 25% of positive RPR test results were not confirmed by the TPHA. This suggests that ~ 25% of patients receiving a diagnosis of syphilis using RPR as a screening test (and not confirmed by TPHA) were treated unnecessarily. More data will need to be obtained via random TPHA confirmatory testing among patients with positive RPR test result to determine whether all

patients with positive RPR test result should have the diagnosis confirmed by TPHA testing before receiving penicillin treatment, which can cause allergic drug reactions in susceptible hosts. The rate of asymptomatic patients with positive RPR tests does, however, provide public health justification for continued syphilis screening by routine RPR testing at entry to care.

HBV infection is also common among HIV-infected adults in Botswana; 58.2% of our initial cohort had antibody evidence of past infection, and 10.6% had circulating hepatitis B surface antigen, indicating chronic hepatitis. The 10% rate of chronic HBV infection coupled with 40% of these carriers also being e antigen positive suggest that the long-term complications of HBV may become a greater problem as many more persons receive HAART as part of Botswana's National ARV treatment program. Emtricitabine, lamuvidine, and tenofovir each have activity against both HIV-1 and HBV and the discontinuation of these drugs may potentially cause significant hepatocellular damage which results from a flare of HBV disease [28]. With very large numbers of adults now receiving lamuvidine-containing HAART in Botswana (and in the region as a whole), those persons who experience failure of first-line therapy, and therefore requiring full HAART regimen switches will need to be monitored for clinical flares of HBV infection, especially during the first 6-8 weeks following discontinuation of therapy.

Fortunately, no patients were found to be infected with HCV, which is a significant cause of additive hepatotoxicity among HIV-1 subtype B- infected adults receiving HAART in developed nations. However, it will be important for Botswana to perform periodic surveillance to assure that its penetration into the population will not be missed, especially because the chronic hepatic complication rates associated with HCV infection are more significant. The absence of HCV infection most likely reflects the fact that the overwhelming majority of HIV-1 subtype C transmission in Botswana is through heterosexual contact, with little documented intravenous drug use.

Serological evidence of prior infection with *T. gondii* was quite uncommon in our population (infection rate, 6.5%), and clinical toxoplasmosis encephalitis is rare. On the other hand, prior CMV infection (indicated by anti-CMV IgG antibody and lack of IgM antibody) was almost universal and CMV retinitis does occur. This high frequency of CMV infection is common for most developing nations, where infection is acquired early in childhood. Both of these organisms, upon re-activation, may cause serious central nervous system complications, although, at present, in the AIDS population in sub-Saharan Africa, cryptococcal meningitis, Mycobacterium tuberculosis, and AIDS dementia complex [29-31] are the most common causes of central nervous system disease.

This study potentially over-estimates the prevalence of prior and/or active infection with these HIV-associated pathogens because it was performed among a group of mostly symptomatic patients presenting for outpatient HIV care. Results may have been more reflective of population trends if this seroprevalence study was conducted among at-risk asymptomatic patients. In addition, serological tests for CMV and toxoplasmosis may have poor clinical significance and predictive value among persons with very advanced HIV disease. In addition, not all adults had the full panel of serologic tests performed as part of our screening approach. These serologic tests were not done within the context of a clinical trial and therefore, once ≥ 40 consecutively screened patients had test results negative for a particular serologic test, for cost purposes, that particular test was no longer performed.

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Chapter 5

Establishment of a public antiretroviral treatment clinic for adults in urban Botswana: Lessons learned

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Abstract

Countries in sub-Saharan Africa are under significant pressure to open large-scale public antiretroviral treatment clinics. Many lessons have been learned in Botswana where the first public antiretroviral treatment clinic in Africa was established. The availability of core, well-trained medical staff will be the primary factor that limits a rapid scale-up of antiretroviral treatment programs.

Key words: Antiretroviral treatment, public, Botswana, infrastructure, training.

Introduction

Because of the World Health Organization's 3 x 5 initiative, many developing countries are under mounting pressure to develop comprehensive public antiretroviral treatment programs[1-3]. Convincing preliminary evidence has documented that the rates of response to HAART among individuals in sub-Saharan Africa who are infected with an HIV-1non-B subtype are comparable to the rates of response to HAART among cohorts in the United States and Europe who are infected with an HIV-1B subtype[4-16]. Data are lacking, however, regarding the practical issues of establishing antiretroviral treatment clinics in the developing world.

Background

Botswana, a country with one of the highest documented HIV-1 seroprevalence rates in the world, has demonstrated strong political will in mounting a public response to the HIV/AIDS epidemic. Efforts towards HIV education began in 1987, and a program for the prevention of mother to child transmission of HIV was initiated in 1999. Voluntary HIV counseling and testing services are available at district health clinics and hospitals, and 16 free-standing centers for voluntary HIV counseling have been established to provide such services to >175,000 clients as of January 2005[17]. In 2000, the government of Botswana entered the African Comprehensive HIV/ AIDS Partnership (ACHAP), a public-private collaboration with the Merck Company Foundation/Merck & Co. Inc. and the Bill and Melinda Gates Foundation to assist in the launching of an antiretroviral program. In 2001, the government of Botswana committed itself to providing HAART to all qualifying citizens through the national antiretroviral treatment program, referred to as "MASA" (Masa is a Setswana word that means "new dawn', signifying hope). The present report summarizes the key lessons learned from the establishment of the first large public HAART clinic in Botswana.

Princess Marina Hospital (PMH). PMH is a tertiary referral hospital that serves southern Botswana in the capital city of Gaborone. The wards of PMH consistently operate at nearly twice their intended capacity, and recent data estimates that up to 80% of all admissions to the medical and pediatric wards of PMH are associated with HIV/AIDS. A nursing deficit of 47% has resulted in significant overburdening of

the staff, and morale is exceedingly low among staff in the medical wards, where 20-25 patients are admitted to a single house officer each night, and where 3-5 patients, on average, do not survive until the morning after admission.

Infectious Disease Care Clinic (IDCC). As the number of patients in dire need of HAART significantly increased, space was made available for the creation of the IDCC, a clinic dedicated to the care of adults and pediatric outpatients with HIV/ AIDS. Because of the presence of on-site HIV physician specialists and a fully operational laboratory capable of performing HIV-1 ELISAs, toxicity monitoring chemical analysis, complete blood counts, HIV-1 DNA PCR analysis, plasma HIV-1 RNA quantification and CD4+ cell counts, the government decided to begin a pilot program of antiretroviral treatment that would enabled local medical officers to work alongside HIV physician specialists. The pilot programme was instrumental in preparing medical officers to deal with rapidly escalating patient demands.

Enrollment of patients into the pilot program lasted 8 months. During that time local medical officers, nurses, pharmacy staff, and counselors received practical training; patient education tools and material assessing patient adherence to treatment were made available; and laboratory requisition materials, tracking forms and clinic-based medical records were developed. Officers collected patient outcome data for all HAART treated adults. Treated patients primarily received stavudine (D4T) and didanosine (DDI) with either nevirapine or efavirenz because of the low cost and availability of these drugs[9].

After the pilot program was completed, the first national site for antiretroviral treatment was opened at the IDCC on January 21st, 2002 . Because of the huge demand for treatment, the names of patients were placed on waiting lists, and many patients waited for as long as 4-5 months to initiate HAART. One screening clinic that measured CD4+ cell counts was initially established using existing infrastructure within Gaborone. Within the next year, 3 additional screening clinics that measured CD4+ cell counts, all of which provided pre-referral care that consisted of disease prophylaxis (with isoniazid and trimethoprim-sulfamethoxazole used for prophylaxis of tuberculosis and *Pneumocystis jiorveci* [formerly *'carinii'*] pneumonia respectively), social support, and nutritional services, were opened in

Gaborone. Regular communication between the staff at the screening clinics that assessed CD4+ cell counts and the staff at the IDCC ensured that patients with CD4+ cell counts < 50 cells/mm^3 were given top priority for referral.

Challenges and lessons learned regarding space. Limited clinic space presented the first challenge. Initially, space was mainly needed for consultation while these often significantly ill patients underwent screening and initiated HAART. As the number of patients rapidly escalated, there soon became a need for adequate space to provide counseling on adherence to treatment. Given the overcrowded hospital conditions, the only available clinic space was an unused isolation ward located on the grounds of the hospital. Initially, 4 consulting rooms, 3 counseling rooms, a waiting room, and an administrative room as well as secure pharmacy space with a dispensing area, were made available. This facility soon reached capacity at 4,000 patients. This facility soon reached its capacity (4000 patients), even as more administrative space and 2-3 more consulting rooms were made available. At present, to accommodate the > 10 000 adult patients and 1000 pediatric patients who are receiving longitudinal care, adjacent structures have been added. These structures include an~6100 m^2, pre-fabricated outpatient clinic for adult patients and the state-of-the-art Botswana-Baylor Center Of Excellence for pediatric care. The next main objective is to decentralize the IDCC by establishing the capacity to provide antiretroviral treatment at existing peripheral city council medical clinics. Decentralization will significantly reduce patient volumes at the IDCC. Peripheral city council medical clinics are in closer geographic proximity to the patient, which allows patients to receive comprehensive care at one clinic; this is in contrast to the present system, in which patients receive preventive therapy or treatment for tuberculosis, as well as social services and routine medical care, at peripheral clinics, although they attend the IDCC to receive HAART. This decentralization project is still in the very early stages of development.

Challenges and lessons learned regarding staffing. Initially, hospital administrators sought to rotate medical officers from the inpatient medical wards to the clinic, so that each officer would be responsible for providing 1-2 half days of outpatient care per week. This decision was ultimately modified to allow for the creation of a core-

team of HIV physician specialists dedicated to providing care for outpatient with HIV infection. These HIV physician specialists are critical to program success because they (1) allow for continuity and consistency of care for a large number of patients, and (2) are dedicated to intensively training other 'junior' medical officers as care providers for patients with HIV infection, thus ensuring the sustainability of the program. The concept of a core team may have initially restricted the numbers of medical officers involved with providing antiretroviral treatment, but this dedicated core team has subsequently been integral to training medical officers during the roll out of the national program.

The directorship of the clinic has been fully transferred from expatriate HIV physician specialists to fully-trained local Botswana HIV physician specialists. The initial core tam consisted of 4-5 full-time physicians and 8-10 nurses. These nurses are presently paired with medical officers and focus primarily on adherence counseling, education, translation, and problem identification. These experienced nurses can and should play a key role in caring for stable patients who are receiving antiretroviral therapy; the role of nurses is especially important in this region with preexisting shortage of medical officers. Allowing antiretroviral treatment clinics to be primarily run by nurses would significantly enhance the capacity of such clinics, because nurses could care for the majority of patients with minimal supervision.
At first, the concept of a core team was met with criticism, because all hospital-based medical officers wanted to take part in the program. Administrative staff addressed this issue by having core teams assigned to the IDCC on a rotational basis, to ensure that training was adequate, the teams were to serve for at least 6-9 months.

Challenges and lessons learned regarding training. All staff involved in the care of patients receiving antiretroviral treatment with HAART, including physicians, nurses, counselors, and pharmacy staff, were required to receive standardized theoretical training. KITSO theoretical training consists of a 12-lectures series that includes presentations and case-based learning supplemented by relevant scientific articles and a CD-ROM that provides additional reading (table 1).

Medical officers at all national antiretroviral treatment sites participating in the national program receive on-site supportive training that is led by mentors who are called 'preceptors.' Medical officers at the IDCC were paired with HIV experts from the Botswana-Harvard School of Public Health AIDS Initiative Partnership for HIV Research and Education, the world Health Organization, and the ACHAP to receive intensive practical training. Within 1 month of initiation of training, the medical officers were able to work independently, with ongoing support provided via the preceptorship program that was established in the clinic (table 1).

Conclusions: The availability of a core team of well-trained medical staff continues to be the main challenge to providing sustainable antiretroviral therapy in this urban Botswana antiretroviral treatment clinic. In the program reported here, the lack of adequate clinic space was a significant initial constraint that was overcome either through the construction of new clinics by public-private partnerships or by government purchase of pre-fabricated structures. In addition, given that survival of patients depends on the experience of the patients' physicians in treating HIV-infected persons[18], preceptorship programs and continuous medical education deserve continued attention. Experienced nurses should also play a significant role as primary care-givers; however, this will require time and flexibility in a system that is rigid in terms of structure, responsibilities, and salary scales.

In Botswana many lessons have been learned that are relevant for others who are contemplating similar initiatives. Despite many difficulties, we have witnessed the exceptional effectiveness of HAART, even among individuals with very advanced disease[14-15]. The political will and foresight of the government of Botswana are definitely worthy of praise and imitation throughout sub-Saharan Africa. The many committed governmental and external funding bodies, however, must act together decisively to hire urgently needed health professionals and to circumvent the bureaucracy that hampers more rapid expansion of this program. This concerted effort will enable Botswana and other countries to successfully provide antiretroviral therapy to the public on a large-scale basis and curb AIDS epidemic that threatens the lives and endangers the future of so many individuals in this region of the world.

Availability of Training Materials. All KITSO AIDS training program materials used for the theoretical and practical antiretroviral medications training of health professionals in Botswana who are providing antiretroviral medications are available on written request. Please address these requests to:
Christine Bussmann or KITSO AIDS Program coordinators,
Botswana-Harvard School of Public Health AIDS Initiative Partnership for HIV Research and Education, Gaborone, Botswana (e-mail: cbussmann@bhp.org.bw).

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Chapter 6

Response to zidovudine/didanosine-containing combination antiretroviral therapy among HIV-1 subtype C-infected adults in Botswana: Two-year outcomes from a randomized clinical trial

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Abstract

Background: Numerous national antiretroviral (ARV) treatment initiatives offering protease-inhibitor (PI)-sparing combination antiretroviral therapy (cART) have recently commenced in southern Africa, the first of which began in Botswana in January 2002. Evaluation of the efficacy and tolerability of various PI-sparing cART regimens requires intensive study in the region, as does investigation of the development of drug resistance and the optimal means of sustaining adherence. The *'Tshepo'* Study is the first large-scale randomized clinical trial that addresses these important issues among HIV-1 subtype C-infected, ARV-treatment naïve adults in southern Africa.

Methods: The *Tshepo* study is a completed open-labeled randomized study that enrolled 650 ARV-naïve adults between December 2002 and December 2004. The study is a 3 x 2 x 2 factorial design comparing the efficacy and tolerability among factors: (1) three combinations of nucleoside reverse transcriptase inhibitors (NRTIs): zidovudine (ZDV) + lamivudine (3TC); ZDV + didanosine (ddI); and stavudine (d4T) + 3TC; (2) two different Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs): nevirapine (NVP) and efavirenz (EFV); and (3) two different adherence strategies: the current national "standard of care' versus an "intensified adherence strategy", incorporating a "community-based directly observed therapy". Study patients were stratified into two balanced CD4+ cell count groups: less than 201 cells/mm^3 versus 201-350 cells/mm^3 with viral load greater than 55,000 copies/mL. Following Data Safety Monitoring Board recommendations in April 2006, ZDV/ddI-containing arms were discontinued due to inferiority in primary endpoint, namely, virologic failure with resistance. We report both overall data and pooled data from patients receiving ZDV/ddI- versus ZDV/3TC- and d4T/3TC-containing CART through 1 April 2006.

Results: Four hundred fifty-one (69%) females and 199 males with a median age of 33.3 years were enrolled into the study. The median follow-up as of 1 April 2006 was 104 weeks, and loss to follow-up rate at two years was 4.1%. The median baseline CD4+ cell count was 199 cells/mm^3 [IQR 136-252] and the median HIV-1 plasma viral load was 193,500 copies/mL [IQR 69-250, 472-500]. The proportion of

participants with virologic failure and genotypic resistance mutations was 11% in those receiving ZDV/ddI-based cART versus 2% in those receiving either ZDV/3TC- or d4T/3TC-based cART (p=0.002). The median CD4+ cell count increase at one year was 137 cells/mm^3 [IQR 74-223] and 199 cells/mm^3 [IQR 112-322] at two years with significantly lower gain in the ZDV/ddI arm. At one and two years, respectively, 92.0% and 88.8% of patients had an undetectable plasma HIV-1 RNA level (≤400 copies/ mL). Kaplan-Meier survival estimates at one and two years were 96.6% and 95.4%. One hundred twenty (18.2%) patients had treatment modifying toxicities, of which the most common were lipodystrophy, anemia, neutropenia, and Stevens-Johnson Syndrome. There was a trend towards difference in time to treatment- modifying toxicity by pooled dual NRTI combination and no difference in death rates.

Conclusions: The preliminary study results show overall excellent efficacy and tolerability of NNRTI-based cART among HIV-1 subtype C-infected adults. ZDV/ddI-containing cART, however, is inferior to the dual NRTIs d4T/3TC or ZDV/3TC when used with an NNRTI for first-line cART.

Key words: HIV/AIDS, CART, Africa, Randomized clinical trial

Introduction

In recent years, combination antiretroviral therapy (cART) has been introduced on a large scale throughout sub-Saharan Africa. Initial reports from antiretroviral (ARV) pilot studies in Côte d'Ivoire [1], Senegal [2], Uganda [3], Khayelitsha, South Africa [4], and Botswana [5] and preliminary data from larger public cART initiatives in Malawi [6,7] and Zambia [8] have been very encouraging and reported impressive clinical, immunologic, and virologic responses among the vast majority of cART-treated individuals. These African cART outcomes have surpassed previous expectations and offer hope for the vast numbers of people in dire need of ARV treatment [9-12].

These published outcomes also raise numerous issues that need to be addressed to further improve ARV treatment protocols. Important considerations include the selection of optimal regimens, especially with regards to tolerability and efficacy; the determination of the optimal time for cART initiation; the optimal means to promote and sustain ARV medication adherence; and the issue of drug resistance among persons infected with non-B subtypes [13-15]. Data from randomized clinical trials conducted in Africa to evaluate these critical questions are very much needed [16-18].

The 'Tshepo' Study was begun in December 2002 in urban Botswana to compare six different cART regimens with regard to their efficacy and tolerability and to describe the development and overall kinetics of drug resistance. The study was also designed to identify the optimal means of promoting adherence by evaluating an intensified adherence strategy [community-based directly observed therapy (Com-DOT)] versus the standard of care SOC). We herein report outcomes among adults randomized to zidovudine (ZDV)/didanosine (ddI)-based cART versus ZDV/lamivudine (3TC)- (given as Lamzid) or stavudine (d4T)/3TC-based cART after the discontinuation of the ZDV/ ddI-containing study treatment arms due to documented inferiority in efficacy as part of the study's third interim Data Safety Monitoring Board (DSMB) review. The word *Tshepo* means 'hope' in Setswana.

Methods

Study Design

The Tshepo Study is an open-label, randomized, 3 x 2 x 2 factorial design study conducted at Princess Marina Hospital (PMH) in Gaborone, Botswana. This study was designed to address many important questions by evaluating the efficacy, tolerability, and the development of drug resistance of six different first-line cART regimens. The evaluated regimens were as follows: ZDV/3TC/nevirapine (NVP) (arm A); ZDV/3TC/efavirenz (EFV) (arm B); ZDV/ddI/NVP (arm C); ZDV/ddI/EFV (arm D); d4T/3TC/NVP (arm E), and d4T/3TC/EFV (arm F). The study also compared two different adherence strategies: SOC versus SOC plus a community-based supervision (Com-DOT) to determine the better method for promoting adherence among treatment-naïve adults initiating cART.

The primary end points of the study were as follows: (1) the development of virologic failure with significant drug resistance and (2) the development of treatment-related toxicity, as defined by first incidence of a grade 3 or higher adverse event. Secondary endpoints were the time-to-occurrence of AIDS-defining events and/or death for any reason.vThe study was approved by the Institutional Review Boards of the Botswana Ministry of Health (Health Research Development Committee) and the Harvard School of Public Health (Human Subjects Committee) and written informed consent was obtained from all participants.

Study Population

Adult (18 years and older), HIV-infected, cART-naïve Botswana citizens who attended one of the five ARV treatment- screening clinics in Gaborone were approached for possible enrollment. All potentially eligible adults had to qualify for cART, based on existing Botswana National ARV Treatment guidelines [19] of having an AIDS-defining illness and/or CD4+ cell count of equal or less than 200 cells/mm^3 or meet this study's upper CD4 stratum eligibility criteria which was a CD4+ cell count between 201 and 350 cells/mm^3 with plasma HIV-1 RNA level greater than 55,000 copies/ mL. Potentially eligible adults also had to reside within the study catchment area for the duration of the study. Other inclusion criteria were as follows: hemoglobin value greater than 8.0 grams/dL; absolute neutrophil count greater than or equal

to 1.0 x 103/mm^3; aminotransferase levels less than five times the upper limit of the normal; serum alkaline phosphatase level of less than three times the upper limits of the normal; and for women of child-bearing potential, a willingness to maintain active contraception throughout the duration of the study and a negative urine pregnancy test within 14 days of study enrollment. Exclusion criteria were: poor Karnofsky performance score (40 or below); an AIDS-related malignancy other than mucocutaneous Kaposi's sarcoma; grade 2 or higher peripheral neuropathy; a major psychiatric illness; and for women, actively breastfeeding and/or less than six months post-partum. Additional treatment steps, defined as protease-inhibitor (PI)-containing regimens, were available for all participants with confirmed virologic failure, toxicities, or concomitant medical conditions that required the use of PI's.

Data Collection and Follow-up

Clinical and adherence assessment were done monthly at the study clinic. To monitor treatment efficacy, CD4+ cell counts (FACS Calibur flow cytometer, Becton Dickinson, San Jose, CA, USA) and plasma HIV-1 RNA levels (Amplicor HIV-1 Monitor test, version 1.5 Roche Diagnostics Systems, Branchburg, NJ), were obtained at enrollment and then every two months for the duration of the study. Laboratory safety monitoring included comprehensive chemistry and full blood count (hematology) specimens at study enrollment, then every month for the first six months of the study, every two months during months 6-12 of study participation, and every four months during the remainder of participation, for safety monitoring purposes. In addition, all patients had lipid chemistries (total, low-density lipoprotein, high-density lipoprotein, cholesterol, glucose, and serum triglycerides) performed at the time of study initiation and then every six months for the duration of the study. Laboratory values were graded according to the 1994 Division of AIDS (DAIDS) laboratory grading scale, except lipid chemistry values that were graded using the DAIDS December 2004 grading scale. Additional routine clinical assessment included peripheral neuropathy assessments every two months, lipodystrophy and performance assessments every six months, and annual screening for the presence of other sexually transmitted infections (hepatitis B and syphilis), proteinuria/ glycosuria, and chest x-ray abnormalities. Patients also received an ophthalmologic evaluation at baseline which was repeated at least every two years on study. All

female patients had baseline *Papanicolau* smears performed which were repeated at least annually or more frequently, as clinically indicated. Colposcopic examinations were performed when clinically indicated. All women of reproductive potential had a monthly urine pregnancy test done. Comprehensive care for study participants was provided in accordance with existing national policy and free-of charge [20, 21]. Opportunistic infections were diagnosed using available laboratory, imaging, and histopathologic services and specialist consultation. Pulmonary tuberculosis (TB) and extrapulmonary TB were diagnosed using acid fast bacilli staining, cerebrospinal fluid microscopy, and radiographic imaging (chest x-ray, computed tomography scanning, and ultrasound). *Pneumocystis jiroveci* (formerly *carinii*) pneumonia was diagnosed using clinical expertise and radiographic imaging (chest radiography). Cryptococcal meningitis was ascertained by india ink staining of the cerebrospinal fluid.

Cytomegalovirus retinitis was diagnosed by fundoscopic examination, which was performed by specially trained ophthalmologists. Malignancies (invasive cervical cancer, Kaposi's sarcoma and non-Hodgkin lymphoma) were diagnosed by histology and expert pathologist and/or oncologist review. Clinical expertise alone was used to diagnose Candida esophagitis and herpes zoster. Prophylaxis for OIs included 6 months of isoniazid plus pyridoxine (vitamin B6) preventative therapy if determined that participant was without clinically active TB and one oral double-strength cotrimoxasole tablet three times per week (or once daily) for the prevention of *Pneumocystis jiroveci* pneumonia if the CD4+ cell count was less than 200 cells/ mm^3.

ARV medication adherence was defined as being 'excellent' (greater than 90 percent) based on a composite adherence measure which included the following: (1) patient four day recall, (2) patient 1 month recall, (3) patient ARV self-demonstration (including verbal reporting on timing of doses, number of tablets per dose, and food requirements), (4) ARV pill counts. Initially, virologic failure was defined as a confirmed plasma HIV-1 RNA level of greater than 5,000 copies/ mL at 16 or more weeks following cART initiation. If the repeat plasma HIV-1 RNA level following an intensified adherence intervention still exceeded 5,000 copies/

mL, the patient underwent a step change and was initiated on two different NRTIs and a PI in accordance with the 2002 and 2005 Botswana National ARV Treatment guidelines [19, 21]. Effective 1 April 2006, the study virologic failure definition was changed to any confirmed viremia (greater than the lower limit of detection, which is 400 copies/mL) in accordance with new literature [22-24]and existing national guidelines [21]. All participants with confirmed virologic failure underwent an intensified adherence intervention which included an initial adherence assessment and adherence education, followed by a two-to-four week period of Com-DOT. A repeat viral load measurement was done at the end of the intervention. Genotypic resistance testing was done using Roche ViroSeq v 2.0, an integrated system for sequence-based analysis of drug resistance mutations in HIV-1, as per the manufacturer's instructions. In cases of first line cART regimen virologic failures, the study physician was blinded to genotypic resistance results.

On 6 April 2006, as part of the third interim analysis, our independent DSMB recommended discontinuing the ZDV/ddI-containing study treatment arms due to inferiority in efficacy, specifically higher virologic failure rates among cohort-treated participants receiving ZDV/ddI-containing cART compared with those receiving ZDV/3TC- and d4T/3TC-containing CART regimens. The Board recommended that all cART-treated patients who were receiving the dual NRTI combination of ZDV/ddI be switched to ZDV/3TC.

Statistical Analysis

All primary analyses and the majority of secondary analyses were performed on an "intent-to-treat" basis. Time-to-event methods (Kaplan-Meier (K-M) survival curves including 95% confidence intervals (CIs) at one and two years and Cox proportional hazards models were used to compare study participants receiving ZDV/ddI-containing cART with patients who received cART regimens that did not contain ZDV/ddI, with respect to event rates for virologic failure, death, toxicities, OIs, and non-adherence. For our analyses, observations were censored on 1 April 2006 or when the participant died or was lost to follow-up, if before that date. All statistical analyses were conducted using SAS software.

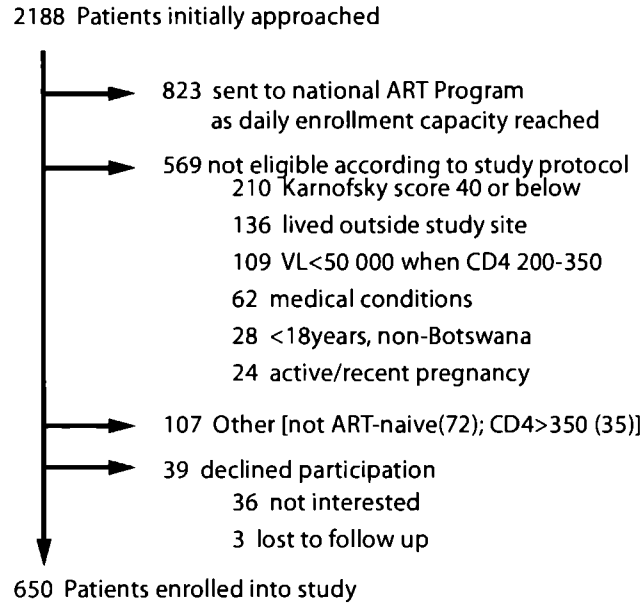


Fig 1. Recruitment flow chart

RESULTS

Study Recruitment

Between December 2002 and December 2004, 2188 patients were screened for possible study enrollment at the adult Infectious Disease Care Clinic (IDCC) of PMH and four designated local Gaborone City Council "CD4+ screening" clinics. In total, 650 adults were eligible, consented, and enrolled in the study. Figure 1 summarizes the main reasons for non-enrollment into the study. In short, 823 patients were referred to the Botswana National ARV Program at the adjacent adult PMH IDCC for continued longitudinal care due to our study team reaching its' daily limit in total number of enrolled patients. 569 patients were not eligible according to the study protocol. Of these 569, 210 patients had a Karnofsky score equal or below 40, and 62 patients were deemed ineligible based on the presence of the following active medical conditions and/or laboratory abnormalities; namely neutropenia (19), anemia (18), active TB not yet on appropriate therapy (9), and other health related conditions (16) such as grade 2 or greater peripheral neuropathy or elevated liver enzymes and active/recent pregnancy. An additional 109 patients were not eligible due to virologic criteria, namely having a CD4+ count between 201 and 350 cells/ mm^3 but having a plasma viral load below 55,000 copies/ml, as our viral load cutoff was based on existing World Health Organization (WHO) and Department of Health

and Human Services guidelines at the time the study was designed. 107 patients had CD4+ count of greater than 350 cells/mm^3 (35), or were not ART-naive (72). 39 patients declined study participation (36) or were lost to follow-up (3) during the screening process.

Baseline Characteristics

Of the 650 enrolled adults, 451 (69.4%) were female. Forty-three percent had advanced WHO clinical disease (stage 3 or stage 4). Table 1 summarizes the key baseline characteristics. Baseline characteristics of patients in the ZDV/ddI arms versus the other four arms (d4T/3TC- and ZDV/3TC-containing) were evenly balanced at entry. Three hundred twenty-five participants were randomized to the intensified adherence (Com-DOT) arm. Three hundred thirty (50.9%) patients were enrolled in the lower CD4+ cell count stratum with a median CD4+ cell count of 137 cells/mm^3. Three hundred twenty (49.1%) patients were enrolled in the upper CD4+ cell count stratum (CD4+ cell count value between 201 and 350 cells/mm^3 and plasma HIV-1 RNA above 55,000 copies/mL) with a median CD4+ cell count of 252 cells/mm^3.

Follow-Up

The amount of study follow-up was approximately 1,308 person-years, with a median follow-up time of 104 weeks [interquartile ratio (IQR) 78-136]. Ninety-eight percent of all scheduled follow-up visits were attended. As of 1 April 2006, four years and three months into the study, 31 of the 650 enrolled patients were lost to follow up with regard to primary endpoint information. Eighteen (58%) of the 31 had moved out of the study catchment area, six (19%) declined further participation, and for seven (23%), no further information was available despite repeated attempts by the study team to contact them. The overall loss to follow-up rates were 2.4% [CI: 1.4%, 3.9%] and 4.1% [CI: 2.6%, 5.2%] at one and two years, respectively. The sociodemographic and clinical characteristics of participants who were lost to follow-up did not differ from those adults who completed the trial.

Table 1 Baseline Characteristics of Study Population

		ZDV/ddI	non-ZDV/ddI	Overall
Gender N (%)	female	157 (24 1)	294 (45 2)	451 (69 4)
	male	59 (9 1)	140 (21 5)	199 (30 6)
Age in yrs, median (IQR)		32 9 (29, 38 1)	33 6 (28 8, 38 8)	33 3 (28 9, 38 7)
Weight in kg, mean (SD)		59 7 (12 5)	59 3 (12 4)	59 4 (12 5)
BMI, median (IQR)		21 1(19 4, 24 9)	21 4(19 1, 24 1)	21 2(19 2, 24 3)
	F	22 (19 7, 26 7)	21 7 (19 3, 25)	21 8 (19 4, 25 3)
	M	20 5 (18 5, 21 5)	20 8 (19, 23 3)	20 7 (18 8, 22 7)
WHO clinical staging, N (%)	I	59 (9 3)	139 (22)	198 (31 3)
	II	50 (7 9)	111 (17 5)	161 (25 4)
	III	85 (13 4)	131 (20 7)	216 (34 1)
	IV	14 (2 2)	44 (6 9)	58 (9 2)
CD4 in cells/µL, median (IQR)	Overall	197 (140, 258)	199 (134, 249)	199 (136, 252)
	CD4 strata ≤200	140 (86, 171)	135 (79, 169 5)	137 (81, 170)
	CD4 strata 201-350	259 (225,288)	250 (222, 294)	252 (222 5, 293)
CD4, N (%)	<50	14 (2 15)	30 (4 6)	44 (6 8)
	50-200	96 (14 8)	190 (29 2)	286 (44)
	201-350	106 (16 3)	214 (32 9)	320 (49 2)
Viral Load in copies/ mL, median (IQR)		207 000 (79 000, 499 000)	190 000 (64 900, 460 000)	193 500 (69 250, 472 500)
Opportunistic Infections, N (%)				
Wasting syndrome** N (%)		36 (5 6)	85 (13 2)	121 (18 7)
Tuberculosis (any site)		33 (5 1)	73 (11 2)	106 (16 3)
Anemia HB<10 g%		34 (5 3)	67 (10 5)	101 (15 9)
HB 10-12 g%		93 (14 6)	170 (26 7)	263 (41 3)
Herpes zoster (VZV)		32 (4 9)	55 (8 5)	87 (13 4)
Peripheral Neuropathy		29 (4 5)	44 (6 8)	73 (11 2)
Esophageal candidiasis		5(0 8)	10 (1 5)	15 (2 3)
Chronic, recurrent diarrhea		2 (0 3)	10 (1 5)	12 (1 9)
Kaposi's sarcoma		3 (0 5)	8 (1 2)	11 (1 7)
Pneumocystis jiroveci pneumonia		1 (0 2)	5 (0 8)	6 (0 9)
Cryptococcal meningitis		2 (0 3)	1 (0 2)	3 (0 5)

Highly Active Antiretroviral Therapy Outcomes:

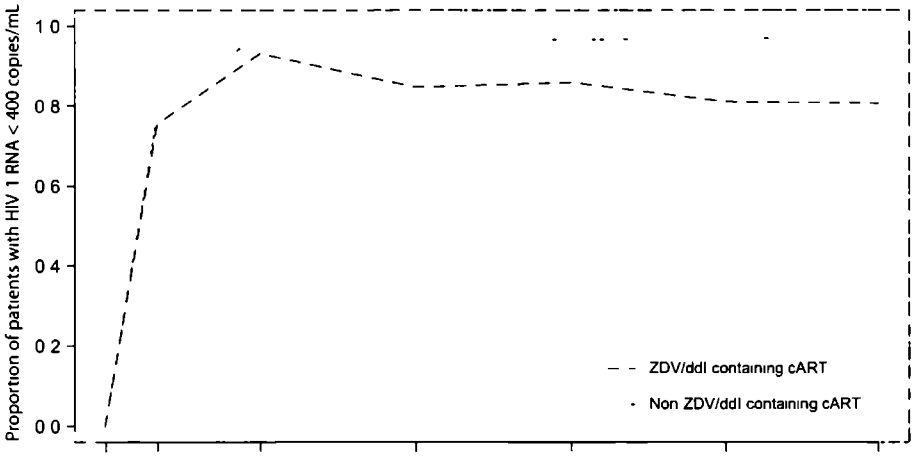
Immunologic:

The median increase in CD4+ cell count was 137 cells/mm^3 at one year [IQR 74-223] and 199 cells/mm^3 at two years [IQR 112-322]. There was a significant difference by treatment arms with a median CD4+ cell gain from baseline in ZDV/ddI arm of 106 (IQR 34-176) and in the non- ZDV/ddI arm of 156 (94,242) at one year and 163 (70,250) and 238 (138-333) at two years respectively (p=0.0001). CD4+ cell count increases were significantly higher in women (p=0.0094). No significant difference was found in relation to anemia (baseline hemoglobin), body mass index, or age greater than 40 years. Concordant virologic and immunologic responses were found in 75%, 76% and 81% of participants at 6, 12, and 24 months respectively. At 12 and 24 months, significantly more patients in the ZDV/ddI group showed a discordant responses. Additional analyses are planned to look more in-depth at potential reasons for a small subset of patients who have discordant virologic and immunologic responses.

Virologic:

Figure 2 shows the proportion of all study participants who had undetectable plasma HIV-1 RNA levels at weeks 8, 24, 48, 72, and 96; stratified by whether they were receiving ZDV/ddI-containing cART or non-ZDV/ddI-containing (ZDV/3TC- or d4T/3TC-based) cART. Three hundred sixty-one (56%) of study participants had undetectable (<400 copies/mL) plasma HIV-1 RNA levels at four weeks after cART initiation. Of those whose HIV-1 RNA suppressed to undetectable levels at eight weeks following cART initiation, 70.8% (64.1%, 76.4%) receiving ZDV/ddI based CART and 85.6% (81.9%, 88.7%) receiving non-ZDV/ddI based cART remained consistently suppressed at one year. At two years, the percentages remaining consistently suppressed were 57.6% (50.4%, 64.2%) for ZDV/ddI arms and 78.5% (74.0%, 82.2%) for the non-ZDV/ddI arms. Following DSMB recommendations, the ZDV/ddI-containing CART arms were discontinued (after the cutoff date for this analysis); and all patients substituted 3TC for ddI due to inferiority in primary endpoint, namely virologic failure.

Fig 2. Proportion of patients with HIV-1 RNA level of less than 400 copies per millileter



Number with Values

ZDV/ddI	216	208	202	196	176	137	88
Non ZDV/ddI	434	414	408	390	362	260	167

As of 1 April 2006, 55 (8.5%) study participants had developed virologic failure, as defined by the study protocol of whom 52 cases were successfully genotyped. In two cases no genotyping data was available due to a missed confirmatory visit, in one case amplification was unsuccessful.Thirty-eight (70%) of available 52 genotypes were found to have virologic failure with primary genotypic resistance mutations. Fourteen cases had virologic failure without resistance mutations. Figure 3 is a Kaplan-Meier plot of time to first virologic failure with resistance. Rates of virologic failure with resistance were significantly higher in the ZDV/ddI-containing treatment arms when compared to the non-ZDV/ddI-containing treatment arms (p-value <0.0001). At one year, 5.3% (3.0%, 9.4%) of patients receiving ZDV/ddI cART had virologic failure with resistance, compared to 1.0% (0.1%, 1.7%) of those receiving non-ZDV/ddI cART. At two years, 13.5% (9.2%, 19.4%) of those receiving ZDV/ddI cART and 3.2% (1.8%, 5.8%) of patients receiving non-ZDV/ddI cART had virologic failure with resistance.

Out of 38 cases of virologic failure with resistance mutations 25 cases occurred

in NVP-containing regimens versus 13 cases of EFV-containing regimens. NRTI mutations were present in 27 cases. Of note, a unique pattern of TAM mutations, namely the 67N 70R 215Y pathway was found among patients on first-line ZDV/ddI-based cART at the time of virologic failure [Novitsky, 2007 #4919]. Among all ZDV/ ddI failing regimens the two most common resistance genotypes were the 67N 70R 215Y gennotype, present in 7 of 19 (37%) of cases, and a single T215Y mutation, present in 5 of 19 (26%) of cases. No 210W nor 219Q genotypes were present among analyzed failures. With the exception of three virologic failure cases, namely, one case of the 67N 70R 215Y genotype and two cases of M41L mutation, all other failing patients having primary NRTI mutations had also NNRTI class mutations.
As of April 2006, 43 patients had been changed from first-line study treatment to PI-based cART. Reasons for the change to second- or third-line PI-based cART included the following: virologic failure (63%), pregnancy protection (23%), and severe ARV-related toxicity (14%), the majority of which were due to moderate-severe symptomatic hyperlactatemia or lactic acidosis syndrome 142.

Clinical

The mean body weight increase after two years of cART was 6.2 kilograms for patients with pre-existing wasting syndrome and 3.0 kilograms for patients without existing HIV-associated wasting syndrome at the time of study enrollment.

Survival

As of 1 April 2006, 32 (5%) of enrolled study participants had died. Eight (25%) of these 32 deaths occurred within the first three months following CART initiation. Among theses 8 cases 4 cases were due to advanced AIDS, 2 cases were non-AIDS related and 2 cases were possibly related to ARV treatment. Overall, of the 32 deaths, seven (23%) were deemed "possibly related to ARV treatment, with four related to lactic acidosis/pancreatitis, two related to traditional medication use/ abuse with possible underlying pancreatitis, and one from nevirapine-associated fulminant hepatic failure potentially exacerbated by isoniazid that the patient was taking for TB prophylaxis. 12 (37.5%) of the 32 deaths were due to an OI.

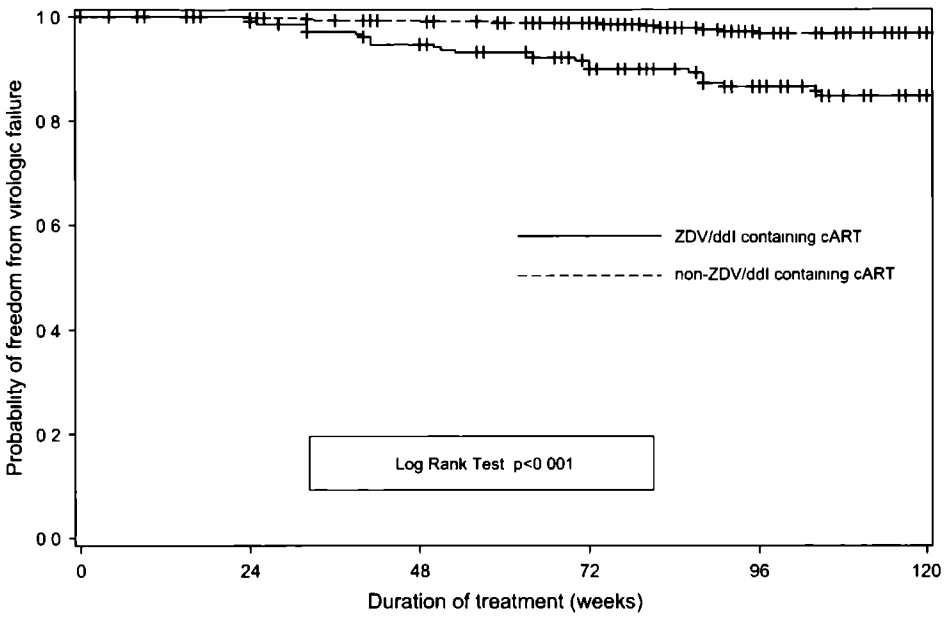


FIGURE 3. K-M curves for virologic failure with resistance by treatment arms.

The Kaplan-Meier one-year and two-year survival estimates were 96.6% [CI: 94.8%, 97.7%] and 95.4% [CI: 93.5%, 96.8%], respectively, for the entire cohort (Figure 4). There were no statistically significant differences across the two pooled treatment groups, ZDV/ddI treated versus non-ZDV/ddI treated (P=0.93). In univariate analysis, death was significantly related to anemia (baseline hemoglobin value <10.0 g/dL), poor performance status (Karnofsky score less than 90), and HIV-associated wasting (BMI <18.5).

Safety and Tolerability

Overall, 208 clinically relevant grade 3 and 4 serious adverse events occurred in 99 patients, excluding laboratory events that did not change clinical management such as amylase, gammaglutaryl transaminae (GGT), and alkaline phosphatase elevations. These serious adverse events were distributed equally among the pooled treatment groups (ZDV/ddI-treated versus ZDV/3TC- and d4T/3TC-treated, log-rank p=0.2575). At one and two year, 11.3% [9.1%, 14.0%] and 14.5% [11.9%,

17.5%] of patients had had a grade 3 or 4 serious adverse event, respectively

Table 2. Adverse Events Resulting in Treatment Modification

Cause	ZDV-ddI patients N (% of 216)	Non ZDV-ddI patients N (% of 434)	Total N (% of 650)
Lipodystrophy	9 (4 2)	41 (9 4)	50 (7 7)
Neutropenia	5 (2 3)	18 (4 1)	23 (3 5)
Anemia	8 (3 7)	14 (3 2)	22 (3 4)
Rash/Hypersensitivity Reaction	5 (2 3)	11 (2 5)	16 (2 5)
Hepatoxicity	2 (0 9)	6 (1 4)	8 (1 2)
Pancreatitis	5 (2 3)	2 (0 5)	7 (1 1)
Lactic Acidosis	0	4 (0 9)	4 (0 6)
Neuropsychiatric Symptoms	1 (0 5)	4 (0 9)	5 (0 8)
Peripheral Neuropathy	0	2 (0 5)	2 (0 3)
Diarrhea	1 (0 5)	0	1 (0 2)
Vomiting	1 (0 5)	0	1 (0 2)
Pancytopenia	0	1 (0 2)	1 (0 2)
Total number of patients*	31	89	120

* Columns do not add correctly because a patient may have more than one treatment-modifying toxicity

Thirty-one (14.8%) patients in the ZDV-ddI group and 89 (19.8%) patients in the non-ZDV/ddI group had 140 treatment modifying toxicities (log-rank p=0.0647), with lipodystrophy (50), neutropenia (23), anemia (22), and cutaneous hypersensitivity reactions (16) being the most common (Table 2).

At one year, 8.9% [5.8%, 13.6%] of patients receiving ZDV/ddI cART had a treatment-modifying toxicity, compared with 12.2% [9.5%, 15.7%] of those who received non-ZDV/ddI cART. At two years, 12.6% [8.8%, 18.0%] of patients who received ZDV/ddI cART and 16.7% (13.8%, 20.7%) of those receiving non-ZDV/ddI cART had a

treatment-modifying toxicity.

Incident Opportunistic Infections

One hundred and six incident OIs were diagnosed in 93 study participants. The most common OIs included (1) varicella zoster virus infection (40 patients), (2) pulmonary TB (36 patients), and (3) extra-pulmonary TB (13 patients, 11 with miliary/disseminated TB, and two with TB meningitis) (Table 3). At one year 12.8% [9.0%, 18.1%] of patients receiving ZDV/ddI cART had had an OI, compared to 7.5% [5.4%, 10.5%] of patients who received non-ZDV/ddI cART. At two years, 16.6%

Table 3: Incident Opportunistic Infections on cART

	0-3 months on CART N (% of 106)	4-6 months on CART N (% of 106)	> 6 months on CART N (% of 106)	Total N (% of 106)
Herpes Zoster (VZV)	9 (8.5)	18 (17)	13 (12.3)	40 (37.7)
Pulmonary TB	11 (10.4)	19 (17.9)	6 (5.7)	36 (34)
Extra-pulmonary TB	2 (1.9)	2 (1.9)	9 (8.5)	13 12.3)
Malignancies Kaposi's sarcoma Invasive cervical carcinoma Squamous cell penile carcinoma Non-Hodgkin's lymphoma	 2 (1.9) 1 (0.9) 0 0	 0 1 (0.9 0 1 (0.9)	 0 1 (0.9) 1 0	 2 (1.9) 3 (2.8) 1 (0.0) 1 (0.9)
Cryptococcal Meningitis	1 (0.9)	3 (2.8)	0	4 (3.8)
Pneumocystis jiroveci (carinii) Pneumonia	1 (0.9)	2 (1.9)	0	3 (2.8)
Esophageal Candidiasis	0	2 (1.9)	0	2(1.9)
CMV Retinitis	0	0	1 (0.9)	1 (0.9)
Total	27 (25.5)	48 (45.3)	31 (29.2)	106 (100)

[12.0%, 22.2%] of patients receiving ZDV/ddI cART and 11.9% [9.1%, 15.6%] of patients who received non-ZDV/ddI cART had had an OI. The log-rank test for treatment group was statistically significant (p=0.042), with ZDV/ddI-treated patients having a shorter time to first OI compared to non-ZDV/ddI-treated patients.

Adherence

Medication adherence was reported to be excellent (i.e. greater than 90% at all measured time-points, as per monthly clinic adherence assessments) in 89.8 % of study participants after one year of follow-up and 81.2 % after two years of study follow-up. There was a statistically significant difference by dual NRTI

combination,with ZDV/ddI-treated patients having a shorter time to first report of non-adherence when compared to those receiving ZDV/3TC- and d4T/3TC-based cART regimens (p=0.03) and anecdotally, study participants frequently expressed difficulties in following the specific food-related instructions when taking non-enteric-coated ddI.. Pooled treatment group analysis also showed statistically significant differences in adherence by sex, with males having a shorter time to non-adherence (p=0.006).

Discussion

We report herein our two-year findings among adult patients enrolled in Botswana's ongoing Tshepo Study, one of the first large-scale randomized clinical trials focusing on cART outcomes to be conducted in sub-Saharan Africa. Our interim study results, backed by an impressive retention rate of study participants during the two years of follow-up, demonstrate an overall excellent immunologic and virologic response to NRTI/NNRTI regimens among HIV-1C infected adults. The low virologic failure rate with resistance mutations, 1.0% at year 1 and 3.2% at year 2, in the four non-ZDV/ddI-containing arms is superior to results obtained from cART-treated cohorts in industrialized countries [26].

Our data clearly demonstrate the inferiority of the dual NRTI combination of ZDV/ddI compared to the dual NRTI combinations of ZDV/3TC and d4T/3TC when given together with either NVP or EFV in HIV-1C infected adults. These findings prompted the closure of the ZDV/ddI containing treatment arms as recommended by our DSMB. Interestingly, a novel reverse transcriptase 67N 70R 215Y genotype was the predominant TAM pathway among HIV-1C infected individuals treated with ZDV/ddI as part of their first-line regimen. This mixture of a TAM-1 (41L/210W/215Y) and TAM-2 (67N/70R/215F/219Q) pathways might represent an HIV-1 subtype C specific resistance pathway to first line ZDV/ddI containing regimens [27].

Although recommended as an alternative first-line regimen by WHO guidelines at the time our randomized trial began, information on the efficacy of the dual NRTI combination of ZDV/ddI in NNRTI-containing cART regimens has been scarce [28-32]. Our findings of a novel TAM resistance pathway selected by HIV-1C in the presence

of ZDV/ddI make a compelling case against the use of this dual NRTI combination for first-line cART in the developing world and support the most recent WHO [33] and international guidelines [34] which now recommend that lamivudine (3TC) (or emtricitabine [FTC]) be given with either tenofovir (TDF) or zidovudine (ZDV) for first-line cART with NVP or EFV.

ART programs are rapidly scaling-up in resource-limited settings in sub-Saharan Africa. Most programs have chosen either *Triommune*™ (d4T/3TC/NVP)-based or *Combivir/Lamzid*™ (ZDV/3TC)-based cART as the first-line regimen [35, 36] . Didanosine still plays an important role for second line cART regimens [37], especially where newer NRTIs (abacavir) and nucleotide reverse transcriptase inhibitors (tenofovir) are not uniformly available, largely due to high cost. Additional studies are needed to characterize the activity of ZDV/ddI-based cART regimens given with a PI as the second-line regimen in settings where d4T and 3TC combinations are used as the NRTI backbones in first-line regimens [38]

The one-year (96.6%) and two-year (95.4%) survival outcomes among cART-treated adults in this large cohort are impressive when compared to data from other cohorts in resource-limited settings [39]. Our study did not include patients that were severely ill at baseline which may have certainly influenced our overall favorable clinical outcomes (i.e. low mortality rates), but as a team we did make every effort to include ill patients as evidenced by the numbers with advanced immunosuppression, advanced WHO clinical stage (3 and/or 4), and high plasma HIV-1 RNA levels at baseline. Nearly a quarter of all deaths were deemed "possibly related to study treatment" with the majority of ARV-related deaths related to severe mitochondrial toxicities, especially lactic acidosis and/or pancreatitis emphasizing the importance of replacing d4T with safer alternatives. Cohort data are still needed to characterize long-term cART outcomes in the region especially as co-morbid medical illness, and particularly cardiovascular causes of death, comprise an increasing percentage of deaths, especially among predominantly NNRTI, non-PI-treated adults.

The sustainability of ART programs largely depends on the tolerability of ARV medication. Although a number of studies in Africa have reported toxicity outcomes

and regimen switch rates, these studies used different regimens, observation times, and severity grading systems. A comprehensive analysis of toxicities related to the individual ARV drugs used in this study is currently being performed. The interim two-year outcomes data of this controlled clinical trial show that all six first-line cART regimens are well tolerated. The overall rate of treatment modifying toxicity was low when compared with results from observational studies followed in the region [40-43]. This may, in part, reflect the fact that many Tshepo study participants initiated cART at higher baseline CD4+ cell counts than in other cohorts. Didanosine- related toxicities such as pancreatits, peripheral neuropathy and diarrhea were rarely a reason for treatment modification in the ZDV/ddI containing treatment arm. Importantly, the occurrence of lipodystrophy and lactic acidosis was disproportionately more frequent among in the pooled, d4T- containing treatment arms. This finding is consistent with the result of other studies that have found a high risk of mitochondrial toxicities among d4T –treated patients [41, 44].

Incident OI rates are important markers for understanding the clinical course of treated HIV disease and for the development of treatment guidelines and planning of health services. Unfortunately, these conditions have often not been reported in a standardized fashion. Limited diagnostic facilities, high TB rates, and difficulties defining immune reconstitution inflammatory syndrome make the task more difficult for African settings [4, 5, 40]. Patients on ZDV/ddI containing cART experienced significantly higher OI rates in the first two years of ARV treatment as compared to those on non-ZDV/ddI regimens. This finding is most probably a consequence of the higher virologic failure rate and the poorer immunologic recovery among patients in the ZDV/ddI arm of the study.

Of note, seven of our intensively monitored participants developed cancer-related diagnoses. As cART-treated adults survive longer in the region, improved diagnostic capacity at the referral hospital level and education and training to enable healthcare providers to more efficiently diagnose and manage these potentially life-threatening conditions will be important regional needs over the next 5-10 years.

We also report excellent one-year adherence rates, which support rates reported

by other ART programs in Africa [8, 11, 45-47]. However, the decline in medication adherence in the second year, especially among male participants, is a concern. As the long-term sustainability of cART programs in Africa largely depends upon sustained cART adherence rates, it is of paramount importance for healthcare personnel to provide ongoing ARV adherence support. This support must be continually adapted to meet patients' needs, while at the same time addressing the needs of those most at risk for poor adherence, with particular attention paid to men and patients with ongoing psychosocial, financial, or physical needs.

In summary, interim results from the Tshepo Study show the importance and feasibility of conducting large clinical research initiatives in resource-limited settings. Our preliminary findings document remarkable immunologic and virologic responses to first-line PI-sparing cART regimens and excellent cohort retention rates. These outcomes surpass many reported in western Europe and the United States. Continued long term cohort follow-up is needed, as are new randomized clinical trials designed to address important regional considerations such as optimal first-line cART regimens, the optimal timing of cART initiation, and the kinetics and development of drug resistance which appears to differ from what has been reported among HIV-1 subtype B infected, cART-treated individuals.

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Chapter 7

Pregnancy rates and birth outcomes among women on efavirenz-containing highly active antiretroviral therapy in Botswana

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Abstract

Background: Millions of HIV-infected women in developing countries are in need of safe and highly effective antiretroviral therapy. Pregnancy rates are usually high in developing countries and Efavirenz (EFV) use in women of childbearing age is of concern due to its potential teratogenicity.

Methods: As part of a prospective study comparing six initial Highly Active Antiretroviral Therapy (HAART) regimens, three of which contained EFV, pregnancy and birth outcomes were evaluated among females enrolled in a randomized clinical trial in Botswana. Before enrollment, all female participants indicated a willingness to avoid pregnancy for the 3-year duration of the study. Monthly urine pregnancy testing and regular contraceptive education and counseling were given to all females on study.

Results: Four hundred and fifty one (69.4%) of 650 enrolled study participants were female and experienced 71 pregnancies, for a rate of 7.9 per 100 person-years during the study. The mean time from HAART initiation to time of first pregnancy was 385 days. The median birth weight of 2950g (interquartile range: 2700- 3250g), the gender (24 females and 15 males) and occurrence of early pregnancy loss (42%) and stillbirths (3%) did not differ between EFV and non-EFV exposed pregnancies (p=0.7). First trimester EFV exposure occurred in 38 (53.5%) of the 71 pregnancies; 22 (57.9%) of these 38 pregnancies resulted in live births. One infant of the 22 EFV-exposed live births (4.5%) had a congenital abnormality with right limb shortening that was assessed to be unrelated to EFV exposure.

Conclusions: The restoration of health and longevity in many HAART-treated women, is often accompanied by childbearing as evidenced by the large fraction of women in our cohort who became pregnant despite their initial statements of intent to avoid pregnancy. Out of 22 first trimester EFV exposed live births one neonate was found to have a major congenital abnormality, however this defect which was unrelated to EFV exposure. The small sample size is insufficient to accurately estimate accurately the underlying risk of congenital malformation after exposure to EFV in early pregnancy, underscoring the importance of reporting to the existing international Antiretroviral Pregnancy Registry. In addition to accessing safe and effective HAART regimens, HIV-infected women require access to comprehensive family planning services, including contraception and procreation counseling.

Millions of HIV-infected women in developing countries are in need of safe and highly effective antiretroviral therapy. In sub-Saharan Africa, Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI)-based HAART regimens are the preferred first- line treatment, largely because of factors such as cost, the potential for drug-drug interactions, and pill burden considerations. When used with two Nucleoside Reverse Transcriptase Inhibitors (NRTIs) as initial treatment, the Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs), EFV and nevirapine (NVP), seem to be equipotent in terms of virologic activity [1]. Although EFV does possess a more favorable side effect profile and a lower pill burden than NVP, these advantages need to be carefully weighed against its potential teratogenicity, especially in resource-poor settings with limited contraceptive and family planning services.

Rates of congenital malformations after HAART- exposed pregnancies seem to be comparable to rates of congenital abnormalities which have been reported in the general (HIV-1 negative) population. In the latest update, the international Antiretroviral Pregnancy Registry has reported a rate of 2.6 (95% CI: 2.1, 3.0) birth defects per 100 live births among pregnancies exposed to HAART at any time during pregnancy (132 birth defects in 5169 HAART exposed pregnancies). Similar prevalence rates were observed in two large European studies [2, 3]. Also, no excess of congenital abnormality has been reported following EFV exposure in early pregnancies. Out of 223 early EFV exposed pregnancies, the Antiretroviral Pregnancy Registry has observed only five birth defects, none of which was a neural tube defect [2.2% (0.7, 5.1%) [4] . Four retrospective human case reports of central nervous system malformations in neonates born from women exposed to EFV [5, 6] have recently led the manufacturer to modify the "pregnancy risk" warning label on EFV from Category C ("risk cannot be ruled out") to Category D ("positive evidence of risk"), however [7] . The earlier Category C pregnancy warning for EFV was based primarily on pre-clinical primate studies [8] .

Consensus ARV treatment guidelines vary somewhat in their NNRTI selection for first-line HAART. Botswana's national policy since the beginning of the public national ARV program in January 2002, has been to place all women with reproductive potential on zidovudine (AZT) and lamuvidine (3TC) with nevirapine

(NVP), reserving EFV combined with the two NRTIs for males and for females without reproductive potential [9] . Other national ARV guidelines such as those of Tanzania [10] recommend stavudine (d4T) and 3TC plus NVP as first line HAART for all qualifying HIV-infected adults. In Botswana, within the context of a large randomized clinical trial, we have intensive longitudinal follow-up from 650 HAART-treated adults, 69.4% of whom were female. All women enrolled in the study received extensive education and counseling, pregnancy screening, and access to family planning services. We report our preliminary data on the pregnancy and birth outcomes of all female study participants, 38 of whom were briefly exposed to EFV during their first trimester of pregnancy.

Methods

Study Population

The Adult Antiretroviral Treatment and Drug Resistance Study (also known as the "Tshepo Study") is a randomized clinical trial that compares the efficacy, tolerability, development of drug resistance, and adherence rates of six different NNRTI-based first-line HAART regimens in urban Botswana. Because three of the six initial HAART regimens under study included EFV, all female participants were counseled extensively regarding the potential harmful effects of EFV on pregnancy; before study enrollment, all indicated a willingness not to become pregnant while on study. All female participants also received counseling before study enrollment and throughout study participation regarding the importance of dual protection by using both condoms and a non-barrier contraceptive method.

During each monthly study visit, reproductive health issues were raised including the following: the status of their current sexual relationships (if applicable), contraceptive usage, and menstrual history. In addition, in accordance with the study protocol, all women with reproductive potential had rapid urine pregnancy tests [Pregwise OneStep hCG (Pregnancy) Rapidip Strip™, Pharma Chem Pharmaceuticals, Republic of South Africa] performed during each scheduled monthly study visit and when clinically indicated. Participants in need of non-barrier contraceptive methods (depomedroxyhydroprogesterone injections [DMPA], oral contraceptive pills, and intrauterine contraceptive devices) were all initially referred to easily accessible

local government health clinics where contraceptive services are provided free of charge [11] . Beginning in May 2005, family planning services became available on-site at the study clinic. Ascertainment of congenital abnormalities were determined from examination of hospital neonatal records and were confirmed by a specialist pediatrician when infants were examined at least once by 3 months of age. No morphologic assessments of cases involving fetal loss were available. This study was approved by the Health Research and Development Committee of the Botswana Ministry of Health and the Harvard School of Public Health Human Subjects Committee.

Statistical Methods

Descriptive univariate analyses included means and medians for normally distributed continuous data, percentages for categorical data, and quartiles for ordinal or non-normal continuous data. To evaluate for potential differences in continuous outcomes by EFV exposure, analyses were performed with the Statistical Product and Service Solutions software, version 14.0 (SPSS Inc., Chicago, Illinois, USA) using the Kruskal-Wallis one-way analysis of variance test. The Chi Square test was used to compare differences between the groups for categorical and ordinal outcomes. P-values less than 0.05 were considered to be statistically significant.

Results

Contraceptive Uptake

According to data collected during routine study visits on case report forms, effective and consistent non-barrier contraceptive methods were used by 80 (19%) of our female study participants. Of these 80 female study participants consistently using non-barrier contraceptives, 41(51.3%) used oral contraceptive pills, 36 (45%) received DMPA injections every three months, and 3 (3.7%) opted for an intrauterine contraceptive device (IUCD).

Pregnancy and Birth Outcomes

Four hundred and fifty one females and 199 male participants were enrolled in the clinical trial between 22 December 2002 and 17 December 2004. As of 01 January

2006, 71 pregnancies had occurred during 900 person-years follow-up among women with reproductive potential, corresponding to an overall pregnancy rate of 7.9 per 100 person-years. The mean time from HAART initiation to time of first pregnancy was 385 days (IQR 232, 562). Four women became pregnant twice. Four urine pregnancy test results were interpreted as false positive and were therefore excluded from the analysis. The median CD4+ cell count value at time of pregnancy diagnosis was 348 cells/mm^3 (IQR 244, 439), reflecting a median gain of 206 cells/mm^3 from the time of HAART initiation (Table 1). All infant Polymerase Chain Reaction (PCR) results obtained up to the time of this report were negative for HIV-1 infection.

TABLE 1. Tshepo Study Participants Becoming Pregnant During Observation Period*

Overall no. pregnancies	71
Pregnancy rate (per 100 person-years)	7.9
Mean time to pregnancy in days (IQR)	385 (232–562)
Median CD4 count (cells/mL) at baseline (IQR)	142 (98–247)
Median CD4 count (cells/mL) at pregnancy diagnosis (IQR)	348 (244–439)
No. pregnancies resulting in live births (%)	39 (54.9)
Female/male	24/15
Median birth weight in grams (IQR)	2950 (2700–3250)
No. pregnancies resulting in stillbirth (%)	2 (2.8)
No. pregnancies resulting in abortion (%)	30 (42.3)
Elective abortion (% of all pregnancies)	4 (5.6)
Unexplained abortion (% of all pregnancies)	26 (36.6)

*December 22, 2002 to December 31, 2005, covering 900 women-years of follow-up.

As of 1 October 2006, all 71 pregnancies have been completed and resulted in 39 live births and 32 pregnancy losses. The 39 pregnancies resulting in live birth (24 females, 15 males) were all singleton, with a median birth weight of 2950 grams (IQR 2700, 3250). Four (10.3%) of the 39 live births were delivered via Cesarean section. Of the pregnancy losses, 26 (81.3%) were due to abortions of unknown etiology, four (12.5%) were attributed to elective abortions (two performed outside of the country and two performed within the country for maternal medical indications), and two (6%) of the pregnancies ended in stillbirth.

TABLE 2. Pregnancy Outcome by HAART Exposure (N = 71)

Pregnancy Outcome	Non–EFV	Exposed EFV Exposed
	(n = 33)	(n = 38)
Live births (female/male)	17 (11/6)	22 (13/9)
Birth weight in grams, median (IQR)	2785 (2408–3140)	3000 (2737–3263)
Prematurity	0	2
Fetal abnormalities	Polydactyly (1), umbilical hernia (1)	Bone dysplasia (1)
Pregnancy loss	16	16
Stillbirth	2	0
Elective TOP	2	2
Unexplained miscarriage	12	14

No significant difference when comparing outcomes of EFV-exposed and non–EFV exposed pregnancies (P = 0.7034).
TOP indicates termination of pregnancy.

Among 38 EFV-exposed pregnancies (all during the first trimester) (Table 2), 22 (57.9%) resulted in live births, and 16 (42.1%) resulted in abortions (two elective, 14 unknown etiology). The median time of EFV exposure was 43 days (IQR 31, 60). All women were switched to non-EFV containing

HAART the same day pregnancy was diagnosed and were given the option to switch back to EFV following the completion of the pregnancy, provided that adequate contraception was practiced. Two (11.8%) minor congenital abnormalities - one infant with polydactyly and one with an umbilical hernia - were detected among 17 live born non-EFV exposed infants. One (4.5%) major congenital anomaly was detected among 22 live born EFV-exposed infants. This infant has congenital shortening of the right lower extremity; radiographic imaging studies documented a shortened right femur with mid-shaft cortical thickening, absence of right femur head epiphysis, and dysplastic right fibular head.

Discussion

Our study is one of the first to document a pregnancy rate among HAART-treated women in sub-Saharan Africa [12] . Our observed pregnancy rate of 7.9 per 100

person years is similar to rates reported among HIV-infected women in the USA [13] and Western Europe [14], but lower than the pregnancy rate among the general Botswana population which recently has been estimated to be approximately 11 % [15, 16] . Notably, at the time of study enrollment (e.g. HAART initiation) all of our female participants verbally agreed to defer childbearing for the duration of the three year study. As per study protocol half of the women were randomized to EFV-based HAART. Because these participants received extensive counseling and education regarding the potential teratogenicity of EFV, even higher pregnancy rates might occur among HAART-treated women cared for in a non-research setting.

There are likely several reasons for the relatively high pregnancy rate, which was independent of the NNRTI being taken (NVP or EFV), in our HAART-treated cohort. First, childbearing is often perceived as a visible and unmistakable expression of health. Although reports from developed countries indicate that pregnancy rates are lower in seropositive women when compared to their seronegative counterparts, HIV-infected women with high CD4+ cell counts not yet on HAART and those with robust immune reconstitution following HAART initiation [17, 18] are more likely to become pregnant [19] . Second, the advent of HAART has also drastically improved the quality and quantity of life for HIV-infected individuals [20, 21]. It is reasonable to anticipate that increasing access to public HAART will influence the reproductive attitudes and behavior of considerable numbers of HIV-infected women in the region.

Third, and most significantly, despite extensive and consistent family planning and risk reduction counseling, uptake of dual contraceptive methods by females in our study was limited. Nationally, Botswana has a long established, easily accessible national family planning service which is available at local government clinics [11]. Recently, however, in the context of widespread acceptance and use of barrier contraceptives (predominantly male condoms), the reported uptake of non-barrier methods has dropped substantially [22]. Contraceptive choice data from our HAART-treated cohort data are consistent with these unfortunate trends, in which study participants report high male condom use (~100%) with few women reporting use of non-barrier contraceptive methods, even in the setting of our clinical trial where

pre-conception counseling and pregnancy surveillance were stringent. Based on anecdotal reports from our study participants, the contraceptive advice that HIV infected individuals often receive at family planning clinics is brief and often limited to advising them to abstain and use condoms. In response to these concerns and to our higher than expected pregnancy rates, our study team also made routine family planning services available to all study participants at the study clinic site beginning in May 2005 .

One other issue that has surfaced during discussions with our study team is that women may feel more comfortable using condoms alone fearing that concomitant use of a non-barrier method may compromise their ability to negotiate the use of condoms. This is especially important in a setting such as ours where condom use may only be viewed as being necessary to prevent HIV transmission to the index partner rather than being seen in a larger context, i.e. family planning and reproductive counseling. Additional research is certainly planned to look more in-depth at the individual social and behavioral factors influencing family planning and reproductive counseling decisions. These studies will include the opinions and attitudes of both men and women in an attempt to identify all potential barriers limiting the use of more efficient contraceptive methods.

To date, data from HAART-exposed females from Western Europe [23] and the United States [4] have consistently documented congenital abnormality rates ranging from 2-4% rates that are similar to those reported in the general (non-HIV-1 infected) population [4] . However, among the reported HAART regimens, only studies evaluating ARV's commonly used in pregnancy (zidovudine, lamivudine, stavudine, nevirapine and nelfinavir) have been sufficiently powered to detect a doubling of the rate of overall birth defects.

Data on pregnancy outcome following EFV exposure are scarce, as EFV-containing HAART is generally avoided in pregnancy due to its potential teratogenic risk. The few prospective studies [3] (including ours) have insufficient sample size to accurately estimate the underlying risk of congenital malformation following exposure to EFV in early pregnancy, a fact that underscores the importance of

prospective reporting to a registry, such as the existing Antiretroviral Pregnancy Registry. Three neonates with meningomyeloceles and one case of a Dandy–Walker malformation in an aborted fetus have been documented following early pregnancy EFV exposure. Within our HAART-treated cohort, the one infant with a major congenital abnormality (shortening of the right limb/femur) was exposed to EFV for 31 days, starting from the first day of the mother's last menstrual period. EFV was discontinued before the critical period of limb development [23, 24] and, thus, it is very unlikely that this defect was related to EFV-exposure. Our study team circulated the details of this case to international experts who helped formulate "ARV medication relatedness" conclusions. Unfortunately, neither country-specific nor regional data on the background frequency of similar anomalies are available. Our observed rate of 26 (36.6%) unexplained early pregnancy losses from 71 total pregnancies is certainly higher than the rate one would expect from spontaneous abortions alone [25] . All early pregnancy losses in our study occurred within the first three months following the date of last reported menstrual period. These early pregnancy losses may be due to multiple factors.

First, underlying congenital malformations may have resulted in spontaneous abortion. Unfortunately, the lack of medical information on these early pregnancy losses precludes definitive conclusions regarding such an etiology.

Second, these early losses may be due to HAART alone, yet we are unaware of any data linking individual ARV medications or classes of ARV medications to early pregnancy loss. Reports on increased rates of premature deliveries among HAART-treated women are inconsistent [26-29] and also would involve pathophysiological mechanisms different from those leading to abortions.

Third, although we cannot fully exclude that our high abortion rate is causally related to ARV treatment, we can note that a significant proportion of unexplained miscarriages in our study are most likely due to elective abortions. Based on feedback that research nurses obtained, a substantial proportion of females stated that their pregnancies were unwanted. This information was obtained at the time when their urine pregnancy test first became positive—when the study nurses were reviewing

the positive rapid urine pregnancy tests with them. Current Botswana statutes do not allow for the termination of pregnancy except in special circumstances, including cases posing a significant health risk to the mother and/or fetus with prior documentation by two medical physician [30, 31] . Of note, maternal HIV infection is not generally regarded as basis to meet these requirements and women may have obtained abortions outside of the existing government healthcare system in order to avoid social and legal consequences of elective pregnancy termination. In a larger context, however, we also realize that child wish is a strong desire for all women with reproductive potential and certainly may have been present but not verbally expressed to our study staff. A fraction of these pregnancies occurred among HAART-treated female study participants who had now experienced excellent clinical response and impressive CD4+ cell count gain and were now eager to begin families and solidify existing relationships. The recently-reported high incidence of induced abortion in Uganda [32] further underscores the need to conduct more detailed behavioral studies to better understand the factors influencing female contraceptive and reproductive choices.

The manufacturer's warning that treatment with EFV should be avoided in the first trimester of pregnancy due to the risk of teratogenicity [7] has led to the recommendation to avoid EFV use in women of reproductive potential unless effective contraception can be ensured [33] . With the rapid expansion of HAART programs in developing countries, EFV will become a choice for women with reproductive potential in whom NVP is contraindicated (e.g. prior NVP toxicity or among women initiating HAART with CD4+ cell counts > 250 cells/mm^3), particularly in settings where access to protease inhibitor drugs is limited. It will be important, therefore, for HAART programs to also develop parallel family planning services that are staffed by personnel uniformly trained to provide accessible and comprehensive counseling and education for HAART- (including EFV-) treated women who became pregnant. The management of EFV-exposed pregnancies when identified during later stages of pregnancy (second or third trimester) is still done on a case-by-case basis. Routine screening for neural tube defects via ultrasound imaging and/or serum alpha fetoprotein levels is of limited availability in the region and, therefore, is not routinely practiced.

Conclusions

The risk of birth defects after early EFV exposure precludes the widespread use of EFV for many women in need of convenient, efficacious and safe HAART in resource limited settings. Numerous HIV-infected and HAART-treated women are likely to receive EFV in the wake of the expansion of national ARV treatment programs worldwide. Evidenced-based data on risk of EFV in pregnancy can only be strengthened if all care providers in the field prospectively report all HAART (and EFV) exposure outcomes to a pregnancy exposure registry, such as the international Antiretroviral Pregnancy Registry. Those involved in ARV treatment program monitoring and evaluation need to ensure that family planning and reproductive health counseling needs are met, especially as they relate to physical manpower and training requirements. To advise and educate burgeoning numbers of HAART-treated women and men in sub-Saharan Africa, the state of existing family planning services including contraception and procreation counseling warrants in-depth evaluation.

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Chapter 8

Adult combination antiretroviral therapy in sub-Saharan Africa: lessons from Botswana and future challenges

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Abstract

Numerous national public initiatives offering first-line combination antiretroviral therapy (cART) for HIV infection have commenced in sub-Saharan Africa since 2002. Presently, 2.1 million of an estimated seven million Africans in need of cART are receiving treatment. Analyses from the region report favorable clinical/ treatment outcomes and impressive declines in AIDS-related mortality among HIV-1-infected adults and children receiving cART. While immunologic recovery, virologic suppression and cART adherence rates are on par with resource-rich settings, loss to follow-up and high mortality rates, especially within the first 6 months of treatment, remain a significant problem. Over the next decade, cART coverage rates are expected to improve across the region, with attendant increases in healthcare utilization for HIV- and non-HIV-related complications and the need for expanded laboratory and clinical services. Planned and in-progress trials will evaluate the use of cART to prevent primary HIV-1 infection with so-called 'test and treat' expansions of coverage and treatment. Education and training programs as well as patient-retention strategies will need to be strengthened as national cART programs are expanded and more people require lifelong monitoring and care.

Key words: adherence; cART; combination antiretroviral therapy; efficacy; HIV/ AIDS; mortality/survival; sub- Saharan Africa; tolerability/toxicity

Update

HIV/AIDS in Africa

As of December 2007, there were an estimated 33 million persons (range: 30–36 million) living with HIV-1 worldwide [1]. Globally, the HIV-1 epidemic appears to have stabilized despite staggering rates of new infections (estimated at 2.7 million [range: 2.2–3.2 million]) and deaths (estimated at 2.0 million [range: 1.8–2.3 million]) in the year 2007 alone [1]. An estimated 1.9 million (range: 1.6–2.1 million) people were newly infected with HIV-1 in sub-Saharan Africa in 2007, bringing the regional total 22 million (range: 20.5–23.6 million) persons living with HIV-1, and representing 67% of the global burden [1].

Certain individuals appear to be at a heightened risk for specific medication-related toxicities, many of which are life-threatening. Preliminary evidence suggests the incidence and patterns of genotypic drug-resistance mutations in sub-Saharan Africa differ from western Europe and North America, perhaps due in part to a higher prevalence of HIV-1 subtype C infection. Opportunistic infections and malignancies, especially TB, continue to cause significant morbidity and mortality in sub-Saharan Africa. The epidemic of HIV-1 infection in sub-Saharan Africa varies significantly from country to country in scale, scope and in dominant circulating HIV-1 subtype [1]. The reported adult national HIV-1 prevalence is less than 2% in many west and central African countries, while in 2007 the prevalence rate exceeded 15% in seven southern African countries (Botswana, Lesotho, Namibia, South Africa, Swaziland, Zambia and Zimbabwe), and was greater than 5% in seven other countries (Cameroon, the Central African Republic, Gabon, Malawi, Mozambique, Uganda and Tanzania) [1]. In Botswana, reductions in HIV-1 prevalence among pregnant 15–19-year-olds (from 25% in 2001 to 18% in 2006) suggest that the rate of new HIV-1 infections is on the decline in that nation [1,2]. The epidemics in Malawi and Zambia also appear to have stabilized, amid some evidence of favorable behavior changes [1,3] and signs of declining HIV-1 prevalence among women presenting for routine antenatal care [1, 4-7]. HIV-1 surveillance data from antenatal clinics in South Africa also suggest that the country's epidemic is stabilizing [1,8], but there is little evidence to date of major behavioral changes among those most 'at-risk' of acquiring HIV-1.

In Rwanda, Kenya, Zimbabwe and Uganda, significant changes in sexual behavior have led to declines in the number of new HIV-1 infections and contributed to the global stabilization of new infection rates among adults aged 15–49 years which began in the late 1990s [1]. These gains, however, have not been consistent within or between regions. Favorable epidemiological and behavioral trends [1] have not been sustained in some countries, and the number of new infections is increasing in several areas, including Mozambique [1]. In Lesotho, Namibia, South Africa and Swaziland, HIV-1 prevalence rates have reached extraordinarily high plateaus [1].

The rate of combination ARV therapy (cART) regimen switching due to treatment failure is expected to increase, but at present there are few second-line, and frequently no third-line, options in most resource-limited settings. Ritonavir-boosted lopinavir is the only widely available generic, co-formulated, heat-stable protease inhibitor (PI), and bringing additional agents to market is a priority of international health organizations. Newer ART drug or antiretroviral (ARV) classes such as integrase inhibitors and CCR5 antagonists are difficult to implement in resource-limited settings, and are not expected in generic formulations in the near future. The criteria for clinical and immunologic treatment failure criteria used in many cART programs perform poorly for detecting virologic failure, increasing the risk of treatment resistance. Large clinical trials investigating the use and cost–effectiveness of routine monitoring of HIV-1 plasma HIV-1 RNA in clinical care are in progress in resource-limited settings. Physician-centered care models are not sustainable in sub-Saharan Africa and escalating manpower constraints will require the evaluation and adoption of novel 'taskshifting' approaches to care.

Combination antiretroviral therapy in Africa

The recent increase in access to cART has been impressive in many African countries. As of December 2007, an estimated 3 million people in low- and middle-income countries were receiving cART [1]. Although this represents only 31% of those in need of treatment, it represents a 45% increase in the total number of patients receiving cART in 2006 [1].

Beginning in 2002, ARV therapy (ART) treatment programs have been rolled out in public sector health facilities in sub-Saharan Africa. In January 2002, ART became available in Botswana in public-sector health facilities through the national ARV treatment program called Masa ('new dawn' in Setswana). The Masa program now provides public ART to more than 115,000 people at more than 32 designated national outpatient treatment sites. This success translates to a current cART coverage rate exceeding 90% of estimated HIV cases, one of the highest in the world. Similar programs have been implemented in other countries supported by the WHO and the President's Emergency Plan for AIDS Relief (PEPFAR). In Namibia, where the cART coverage rate was below 1.0% in 2003, 88% of persons in need of treatment were receiving cART as of 2007 [1]. While Rwanda ranked 161st out of 177 countries in the Human Development Index [1,9] and is still recovering from the 1994 genocide, it has increased its cART coverage rates from 1% in 2003 to almost 71% in 2007 [1]. Such impressive expansion in HIV care delivery was made possible by a 40-fold increase in the number of Rwandan ARV treatment sites during this period [1].

With a few exceptions (Botswana, Namibia, Rwanda and Senegal), the majority of African nations still have less than 50% cART coverage. The sub-Saharan African region, as a whole, however, has made significant strides in increasing access to prevention of mother to child transmission (PMTCT) programs. Five countries (Kenya, Namibia, Rwanda, South Africa and Swaziland) have improved coverage to 50–75% of those in need of services, and one country, Botswana, has achieved over 90% coverage (Box 1) [1].

Box 1
Combination antiretroviral therapy coverage rates of qualifying (i.e., those with advanced HIV/AIDS) adults and children in Africa*

Less than 25% coverage	• Mozambique	• Lesotho
• Burundi	• Sierra Leone	• Malawi
• Central African Republic	• Somalia	• Mali
• Chad	• Sudan	• Morocco
• Democratic Republic of Congo	• Zimbabwe	• Nigeria
		• South Africa
• Djibouti	**25–49% coverage**	• Swaziland
• Egypt	• Angola	• Uganda
• Eritrea	• Benin	• Tanzania
• Gambia	• Burkina Faso	• Zambia
• Ghana	• Cameroon	
• Guinea–Bissau	• Côte d'Ivoire	**50–75% coverage**
• Liberia	• Equatorial Guinea	• Rwanda
• Madagascar	• Ethiopia	• Senegal
• Mauritania	• Gabon	**Greater than 75% coverage**
• Mauritius	• Guinea	
	• Kenya	• Botswana
		• Namibia

Breakdown by quartiles (n = 106)
Data adapted from [1]
*All values are based on need estimates using Joint United Nations Programme on HIV/AIDS/WHO methodology. Includes all countries for which the number of adults and children on antiretroviral therapy was reported for 2007, except countries for which UNAIDS/WHO need estimates are not available, or with need estimates less than 500.

Current cART recommendations

In resource-rich settings, the current gold-standard first-line ART regimen is a combination of the nucleoside reverse transcriptase inhibitors (NRTIs) tenofovir (TDF) plus emtricitabine (FTC) with the non-NRTI (NNRTI) efavirenz (EFV) [10,201]. Other options include the use of two NRTIs, TDF plus FTC or alternatively abacavir (ABC) plus lamuvidine (3TC), given with a ritonavir-boosted PI such as atazanavir/ritonavir (ATV/r), fosamprenavir/ritonavir (FPV/r), darunavir/ritonavir or lopinavir/

ritonavir (LPV/r) [201]. In multiple adult head-to-head clinical trials, ART-treated persons receiving EFV with zidovudine (ZDV) plus 3TC, and more recently with TDF plus FTC, have experienced the most favorable virological outcomes [11-13,201].

The current standard recommendations for first-line adult ART in sub-Saharan Africa consists of two NRTIs plus one NNRTI [14,202]; with the vast majority of ART-treated adults receiving either stavudine (d4T) and 3TC or ZDV and 3TC with either nevirapine (NVP) or EFV. PIs are primarily reserved for second-line treatment, owing to issues of cost, dosing frequency, drug–drug interactions, potential for long-term side effects and higher pill burden. Persons failing first-line regimens in sub-Saharan Africa are usually switched to a regimen of two NRTIs (at least one of which is new) plus a boosted PI, typically LPV/r [14,202].

When to start cART

Criteria for cART initiation differ between settings and by national guidelines [14]. For example, the current International AIDS Society USA guidelines for treatment of HIV-1 infection in adults [15,16] recommend that cART should be considered in asymptomatic adults once their CD4+ cell count declines below 350 cells/mm^3 and initiated in all patients whose CD4+ cell count values are less than 200 cells/mm^3. In resourcerich settings, the scientific evidence of advantages of starting earlier cART is growing [17], with the standard-of-care evolving to cART initiation as early as the initial HIV-1 diagnosis.

By contrast, the 2002 WHO guidelines, which are still used in some countries, recommend cART only for patients with WHO clinical stage 4 disease or a CD4+ cell count of less than 200 cells/mm^3. These recommendations were revised in 2003 and now state that cART should also be initiated in patients with both WHO clinical stage 3 disease and a CD4+ cell count between 200 and 350 cells/mm^3 [203].

The vast majority of national guidelines currently rely on the identification of WHO clinical stage 3 or 4 for cART initiation criteria, rather than using alternative CDC classification definitions. Please refer to Box 2 for a detailed list of WHO clinical stages for HIV/AIDS.

Data from adult cART-treated programs in Botswana have shown a significantly increased riskof mortality among persons initiating cART with more advanced immunosuppression. In one such study patients initiating cART with a base-line CD4+ cell count of less than 50 cells/ mm^3 had a 3.2-fold higher mortality rate (p = 0.004) compared with patients with a CD4+ cell count between 51 and 200 cells/ mm^3 at the time of cART initiation [18].

All patients had advanced immunosuppression at the time of cART initiation (n = 153, median CD4+ cell count: 96, interquartile range [IQR]: 33–165, 31% with CD4+ cell count <50 cells/mm^3). At 48 weeks the mean CD4+ increase was 204 cells/mm^3 and 78.8% had achieved an undetectable plasma HIV-1 RNA level [18].

Recently released data from the ongoing CIPRA HT 001 trial Data Safety and Monitoring Board (DSMB) [204] has shown that in Haiti, starting cART in adults (n = 816) when their CD4+ cell counts are between 200 and 350 cells/mm^3 improves survival when compared with deferring cART until their CD4+ cell count drops below 200 cells/mm^3. In addition, interim analysis showed that among patients initiating cART without TB at baseline, 18 persons in the early treatment (CD4+ cell count of 200–350 cells/mm^3) group developed incident TB compared with 36 persons in the standard-of-care (CD4+ cell count <200 cells/mm^3) group, a statistically significant difference [204]. Certainly, longer term follow-up and additional study data are needed, but such data do have the potential to change the standard-of-care recommendations for 'when to start' cART in resource-limited settings.

Box 2

WHO clinical staging
Clinical stage 1: asymptomatic
• Asymptomatic
• Persistent generalized lymphadenopathy
Clinical stage 2: moderate disease
• Unexplained moderate weight loss of less than 10% of baseline weight
• Recurrent upper respiratory infections (sinusitis, otitis media, tonsillitis, pharyngitis)
• Mono-dermatomal VZV (shingles)
• Recurrent oral ulceration
• Papular pruritic eruptions/dermatitis
• Seborrheic dermatitis
• Fungal nail infections
Clinical stage 3: advanced disease
• Unexplained weight loss above 10% of baseline
• Unexplained chronic diarrhea for more than 1 month
• Unexplained persistent fever (>37 5°C, intermittent or constant) for more than 1 month
• Persistent oral candidiasis
• Oral hairy leukoplakia
• Pulmonary TB
• Severe bacterial infections (e g , pneumonia, meningitis, PID*, bone/joint infection, bacteremia)
• Multidermatomal, recurrent mono-dermatomal or ophthalmic VZV*
• Necrotizing ulcerative gingivitis, peridontitis or stomatitis
• Unexplained anemia (<8 0 gr/dl), neutropenia (<500/µl) and/or thrombocytopenia (<50,000/µl)
Clinical stage 4: severe disease
• HIV-1 wasting syndrome
• Pneumocystis jiroveci (formerly carinii) pneumonia
• Recurrent severe bacterial pneumonia
• Chronic HSV infection (orolabial, genital or rectal for more than 1 month or visceral at any site)
• Esophageal candidiasis (or candidiasis of trachea, bronchi or lungs)
• Extrapulmonary TB
• Kaposi's sarcoma
• Cytomegalovirus retinitis or infection of other organs
• CNS toxoplasmosis
• HIV-1 encephalopathy (AIDS dementia complex)
• Extrapulmonary cryptococcosis, including meningitis
• Disseminated non-TB mycobacterial infection
• Progressive multifocal leukoencephalopathy
• Chronic diarrhea due to cryptosporidiosis and/or isosporiasis
• Disseminated mycosis
• Recurrent septicemia

• Lymphoma (cerebral or non-Hodgkin's)
• Invasive cervical carcinoma
• Atypical disseminated leishmaniasis
• Symptomatic HIV-associated nephropathy and cardiomyopathy

HSV: Herpes simplex virus; PID: Pelvic inflammatory disease, VZV. Varicella zoster virus.
*Not part of international WHO staging, but added as frequent Botswana-specific HIV-related 'advanced' conditions meriting combination antiretroviral therapy initiation.
Recently released data from the ongoing CIPRA HT 001 trial Data Safety and Monitoring

What to start

Current US Department of Health and Human Services (DHHS) guidelines [201] recommend that qualifying HIV-1-infected adults be initiated on two NRTIs plus one NNRTI or two NRTIs plus one boosted PI. The preferred agents are co-formulated TDF/FTC, EFV, and ritonavir-boosted atazanavir, lopinavir, darunavir or fosamprenavir. Alternatives include the dual NRTI combination of ABC plus 3TC, the NNRTI NVP (only in women with a baseline CD4+ cell count of less than 250 cells/mm^3 or men with a CD4+ cell count less than 400 cells/mm^3 due to the heightened risk for hepatotoxicity), and the unboosted PIs of atazanavir or fosamprenavir [201].

The preferred first- and second-line cART regimens for sub-Saharan Africa are a subject of considerable debate. Policymakers need to consider a range of issues when formulating national guidelines, including cost, efficacy, tolerability, the potential for drug resistance and drug–drug interactions, as well as short-term and longer term ARV treatment adherence. Several national initiatives offering public NNRTI-based cART have recently commenced in sub-Saharan Africa. Preliminary outcomes data from ARV pilot studies in Côte d'Ivoire [19], Senegal [20], Uganda [21], Khayelitsha, South Africa [22] and Botswana [23], as well as preliminary data from larger public cART initiatives in Malawi [24,25], Botswana [26] and Zambia [27], have documented favorable outcomes among the vast majority of cART-treated adults.

Nucleoside reverse transcriptase inhibitors

The WHO guidelines issued in 2003 recommended a five ARV formulary approach for first-line use: 3TC plus ZDV or d4T plus NVP or EFV [203]. In 2006, this recommendation was revised to include TDF or ABC as alternative first-line NRTIs

while advising policymakers to 'move away' from d4T-containing regimens in an attempt to minimize or avoid the potential mitochondrial toxicities associated with this ARV medication. In sub-Saharan Africa, the majority of cART-treated adults are presently receiving d4T (or ZDV) and 3TC plus either NVP or EFV. Women with reproductive potential are initiated on NVP-based cART owing to the potential teratogenicity of EFV.Most recently, many countries in the region, including Botswana, Zambia and, on a smaller scale, Nigeria, have switched to TDF-based cART, administered with FTC as the co-formulation Truvada™. This regimen is in line with resource-rich recommendations and avoids the potential toxicities associated with ZDV and d4T use.

There are, however, a few smaller case series that have reported poorer virologic outcomes among adults receiving first-line TDF, FTC and NVP [28], which warrants consideration and close monitoring of the initial group of adults treated with this regimen in sub-Saharan Africa. In the analysis of data from the ART-LINC collaboration [29], which included adults receiving cART at 17 programs in 12 countries in sub-Saharan Africa, South America and Asia (n = 36,715), median CD4+ cell count at the time of cART initiation (baseline) increased in Africa in recent years (2005–2006) to 122 cells/mm^3 [IQR: 53–194] where the majority of cART-treated patients (56%) received d4T/3TC and NVP. In all regions, females had higher median CD4+ cell counts than men at the time of cART initiation, with an average of 22 cells/mm^3 higher in Africa [29].

ZDV/didanosine inferiority

Interim data from a large randomized clinical trial conducted in Botswana has shown that the proportion of participants with virologic failure and genotypic resistance mutations was 11% in those receiving ZDV/didanosine (ddI)-based cART versus 2% in those receiving either ZDV/3TC- or d4T/3TC-based cART (p = 0.002) [23]. The median CD4 T-cell count increase at 1 year was 137 cells/mm^3 (IQR: 74–223) and 199 cells/mm^3 (IQR: 112–322) at 2 years with a significantly lower gain in the ZDV/ddI arm [23]. At 1 and 2 years, 92.0 and 88.8%, respectively, of patients had an undetectable plasma HIV-1 RNA level (≤400 copies/ml) [23].

Additional analyses suggested a trend towards poorer adherence among ZDV/

ddI-treated participants, and anecdotally study participants frequently expressed difficulty following the specific food-related instruction (i.e., the need to take it first thing in the morning on an empty stomach) when taking the non-enteric coated ddI [23]. How these findings relate to the efficacy of ZDV/ddI as the dual NRTI backbone for second-line cART regimens remains unknown and a necessary area of future research.

TDF considerations

Owing to the potential of TDF for bone mineral density changes, including demineralization and bone porosity effects, TDF is currently not recommended in pregnancy. Consensus regional recommendations state that all women identified as pregnant should be immediately switched to ZDV/3TC plus NVP if they have a CD4 cell count of less than 250 or 200 cells/ mm^3, depending on existing national ARV guidelines. Women often continue their ZDV-based regimen post-partum if they have not experienced any ZDV-related or exacerbated side effects [202].

Tenofovir also has the potential to cause renal insufficiency or failure from renal tubular insult, and therefore creatinine clearance must be calculated and recorded at baseline, 3 and 6 months following cART initiation, and if stable, every 6 months thereafter. Reliance on serum creatinine as a surrogate marker for creatinine clearance is inappropriate, as significant glomerular filtration rate reductions can occur before serum creatinine values become abnormal [202]. Creatinine clearance can be estimated from formulas using age, gender, body mass and serum creatinine. If a person's baseline creatinine clearance is less than 60 ml/min, TDF should not be initiatied. If a person's creatinine clearance reduces to less than 50 ml/min while receiving TDF, an HIV clinician should be immediately consulted so the appropriate casemanagement steps can be taken [202].

Non-nucleoside reverse transcriptase inhibitors

Based on available data from numerous clinical trials [11,12,30-35], EFV is the NNRTI of choice in resourcerich settings and is 'preferred' for first-line cART along with two NRTIs, usually TDF and FTC [33-35,201]. This recommendation is based on impressive efficacy and more favorable tolerability data [11,12,27-35,201]. In resourcelimited

settings, the majority of cART-treated adults are female [22,26,27,36,37] and have been placed on NVP-based cART regimens due to the potential teratogenic effects of EFV. In addition, recent data have also shown that maternal NVP efficacy may be significantly compromised when administered to women within 6 months of receiving singledose (sd) NVP for PMTCT purposes [38]. NVPbased cART regimens are available generically as Triommune™, a coformulated tablet containing NVP, d4T and 3TC [1,16,203].

Compromised efficacy of NVP-based cART among women with prior/recent sd NVP exposure

Preliminary efficacy data are available from women enrolled in the Adult AIDS Clinical Trials Group (AACTG 5208/OCTANE) study [39] from eight sites in sub-Saharan Africa. In this study, 243 women with prior sd NVP exposure at least 6 months previously and 502 women without prior sd NVP exposure received TDF/ FTC with either NVP or LPV/r [39]. Analysis of the data of women with prior sd NVP exposure revealed that significantly more women in the NVP arm (n = 31 [26%]) than the LPV/r arm (n = 10 [8%], p = 0.0007) reached the primary end point of virologic failure. This effect was greatest among women initiating cART within 6–12 months of sd NVP and appeared to diminish with increasing time between sd NVP receipt and cART initiation. Investigators concluded that TDF/FTC plus LPV/r is superior to TDF/ FTC plus NVP in women with prior exposure to peripartum sd NVP [39].

Studies in Botswana [38] show that women recently receiving sd NVP for PMTCT had higher rates of virologic failure when subsequently treated with NVP-based cART compared with women treated with NVP-based cART without recent sd NVP exposure. However, this applied only when NVP-based cART was initiated within 6 months following sd NVP [38].

Based on these results, many countries in the region now specify that nonpregnant women qualifying for cART initiation who have had sd NVP exposure for PMTCT within the previous 6 months should be initiated on TDF/FTC (or 3TC) plus LPV/r [38-41,202]. Non-pregnant women initiating cART who received sd NVP for PMTCT

greater than 6 months ago should be initiated on standard first-line cART of TDF/FTC or ZDV/3TC with either NVP or EFV, but must be closely monitored for evidence of virologic failure [202]. One additional safeguard adopted in Botswana and incorporated into the most recent national ARV treatment guidelines in November 2008 specifies that all women who have received sd NVP for PMTCT at any time in the past and are initiated on NNRTI-based cART require close monitoring for virologic failure [202].

NVP versus EFV outcomes

The 2NN trial [42], a large adult randomized trial, compared HIV-1-infected adults (n = 1216) receiving the dual NRTI combination of d4T plus 3TC with either NVP or EFV in North and South America, Australia, Europe, South Africa and Thailand. They found no significant differences in efficacy with NVP-versus EFV-treated patients experiencing similar rates of virologic failure. At week 48, 70% of patients taking EFV, 65% of those taking twice-daily NVP and 70% of those taking once-daily NVP had achieved undetectable plasma HIV-1 RNA levels using a lower plasma HIV-1 RNA cutoff of less than 50 copies/ml [42]. Additional 2NN analyses [42], however, did demonstrate that NVP was associated with higher rates of serious (i.e., grade 3 or higher) toxicities.

At or near the time the 2NN trial was completed, Boehringer-Ingelheim Pharmaceuticals, Inc. issued a letter to healthcare professionals [43] detailing these and related findings with advice against the use of NVP-based cART to women with baseline CD4+ cell counts of more than 250 cells/mm^3. This warning was based on an observed increased risk (up to 12-fold) for NVP-associated hepatotoxicity. similar recommendations were made for men, but the baseline CD4+ cell count threshold was set higher at more than 400 cells/mm^3 [43]. A recently published study among private-sector-treated adults from nine sub-Saharan Africa countries [44] suggests EFV superiority when compared with NVP, a result that is similar to reports from resource-rich settings. In this observational cohort of approximately 2800 adult patients, multivariate analysis showed that NVP-treated patients had a greater risk of virologic failure (hazard ratio [HR]: 1.52 [95% CI: 1.24–21.86]), death (HR: 2.17 [95% CI: 1.31–33.60]) and regimen discontinuation (HR: 1.67 [95% CI:

1.32–32.11]) when compared with EFV-treated patients [39]. Recently presented data [36] from the Adult Antiretroviral Treatment and Drug Resistance ('Tshepo') study in Botswana, which random randomized 650 ARV-naive adults to NVP or EFV, documented no significant difference by assigned NNRTI in the time to virologic failure with resistance (log-rank p = 0.14; NVP versus EFV risk ratio = 1.54 [95% CI: 0.86–2.70]). Rates of virologic failure with resistance were 9.6% for NVP-treated patients (95% CI: 6.8–13.5) versus 6.6% for EFV-treated patients (95% CI: 4.2–10.0) at 3 years [36]. NVP-treated females had a statistical trend towards higher virological failure rates compared with EFV-treated females (Holm corrected log-rank p = 0.072; NVP vs EFV risk ratio = 2.22 [95% CI: 0.94–5.00]). There were no differences among males [36].

Genotypic drug resistance patterns & differences

The rates and patterns for the development of genotypic drug resistance mutations appear to differ in HIV-1 nonsubtype B-infected adults who predominantly reside in resource-limited settings of the world compared with HIV-1 subtype B-infected adults that predominantly reside in resource-rich settings.

Major NRTI mutations

Recent data among HIV subtype C-infected adults in Malawi have documented that 19% of persons who met WHO clinical or immunological criteria for failure and had plasma HIV-1 RNA levels above 400 copies/ml showed the following: 19% had the Q151M mutation, 56% had thymidine analog mutations and 24% had the K65R or K70E mutation [45].

In addition, the reverse transcriptase mutation K65R can be selected by TDF, ddI, ABC and d4T, and leads to reduced susceptibility to all clinically used NRTIs except ZDV [46-50]. Wainberg et al. previously described the rapid emergence of the K65R mutation by week 12 in subtype C virus in cell culture when exposed to TDF compared with other subtypes (A, B, D, CRF_AE, CRF2_AG, and HIV-2). The group showed that subtype C isolates had unique polymorphisms in RT codons 64, 65 and 66, which were absent in other subtypes [51].

Additional studies have highlighted the preferential emergence of K65R in subtype

C-infected patients failing d4T/ddI-based cART regimens in Botswana (30%), and d4T/3TC-based regimens in South Africa and Malawi (7–20%) [51-56]. By contrast, K65R was present in only 1.8% of HIV-1 subtype B-infected patients failing d4T-based regimens in the Stanford HIV Resistance Database. It appears to be most common in patients failing TDF-based regimens, where it is found in up to 15% of these patients [52,56-59]. The underlying reason for the more rapid selection of the K65R mutation among subtype C-infected adults does not appear to be related to enzyme-based mechanisms [52] but more research is needed. Based on these studies, HIV-1 subtype C-infected adults treated with TDF-based regimens will need to be carefully monitored for the possible selection of K65R [52,53].

Interim data from a large randomized clinical trial conducted in Botswana have shown inferiority in primary end point among adults treated with ZDV/ddI-containing cART when compared with those treated with either ZDV/3TC- or d4T/3TC-containing cART [22,60]. The 67N 70R 215Y genotype with wild-type mutations at codon positions 41 and 210 was a dominant pattern of NRTI-associated mutations at the time of virologic failure [60]. Although limited by small numbers, these data on the 67N 70R 215Y genotype with wild-type amino acids at codon positions 41, 210 and 219 in HIV-1 subtype C infection suggest that the evolution of ARV-associated mutations and thymidine analog mutation pathways may be unique in non- B subtypes treated with certain cART regimens [60].

Major NNRTI mutations

Research has shown HIV-1 subtype C variants, the prevalent circulating subtype in southern Africa, contain a valine codon 106 polymorphism (GTG) that facilitates a V106M transition (GTG←--ATG) following selection with EFV [61]. Prior to this report, V106A was listed as a NVP-specific mutation while V106M was not recognized. It is now widely believed that this V106M mutation represents a signature mutation among HIV-1 subtype C-infected persons treated with EFV, which possesses the potential to confer high-level multi-NNRTI resistance [61].

The most common major NNRTI mutations identified in patients with virologic failure in the recently completed Adult Antiretroviral Treatment and Drug Resistance ('Tshepo') study in Botswana were as follows: K103N (34.8%), G190A (28.3%), V106M (17.4%), Y181C (13.0%) and V108I (4.8%) [36].

Survival/mortality

Despite the recent successes of the rapidly expanding number of ARV treatment sites in sub- Saharan Africa, an analysis by the ART–lower income county (ART-LINC) collaboration found that the adjusted HR (AHR) of mortality among cART-treated adults in resource-limited settings was 4.3 (95% CI: 1.6–11.8) during the first months of cART compared with those treated in resource-rich settings [62]. The AHR dropped from 4.3 during the first 6 months following cART initiation to 1.5 (95% CI: 0.7–3.0) during months 7–12 [62]. Additional data from this group [63] evaluating 5491 adult patients found that the incidence of early patient loss increased when programs were scaled-up and were associated with fee-for-service and advanced immunosuppression at baseline. Overall, 3.8% of patients had no follow-up, 16.0% were lost to follow-up and 2.8% were known to have died in the first 6 months following cART initiation [63]. Another study by Bisson et al. in Botswana found that over half (58.8%) of patients deemed 'lost to follow-up' were confirmed dead after intensive tracing. In addition, they concluded that a significantly increased risk of death following cART initiation among men (AHR: 1.74; 95% CI: 1.05–02.87) would have been missed had these patients not been traced in their respective communities [64].

Lawn *et al.* have documented that between 8 and 26% of patients receiving cART in sub- Saharan Africa die within the first year of treatment, with the majority of these deaths occurring in the first few months of treatment [65,66]. Baseline characteristics that are independently associated with early mortality risk include low CD4+ cell count, advanced WHO clinical stage disease (stage 3 or 4 disease), low BMI (<18.5), anemia and male gender [65]. In numerous programs in Africa [65,66], the median CD4+ cell count values at the time of cART initiation are low, in the range of 100 and 150 cells/mm^3 [65-72], and this has been highlighted as a significant contributing factor to high early mortality rates [65,66]. In their most

recent analysis of 2423 adult patients, Lawn et al. documented mortality rates for up to 5 years of follow-up at 8.1%, and a multivariate analysis showed that patients who had a baseline CD4+ cell count of less than 100 cells/mm^3 had significantly higher cumulative mortality estimates at 1 and 4 years (11.6 and 16.7%, respectively) compared with patients whose baseline CD4+ cell count was at least 100 cells/mm^3 (5.2 and 9.5%, respectively). Investigators contend that these discrepancies were largely due to greater cumulative persontime being spent in what is now being referred to as the 'death zone' (i.e., CD4+ cell count values <200 cells/mm^3) [65,66].

Bussmann *et al.* recently published results from a trial of 633 public cART-treated adults in Botswana [26] with Kaplan–Meier survival estimates at 1, 3 and 5 years of 82.7, 79.3 and 79.0%, respectively. Adjusted mortality rates showed similarly high early mortality rates when compared with other cohorts of adults initiating cART with severe baseline immunosuppression [31,66-74]. Mortality in this cohort was highest in the first year, with 50% dying in the first 3 months and approximately 86% of all deaths occurring within the first year. The majority of deaths were secondary to advanced AIDS, with only a small fraction attributed to ARV-related toxicities. In addition to the high early on-treatment mortality, another concern was the significant number of patients that qualified to receive cART but died prior to cART initiation, further emphasizing that a swift and decentralized roll-out of ART programs in high-prevalence countries is urgently needed [75,76].

Tolerability/toxicity Rates of ARV-related toxicities in sub-Sahara n Africa

A recent meta-analysis review of 28 articles and abstracts from 14 African countries [77] found that an overall median of 21.2% of patients had experienced drug toxicity, although there were few cases of grade 3 or 4 events. A Swiss cohort study [78] found that 47% of patients presented with clinical adverse events while on treatment with 'PI-sparing' cART regimens, which most of the African studies employed. The most commonly reported toxicities were emesis, mood disorders, elevated amylase levels, elevated glucose levels, lactic acidosis, neutropenia and elevated alkaline phosphatase levels [78]. Using data from 153 patients enrolled in Botswana's public ART pilot program [18], a Kaplan–Meier estimate of toxicity within the first year of ART was 23.8% (95% CI: 15.5–31.2%), with a Kaplan–Meier

estimate of developing a treatment-modifying toxicity by year one of 32.2% (95% CI: 23.0–40.4%). In total, 47 of the 153 (30.7%) patients had treatment-modifying toxicities within the first year on treatment [18]. The study also found that 29% of these treatment-modifying toxicities were for severe peripheral neuropathy, 6% for hepatotoxicity, and 4% for ddI-related pancreatitis and NVP-related cutaneous hypersensitivity.

Additional preliminary regional data from Botswana evaluating 306 ART-naive adults treated with PI-sparing public ZDV-based cART showed that 8.0% developed severe (grade 3–4) anemia (mean time to development = 11.6 weeks), 2.7% developed Stevens-Johnson syndrome (mean time to development = 28.0 days on ART) and 3.4% developed grade 3–4 liver function test abnormalities (mean time to development = 12.6 weeks) [79]. This initial group of cART-treated patients was significantly immunocompromised at the time of ART initiation, with a median CD4+ cell count of 81 cells/mm^3 and a median plasma HIV-1 RNA level of 442,000 copies/ml, with 89.2% of patients having WHO clinical stage 3 or 4 disease.

Data of longer term ART outcomes from Khayelitsha, South Africa [80] have shown similar toxicity rates from 287 adults with advanced baseline HIV-1 disease (median CD4+ cell count of 43 cells/mm^3 and mean plasma HIV RNA level of 151,000 cop ies/ml) treated with ZDV/ 3TC-based cART with either EFV (60%) or NVP (38%). In this cohort, the cumulative probability of changing a single ARV medication by 24 months was 15.1% due to toxicity or contraindications, and 8.4% due to toxicity alone. Most changes occurred soon after cART initiation (median time: 42 days; IQR: 28–56 days). Additional data from this cohort showed that after 24 months of therapy a similar proportion of patients had switched from d4T, ZDV and NVP due to toxicity (8.5, 8.7 and 8.9%, respectively) [81].

By contrast, only 1.7% had switched from 3TC. Most drug regimen changes (36 of 44, or approximately 82%) were attributable to anemia among ZDV-treated patients. Data from Uganda (n = 137) among adults receiving d4T- and 3TC-based cART with primarily NVP (77%) or EFV (14%) showed that 55% of patients experienced some level of discomfort, with 51% reporting pain, numbness, tingling of the hands or

feet and skin rash with dryness or pruritus. Rash was reported in 49% of the 125 patients treated with an NNRTI-containing regimen [82]. Interim data from a large cohort of ART-treated adults in Botswana receiving ZDV/ddI, ZDV/ 3TC or d4T/3TC with either NVP or EFV (n = 650 total) showed that 17.7% of patients experienced a treatment modifying toxicity [83]. The most common toxicities were anemia (3%), lipodystrophy (3%), grade 3 hypersensitivity cutaneous reactions or Stevens–Johnson syndrome (3%), neutropenia (2%), lactic acidosis (1%), moderate-to-severe symptomatic hyperlac tatemia (1%), hepatotoxicity (1%), neuropsychiat ric symptoms (1%) and pancreatitis (1%).

Completed 3-year study data from this study showed that 140 patients had 178 treatment-modifying toxicities (27.7% NVP vs 15.7% EFV; p = .0001). Pertinent treatment-modifying toxicities include the following: 20 (6.2%) NVP-treated patients developed cutaneous hypersensitivity reactions, seven (2.2%) EFV-treated patients developed neuropsychiatric symptoms, and 11 (3.4%) of NVP-treated and three (0.9%) EFV-treated patients developed hepatotoxicity, excluding lactic acidosis [36].

Preliminary data from southern Africa [77,80-84] have shown differences in the patterns and rates of toxicities among HIV-1 subtype C-infected adults when compared with HIV-1 subtype B-infected, ART-treated counterparts in resource-rich settings. Keiser et al. compared cART-treated adults in Cape Town, South Africa (n = 2348) to those treated in a Swiss Cohort (n = 1016) [85]. Treatment changes due to toxicity in the first 3 months following cART initiation were more frequent in Switzerland than in South Africa, despite the fact that Switzerland had greater first-line medication options, with more favorable toxicity profiles.

The type of treatment-modifying toxicities were fairly similar in the two settings, with the exception of symptomatic hyperlactatemia or lactic acidosis, which was documented in 32 South African patients, but not observed in Switzerland [85]. Preliminary regional data reveal a higher than expected rate of lactic acidosis (1.0–1.1%) among ART-treated adults, and its development appears to be related to female gender, body habitus (being overweight, BMI >25 or body weight >75 kg), and use of one or more 'D' drugs (d4T or ddI). Reasons for the higher rate

of lactic acidosis remain to be fully elucidated but most likely involve host genetic factors. More in-depth studies are underway. Early on (2002–2004) in public-ART-treated cohorts, there were significant rates of ZDV-associated anemia, as high as 8%. Anemia can pose a significant problem, especially in more remote areas where patients travel long distances to reach a district or referral hospital and where blood sup plies are low. Preliminary data have also shown that ART-treated adults in Africa appear to have higher than expected rates (as high as 2.7%) of NVP-associated cutaneous hypersensitivity reactions including Stevens-Johnson syndrome and lipodystrophy when compared with subtype B-infected patients [77,79,86].

Rates of lipid abnormalities, defined as cholesterol and triglyceride elevations, are not yet known, as few patients have received PI-based ART for prolonged periods of time in sub- Saharan Africa. Patients may develop degrees of lipid profile changes secondary to d4T or EFV exposure, and cART-treated adults, the majority of whom are female in sub-Saharan Africa, have experienced significant rates of body habitus changes – namely lipoatrophy involving the buttocks, thighs and face – especially those who have been on cART for more than 2 years. These body habitus changes have been attributed to d4T use and, along with the higher than expected rates of lactic acidosis, are prompting many policymakers to reconsider first-line cART options for their public ART programs. In the near future, public cART programs will move away from ZDV-or d4T-based cART regimens and switch to TDF-based first-line ART regimens, which have more favorable tolerability profiles.

Opportunistic infections Hepatitis B co-infection

Emtricitabine, 3TC and TDF all have significant activity against both HIV-1 and HBV infection [201]. Discontinuation of any of these ARV medications, however, may cause serious hepatocellular damage resulting from reactivation of HBV [87-89,201]. One study evaluating consecutively screened adults prior to cART initiation in Botswana [90] documented that 15 (10.6%) of 141 patients tested positive for hepatitis B surface antigen, with hepatitis B e antigen detected in six (40%) of the 15 hepatitis B surface antigen-positive adults. Of 140 evaluated patients, 82 (58.2%) tested positive for core IgG antibody, and 52 (37.1%) had positive results for surface antibody.

Of note, in this Botswana study, HCV antibodies were not detected in any of the 50 patients screened [90]. However, another larger (n = 1,779) study conducted in HIV-1-infected individuals in Nigeria identified 11.9% of patients as positive for hepatitis B surface antigen and 4.8% tested positive for HCV antibodies [91]. These data suggest that periodic HCV surveillance should be undertaken, especially in 'higher-risk' areas where HCV transmission may be more likely to occur, including prevalent intravenous drug use settings, settings where scarification/other 'at-risk' practices are widespread and among blood transfusion recipients where blood banks have very limited or unreliable HCV screening capacity.

Mycobacterium tuberculosis co-infection Timing of cART initiation

In HIV-1-infected adults with TB who have CD4+ cell count values below 200 cells/ mm^3, cART improves survival [92] but can be complicated by toxicity, especially hepatic and cutaneous toxicity, and the development of immune reconstitution inflammatory syndrome (IRIS). One study analyzing hypothetical cohorts, each having 1000 patients, evaluated 1-year mortality rates as well as the development of incident AIDS-defining conditions, severe IRIS and/or severe ARV medication-related toxicity in three groups of patients:

- 'Early integrated' treatment: cART initiated as soon as possible after commencing TB treatment (within 2 months);
- 'Later integrated' treatment: cART initiated once the intensive (2-month) phase of TB treatment has been completed, typically in months 3–4 of TB treatment;
- 'Sequential treatment': for this group, cART was only initiated once the TB treatment had been completed, typically 6–8 months after starting TB treatment [92].

When evaluating rates of mortality, the pri primary outcome, early cART was favored, even with the highest rates of IRIS (70%) and severe ARV medication-related toxicity (56%). In this model, deferred cART was favored over early cART only if the IRIS-related mortality rate in the early cART-treated group exceeded 4.6%. These results favor the early initiation of cART in HIV-1 and TB co-infected adults with advanced immunosuppression (CD4+ <200 cells/mm^3), except when IRIS-related mortality exceeds 4.6%. Preliminary regional data suggest that although TB IRIS mortality

rates are not insignificant, they do not presently appear to exceed 4.6%. A research group in Brazil commented on the lack of definitive data answering this important question [93]. Brazilian national standard-of-care is currently to initiate cART 4 weeks after TB treatment has been started and to include rifampicin in the first-line TB regimen. In results from a study that treated patients with culture-proven TB between January 2000 to August 2006 with anti-TB therapy (ATT), this group reported 19.8% TB-related deaths, with 50% of these deaths occurring within the first 3 months following TB diagnosis. They also reported lower rates of paradoxical reactions (6.6%) than what has been reported elsewhere. They observed no increased incidence of TB IRIS and did not record any TB IRIS-related deaths [93].

Another study, the Starting Antiretroviral Therapy at Three Points in Tuberculosis Therapy (SAPIT) study, is a randomized open-label trial that recruited 645 adults diagnosed with acid fast bacilli smear-positive TB in South Africa [205]. Study participants were randomized to commence once-daily cART consisting of ddI/3TC and EFV at one of three specific times during their course of TB treatment. The three time frames were early integrated, later integrated and sequential treatment, as described previously.

The study's DSMB decided to terminate the sequential treatment arm after an interim analysis showed that patients in the other two arms (early integrated and later integrated) had a 55% lower death rate when compared with sequential treatment arm-treated patients ($p = 0.005$). There were no significant baseline characteristic differences between study groups. Based on these interim DSMB findings, all patients initially randomized to the sequential treatment arm were offered cART. Patients in the two integrated treatment arms continued in active followup to determine whether there was any significant outcome difference between these 'integrated treatment' strategies [205].

Based on this and other data, patients who are receiving cART, and who develop active TB, can continue cART while ATT is initiated, with close monitoring for any potential toxicity, especially hepatotoxicity and rash [202]. For HIV-infected adults who have active TB and who have not yet been initiated on cART, it is often prudent

to just treat the TB first [202] if their CD4+ cell count is greater than 250 cells/mm^3 and they have not experienced a WHO clinical stage 3 or 4 event. Several other ongoing studies will address this critically important question of optimal timing of cART initiation in HIV-1 and TB co-infected adults requiring treatment for both diseases (i.e., Adult Clinical Trials Group A5221 and the ANRS sponsored Cambodian CAMELIA study), but until such results become available it is prudent to continue to individualize such decisions, taking into consideration both immunologic and clinical status. The clinical management strategies described later are from the latest version of the Botswana national ARV treatment guidelines [202], stating that patients should be treated on a case-by-case basis, taking into consideration both immunologic and clinical status. All TB patients who have a CD4+ cell count of over 250 cells/mm^3, but who have another active WHO stage 3 or 4 condition, should be initiated on cART at 2 months following ATT initiation.

If the WHO stage 3 or 4 condition is life-threatening, cART should be initiated much sooner following ATT initiation, with the exact timing of cART initiation depending upon the seriousness of the underlying medical condition. All multidrug-resistant TB patients who have a CD4+ cell count value of more than 250 cells/mm^3 can be initiated on cART at 2 months after ATT initiation, with close monitoring for additive medication toxicities, especially hepatotoxicity [202]. If the CD4+ cell count is below 100 cells/mm^3, cART may be started as early as 2 weeks following the initiation of ATT, especially if the patient's clinical condition is poor. If the CD4+ cell count is between 100 and 250 cells/mm^3, cART can be started within 2 months following ATT initiation, after the intensification phase. If the patient's clinical condition is poor, cART can be initiated earlier at 2–4 weeks after TB treatment has commenced, although treatment should be handled on a case-by-case basis. Adults who have other serious AIDS-defining or HIV-related events may be initiated on cART as early as 2 weeks following ATT initiation, but must be monitored for hepatotoxicity and TB exacerbation, especially during the first 6 months after cART initiation when IRIS is most likely to occur. All adults with active TB should be initiated on cotrimoxazole for Pneumocystis carinii pneumonia prophylaxis, one double strength tablet (160/800 mg) administered once daily.

One large, observational study from South Africa evaluated virologic responses at 6 months in patients treated with an NNRTI-based regimen with or without TB treatment that contained rifampicin. Among the NVP-treated patients, the rate of virologic failure was higher among those with TB compared with those without TB (16.3 vs 8.3%; adjusted OR: 2.1; 95% CI: 1.2–3.4). No difference in virologic response as seen when comparing TB versus non-TB patients who were started on EFV-based regimens [94]. NVP drug–drug interactions with rifampicin coupled with the heightened risk for hepatotoxicity among co-infected and treated adults also restricts the use of NVP among adults with active TB, although recently published data demonstrate noninferior outcomes among large numbers of HIV-1 and TB co-infected adults receiving NNRTI-based cART in Botswana [95].

Immune reconstitution inflammatory syndrome

Some patients while on treatment for active TB will develop IRIS, which is characterized by findings such as fever, new or worsening lymphadenopathy, worsening of pulmonary infiltrates and pleural effusion. Owing to the lack of standardized IRIS case definitions, the International Network for Study of HIV-Associated IRIS team recently published case definitions that can be utilized by healthcare providers in resource-rich as well as resource-limited settings [96]. IRIS reactions may occur in the absence of HIV-1 infection and without cART, but are more common after the initiation of cART in patients with active TB disease as a consequence of immune reconstitution. One review stated that IRIS had been reported in 8–43% of patients with HIV/TB co-infection and may contribute to the higher mortality from ART in the first year of treatment [97].

Risk factors for the development of IRIS include advanced immunosuppression (CD4+ cell count of <50 cells/mm^3), severe TB disease with high pathogen burden, and interval between initiation of TB and HIV treatment of less than 30 days [97-102,201]. Most IRIS in HIV-1 and TB co-infected individuals occurs within 3 months of the initiation of TB treatment. Delaying the start of cART for 2–8 weeks may reduce the incidence and severity of IRIS but must be balanced against the potential benefit of earlier cART initiation in improving immune function and preventing HIV-1 disease progression.

Interim findings from the Tshepo study team in Botswana documented 106 incident opportunistic infections (OIs) among 93 study participants [23,83]. A total of 44 patients had a total of 50 incident OIs within the first 6 months following cART initiation; these are believed to be linked to IRIS. When stratified by baseline CD4+ cell count of under 51, 51–200 or 201– 350 cell/mm^3, a marginally significant difference was found in the number of patients with an OI within 6 months (p=0.05) of cART initiation, with those in the lowest baseline CD4+ cell count group experiencing more OIs than the other two CD4+ cell count groups [23,83]. The most frequent IRIS reactions were pulmonary TB (39%) and cutaneous zoster (37%). High rates (15–45%) of IRIS among cART-treated adults with pre-existing OIs have been reported in resource-limited settings [20,103-106]. While Mycobacterium tuberculosis co-infection is prevalent in this setting [103], the lower than expected frequency of IRIS could be explained by the high rate of enrolled patients (16.2%) who had received recent full courses of ATT, as well as the overwhelming majority of other patients without recent active TB who received 6 months of TB preventative therapy with isoniazid.

Incident opportunistic infections

Incident OI rates are important markers for understanding the clinical course of treated HIV-1 disease and developing treatment guidelines and planning health services. Unfortunately, these conditions have often not been reported on in a standardized fashion. Limited diagnostic facilities, high TB rates and difficulties defining IRIS make the task more difficult in African settings [22,23,107,108].

A few regional studies have evaluated rates of incident TB among cART-treated adults. One study evaluating a 346-person cART-treated cohort in South Africa between 1996 and 2005 showed that the TB incidence density rate ranged from as high as 3.5/100 person-years during the first year of cART down to 1.01 per 100 person-years during year 5 of cART [109]. This study revealed that incidence of TB was highest in adults with the following baselinecharacteristics: CD4+ cell counts less than 100 ells/mm^3 [109] (adjusted risk ratio (ARR): 2.38; 95% CI: 1.01–5.60), WHO stage 3 or 4 clinical disease (ARR: 3.60 [95% CI: 1.32–9.80]) and age younger than 33 years (ARR: 2.86 [95% CI: 1.29–6.34]). The risk of incident TB was not independently

associated with plasma HIV-1 RNA level, previous history of TB, low socioeconomic status or gender [109]. Despite similar virologic responses to cART, CD4+ cell count increases were significantly less pronounced among patients developing incident TB compared with those patients who remained TB free [109]. In addition, Lawn et al. evaluated a community-based cohort of 1480 adults in South Africa for up to 4.5 years and identified two to three incident TB cases during 2785 person-years of follow-up (overall incidence = 7.3 cases/100 person-years) [110]. During person-time accrued with specific CD4+ cell count strata, specifically 0–100, 101–200, 201–300, 301–400, 401–500 and more than 500 cells/ mm^3, unadjusted TB incidence rates were 16.8, 9.3, 5.5, 4.6, 4.2 and 1.5 cases/100 person years of follow-up, respectively ($p < 0.001$). They also found that patients with low base-lineCD4+ cell counts (0–200 cells/mm^3) had a 1.7-fold higher TB incident rate during early cART treatment (with 'early' being defined as the first 4 months following cART initiation) than during long-term cART treatment ($p = 0.026$) [110]. The authors concluded that TB risk could be reduced by developing cART program policies that limited the time cART-treated adults remained most at risk, by having CD4+ cell count values of less than 500 cells/mm^3 [110].

Adherence

In resource-limited settings and in particular sub-Saharan Africa, preliminary ARV adherence statistics report that cART-treated persons are generally taking more than 90% of their prescribed doses [22,68,77,111,112] – rates which are certainly comparable to those reported among cART-treated adults in resource-rich settings. However, there is surely a publication bias, as adherence data are not collected routinely in reportable PEPFAR statistics. Despite rapidly escalating numbers of adults receiving cART in the region, certain social and cultural barriers remain that may negatively impact ARV adherence rates in the future.

As part of the interim findings from the Tshepo study in Botswana, Bussmann et al. reported excellent overall medication adherence rates in this randomized study of 650 cART-treated adults [23,83]. Medication adherence rates of greater than 90% at all measured time points in monthly clinic adherence assessments were reported in 89.8% of study participants after 1 year of follow-up and in 81.2% of participants

after 2 years of study follow-up [23]. Pooled treatment group analysis documented statistically significant differences in adherence rates when analyzed by gender, with males having poorer overall adherence rates ($p = 0.006$) [23]. In reporting 5-year outcomes data of adults receiving national program cART in Botswana, Bussmann et al. have also demonstrated excellent overall ARV medication adherence rates as evidenced by high rates of virologic suppression (90% or greater), a low virologic failure rate (<10%) and superior monthly ARV medication refill rates [26]. However, the decline in medication adherence over time, especially among male participants, gives rise to concern. As the long-term sustainability of cART programs in Africa will largely depend on excellent sustained cART adherence rates, it is of paramount importance for healthcare personnel to provide ongoing ARV adherence support that continually adapts to meet the needs of those involved. Adherence counseling must also specifically address the needs of males, patients with ongoing psychosocial, financial or physical problems, and others who are most at risk for poor adherence.

The issues of stigma and discrimination are important influences on treatment access and adherence. Anecdotally, there are numerous cases of cART-treated adults who do not disclose their positive HIV-1 status to their partner for fear of stigma and discrimination. Do et al. evaluated 300 adult cART-treated adults in Botswana's national ARV treatment program between April and May 2005 [Do N, PHIRI K, BUSSMANN H, FOYA K, MARLINK RG, WESTER CW: Social factors influencing antiretroviral medication adherence rates among HAART-treated adults in Botswana (2009), manuscript in preparation]. Using a comprehensive 87-item survey, they documented an overall 81.3% ART adherence rate, which was based on 4 day and 1 month patient recall and on clinic attendance for ART medication refills during the previous 3 months. Adults receiving cART for 1–6 months were the least adherent (77% overall) followed by those who had been receiving cART for greater than 12 months (79%). Alcohol use and abuse, the presence of depression and not disclosing positive HIV-1 status to one's partner were all predictive of poor adherence rates ($p < 0.02$) [Do N, Phiri K, Bussmann H, Foya K, Marlink RG, Wester CW: Social factors influencing antiretroviral medication adherence rates among HAART-treated adults in Botswana (2009), manuscript in preparation]. In addition, a regional study from Lesotho, Swaziland, Malawi, South Africa and Tanzania [113]

evaluated 1457 HIV-infected adults (698 [48%] of whom were cART treated) and found a significant relationship between perceived HIV stigma and self-report of missed ARV medications over time ($p < 0.001$). Individuals who reported missing more ARV medication doses also reported higher levels of HIV-associated stigma [113].

National cART program staff in sub-Saharan Africa will need to provide ongoing counseling and education to help patients recognize and overcome HIV-associated stigma. Counselors must also continue to stress the need for life-long adherence to therapy, especially when cART-treated adults may believe they no longer need their medications in the wake of significant weight gain coupled with the ability to resume normal activities. Community, school, church/ mosque and political venues must be targeted to change community norms.

Future challenges

Cost/sustainability of cART programs

In 2002, the cost to treat an HIV-1-infected adult with cART for 1 year was approximately US $10,000 [114]. In Haiti, the annual cost in 2002 was $700 per patient [114]. The lowest possible price per year to treat an HIV-1-infected adult in resource-limited settings [114] in 2002 was approximately $300, using the lowest priced generic formulations. In low- and middle-income countries, the prices of most first-line medicines decreased by 30–64% from 2004 to 2007 and by 10–40% from 2006 to 2007 [206]. These reductions in ARV medication prices have contributed significantly to the increased availability of cART [206]. The median price paid for first-line treatment (prequalified by WHO) in low-income countries in 2007 ranged from $92 per person per year for the fixed-dose combination of d4T, 3TC plus NVP (the most widely used combination) to $294 for the fixed-dose combination ZDV/3TC plus EFV [206]. The weighted average median price of the four combinations most widely used in first-line treatment (representing 86% of the prescribed first-line treatments in low-income countries) was $170 per person per year in 2007 [206]. The Clinton Foundation has just recently negotiated the cost of annual generic cART down to $90 per year in 2009. In many sub-Saharan African countries heavily impacted by the HIV epidemic, including Kenya, Malawi,

Nigeria and Zambia, the annual per capita spending on healthcare of any kind is less than $100 [1]. Even with reduced drug costs, cART requires significant health expenditure in the form of maintenance or expansion of physical health infrastructure, healthcare worker training and retention, laboratory monitoring and other related costs. Fiscal constraints will remain a challenge for some countries as cART coverage rates increase over the next decade. Numerous national cART programs will continue to depend on strong financial support from PEPFAR and the Global Fund, in particular, with added support from the Bill and Melinda Gates Foundation, the Clinton Foundation and governments themselves.

Optimizing patient retention

As national cART programs continue to burgeon in the region, data have shown that increasing numbers of patients are being lost to follow-up [115]. ART-LINC and IeDEA investigators using data on 5491 adult patients initiating cART in 15 treatment programs in Africa, Asia and South America found that 3.8% of patients had no follow-up, 16.0% were lost to follow-up and 2.6% were known to have died during the first 6 months following cART initiation [116]. The probability of no follow-up was higher in 2003–2004 than it was in 2000 (OR: 5.06 [95% CI: 0.28–20.0]) [115] as was lost to follow-up (HR: 7.62 [95% CI: 4.55–12.8]), but not recorded death (HR: 1.02 [95% CI: 0.44–2.36]). In this large study, compared with having a baseline CD4+ cell count of 50 cells/mm^3 or greater, having a baseline CD4+ cell count of less than 25 cells/mm^3 was associated with having a higher probability of no follow-up (OR: 2.49 [95% CI: 1.43–4.33]), loss to follow-up (HR: 1.48 [95% CI: 1.23–1.77]) and death (HR: 3.34 [95% CI: 2.10–5.30]) [115]. A study from Durban, South Africa, revealed similar findings, with 81.4% of 501 registered patients still being in care at 3 months following cART initiation, and 82 (16.4%) lost to follow-up; 28 (34.1%) of whom had died and 32 (39%) of these being unreachable by phone despite multiple attempts [117]. Lower baseline CD4+ cell counts (<100 cells/mm^3) and unemployment were independently associated with being lost to follow-up [117]. As public cART programs continue to expand in the region, it will be of paramount importance to continue to identify patients most at-risk of loss to follow-up and to develop novel measures with the necessary staff resources to maximize ART program retention in resource-limited countries.

Expanded cART for the purposes of primary HIV-1 prevention (cART as prevention)

There is growing interest in using cART as a prevention strategy, with clinical trials evaluating the use of cART in HIV-1-infected persons in serodiscordant relationships and pre-exposure prophylaxis (PrEP) in 'at risk' young HIV-1-infected individuals. Proponents of the first strategy argue that cART may be a cost-effective approach to reduce HIV transmission by reducing plasma HIV-1 RNA levels, regardless of CD4+ cell count. A South African WHO mathematical modeling study incorporating universal HIV testing and immediate cART initiation (regardless of CD4+ cell count) predicted that HIV-1 incidence and mortality rates could be reduced to below 1% within 50 years [118,119]. Data from a Zambian and Rwandan observational study, in which the HIV-1-infected partner in a sero-discordant relationship was given cART, showed a reduction in HIV-1 incidence of 79% in the uninfected partner [118, 120]. Such an aggressive 'cART as prevention' approach may save considerable money over the next decades if it were to result in fewer cases of HIV infection and, therefore, fewer additional adults requiring lifelong cART. However, the impact on the level of population level resistance is unknown, and it is uncertain whether lifelong therapy is practical when we currently struggle to treat people who meet current guidelines.

Prevention of HIV-1 transmission or viral shedding may be especially important as some preliminary data [ESSEX ME, UNPUBLISHED DATA] suggest that 25–30% of HIV-1 subtype C acutely infected adults may have prolonged viremia, defined as extremely high plasma HIV-1 RNA levels for as long as 9–12 months. Prolonged viremia also supports the community-based cART as prevention approach. Other ongoing and planned trials evaluating similar concepts include the HPTN 052 (ACTG 5245) discor discordant couples study in which half of enrolled participants in serodiscordant relationships will receive cART earlier in the course of their HIV-1 infection, when CD4+ cell count values are in the 350–550 cells/mm^3 range. Botswana is beginning to offer universal cART in pregnancy as a means to drastically curtail and hopefully eradicate mother-to-child transmission (MTCT) of HIV [202]. Other promising preliminary data regarding strategies to reduce MTCT rates were recently presented at the 16th Conference on Retroviruses and Opportunistic Infections [121]. Two of these approaches were

maternal cART during breastfeeding and extended infant ARV prophylaxis. The results of ongoing clinical trials including the KiBS (Kenya), Mma Bana (Botswana), MITRA-Plus, ZEBS (Zambia) [122], EARL, BAN, PEPI [123] and planned large PROMISE (IMPAACT) studies will largely inform PMTCT policy in the region [121].

Another potentially promising approach using cART as prevention involves the use of PrEP, and a few ongoing placebo-controlled, randomized African PrEP trials [124] should soon yield important data. Two African trials warranting specific mention at present include: the TDF-2 trial in Botswana [125], a Phase I/II randomized double-blind placebo-controlled trial (n = 2000 individuals) of daily TDF plus FTC for healthy HIV-1-negative adults aged 18 to 39 years in Botswana and South Africa; and the Partners PrEP trial (n=3900 African discordant couples) of TDF/FTC, TDF alone or placebo for 36 months. These cART PrEP trials are especially important as data have shown that greater than 50% of new HIV-1 transmissions occur in stable couples. One potential concern with PrEP, based on data from Wainberg's group in McGill and Botswana [51], is the possibility of rapid selection and the emergence of the K65R mutation. This emphasizes the importance of monitoring for primary HIV-1 genotypic resistance in cART PrEP trials. At present, most of the research in the region has shown very little major genotypic resistance mutations to first-line cART, although continued surveillance is warranted.

Availability of second- & third-line cART regimens

As patients receiving cART live longer in resource-limited settings, the need for second- and third-line treatment options will increase. A 2006 WHO survey of low- and middle-income countries found that 96% of patients were still reported to be on a first-line regimen [126, 127]. However, the annual rate of switching to second-line cART is forecast to increase from 5% in 2005 to 12% in 2010, representing between 500,000 and 800,000 individuals [128, 207].

The WHO recommends a PI-based second-line regimen for patients failing NNRTI-containing initial regimens [128]. Only ritonavir-boosted PIs were recommended in the 2006 WHO treatment guidelines, and subsequent publications gave preference to LPV/r, ATV/r or FPV/r. The selection of ritonavir-boosted indinavir or saquinavir

was discouraged owing to concerns about poor tolerability and adverse events, especially in hot climates [116,129,130]. Currently, LPV/r is the only generic heat-stable fixed-dose combination PI widely available in resource-limited settings [131]. A fixed-dose, heat-stable formulation of ATV/r is expected to have an efficacy and tolerability profile similar to LPV/r, but at a lower pharmaceutical ingredient cost [132]. Voluntary licenses from the innovator company (Bristol–Myers Squibb) have been granted for generic production of ATV, and the WHO has declared the wide availability of a co-formulated heat-stable ATV/r to be a priority [128,207].

Most first-line cART in sub-Saharan Africa incorporates 3TC and AZT or d4T, and the WHO recommends the combination of TDF plus 3TC or ABC plus ddI as second-line NRTIs for patients failing thymidine analog-containing regimens [128]. However. recent reports from Botswana, South Africa and Malawi suggest that these second-line regimens may be less effective than anticipated owing to the presence of K70E or K65R mutations at clinical or immunological treatment failure [52-55]. TDF has been selected as a first-line agent by some national HIV-treatment programs despite higher costs [133], supported in part by evidence for lower adverse event rates (especially anemia) and a more favorable resistance profile at treatment failure. For patients failing a TDF-containing first-line treatment, the WHO recommends a second-line NRTI combination of AZT plus 3TC.

Newer ARV agents may have a future role in resource-limited settings, but high cost, lack of generic alternatives, the need for expensive pre-treatment testing and poor heat stability prohibit their extensive use at present. Etravirine is a new, PI-sparing, NNRTI option for patients failing NVP or EFV and shows increased in vitro potency (ten- to 500-fold) in comparison to EFV in the presence of combination mutations, including K103N with Y181C or L100I [134,135]. Darunavir and tipranavir are second-generation PIs with activity against HIV strains with multiple PI resistance mutations, and could be important treatment options for patients failing current LPV/r-based second-line regimens [125,136,137]. Raltegravir is the first member of the integrase inhibitor ARV class and initial studies have demonstrated efficacy in heavily treatment-experienced patients with multiclass ARV resistance [138]. The appropriate use of raltegravir is still being defined in resource-rich countries,

but it may be a potent third-line or beyond treatment option. Maraviroc is a CCR5 chemokine receptor inhibitor that prevents viral entry and has shown efficacy in heavily treatment-experienced patients [139]. Its use in resource-limited settings is constrained by the need for a complex and relatively high-cost HIV-1 tropism assay to identify patients with susceptible virus. Enfuvirtide (T-20) is a biometric peptide that prevents fusion of the viral and host cell membranes [140]. It is primarily reserved for salvage therapy in resource-rich settings, and its use in resource-limited settings is constrained by high cost, poor heat stability and subcutaneous administration. All of these newer ARV agents were patented and approved for use by the US FDA since 2003, and generic versions are not expected to reach the market in the near future.

Treatment monitoring & criteria for switching to second-line cART

The measurement of HIV-1 RNA levels (i.e., viral load [VL]) is recommended to determine the response to cART in developed countries [10,16], but the high cost and sophisticated laboratory equipment necessary to perform VL testing currently prohibits the use of this technology in many resource-limited areas. When VL testing is available, current WHO guidelines recommend a switch to second-line cART if the VL rises above 10,000 copies/ml. This threshold is based on limited evidence of minimal CD4+ cell count decline and disease progression in patients with detectable viremia below this level [141,142]. Delaying cART switching in areas with limited second- or third-line options may preserve viable treatment regimens and improve long-term outcomes, but further data on the accumulation of resistance mutations are needed.

In areas without access to VL testing, the WHO has proposed the use of clinical and CD4+ cell count-based criteria to guide treatment decisions [207], but the performance of these criteria has been poor. An assessment of 1133 patients in rural Uganda found a composite sensitivity of 23–28% and a specificity of 90% for detecting virologic failure (depending on the VL threshold) [143]. A study in South Africa among 324 patients reported a sensitivity of 21% and a specificity of 96% in detecting a VL greater than 10,000 copies/ml [144], while a study in Nigeria of 395 'high-risk' patients (treatment exposed, poorly adherent or with evidence

of immunologic or clinical deterioration) reported a sensitivity of 39–59% and a specificity of 59–80% (depending on the criterion) to detect a VL greater than 400 copies/ml [145]. Similar reports from British Columbia [146], South Africa [147], Thailand [148] and Uganda [149]highlight the need for improved treatment failure algorithms in the absence of VL monitoring. Arguments to minimize VL monitoring [150] must be weighed against the health effects and unnecessary costs associated with unreliable and inaccurate diagnostic tools [151]. A computer simulation model of routine VL monitoring compared with clinical or CD4+ cell count-based monitoring found a moderate survival benefit, but at an increased cost of approximately $3500.00 per life-year gained [152]. Cost–effectiveness analyses have reported high incremental cost–effectiveness ratios for VL monitoring compared with CD4+ cell count monitoring in resource-limited settings [153,154], but potential long-term savings [155].

The benefit of routine VL monitoring in resource-limited settings is uncertain because of the high costs associated with the test and the limited cART regimens. A trial in Zambia(NCT00929604) will assess mortality at 36 months among approximately 2100 cART-naïve patients initiating therapy and receiving care at facilities with access to routine HIV VL testing (at cART initiation, at 3 months and at every 6 months thereafter) compared with those initiating first regimens and receiving care at facilities with 'discretionary' VL testing (i.e., owing to clinical failure or immunologic failure as defined by local criteria). In Uganda, a trial (NCT00434070) of combined VL and CD4+ cell count treatment monitoring versus CD4+ cell count alone is recruiting participants and will assess the development of resistance mutations at 36 months associated with each strategy. These targeted evaluations will provide critical information on the potential survival benefits and effect on the development of drug resistance in resource-limited settings, in addition to urgently needed information on feasibility, acceptability and cost–effectiveness.

Task-shifting

Sub-Saharan Africa has only 3% of the world's healthworkers and accounts for less than 1% of global health spending [1,156,157], although it has almost 12% of the world's population. To illustrate this point further, there are currently 347

physicians available for every 100,000 persons in Norway, yet there are only two physicians for every 100,000 persons in Malawi or Tanzania [1,156,157]. Numerous factors contribute to the significant manpower constraints that exist in resource-limited settings. Some of these factors include the weakness of national medical education and training programs, limited implementation of national human resource management policies, and the well-documented 'brain drain' of health professionals who migrate from lower paying jobs in their home countries to 'more remunerative' or 'more rewarding' work in higher-income or neighboring countries [1,158,159].

As of 2009, more than 110,000 persons are receiving public-sector-supported cART in Botswana. It is anticipated that the number of persons receiving public cART as part of the national ARV treatment ('Masa') program will double within the next 10 years. Similar trends and projections exist in the vast majority of sub-Saharan African countries. In Botswana and some other regional settings, longitudinal care is largely provided by physicians or medical officers who are paired in medical consultation rooms with nurses. Nurse/physician visits are scheduled approximately every 3–6 months for cART-treated persons. Presently, patients on cART also attend outpatient clinics on a monthly basis for ARV dispensing and therefore interact most consistently with pharmacy staff. Pharmacy staff provide limited screening for toxicities but primarily focus on providing adherence counseling, education and evaluation for potential drug–drug interactions. Since the majority of patient visits are clinically uneventful and there is a limited number of physicians and medical officers in the region, most patients could be fully managed by specially trained nonphysician staff such as nurses, counselors and pharmacy staff. Innovative approaches of healthcare service delivery (i.e., nurse-centered care) will need to be designed and validated in order to meet rapidly expanding patient care demands while ensuring quality care.

Recent literature and past experiences in other resource-limited settings have shown that necessary skills once possessed only by specialized physicians can be successfully transferred to nurses, general clinical workers and even community workers [157,158]. The Medicins sans Frontieres programs in Khayelitsha and

Lusikisiki, South Africa, are largely managed by nurses [160,161]. In the Khayelitsha program, a clinical team typically consists of one physician or medical officer, two nurses and two counselors. Physicians and medical officers play a prominent role at the time of cART initiation, but routine longitudinal care visits are primarily staffed by nurses and involve physicians and medical officers only if needed. At the Lusikisiki site, nurses are in charge of the cART program and physicians and medical officers rotate through the clinics on a biweekly basis [160,161]. Other nurse-centered programs, such as the Zambian National ARV program, have also reported similar anecdotal successes [162].

With large numbers of available locally trained nurses and uniform training and education mechanisms already in place, the majority of sub-Saharan African countries presently have the capacity to shift towards a more cost-effective and sustainable nurse-centered care approach. One possible permutation of the nurse-centered care approach would be to have four nurses trained specifically in HIV/AIDS and cART management see patients under the supervision of one on-site physician who could be available for immediate consultation. The more experienced physicians could also supervise and mentor junior physicians who are seeing patients at the same time, which would facilitate skills transfer over time and provide a more sustainable model of healthcare delivery.

To circumvent these manpower shortages, numerous sub-Saharan African countries have already switched in varying degrees to nonphysician care models, and the preliminary results are promising. Regional public programs adopting nurse-centered approaches to healthcare delivery in Haiti and Rwanda report very low lost to follow-up rates, high ARV treatment success rates and mortality rates that are comparable to those among cART-treated persons residing in resource-rich settings [1,163].

Survival/mortality

Early mortality among cART-treated adults needs to be a primary focus in the region over the next few years. Rates of pulmonary and extrapulmonary TB, both IRIS related and non-IRIS related, are high, especially among those initiating cART with

advanced immunosuppression and baseline CD4+ cell count values of less than 100 cells/mm^3. Planned public-health approaches to address this include the initiation of empiric ATT as part of a clinical trial in an attempt to alleviate the morbidity and mortality associated with resistant TB infections. Novel therapeutic interventional strategies to treat IRIS will be an area of focus. In addition, public hospitals will need to expand laboratory, diagnostic, microbiologic and surgical pathologic capacity providing evidence-based data that will inform public policy and help healthcare providers give high quality care to patients. These trials will also increase our understanding about the scope of resistant (multidrug resistant-TB/extensively drug resistant-TB) infections, antibiotic resistant respiratory and enteric infections, cotrimoxazole and screen for preventable cancers.

Opportunistic infections

Over the next 5–10 years, expanded cART coverage and isoniazid preventative therapy to all HIV-1-infected adults will hopefully result in reduced TB incidence rates. The issues of how best to manage adults with incident TB who are receiving second-line cART needs to be an area of focus, as issues of overlapping toxicity (hepatotoxicity) and drug–drug interactions remain.

Co-formulated LPV/r is widely used for second-line ART in both resource-rich and resource-poor settings. Owing to cost and supply chain constraints, rifabutin has largely been unavailable in many resource-limited settings [164]. However, reliable stocks of rifabutin have recently become available in India, and in March 2009, the WHO placed rifabutin on it its list of 'essential medicines' but only for use in patients with HIV-1 on PI-containing regimens [165]. As part its recent meetings, the WHO's 17th Expert Committee on the Selection and Use of Essential Medicines plans to approve the use of rifabutin for the treatment of TB in the place of rifampicin among patients receiving concomitant PI-based cART. These recommendations state that dose-reduced rifabutin can be administered with normal doses of LPV/r, ATV/r, ritonavir-boosted darunavir and FPV/r [166]. Of note, the recommendations for rifabutin dosing, namely the 75% dose reduction, among HIV-1-infected adults receiving concomitant LPV/r are based on extrapolations from healthy HIV-1-negative adults receiving foasamprenavir/ritonavir [166].

The March 2009 WHO essential medicine list recommendations for rifabutin, which allows it to be used in HIV-1-infected persons receiving PI-containing cART regimens, will increase the use of rifabutin or treatment of resistant TB in HIV-infected persons treated with LPV/r. There is an urgent need for data to directly confirm or refute the WHO recommendation. Pharmacokinetic studies will need to be performed among healthy HIV-1-negative adults receiving dose-reduced rifabutin and LPV/r. Findings from these preliminary pharmacokinetic studies conducted in healthy HIV-1-negative adults will assist with planning larger efficacy and outcomes trials for treating HIV-1/TB co-infection.

Tuberculosis treatment and cART services have not been well integrated in many sub-Saharan African countries where large numbers of HIV-1 and TB co-infected persons reside. Recent statistics from the Joint United Nations Programme on HIV/AIDS (UNAIDS) [1] report that only 42% of countries with generalized HIV-1 epidemics have implemented routine TB screening for HIV-positive patients, and only 27% provide TB preventative therapy in all districts in need. In addition, hundreds of thousands of persons who are co-infected with HIV-1 and resistant TB die unnecessarily each year owing to inadequate TB diagnostic services, failure to deliver affordable medications to those in need and increasing rates of TB drug resistance. Although the question of when best to initiate cART in relation to the timing of ATT will hopefully be answered by ongoing clinical trials in the next 1–2 years, considerable work needs to be done to integrate TB and HIV services, to more rapidly and efficiently diagnose active TB and drug-resistant infections, and to improve infection control practices.

Non-AIDS complications

As persons survive longer on cART in sub-Saharan Africa, similar to trends in resource-rich settings, increasing numbers of cART-treated adults in sub-Saharan Africa will develop non- AIDS-defining events, which will include hepatic, renal, cardiovascular and non-AIDS-related malignancies. As an average of 4–10% of HIV-1-infected adults in the region are HBV co-infected and an unknown percentage are HCV co-infected, increasing numbers of adults will be at risk for the development of end-stage liver disease and hepatocellular carcinoma. Cancer and cardiovascular

disease are the most common causes of death worldwide, and hospitals in the region will need to continually expand their diagnostic services to screen for and effectively manage these complications in HIV-infected patients over time. This will become especially important as the cART-treated populations age across the continent, as at present, a very low proportion of cART-treated adults are older than 50 years of age. This proportion, however, will increase significantly over the next 10–15 years. Cervical cancer screening has been shown to be feasible within an ART clinic context with more than 30,000 women screened in Zambia in just 2 years [123].

Adherence

In addition to providing counseling and education that addresses the issue of stigma in longitudinally cART-treated adults, healthcare providers will also need to screen for the presence of depression and other mental illness, the use of concomitant traditional medications, the lack of disclosure of positive HIV-1 status, and alcohol and other substance use and abuse, all which have been shown to negatively impact ARV-medication adherence rates. In addition, preliminary data have shown that males may be at higher risk for poor adherence. Long-term follow-up, including studies evaluating sociobehavioral aspects of adherence, is still needed and continued adherence counseling and education is warranted. Adherence counselors will need to be diligent as persons experience significant quality-of-life gains and may becoming complacent, feeling that they no longer need their cART regimens.

In addition, much can be learned from the successes of the directly observed therapy (DOT) TB control programs, lessons which can be applied to cART-treated adults. This includes theformal study of ommunity-based DOT strategies, which have been largely utilized in Haiti and Rwanda by Partners in Health. Community-based DOT has been evaluated in Botswana and Southern Africa and preliminarily has not been shown to be beneficial in randomized trials owing to equivalent virologic failure rates between adherence strategies, but more research is needed in this area.

Summary

Over the next 10 years, cART coverage rates will significantly improve across the region, with attendant increases in healthcare utilization for HIV- and non-HIV-related complications and the need for expanded laboratory and clinical services. Results of ongoing trials will greatly inform discussions pertaining to the use of cART for primary HIV-1 prevention. The annual rate of cART regimen switching due to treatment failure will increase, and bringing novel agents to market, in addition to the widely available LPV/r (i.e., ritonavir-boosted darunavir and ATV/r), needs to be a high priority of international health organizations. Newer means for the timely detection of cART treatment failure is an urgent priority and will largely be informed by ongoing trials evaluating the use and cost–effectiveness of routine HIV-1 plasma RNA monitoring in clinical care in resource-limited settings.

Physician-centered care models will not be sustainable in sub-Saharan Africa and escalating manpower constraints will require the evaluation and adoption of novel task-shifting approaches to care. Education and training programs as well as patient-retention strategies will need to be strengthened as national cART programs are expanded and growing numbers of individuals require lifelong monitoring and care.

Executive Summary

Update

- Significant progress has been made in terms of the numbers of total qualifying adults now receiving potentially life-saving combination antiretroviral therapy (cART) in sub-Saharan Africa, with Botswana, Rwanda, Senegal and Namibia achieving over 50% coverage rates
- Despite impressive clinical, immunologic and virologic successes as well as excellent preliminary cART medication adherence rates, high early mortality rates, especially within the first 6 months following cART initiation, remain a significant problem
- Certain adult individuals appear to be at heightened risk for specific medication related toxicities, many of which are life-threatening
- The rate and patterns of genotypic drug resistance mutations also appear to differ when compared with cART-treated adults residing in resource-rich settings, potentially due to a higer prevalence of HIV-1 subtype C infection
- Opportunistic infections continue to cause significant morbidity and mortality in the region with HIV-1 and tuberculosis co-infected patients presenting unique and sometimes difficult clinical management scenarios
- Future challenges
- The use of cART for the purposes of primary HIV-1 prevention (i e , prevention of viral shedding) is likely to expand in sub-Saharan Africa over the next 5–10 years
- Reduction of the strikingly high early mortality following the initiation of cART and the management of HIV/TB co-infection will be priority areas for clinical research
- Rates of non-AIDS complications will increase, necessitating enhanced laboratory and diagnostic capacity within the region

- Physician-centered care models will not be sustainable in sub-Saharan Africa, and escalating manpower constraints will magnify the need to evaluate and adopt novel 'task-shifting' approaches such as nurse-centered care.
- The need for effective second-line and salvage treatment options will increase as the annual rate of switching to second-line cART is predicted to increase from 5% in 2005 to 12% in 2010, especially generic, heat-stable, ritonavir-boosted protease inhibitors.
- Newer antiretroviral agents such as second-generation protease inhibitors, integrase inhibitors and cell entry inhibitors need to be added to the armamentarium of regional public cART programs but implementation is problematic owing to the lack of generic alternatives and the frequent need for ancillary testing.
- There is increasing evidence that the immunological and clinical treatment monitoring criteria used in many resource-limiting settings perform poorly, increasing the risk of resistance development in patients with detectable viremia.
- The role of routine viral load testing in treatment monitoring is controversial given the high cost of equipment and training, and the limited repertoire of second-line treatment regimens in many programs; clinical trials to evaluate this strategy are in progress.
- Primary care infrastructures must be improved for HIV care to be integrated and sustained by African countries, anticipating a day when foreign assistance may diminish. This will also help tackle the many other problems that Africans face and that HIV exacerbates.

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Chapter 9

Hybrid data capture for monitoring patients on highly active antiretroviral therapy (HAART) in urban Botswana

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Abstract

Individual patient care and programme evaluation are pivotal for the success and long-term sustainability of antiretroviral treatment programmes in resource-limited countries. While computer-aided documentation and data storage are indispensable for any large programme, several important issues need to be addressed including which data are to be collected, who collects it and how it is entered into an electronic database. We describe a patient monitoring approach, which uses concise patient encounter forms (in hybrid paper + electronic format) based on optical character recognition technology, piloted at Princess Marina Hospital in Gaborone, Botswana's first public HAART outpatient clinic. Our novel data capture approach collects "key" data for tracking patient and programme outcomes. It saves physician time and does not detract from clinical care.

Key words Medical records; Medical records systems, Computerized; Data collection; Automatic data processing; Botswana.

Introduction

In recent years the international community has responded with unprecedented attention and commitment to the human immunodeficiency virus (HIV)-1 pandemic. Several antiretroviral treatment (ART) initiatives have recently been initiated in sub-Saharan Africa and other areas hardest hit by the HIV/AIDS epidemic in an effort to significantly improve the quality of life for the millions of persons with HIV/AIDS who urgently need ART.1–3 With the rapid scale-up of these programmes, large numbers of patients will require comprehensive care which includes the lifelong provision of highly active antiretroviral therapy (HAART). While issues of antiretroviral (ARV) affordability, ARV production and procurement, training needs and manpower constraints have received most of the attention in the past years,[4–7] the need for reliable documentation and tracking of patient outcomes for ascertaining overall programme success have only recently begun to receive attention.[5,6] There is a paucity of data on the lessons learned and best method to monitor and evaluate the outcomes of patients initiated on HAART in sub-Saharan Africa.[8–11] Well-designed and 'longitudinal' data capture and tracking systems are vital for the successful and sustainable implementation of these programmes at the level of the patient and in the broader public health perspective.

We developed a concise hybrid "paper–electronic" patient documentation system, which makes use of optical character recognition (OCR) technology, at Princess Marina Hospital in Gaborone, Botswana's largest tertiary referral hospital and the first public HAART outpatient clinic. While imposing minimal burden on physicians this system provides critical data documenting patient outcomes that can be used both at the clinic and national programme level.

Background

The Botswana medical record system used countrywide has proven to be an efficient and reliable means of maintaining documentation. It primarily uses paper-based patient-held outpatient department (OPD) cards that contain all pertinent medical data, including outpatient visits, hospitalization summaries, specialist notes, prescriptions and diagnostic results, arranged in chronological order. Medical records detailing hospital admissions are maintained at the individual district and

referral hospitals. Parallel health programmes, such as directly observed anti-tuberculosis therapy, antenatal/obstetrical care and family planning services, use additional patient-held documents. In January 2002, medical staff at the outpatient HIV clinic of Princess Marina Hospital (referred to as the adult Infectious Disease Care Clinic [IDCC]) officially began Botswana's national ART programme by providing HAART to qualifying Botswana citizens in accordance with Botswana National Antiretroviral Treatment Guidelines.12,13 Botswana's ART programme estimated that 6000 patients would need to be placed on HAART in Gaborone alone during the first year of this programme.

The IDCC medical staff were able to rapidly expand HIV/AIDS services and provide care to approximately 1000 qualifying adults by the end of the first six months of the programme using the paper-based data capture method. This consisted of (1) a registration sheet with baseline demographics and contact information collected by clinic administrative staff, (2) a clinic appointment database maintained by clinic administrative staff, (3) medical data on past illnesses and newly emerging problems such as toxicities and incident opportunistic infections collected by the physician/ nurse, (4) laboratory data provided by the laboratory, and (5) ARV prescription (physician) and dispensing data (pharmacy staff).

However as the number of patients grew, efficient monitoring of this huge patient base would be possible only with the help of a computer-based patient tracking system. Recognizing this need and the difficulty of using OPD cards for HIV care and ART due to the regular multiple clinic visits, the Government of Botswana in April 2003 installed a computerized data capturing system in the then four operating sites of Botswana's national ARV (MASA") program, including IDCC, Gaborone. Later in April 2004 the above system was replaced by an integrated patient management system (IPMS) to centralize healthy data. This new comprehensive state-of-the-art health care information technology system based on "real-time" health provider data entry on to a computer workstation in their consulting room is planned to eventually include all 32 ART sites within Botswana's national ARV ("MASA") treatment programme.

Hybrid data forms based on optical character recognition technology for patient tracking and data capture

Before the introduction of the government's computerized data capturing system, we developed and piloted a novel data capture and patient tracking system at Princess Marina Hospital to address the need for electronic documentation. Our system monitored ARV tolerability, ARV drug switches, as well as the occurrence of opportunistic and HIV related illnesses; was a component of visit tracking activities (missed visits): provided lost to follow-up statistics (including deaths and transfers), and ARV drug procurement projections from August 2002 through to March 2003.

Objectives

Our primary objective was to develop a patient tracking system that met the following criteria: (1) captures only key ART-related data, (2) requires very little physician time, (3) does not involve direct "real time" physician data entry, thereby not hindering the patient–physician relationship, (4) requires only one concise sheet to be completed at the baseline and at all subsequent patient visits, (5) has minimal "free text" thus minimizing errors and the need for data queries, and (6) allows data to be summarized on a one-page patient visit summary allowing providers unfamiliar with a patient's medical history to be quickly and comprehensively updated.

Our secondary objective was to use our database (1) for individual patient care (site level) and (2) as a programme monitoring and evaluation tool (national level).

Patient tracking

During their first clinic visit each patient was given a unique patient identification number, and an administrative assistant directly entered each patient's contact and demographic information (name, date of birth, gender, national identification/ passport number, contact address, phone and next of kin) into a confidentially kept electronic database. At this visit, the medical officer completed a one-page "Baseline History" form (Annex A, web versiononly, available at: http://www.who. int/bulletin), which contained a list of major opportunistic infections, other major concomitant illnesses, and the WHO clinical stage. At this and all subsequent visits, a one-page "Longitudinal Visit Form" (Annex B, web version only, available at: http://www.who.int/bulletin) was completed capturing (1) status of ART, (2) toxicity

information, (3) information detailing any opportunistic infections and medications used to treat them, (4) adherence assessment, (5) any ART modifications, and (6) the currently prescribed ARV regimen.

Data entry clerks directly entered laboratory data on haematology, viral load and CD4, using the original laboratory result sheets as source documents. These data appeared in summarized table format in our "Patient Visit Summary" form (described later). An additional form called "Patient Disposition Form" (not shown) was designed to capture events such as death, transfer to other treatment sites and lost to follow-up and would only be completed when such an event occurs. This form is important for transparent patient disposition both for clinic routine as well as programme monitoring and evaluation.

The forms were scanned into an electronic database, and the original paper versions were retained at the clinic as source documents along with individual patient medical charts. Any additional information that the physician deemed important could be added as free text at the back of the Longitudinal Visit Form and be readily retrieved from the chronologically arranged patient medical charts.

These three concise yet comprehensive one-page data capture forms developed using OCR technology (Cardiff TELEforms™ version 8) primarily used a check box format and had minimal free text fields, thus minimizing handwriting errors. These forms took less than one minute to complete, and therefore only minimally detracted from the overall patient–physician relationship. The baseline and longitudinal patient tracking forms are extremely versatile and can be easily modified to capture information deemed as "essential" by the treating medical clinic staff and/or national ARV programme managers to adapt to the varying levels of ART monitoring capacity across different sites within sub-Saharan Africa.

Clinic (site) and programme (national) level monitoring and evaluation

For site-level monitoring, we designed a concise Patient Visit Summary form (Annex C, web version only, available at: http://www.who.int/bulletin). This form efficiently summarized individual patient outcomes and contained all key aspects of patient

care collected from the Baseline History and Longitudinal Visit forms, as well as CD4+ cell count and plasma HIV-1 RNA data. This form would be consistently updated after each scheduled visit. The Patient Visit Summary form, which is retained by the patient until the next scheduled clinic visit, helps to provide patient information to other health workers, such as at the local clinic, who may help provide care for the patient, or those at another ART site if the patient gets transferred.

For programme monitoring and evaluation, we opine that aggregated patient data could be summarized in report formats and regularly reviewed by the clinic's senior medical staff and/or programme managers at the national ART programme level. We could not test this due to the short duration of our pilot study.

We believe that these aggregate reports would help provide the following "key" information: (1) total number of patients newly initiated on ART, (2) total number of patients receiving ART, (3) breakdown of patient numbers on various HAART regimens, (4) number of patients with individual ARV toxicities, (5) mortality statistics, (6) adherence estimates based on plasma HIV-1 RNA levels and patient adherence to clinic visits, and (7) lost to follow-up or transfer statistics. Each aggregate summary could easily be modified and tailored to match the local infrastructure and operations specific for each individual ART site.

Methodology

We piloted this data capturing approach in a setting in which the ART clinic and data management centre were in close proximity to each other. Baseline Medical History and Longitudinal Visit forms were dispatched at the end of each clinic day to the data management centre where they were scanned and verified by an experienced medical student trained in the procedure. These forms were then sent back to the site and filed alongside the patients' medical charts to be available for their next clinic appointment. Patient visit summaries with updated clinical data, ARV prescription and laboratory data (CD4+ cell count and plasma HIV-1 RNA levels) were available within one month (before the next scheduled visit) of the visit. The software program for our tracking system was developed using the basic features of Microsoft Access™.

Personnel needed

At the clinic level, an administrative assistant with basic skills in computer use was trained to manage the registration of new patients, to book future patient appointments, and to oversee the "housekeeping" of individual patient charts. Support staff (students, volunteers) were trained to assist in updating the patient charts, filing of Baseline Medical History, Longitudinal Visit, and Patient Visit Summary forms, carrying the forms to and from the nearby data management centre, and providing the first level of quality assurance by responding to queries not requiring physician supervision and involvement. Treating physicians received training in completion of forms as well as basic information technology skills to readily access and print on demand the individual Patient Visit Summary forms. At the data management centre, an experienced medical student verified all scanned forms for completeness and legibility. Trained volunteers and/or treating physicians answered any questions that the medical student had.

The length of our pilot was approximately seven months and we effectively managed about 3000 patients (who are seen by a physician five times a year on average) using our core staff. We envisage that to sustain a patient volume of up to 6000, 1 or 2 full-time data entry clerks (who could scan 150–200 one-page data capture forms/ day) and a part-time (0.5 full-time-equivalent) data manager to oversee their work and provide the necessary quality assurance would be required. A person may also be required for maintenance of the central database to ensure effective functioning of the data-capture system.

Costing analysis

To effectively and efficiently provide care for approximately 6000 patients using our system, the start-up costs would include the purchase of a scanner (7000 US$), one PC workstation (1000 US$) and software license (1500 US$). Ongoing costs would include salary support for staff indicated above, 300 US$ for about 3 toner (printer) cartridges per year as well as around 500 US$/year for paper/photocopying expenses.

Lessons learned

What data should be collected?

Recently, several opinions detailing the minimum level of patient information needed for effective patient tracking and programme evaluation have been published.[5,14] An electronic medical record system developed and implemented in Kenya used a 8-page initial visit encounter form and a two-page return visit encounter form.9 Our concise one-page Baseline Medical History and Longitudinal Visit forms were well received by treating physicians and significantly reduced costs on consumables (reduced photocopying costs) and quality assurance as they had minimal free text thus generating fewer queries and requiring less data manager supervision. In addition, the majority of the queries were not complicated as they could be handled at the data keying and physician level. We refrained from collecting extensive data on physical examination, signs and symptoms, and diagnostic tests, and instead chose to focus on ARV-associated toxicity, virological failure, adherence assessment and incident opportunistic infections. At our clinic, where individual physicians were routinely examining 25–40 patients per day, the collection of any additional data not considered to be "key" for tracking major patient outcomes was perceived as a burden and was likely to not be reliably collected. We strongly feel that if additional data collection is required, designated research teams should collect it separately as they typically have fewer manpower constraints and therefore can spend more time per patient encounter.

How best to capture the data?

While addressing the question of how best to enter individual and aggregate patient data in electronic format, we focused primarily on developing a physician independent approach by using optical character recognition (OCR) technology.

Why is a physician independent approach necessary?

We believe that an optimal data capture system should be "physician independent", as physicians are often a scarce resource in busy ART clinics, and their time should be reserved for day-to-day clinic administrative duties, patient management and staff education. Many physicians in the ART clinics have competing outpatient clinic and medical ward responsibilities and do not have time during any given work-day to complete multiple-page forms and answer multiple queries that incomplete

filling out of these numerous forms would generate. Even for the newer more expensive IPMS, "physician-driven" data entry is not only time consuming but also likely to detract from the patient–physician relationship as the treating physician spends less time examining and talking to individual patients because they have to constantly look at the workstation on their desk to ensure simultaneous correct data entry.

How does optical character recognition technology help in data capture?

To best accomplish our above-mentioned objectives, we decided to capitalize on existing OCR software technology (Cardiff TELEforms™ version 8) to scan forms into the central database. Our one-page OCR forms were created mostly in check box format (rather than having "free text" fields), allowed us to daily scan these forms into the central database while still being instantly accessible for review in the patient folder, thereby avoiding multiple data entry. The Longitudinal Visit forms could also assist in maintaining a high standard of care and in strengthening adherence to existing national ART guidelines. Another advantage of the OCR format is the possibility of conveniently linking peripheral sites with phone access to a central site via fax.

Other data entry options

Advances in information technology offer a wide array of data capturing methods, which could be considered for specific settings. Innovative technologies such as personal digital assistants, touch-screen computer technology or web-based medical record systems15 are promising avenues that potentially combine ease of data entry and minimal interference with physicians' work. Their use in resource-limited settings, however, has yet to be studied.

Limitations

Our experiences were limited by the relatively short time available for piloting our data capture system because the government launched the centralized IPMS. We, however, believe that the large number of patients that we followed up compensated for this shortcoming. We also could not extend our pilot study to involve one or more peripheral sites but such a system should be tried in more geographically isolated areas where many rural clinics feed into one or more central clinics. In addition, now that more is being reported on the specifics of patient monitoring

and evaluation, any future development of our or other related novel data capture systems would need to harmonize with guidelines that are now available which emphasize standardization of data dictionaries and health data programming as set forth by the HL7 and WHO working groups.6

Conclusion

The number of HIV-1 infected persons receiving lifelong HAART in sub-Saharan Africa is expected to grow exponentially in the next few years. Infrastructure limitations and manpower shortages will constitute major challenges in efficiently and effectively documenting individual patient care and overall ART programme outcomes. While, the traditional method of paper to electronic data transfer by data entry clerks often compromises on the format because it has to suit the health care provider's need for ease of completion and the data entry clerk's need for quick and reliable entry into the electronic database, direct electronic data input by physicians detracts from patient–physician encounters and also require a reliable power supply and on-going technical assistance, which may be scarce in resource-limited settings. We believe that the newly implemented integrated patient management system in Botswana would be costly requiring multiple computer stations at each ART site as well as be highly provider-dependent as it requires health care providers to enter data "real-time" directly onto their workstation in their consultation room and may therefore only be feasible for a few countries among those who will offer ART. We recommend the use of our concise one-page OCR based forms in hybrid paper–electronic format that collects "key" data for individual and overall patient outcomes, combining ease of data entry and saving of limited physician time while not disrupting the patient–physician encounter.

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Annex A

BASELINE HISTORY

PID | DATE OF VISIT (dd / mm / 20 yy) | INITIALS | SITE

INSTRUCTIONS Shade the bubble when applicable For other fields, enter the value as equired

			mm	yyyy
Pulmonary Tuberculosis (Most Recent) ○ SMEAR + ○ SMEAR - ○ UNKNOWN	○ Yes	○ No		
Pulmonary Tuberculosis (Previous) ○ SMEAR + ○ SMEAR - ○ UNKNOWN	○ Yes	○ No		
Extrapulmonary Tuberculosis ○ CNS ○ GI ○ Cardiac ○ Other	○ Yes	○ No		
Cryptococcal Meningitis	○ Yes	○ No		
Kaposi's Sarcoma ○ Cutaneous ○ Palatal only ○ palatal and cutaneous ○ diffuse with lymphatic obstruction ○ diffuse without lymphatic obstruction ○ presumed visceral disease	○ Yes	○ No		
Current peripheral Neuropathy If yes, specify grade ○ Moderate ○ Severe	○ Yes	○ No		
Wasting Syndrome (>10% net loss from baseline) ○ With chronic diarrhea ○ Without chronic diarrhea	○ Yes	○ No		
Chronic Diarrhea (>1 month duration)	○ Yes	○ No		
AIDS Dementia Complex	○ Yes	○ No		
CMV Retinitis	○ Yes	○ No		
Candidiasis ○ Esophageal ○ Oral ○ Both	○ Yes	○ No		
Chronic dermatitis ○ Seborrhoic dermatitis ○ Other ○ Papular pruritic dermatitis	○ Yes	○ No		
PCP Pneumonia ○ Proven ○ Suspected	○ Yes	○ No		
Recurrent genital ulcers	○ Yes	○ No		
Herpes Zoster ○ cutaneous ○ Ophthalmic	○ Yes	○ No		
Other	○ Yes	○ No		

Best Weight (kg) . **Karnofsky score**

Current weight (kg) . **Height (cm)**

WHO clinical stage (Specify):
○ Stage 1 ○ Stage 2 ○ Stage 3 ○ Stage 4

Active/Previous medical history:
○ [redacted] ○ Malignancy
○ Hypertension ○ Diabetes mellitus
○ Renal Disease ○ Traditional Medicine use
○ Psychiatric illness requiring treatment

Clinician's Signature:

Annex B

LONGITUDINAL VISIT FORM

PID | DATE OF VISIT (dd / mm / 20 yy) | INITIALS | SITE

INSTRUCTIONS: Shade the bubble when applicable. For other fields, enter the value as equired

1. Is the patient on ART? ◯ Yes ◯ No ◯ Initiated at this visit

Weight (kg): ___ . ___

2. Indicate any ARV-related toxicities since the last visit:

- ◯ ZDV-Anemia moderate (Hb between 7-7.9g/dL)
- ◯ ZDV-Anemia severe (Hb between 6-6.9g/dL)
- ◯ ZDV-Anemia life threatening (Hb < 6 g/dL)
- ◯ Peripheral neuropathy moderate (discomfort requiring Rx)
- ◯ Peripheral neuropathy severe (incapacitated, disabled)
- ◯ NVP rash moderate (maculopapular)
- ◯ NVP rash severe (blistering, mucosal involvement)
- ◯ EFV CNS symptoms
- ◯ Minor ZDV-related symptoms (headaches/nausea)
- ◯ ddI-related clinical pancreatitis
- ◯ ddI-related chemical pancreatitis
- ◯ Lipoatrophy/Lipohypertrophy
- ◯ Hepatotoxicity
- ◯ Other:

3. Indicate any new HIV-related diagnosis since the last visit:

- ◯ Herpes Zoster
- ◯ Pulmonary Tb
- ◯ Extrapulmonary TB
- ◯ Candidiasis (oral)
- ◯ Candidiasis (esophageal)
- ◯ CMV retinitis
- ◯ PCP pneumonia
- ◯ HIV encephalopathy
- ◯ Wasting Syndrome
- ◯ Cryptococcal Meningitis
- ◯ Diarrheal illness (>7 days duration)
- ◯ recurrent genital ulcers (>1 month)
- ◯ diffuse Lymphadenopathy
- ◯ Other:

4. Indicate OI preventative / therapeutic medication prescribed at this visit or since last visit

- ◯ Amitriptyline
- ◯ Cotrimoxazol (treatment dose)
- ◯ Cotrimoxazol (prophylaxix dose)
- ◯ Ketoconazole
- ◯ Fluconazole
- ◯ ATT
- ◯ IPT
- ◯ Benzathine Penicillin
- ◯ Gancyclovir
- ◯ Acyclovir
- ◯ Nystatin
- ◯ Other:

5. Are there any new drug allergies, previous unreported?

◯ Yes ◯ No Specify.

6. In your opinion, is the patient at least 90% adherent to ART/ (Clinician) ◯ Yes ◯ No

7a Are there any changes in ART at today's visit? ◯ No changes ◯ Modified

7b If dose modification: ◯ Reduced ◯ Increased ◯ Held ◯ Resumed ◯ Drug switch

7c Please give the primary reason for change:

- ◯ Toxicity
- ◯ Virologic failure
- ◯ TB Treatment
- ◯ Dose escalation
- ◯ Pregnancy
- ◯ Other:

8. What is the prescribed regimen today?

NRTIs

- ◯ CBV BD
- ◯ ZDV 300 BD
- ◯ ZDV 200 BD
- ◯ 3TC 150 BD
- ◯ ABC 300 BD
- ◯ ddI 400 OD
- ◯ ddI 300 OD
- ◯ ddI 200 OD
- ◯ d4T 40 BD
- ◯ d4T 30 BD
- ◯ d4T 20 BD

NNRTIs

- ◯ NVP 200 OD
- ◯ NVP200 BD
- ◯ NVP 400OD
- ◯ EFV 600OD
- ◯ EFV 800 OD

PIs

- ◯ NEL 1250 BD
- ◯ IDV 800 TDS
- ◯ RIT/IDV 100/800 BD
- ◯ RIT/SQV 400/400 BD
- ◯ RIT/SQV 100/1000 BD
- ◯ RIT/SQV 100/1600 OD

DATE OF NEXT VISIT: (dd / mm / 20 yy) **Clinician's Signature:**

Annex C

02406 **Patient Initials:** *KS* **Gender:** *F* **Date of Birth:** *16/01/1975*

Allergies: *NONE* **Pre-enrolment ARV History (if any):**

Baseline History: *Wasting Syndrome without chronic diarrhea*
Herpes Zoster (cutaneous) 2000
Pulmonary TB, smear+ 2002

PATIENT VISIT SUMMARY

Date	Wt	on ART	ARV Regimen	ART modified	Adherent	VL	CD4	Toxicities/ OIs	OI Medication
20/12/02	40	no				750000	89	Candidiasis (oral)	CTM (proph dose) Nystatin
03/01/03	39.5	initiated	CBV BD NVP 200 OD	no	Yes				CTM (proph dose)
17/01/03	40	Yes	CBV BD NVP 200 BD	increased	Yes				CTM(proph dose)
13/02/03	42	Yes	d4T 30 BD 3TC 150 BD NVP 200 BD	Yes	Yes			ZDV-anemia (severe)	CTM (proph dose)
10/03/03	45	Yes	d4T 30 BD 3TC 150 BD NVP 200 BD	No	Yes	<400	158		CTM (proph dose)
07/04/03	44	Yes	d4T 30 BD 3TC 150 BD NVP 200 BD	No	Yes			Pulmonary TB	CTM (proph dose) ATT
07/06/03	47	Yes	d4T 30 BD 3TC 150 BD NVP 200 BD	No	Yes				CTM (proph dose) ATT
09/06/03	48	Yes	d4T 30 BD 3TC 150 BD NVP 200 BD	No	Yes				CTM(proph dose) ATT
07/07/03	51	Yes	d4T 30 BD 3TC 150 BD NVP 200 BD	No	Yes				CTM (proph dose) ATT
04/08/03	53	Yes	d4T 30 BD 3TC 150 BD NVP 200 BD	No	Yes			Periph Neuropathy (mod)	CTM (proph dose) ATT Amitriptyline
01/09/03	52	Yes	d4T 30 BD 3TC 150 BD NVP 200 BD	No	Yes	<400	212	Periph Neuropathy (mod)	CTM (proph dose) ATT Amitriptyline
29/09/03	53	Yes	d4T 30 BD 3TC 150 BD NVP 200 BD	No	Yes			Periph Neuropathy (mod)	CTM (proph dose) Amitriptyline
27/10/03	51.5	Yes	d4T 30 BD 3TC 150 BD NVP 200 BD	No	Yes			Periph Neuropathy (mod)	CTM(proph dose) Amitriptyline
24/11/03	53	Yes	d4T 30 BD 3TC 150 BD NVP 200 BD	No	Yes			Periph Neuropathy (mod)	CTM (proph dose) Amitriptyline
22/12/03	52	Yes	d4T 30 BD 3TC 150 BD NVP 200 BD	No	Yes			Periph Neuropathy (mod)	CTM (proph dose) Amitriptyline
19/01/04	52	Yes	d4T 30 BD 3TC 150 BD NVP 200 BD	No	Yes			Periph Neuropathy (mod)	CTM (proph dose) Amitriptyline
16/02/04	52.5	Yes	d4T 30 BD 3TC 150 BD NVP 200 BD	No	No	1450	248	Periph Neuropathy (mod)	CTM (proph dose) Amitriptyline
12/03/04	53	Yes	d4T 30 BD 3TC 150 BD NVP 200 BD	No	Yes				CTM(proph dose) Amitriptyline
12/04/04	54	Yes	d4T 30 BD 3TC 150 BD NVP 200 BD	No	Yes				CTM (proph dose) Amitriptyline
10/05/04	54	Yes	d4T 30 BD 3TC 150 BD NVP 200 BD	No	Yes				CTM (proph dose) Amitriptyline
07/06/04	54	Yes	d4T 30 BD 3TC 150 BD NVP 200 BD	No	Yes				
06/07/04	53	Yes	d4T 30 BD 3TC 150 BD NVP 200 BD	No	Yes				
06/08/04	53.5	Yes	d4T 30 BD 3TC 150 BD NVP 200 BD	No	Yes	< 400	312		

Chapter 10

Five-year outcomes of initial patients treated in Botswana's National Antiretroviral Treatment Program

Hermann Bussmann, C. William Wester, Ndwapi Ndwapi, Nicolas Grundmann, Tendani Gaolathe, John Puvimanasinghe, Ava Avalos, Madisa Mine, Khumo Seipone, M. Essex, Victor deGruttola, Richard Marlink

AIDS 2008, 22 (17): 2303-11

Abstract

Background: Antiretroviral treatment (ART) initiatives have now been established in many sub-Saharan African countries showing early benefits. To date, few results are available concerning long-term clinical outcomes in these treatment programs.

Methods: Response to ART is described in the first HIV–1C infected adults enrolled in the Botswana ART program in 2002. Data analysis was conducted on available longitudinal data up to 1 April 2007.

Results: 633 severely immunodeficient patients with a median CD4+ cell count of 67 cell/ μl were initiated on non-nucleoside reverse transcriptase inhibitor-based combination ART and followed for a median of 41.9 months. The median CD4+ increases were 169 cells μl, 302 cells/μl, and 337 cells/ μl at 1, 3, and 5 years, respectively. The percentages of patients with a viral load of less than 400 copies/ mL at 1, 3, and 5 years were 91.3%, 90.1%, and 98.3%, respectively. 75% of patients did not miss a single, or missed only one, monthly ART pick-up per year with a mean pick-up rate of 92.5%. The Kaplan-Meier survival estimates (95%CI) at 1, 3, and 5 years were 82.7% (81.2%,84.3%), 79.3% (77.6%, 81.0%), and 79.0% (77.3%, 80.7%), respectively. At six months, the risk of treatment modification for anemia was 6.94% (5.9%, 8.0%) for cutaneous hypersensitivity reactions, 1.3% (0.8%, 1.7%), and 1.1% (0.7%, 1.6%) for hepatotoxicity.

Conclusions: This initial group of adults on ART in urban Botswana had excellent sustained immunologic, virologic, and clinical outcomes for up to five years of follow-up with low mortality among those surviving into the second year of antiretroviral treatment.

Key words:HIV/AIDS, Africa, antiretroviral therapy, Botswana, public sector

Introduction

Botswana was the first country in Africa to offer large-scale public antiretroviral treatment (ART) to qualifying citizens. From its inception in January 2002, the Botswana national ART program, commonly referred to as the "MASA program" with MASA meaning "new dawn" in Setswana, has significantly scaled up its efforts. The program currently has more than 90,000 persons receiving ART at 32 designated sites and associated satellite clinics throughout the country [1] . In recent years, other ART initiatives have been established in neighboring sub-Saharan African countries, and the preliminary positive benefits of ART are well documented [2-8]. These outcomes demonstrate excellent immunologic and virologic responses coupled with overall ART adherence rates that are comparable to outcomes reported in industrialized countries.

However, mortality rates, especially during the first six months after ART initiation, are substantially higher in developing countries when compared to mortality rates in industrialized nations [9] . These high early mortality rates are associated with severe immunodeficiency [2], a wide spectrum of co-morbid conditions including poor or reduced nutritional status, anemia, tuberculosis, and other opportunistic infections among adults initiating ART in sub-Saharan Africa [3, 4, 10] . In addition, the reports of high rates of early losses to follow-up (LFU) among those initiated onto ART in southern Africa are concerning and require more efficient patient tracking and monitoring systems [11].

In general, these ART regimens appear to be well tolerated, however, the possibility of higher than expected rates of ARV-associated toxicities, when compared to developed country ART-treated cohorts, warrants more in-depth and long-term study. These toxicities include lactic acidosis [11, 12], nevirapine cutaneous hypersensitivity reactions [12, 13], and lipodystrophy [13-15]. To date, though, very few studies have reported long-term outcomes among patients on ART in the region [8]. We report herein five-year outcomes among the first 633 patients initiated on public ART as part of Botswana's MASA program.

Methods

Patient Population

Princess Marina Hospital (PMH), in the capital city of Gaborone, is the largest referral hospital in the country and serves a population of approximately 800,000 persons residing primarily in southern Botswana. The overwhelming majority of adults and children in Botswana receive health care free-of-charge via the public health care system. Only a small fraction of the population receives their healthcare in the private sector. The Infectious Disease Care Clinic (IDCC) of PMH was established in March 2001 and the first group of patients on ART in Botswana received longitudinal care and treatment at this clinic [3,16]. On 21st January 2002, the IDCC became the first public sector ART site of Botswana's MASA program, providing care to patients from a large geographic catchment area. Between May 2002 and December 2004 31 additional MASA sites were established countrywide. All adult patients with an AIDS-defining illness, a CD4+ cell count of less than 200 cells/μl or both were offered ART, according to the Botswana Guidelines on Antiretroviral Treatment .

For this analysis, the study population consisted of all HIV-1 infected, ART-naïve adults who registered for care at the PMH IDCC between the inception of the public ART program on 21 January 2002 and 7 August 2002.

Treatment Regimens

The Botswana ART guidelines [18] for adults recommend two nucleoside reverse transcriptase inhibitors (NRTIs), zidovudine/lamivudine, and one non-nucleoside reverse transcriptase inhibitor (NNRTI), usually efavirenz, or for women of reproductive potential, nevirapine. Zidovudine and lamuvidine were given initially as co-formulated Combivir and once available, as co-formulated lamzid which was taken twice daily. Following a 14-day lead-in period of 200 mg once daily, nevirapine was maintained at 200 mgs twice daily. The efavirenz dose was 600 mg/day given as three 200 mg tablets and, once available, as a single 600 mg tablet. Therefore, total ARV pill counts ranged from 3 tablets per day for efavirenz-based HAART regimens and 4 tablets per day for nevirapine-based HAART regimens. Patients with pre-existing significant anemia were offered stavudine instead of zidovudine. Patients experiencing virologic failure on first-line regimens were switched to protease inhibitor- based HAART (initially with nelfinavir, and later changed to lopinavir/ ritonavir) with two new NRTIs (didanosine and stavudine or when available

tenofovir or abacavir and lamivudine). Genotypic resistance testing was reserved for second-line failures, complicated first-line switches or both requiring resistance testing such as pregnancy with ongoing viremia.

Patient Visits

At the initial clinic visits, all patients had a comprehensive history taken and received a physical examination. When clinically indicated, chest radiography and sputum microscopy for acid-fast bacilli smears were done to rule out active pulmonary TB. The following baseline laboratory tests were performed: chemistry, hematology, CD4+ cell count, plasma HIV-1 RNA level, hepatitis B surface antigen, and syphilis serology. Serology testing and tracking of results, however, was not routinely done in the early days of the National Antiretroviral Treatment Program . Patients with an active opportunistic infection were treated in accordance with national standards. Oopportunistic infection prophylaxis was offered in the form of 6-month isonaizid/ pyridoxine (B6) to all persons documented to be HIV-1 infected and cotrimoxasole *Pneumocystis pneumonia* (PCP) prophylaxis for all adults having a CD4+ cell count of less than 200 cells/µl.

All eligible patients were initiated on ART within two weeks of initial registration, allowing for opportunistic infection screening and review of baseline laboratory investigations. Nevirapine-treated patients had liver function testing and a scheduled clinic visit prior to dose escalation at 2 weeks. CD4+ cell counts and plasma HIV-1 RNA levels were initially obtained three monthly and after November 2006, six monthly. Chemistry and hematology were obtained at months 1 and 3 and then at three month intervals. In accordance with revised national guidelines from 2005 chemistry and hematology as well as lipase, non-fasting and fasting glucose, lipids, and lactate were done on an as-needed basis.

A confirmatory test was performed for patients with detectable plasma HIV-1 RNA levels (>400 copies/mL). These patients received additional adherence counseling and education. In cases of confirmed virologic failure, patients were switched to second-line protease inhibitor-based ART. All clinical visits within the first year were conducted by physicians trained in HIV care via the National AIDS Training Program, KITSO [16] . Clinically stable patients were subsequently managed by

trained nurses. Since 2005, 103 patients deemed "stable" on their first-line ART regimen beyond one year were transferred to private physicians for care as part of the "public-private partnership" (PPP) arrangement.

Patient Education

At the time of ART initiation, all adult patients were required to have a self-appointed adherence assistant, who would receive adherence education and counseling. The adherence assistants were trained to help with ART adherence and toxicity recognition. All patients returned monthly to the PMH IDCC pharmacy for ARV medication refills, during which time pill counts were performed and patients received further counseling and education.

Laboratory Methods

Plasma HIV-1 RNA levels were quantified using the Amplicor HIV-1 Monitor test, version 1.5 (Roche Diagnostics Systems, Branchburg, NJ) with a lower limit of detection of 400 copies/mL. CD4+ cell counts were determined using the FACSCalibur™ flow cytometer (Becton Dickenson, San Jose, CA, USA) with CD3/4/8/45 Multiset reagents.

Data Collection

The MASA program documentation system has evolved from facility-based paper records of clinical, laboratory, and pharmacy data to an electronic integrated patient management system with direct computer entry of clinical and pharmacy information and transfer of data to a centrally maintained database in the Ministry of Health. Using SAS software, a comprehensive electronic analysis database was created that incorporated data from the following: data abstracted from paper-based clinical records, data from the centralized Ministry of Health electronic database, and data from the Botswana PPP. For all patients who did not return for follow-up, an attempt was made to obtain information on visits by direct phone contact, home visits, or cross-checking with other ART sites.

Statistical Analysis

Data from all patients are included in analyses, irrespective of adherence to or change in ART regimen. Length of follow-up varied because of death, LFU, and transfers to other MASA clinics. Observations were censored at 1 April 2007 or at the time of LFU or transfer. Time to virologic failure and drug changes were censored

at the date of the last clinic visit. Time to laboratory marker events was censored at the date of the last laboratory specimen. Kaplan-Meier estimates and 95% confidence intervals (CI) based on the Greenwood formula were used to describe time-to-events distribution. Log-rank tests were used to compare time-to-event after ART initiation across groups. Likelihood ratio tests from Cox proportional hazard regression models were used to compare time-to-event after ART initiation for continuous variables and multiple outcomes predictors.

For analyses involving CD4+ cell counts and plasma HIV-1 RNA, data were grouped into intervals around the ideal time points. Counting time from treatment initiation, non-overlapping intervals centered at each evaluation time point were created and the measure closest was selected, so that each patient contributed to each interval either one observation or none. CD4+ cell graphs show median changes with interquartile ranges. HIV-1 RNA graphs display percentages with 95% CIs as calculated using the method of Agresti and Coull. Pre-treatment values were measured no more than three months before ART initiation. Patient data were censored at the event of transfer to another public MASA site. We assumed that censoring at transfer was non-informative.

Univariate analyses were performed to identify predictors of death and LFU using a log rank test. The following baseline characteristic were included in the analysis: sex, WHO staging, CD4+ cell count, viral load (less than, greater than, or equal to 200,000 copies/mL), active TB, age (<35 years, 35-45 years, and >45 years), initial ART regimen, and hemoglobin below 8.0 g/dL. To adjust for potential bias arising from LFU, we identified predictors of both death and loss to follow-up. Two variables, anemia and WHO clinical stage 3/4 disease, predicted both death and LFU. Therefore, we performed Kaplan-Meier analyses, stratified by all combinations of predictor variables. Our results from stratified Kaplan-Meier estimates differed from the nonstratified Kaplan-Meier estimates by less then 0.35%, implying low bias from the assumption of non-informative censoring. All values and figures reported are generated from nonstratified analyses.

Ethics

Ethical approval was obtained from the Botswana Ministry of Health's Health Research Development Committee and the Harvard School of Public Health's Human Subjects Committee.

Results

Between 21 January 2002 and 7 August 2002, 871 adult patients were registered at the PMH IDCC. Six-hundred and thirty-three ARV-naïve adults were initiated on ART, with the remaining 238 not initiating ART for the following reasons: 131 were already receiving ART via the private sector, 42 died prior to ART initiation, 38 were lost to follow-up before initiating ART, and 27 patients were not yet eligible by clinical, immunologic or both criteria to receive ART [17] .

Baseline characteristics of the 633 ARV-naïve patients are shown in Table 1. The median age was 34.8 years [interquartile range (IQR) 30.2 - 41.3]; 60.0% were female. At baseline, the vast majority of patients had advanced HIV disease, as evidenced by their WHO clinical stage, the number of recent or active opportunistic infections, and CD4+ cell count and plasma HIV-1 RNA values. The most common first-line ART regimens were zidovudine/lamuvidine and either efavirenz (50.6%; n=320) or nevirapine (44.4%; n=281).

The median duration of follow-up was 41.9 months [IQR 8.3 – 56.9 months], calculated as the time to either death, transfer to non-PMH MASA site, LFU, or 1 April 2007 (Figure 1). The total duration of follow-up was 1822.2 patient-years. The median duration of follow-up for the 290 patients alive and known to be receiving treatment as of 1 April 2007 was 57.2 months [IQR 55.9 – 58.8 months]. The Kaplan-Meier estimates of LFU for all 633 patients at 1, 3, and 5 years were 8.9% (CI 7.7%, 10.1%), 15.4% (CI 13.8%, 17.1%), and 21.8% (CI 19.9%, 23.8%).

Table 1: Baseline characteristics and initial treatment of first national program patients initiated on HAART between January and July 2002 (N=633)

Age, median (IQR) in years	34 8 (30 2-41 3)
Female, Number (%)	378 (59 88)
Weight, mean in kg	52.3
BMI, mean BMI< 18 5, Number (%)	18 8 219 (35)
CD4 cell count, Median (IQR)	67 (28-127)
HIV-1 plasma RNA, Median (copies/mL)	444,000 (173,000- 750,000)
Karnofsky score, Mean ≤ 60, No, (%)	80 4 55 (9)
Hemoglobin, Mean, g/dl Hemoglobin <8 g/dL, No (%)	10.7 62 (9.8)
WHO stage at entry, Number (%) I II III IV	606 (95.5) 18 (3) 74 (12) 271 (43) 243 (38)
Opportunistic Infections n, (%) Wasting Syndrome Pulmonary TB Extrapulmonary TB Herpes Zoster Papular pruritic dermatitis/eosinophilic folliculitis Chronic diarrhea Esophageal candidiasis Kaposis's sarcoma Pneumocystis jiroveci pneumonia (PJP) Cryptococcal Meningitis AIDS Dementia Cytomegalovirus (CMV) retinitis	 407 (64) 244 (39) 42 (7) 110 (17) 106 (17) 103 (16) 48 (8) 46 (7) 39 (6) 33 (5) 29 (5) 14 (2)
Initial ART regimen n, (%) CBV / NVP CBV / EFV d4T / 3TC / NVP d4T / 3TC / EFV d4T / ddI / NVP d4T / ddI / EFV	 281 (44.4) 320 (50.6) 15 (2.4) 11 (1.7) 2 (0.3) 4 (0.8)

The median CD4+ cell count increases were 169 cells/μl, 302 cells/μl, and 337 cells/μl at 1, 3, and 5 years (Figure 2). For individuals with a baseline CD4+ cell count of less than 50 cells/μl, the median CD4+ cell count increases were 200 cells/μl, 315 cells/μl, and 367 cells/μl at 1, 3, and 5 years, respectively. For individuals with a baseline CD4+ cell count of more than 50 cells/μl, the median CD4+ cell count increases were 162 cells/μl, 298 cells/μl, and 326 cells/μl at 1, 3, and 5 years, respectively. The one-year median CD4+ increase in the group of patients initiated on ART with baseline CD4+ cell count of less than 50 cells/μl was 200 versus 162 cells/ μl in the group

initiated with a CD4+ cell count of 50-200 cells/μl (p=0.037). By 4 years, there were no longer any significant differences in absolute CD4+ cell count increases between the groups (p=0.81) The percentages of patients with undetectable viral load levels at 1, 3, and 5 years were 91.3%, 90.1%, 98.3%, respectively (Figure 3).

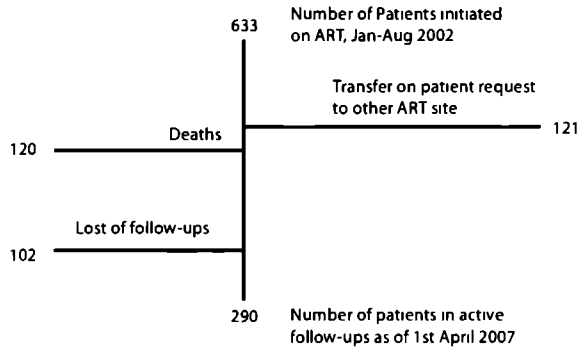
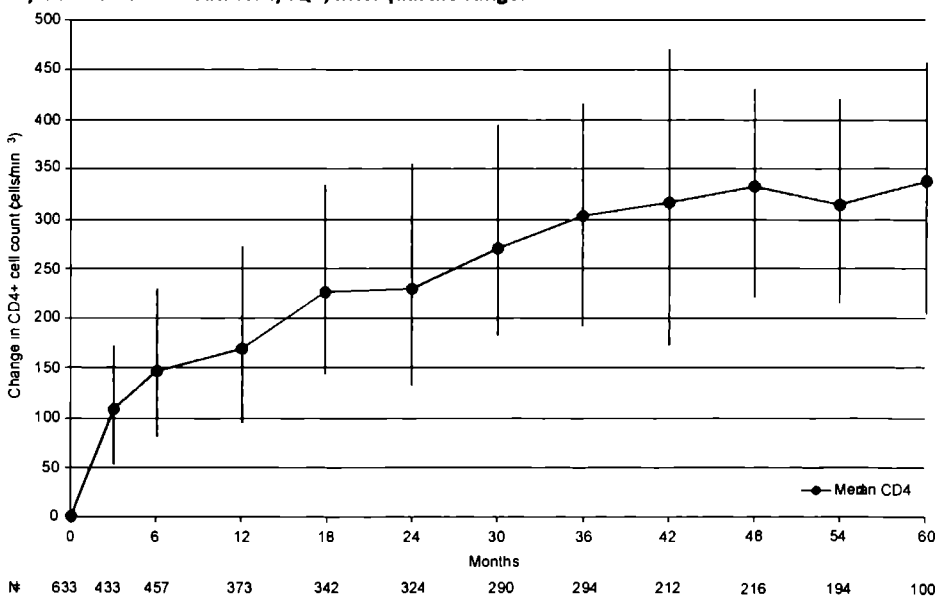


Fig 1. Status of first group of adults initiated on ART in Botswana National Antiretroviral Treatment Program. ART, Antiretroviral

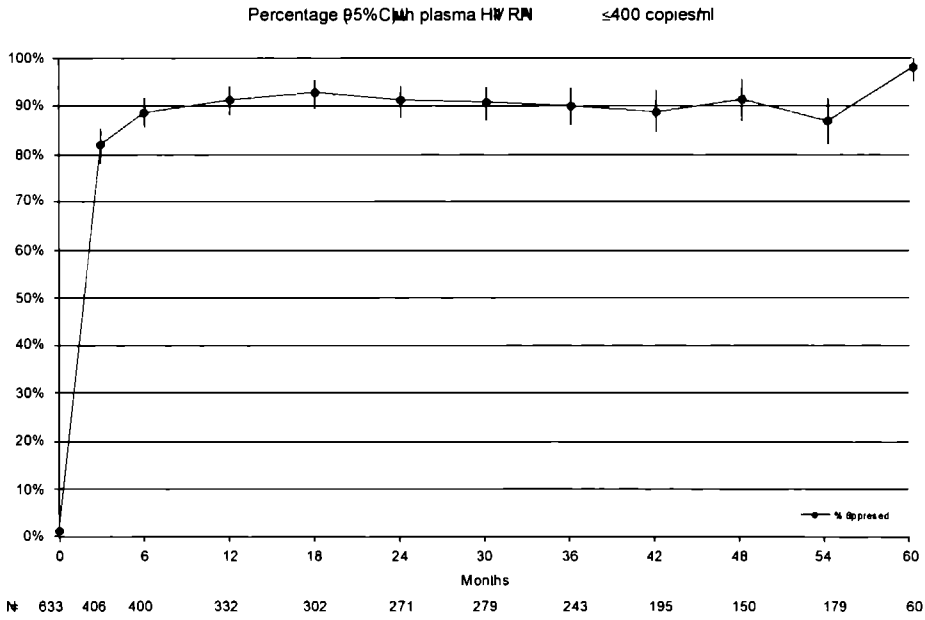
During follow-up, 47 patients experienced virologic failure. The 1, 3, and 5-year Kaplan-Meier estimates of death or virologic failure were 17.6% (CI 16.0%, 19.1%), 22.1% (CI 20.3%, 23.8%) and 30.1% (CI 28.0%, 32.2%), respectively (Figure 4). One-hundred and twenty of the 633 patients died during follow-up. The Kaplan-Meier survival estimates at six months, 1, 3, and 5 years were 87.8% (CI 86.5%,

Fig. 2. Median (IQR) CD4+ cell count increases from ART initiation.
ART, antiretroviral treatment; IQR, interquartile range.



89.1%), 82.7% (CI 81.2%, 84.3%), 79.3% (CI 77.6%, 81.0%), and 79.0% (CI 77.3%, 80.7%), respectively. The Kaplan-Meier 1, 3, and 5-year survival estimates for patients with baseline CD4+ cell counts of less than 50 cells/μl were 74.8% (CI 71.9%, 77.6%), 71.1% (CI 68.1%, 74.1%), and 70.5% (CI 67.4%, 73.5%) versus 87.9% (CI 86.1%, 89.6%), 84.5% (CI 82.5%, 86.5%), and 84.5% (CI 82.6%, 86.5%) for patients with baseline CD4+ cell counts of at least 50 cells/μl. At year 1, there was a 2.3-fold (CI 1.6, 3.4) higher mortality rate among patients initiating ART with a baseline CD4+ cell count of less than 50 cells/μl compared with those initiating ART with baseline CD4+ cell counts of 51-200 cells/μl (p=0.0001). Advanced immunosuppression (i.e. CD4+ cell count of less than 50 cells/μl; log-rank p<0.001)), WHO clinical stage 3/4; (log-rank p=0.003), and baseline hemoglobin below 8.0 g/dL (log-rank p=0.0087) were all significant predictors of mortality. Autopsy data were not available for the majority of deaths. Clinical records and verbal autopsy information, however, identified presumptive causes of deaths in 71 (59.2%) patients. The most common

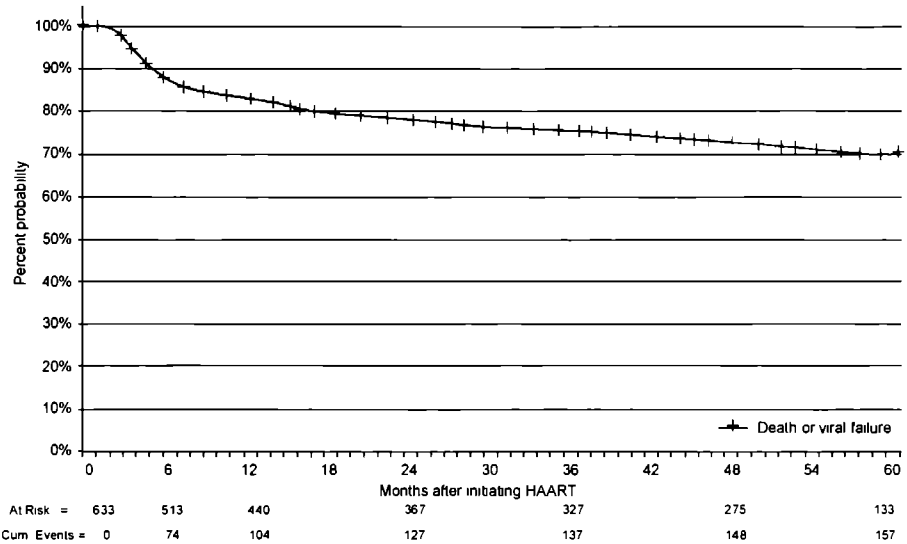
Fig. 3. Percentage (95% CI) with plasma HIV-1 RNA 400 copies/ml or less. CI, confidence interval.



causes of death were advanced AIDS with wasting syndrome (n=41, 34.2%) and pulmonary TB (n=8, 6.7%). Serious, potentially life-threatening clinical, laboratory,

or both events occurring within the first six months following ART initiation included anemia, cutaneous hypersensitivity reactions, and grade 3/4 hepatotoxicity. At six months, the risk of treatment modification for anemia was 6.9% (CI 5.9, 8.0), 1.3% (CI 0.8, 1.7) for cutaneous hypersensitivity reactions, and 1.1% (CI 0.7, 1.60) for hepatotoxicity. Overall, 13 patients had a regimen changed for reasons of

Fig. 4. Survival estimate for death or viral failure



lipodystrophy, with the majority of those switches in stavudine-treated patients. Other reasons for treatment modification during the five years of follow-up included temporary interruption of efavirenz supply, pregnancy, and the development of TB. Adherence was measured by the individual patient's frequency of attended medication refill visits (percent of attended visits vs scheduled visits). Using this method, adherence could only be calculated beginning in July 2002 when the computerized pharmacy record system was introduced. The mean overall monthly pharmacy refill rate was 92.5% (CI 91.2%, 93.6%). Of all patients, 73.7% (CI 70.1%, 77.4%) patients never missed or missed less than one refill visit per year, that is were adhering to more than 90% of refill visits, whereas 11.3% (CI 7.9%, 14.8%) of patients missed two or more visits per year. Nearly half, 49.6% (CI 45.4%, 53.8%), of our patients did not miss any refill visits over the entire reporting period. There were no significant differences in adherence rates by gender (p=0.11).

Discussion

We herein report five-year outcomes of the first group of ART-treated adults in Botswana's public National Antiretroviral Treatment Program. A striking feature of this cohort is the severity of immunodeficiency at the time of ART initiation. Early treatment cohorts in other resource-limited countries were selected in part by financial [19], social, or both criteria [4] in addition to the degree of immunodeficiency, Botswana offered public ART to all qualifying persons [20] . For the first six months of 2002, the PMH IDCC in Gaborone was the only ARV treatment site countrywide. The selection of patients, therefore, was heavily biased by "disease severity", as evidenced by the median CD4+ cell count and viral load values, the high percentage of patients with active or recent opportunistic infections and, advanced WHO clinical stage disease [21] .

The vast majority of ART-treated adults exhibited excellent immune recovery with sustained CD4+ cell gains persisting beyond five years on ART. Although the gain in CD4+ cells observed in this severely immunodeficient population is similar to that reported among adults initiating ART at higher CD4+ cell counts [22, 23], the high mortality rate in this population might have seriously biased this result and highlights the importance of earlier ART initiation.

A paucity of data is available describing the long term virologic outcomes among ART-treated patients in resource-limited settings. The high virologic suppression rate among patients still receiving care in our cohort is encouraging, especially in light of underlying public health concerns that indiscriminate prescription of ART in resource-constrained settings could generate widespread HIV-1 drug resistance. The virologic failure rate is an underestimate considering the high LFU and death rates, but within our cohort, the vast majority of patients alive and continuing ART maintained excellent virologic suppression. Importantly, however, measures to minimize and monitor emergence of treatment-associated drug resistance remain essential [24]. Our adjusted mortality rates showed similarly high early mortality rates when compared with other cohorts of adults initiating ART with severe baseline immunodeficiency [2,9,25-27].

Mortality in this cohort was highest in the first year, with 50% occurring in the first three months and approximately 86% of all deaths occurring within the first year. The majority of deaths were due to advanced AIDS, with only a small fraction attributed to ARV-related toxicities. In addition to the high early on-treatment mortality, another concern is the significant number of patients who were qualified to receive treatment but died before ART could be initiated and indicates that a swift and decentralized plan for the roll-out of ART programs in high-prevalence countries is urgently needed [28,29].

Botswana is one of the few sub-Saharan countries to choose zidovudine-based ART for first line treatment [4,18,30] . Overall rates of early treatment-modifying toxicities were low, but higher rates of grade 3/4 anemia following ART initiation occurred in our cohort of severely immunodeficient adults when compared to healthier patient cohorts [30] . These high rates of anemia are of significant concern for rural Botswana and other sites in the region where blood supplies are limited and alternatives, including better tolerated NRTI-backbones for first-line ART, should be considered. Of note, tenofovir-based first-line ART is now offered in Botswana, Zambia and Nigeria.

The good initial ART adherence rates found among early African cohorts [31] are also demonstrated in our long-term cohort. Even into the fifth year on ART, overall patient adherence rates did not wane. As evidenced by high rates of virologic suppression (90% or greater), a low virologic failure rate (<10%), and excellent monthly ARV medication refill rates, sustained adult ART adherence is possible. There was no difference in ARV medication adherence rates when analyzed by gender, although a separate clinical trial done in this same setting did show poorer adherence rates among men [14]. Long-term follow-up, including studies evaluating socio-behavioral aspects of adherence, is still needed and continued diligence in terms of adherence counseling and education is warranted. We abstained from investigating predictors of virologic failure in our cohort due to the high numbers of death which would bias such analysis, as death informatively censors virologic failure.

Limitations of our analyses include the high number of patients who were LFU or transferred to another ART site following the countrywide roll-out of ART. Transfers to other sites mainly occurred to facilitate patient care in newly established sites in closer geographic proximity to the patient's home. There were no significant baseline characteristic differences between transfers and non-transfers. We, therefore, assume that patients who were censored at transfer have similar clinical course after censoring compared to patients who were not censored. Although the LFU rate in our cohort compares favorably with those from other sub-Saharan cohorts [32-34], it potentially biased the survival analysis. The number of deaths between three and five years of follow-up is likely to be underestimated since a significant number of patients classified as LFU have probably died [11]. The clinical event analysis was also limited due to the varying levels of detail available in the medical records and the reliance on patient self-reporting. Laboratory and pharmacological data, however, were ascertained via multiple systematic crosschecks.

In summary, we present the first five-year ART outcomes from a severely immunodeficient cohort of HIV-1 infected adults receiving treatment in the public sector in southern Africa. The high early mortality rate overshadows excellent immunologic and virologic outcomes and overall ARV medication adherence rates of more than 90%. Outcomes data from these 633 ART-treated adults represent one of the longest follow-up time periods of any public ART program in Africa. These five-year data provide important information for clinicians and policymakers in the region as they begin to evaluate and plan for the future needs of their own rapidly expanding programs.

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Chapter 11

Prevalence of transmitted HIV drug resistance in Botswana: Lessons learned from the HIVDR-threshold survey conducted among women presenting for routine antenatal care as part of the 2007 National Sentinel Survey

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Abstract

The emergence and spread of transmitted drug resistance (TDR) poses a major threat to the success of the rapidly expanding antiretroviral treatment (ART) programs in resource-limited countries. The World Health Organization recommends the use of the HIV Drug Resistance Threshold Survey (HIVDR-TS) as an affordable means to monitor the presence of TDR in these settings. We report our experiences and results of the 2007 HIVDR-TS in Botswana, a country with one of the longest-existing national public ART programs in Africa. The HIVDR-TS and HIV-1 incidence testing were performed in the two largest national sites as part of the 2007 antenatal Botswana Sentinel Survey. The HIVDR-TS showed no significant drug resistance mutations (TDR less than 5%) in one site. TDR prevalence, however, could not be ascertained at the second site due to low sample size. The agreement between HIVDR-TS eligibility criteria and laboratory-based methodologies (i.e., BED-CEIA and LS-EIA) in identifying recently HIV-1 infected adults was poor. Five years following the establishment of Botswana's public ART program, the prevalence of TDR remains low. The HIVDR-TS methodology has limitations for low-density populations as in Botswana, where the majority of antenatal sites are too small to recruit sufficient numbers of patients. In addition, the eligibility criteria (age < 25 years and parity (first pregnancy)) of the HIVDR-TS performed poorly in identifying rec ent HIV-1 infections in Botswana. An alternative sampling strategy should be considered for the surveillance of HIVDR in Botswana and similar geographic settings.

Introduction

Antiretroviral treatment (ART) programs are rapidly expanding in resource-limited countries. As of 2009, it has been estimated that more than 3 million persons have been initiated on ART in sub-Saharan Africa [1] . Reports from existing ART programs show excellent immunologic, virologic, and clinical response to currently recommended first-line combination ART regimens [2-6]. In order to monitor the emergence of transmitted drug resistance (TDR) in resource-limited countries, the WHO has proposed the HIV drug resistance threshold survey (HIVDR-TS), which categorizes transmitted drug resistance as low (less than 5%), medium (5–15%), or high (greater than 15%)[7,8] . The HIVDR-TS has been recommended for use in small geographic areas where at least 20% of combination ART (cART)-eligible HIV-infected adults have been receiving cART for more than 3 years. The methodology is based on binomial sequential sampling of up to 47 eligible samples.

It is recommended that the HIVDR-TS be incorporated in well-established HIV antenatal sentinel surveillance activities, using the following three mandatory eligibility criteria: HIV seropositive status, age (< 25 years), and parity (no prior pregnancy). As long-term HIV infection or previous ARV drug exposure might confound or mask the diagnosis of transmitted drug resistance, additional criteria, when routinely available, are also recommended, such as documented laboratory evidence of seroconversion or recent infection, a CD4+ cell count greater than 500 cells/mm^3, no evidence of WHO clinical stage 3 or 4 events, and/or no known prior exposure to ARV medications. Several countries have conducted HIVDR-TS surveys within existing HIV-1 antenatal sentinel surveillance monitoring using mandatory eligibility criteria [9-15]. The use of laboratory-based evidence of recent infection as eligibility criteria has not been reported in antenatal sentinel surveillance.

Botswana, a sparsely populated country (1.7 million inhabitants) and a high HIV-1 prevalence rate (17.1% among the general population based on the 2004 Botswana AIDS Impact Survey [16]) began offering public ART to qualifying citizens in January 2002. As of mid-2007, 92,000 of approximately 110,000 patients ever registered in the national ARV treatment program were actively receiving ART. The vast majority of cART-treated adults were receiving the following first-line ART regimens as

recommended by existing national guidelines: zidovudine (ZDV), lamuvidine (3TC) plus efavirenz (EFV) for men and all women not having reproductive potential, and zidovudine (ZDV), lamuvidine (3TC) plus nevirapine (NVP) for women with childbearing capacity. In 2007, Botswana included in its national HIV-1 antenatal sentinel survey both the HIVDR-TS and HIV-1 incidence estimation using HIV-1 BED Incidence EIA® (BED-CEIA) and Vironostika® Less Sensitive (LS-EIA) HIV EIA methodology. We herein report our experiences and results from the use of the HIVDR-TS as well as laboratory-based HIV-1 incidence estimations in Botswana in 2007.

Methods

Study Population

In Botswana, national HIV-1 antenatal sentinel surveillance has been performed annually through 2007. According to the national protocol [17], during a period of 12 weeks, all pregnant women were eligible who registered for routine antenatal care at pre-specified clinics. These clinics were selected based on the number of patients presenting for care (clinic volume). Sociodemographic data were collected and residual blood samples from routine antenatal clinic (ANC) syphilis testing were utilized as per established unlinked, anonymous testing policy.

In 2007, routine sentinel surveillance was supplemented by two additional surveys. namely the HIVDR-TS and HIV-1 incidence testing. In short, all sentinel surveillance samples were tested for HIV-1 using the parallel ELISA strategy (Murex® and Vironostika®) as recommended by the Botswana Ministry of Health HIV testing guidelines. Incidence testing using the BED-CEIA and LS-EIA was performed on all HIV-1 positive samples. Prevalence of transmitted drug resistance (TDR) was estimated in two sites, namely Francistown and Gaborone, as these sites are Botswana's 2 largest cities serving close to 400,000 persons or approximately 25% of the country's population. In addition, these sites were established in 2002, and as of early 2007, at least 80% of cART-eligible HIV-1 infected persons were receiving cART at these sites. Approximately 22,000 total patients had been receiving cART for a minimum of 2 years at these two large urban sites.

Study Procedures

An aliquot of plasma from anonymized sentinel surveillance participants at the two sites was sent within 24 hours of sampling to the national Botswana–Harvard HIV Reference Laboratory (BHHRL) in Gaborone where it was stored at -70° C. The HIVDR-TS protocol as recommended by the WHO [18] was followed to estimate TDR prevalence. In summary, women were included if they fulfilled the three mandatory criteria: (1) HIV-1 EIA positive, (2) less than 25 years of age, and (3) pregnant for the first time, and in addition were ARV-naïve as self-reported in the sentinel surveillance questionnaire.

Laboratory Methods

HIV-1 genotypic drug resistance testing and analyses

Eligible samples were listed consecutively. HIV-1 genotypic drug resistance testing was performed using a broadly sensitive in-house genotyping protocol at the International Laboratory Branch, Division of Global AIDS, National Center for HIV/AIDS, Hepatitis, STD, and TB Prevention (NCHHSTP), Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA [19] . Transmitted HIV-1 drug resistance mutations were determined by Calibrated Population Resistance (CPR) version 4.1 beta using the Stanford HIV Drug Resistance Database [20] . The binomial sequential sampling and classification table was used to categorize the prevalence of HIV-1 drug resistance as specified in the protocol.

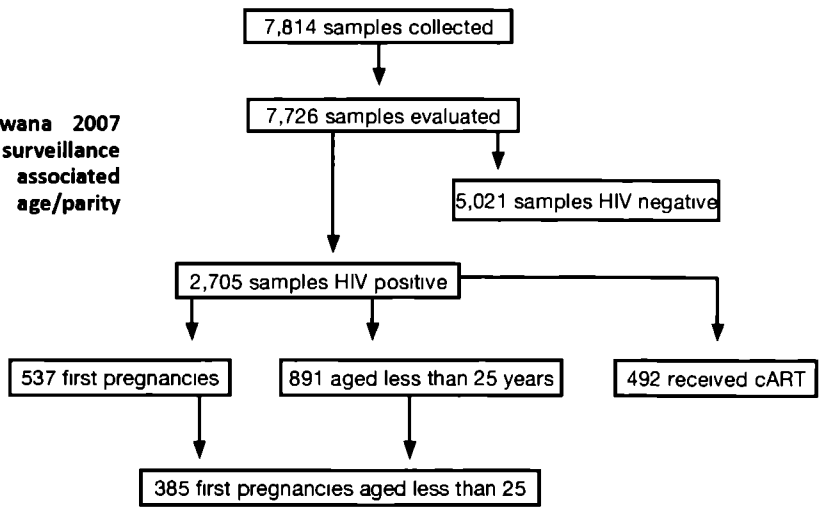

Figure 1: Botswana 2007 sentinel surveillance samples and associated HIV status and age/parity characteristics

Phylogenetic analysis: Generated pol nucleotide sequences were aligned using Muscle [21] with HIV-1 subtypes references from the Los Alamos HIV Sequence Database [22] followed by a BioEdit [23] manual adjustment. The evolutionary model was selected by using the Akaike information criterion in jModeltest 0.1.1 [24] . The parameters of the model (GTR+I+Γ) were as follows: nucleotide frequencies f_A=0.4225, f_C= 0.1645, f_G=0.1838, and f_T=0.2291; estimated value of shape parameter α of the Γ distribution = 0.7590; estimated value of proportion of invariable sites = 0.3720; and R matrix values RA↔C = 1.9835, RA↔G = 8.2960, $R_{A\leftrightarrow T}$ = 0.8602, $R_{C\leftrightarrow G}$ = 0.8400, $R_{C\leftrightarrow T}$ = 11.2101, and $R_{G\leftrightarrow T}$ = 1.0. The identified substitution model was used in PhyML [25] to reconstruct the genealogy of analyzed pol sequences. The maximum likelihood tree was visualized in MEGA v4 [26] . The approximate Likelihood-Ratio Test (aLRT) was used as a statistical test to compute branch supports. The aLRT branch support was significant when it was larger than 0.90. The evolutionary history was also inferred by the Neighbor-Joining method [27] using the Kimura 2-parameter method [28] in MEGA4 [29] . The bootstrap test (100 replicates) was used to support branching topology . The bootstrap values of 80 and higher were considered significant. A total of 39 HIV-1 subtype references from Los-Alamos HIV Sequence Database were represented by 4 A1's, 2 A2's, 5 B's, 4 C's, 4 D's, 4 F1's, 4 F2's, 4 G's, 3 H's, 3 J's, and 2 K's sequences. Three CPZ sequences were used as an outgroup. The recombination analysis was performed by SimPlot [30] .

Determination of "recent" HIV-1 infections: All samples that tested HIV-1 positive in the sentinel survey were additionally tested for recent HIV-1 seroconversion using (i) BED-CEIA (HIV-1 BED Incidence EIA®, Calypte Biomedical Corporation, Portland, OR)) [31] and (ii) LS-EIA (Vironostika Microelisa System®, bioMérieux, Durham, NC) [32] . Patients were considered recently infected if, using the BED-CEIA technique, the normalized optical density (ODn) was less than 0.8, or if, using the LS-EIA technique, the standardized optical density (SOD) was less than 1.0. Definitions: For the purpose of this study, a participant was defined as recently HIV infected when (1) the HIV-1 EIA test was positive, (2) the BED-CEIA or LS-EIA indicated recent infection as defined above, and (3) the participant reported that he/she was not taking antiretroviral therapy in the sentinel survey questionnaire.

Statistical Methods

Agreement between HIVDR-TS eligibility criteria and BED-CEIA or LS-EIA test results were measured using concordance (percent agreement), i.e. the proportion of all participants with concordant results, and -statistic, i.e. an index comparing the observed agreement versus what might be expected by chance.

Ethical Approvals

The study was approved by the Human Research and Development Committee of the Ministry of Health, Botswana. The Human Subjects Committee of the Harvard School of Public Health determined that the study qualified for "exemption" status based on fulfilling the definitions of such research as set forth in the United States code of federal regulations, section 45 CFR 46.101(b) [4].

Results

Between July 9th and September 28th, 2007 (12 weeks), 264 health facilities (28 hospitals and 236 clinics) from all 24 Botswana health districts took part in the national HIV-1 sentinel surveillance. Out of a total of 7,814 collected samples, 7,726 samples (98.9%) were evaluable for analysis (Fig 1). Overall 2,705 (35.0%) of 7,726 samples tested HIV-1 positive. The HIV-1 prevalence rate was 19.9% (537 of 2,705) among women experiencing their first pregnancy and 32.9% (891 of 2,705) among women less than 25 years of age. Both criteria were met by 14.2 % (385 of 2,705) women. 492 (18.2%) of 2,705 patients testing HIV-1 EIA positive reported they were taking ART and were thus excluded from the formal analysis .

HIV-1 subtyping

To determine HIV-1 subtype, newly generated pol sequences were genotyped. The maximum likelihood tree is presented in Figure 2. A total of 71 out of 72 sequences clustered with HIV-1 subtype C references providing evidence that 98.6% of analyzed pol sequences belong to HIV-1 subtype C. Viral sequence from one participant, B0604, clustered with HIV-1 subtype J reference sequences, but branching topology did not support pure subtype J. The follow-up analysis by SimPlot provided evidence that this pol sequence represents a complex recombinant between HIV-1 subtype J and K. Analysis by the Neighbor-Joining method produced similar results (data not shown).

Table 1: Genotypic resistance testing of HIVDR-TS eligible women in 2 major ART sites in Botswana

	Eligible by HIVDR-TS criteria	Successful amplification N (%)	Surveillance drug resistance mutations*
Francistown	44	39 (88.6%) #	None
Gaborone	42	33 (78.6%)	None
Total	86	72 (83.7%)	None

*Surveillance drug resistance mutations as defined by references 22 and 23
one sample contained multiple NRTI and NNRTI drug resistance mutations

Cluster analysis

Phylogenetic analysis of newly generated pol sequences revealed clusters supported by high aLRT values. As shown in Figure 2, two clusters within HIV-1 subtype C are supported by aLRT values of 0.98 and higher: one cluster includes two sequences

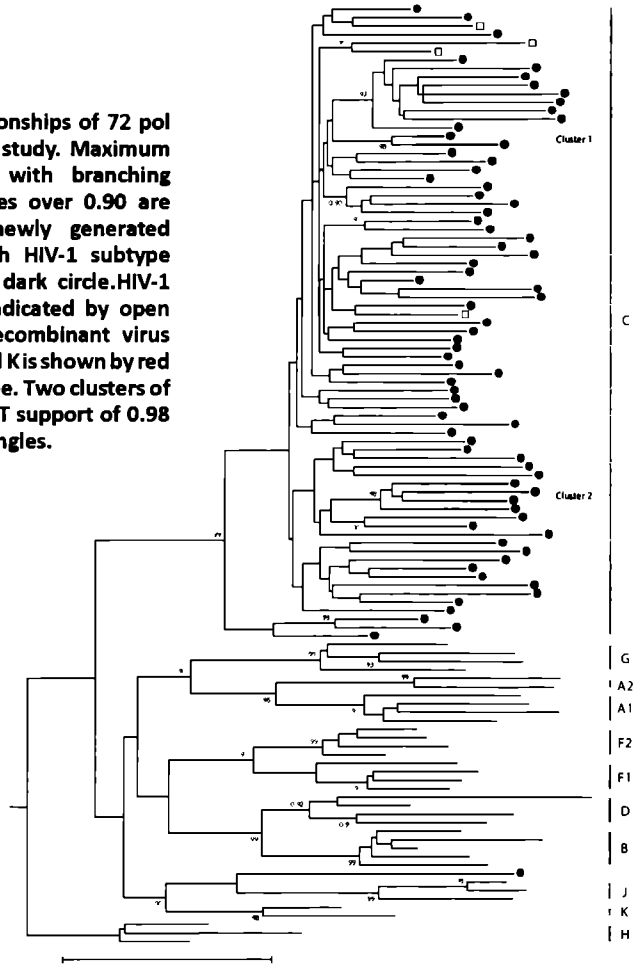

Figure 2: Phylogenetic relationships of 72 pol sequences generated in the study. Maximum likelihood tree is shown with branching support of aLRT (only values over 0.90 are shown). A total of 71 newly generated sequences that cluster with HIV-1 subtype C references are shown by dark circle.HIV-1 subtype C references are indicated by open blue squares. A complex recombinant virus between HIV-1 subtypes J and K is shown by red circle at the bottom of the tree. Two clusters of new pol sequences with aLRT support of 0.98 are highlighted by gray rectangles.

each, while cluster 2 includes four sequences. The same four sequences clustered in the tree inferred by the Neighbor-Joining tree with bootstrap support of 90 (data not shown). It is likely that high aLRT and bootstrap support of branching topology indicates HIV-1 transmission cluster in the local epidemic in Botswana.

2007 HIVDR-TS

The two largest sentinel surveillance sites, Gaborone and Francistown, identified 264 and 225 HIV-1 positive specimens, respectively (Table 1). Of these, 44 (16.7%) and 42 (18.7%) patients, respectively, met the eligibility criteria for HIVDR-TS testing as specified by the WHO. 39 (88.6%) of the 44 samples from Francistown and 33 (78.6%) of the 42 samples from Gaborone could be genotyped. One sample showed multiple NRTI and NNRTI drug resistance mutations and after case review was classified as acquired drug resistance. No significant drug resistance mutations were identified from the other 71 evaluable subjects. Amino acid substitutions which were deemed to be insignificant were found within the reverse transcriptase gene at position 179 [V179I (1)] and within the protease gene at positions 23 and 82 [V82I (2), L23F (1)]. According to HIVDR-TS protocol, the prevalence of TDR was less than 5% in Francistown; the prevalence in Gaborone could not be determined as the required minimum of 34 samples for genotypic analysis was not attained.

Determination of recent infections using BED-CEIA and LS-EIA

All HIV-1 positive sentinel surveillance samples were tested using the BED-CEIA and LS-EIA methodologies. From a total of 467 patients satisfying BED-CEIA criteria for recent infection, 37 were from Gaborone and 40 from Francistown. Of a total of 265 samples meeting V-LS criteria for recent infection, 24 came from Gaborone and 24 from Francistown.

Association between HIVDR-TS criteria compared to BED-CEIA and LS-EIA

The agreement between the HIVDR-TS criteria (less than 25 years of age and first pregnancy) and BED-CEIA and between HIVDR-TS criteria and LS-EIA was marginal, namely (= 0.16, 95% CI = 0.11 - 0.2; concordance = 77.0%) and (= 0.14, 95% CI = 0.09 - 0.19; concordance = 81.7%), respectively (Table 2).

Discussion

Botswana has one of the longest-established public national ART programs in sub-Saharan Africa and the development and propagation of transmitted drug resistance is considered to be the major threat to the success of this ambitious initiative. The 2007 Botswana HIVDR-TS survey completed in one of the largest urban national sites indicated that 5 years following the rapid, countrywide ART rollout, TDR was still very low, i.e. less than 5% according to the HIVDR-TS protocol [33] . No major drug resistance mutations as defined by the consensus list of surveillance drug resistance mutations compiled by an expert panel from the WHO were detected [34,35] . While these data from one single health district should not be extrapolated to the entire country, they are consistent with projections made by Vardavas and Blower [36], who had modeled the evolution of TDR in Botswana and predicted that TDR was unlikely to exceed the WHO threshold by 2009, even while assuming (i) a relatively high rate of acquired drug resistance of 20% per year, and (ii) a 50% reduced fitness of the transmitted virus . Botswana-specific data concerning these two key determinants of TDR are scarce. Program data on the level of acquired drug resistance are also not available as genotypic resistance testing is not routinely performed at the time of first-line regimen failure.

Table 2: Cross-tabulation of HIVDR-TS criteria versus BED-CEIA and LS-EIA results

		BED-CEIA (N=2663)*		LS-EIA (N=2650)*	
		Recent infection	Established infection	Recent infection	Established infection
Eligible by HIVDR-TS criteria	Yes	126	268	78	299
	No	341	1928	187	2086
		k =0.16 c = 77 1%		k =0 14 c = 81 7%	

*Number of samples with available information on both lab-based incidence results and HIVDR-TS eligibility criteria.
kappa statistic (k) and concordance (c)· measures of agreement between HIVDR-TS and laboratory-based criteria

Phylogenetic analysis in this study confirmed previous reports that a vast majority of HIV-1 infections in Botswana are caused by HIV-1 subtype C. Of 72 genotyped individuals, 71 (98.6%) were infected with HIV-1 subtype C, while one participant was infected with a complex HIV-1 J/K recombinant. Excellent five-year virologic outcomes have been reported from the largest ART site in Botswana [3] . Little

is known about the fitness of drug-resistant strains for HIV-1C. A recent report [37] on increased fitness of viruses with specific thymidine analogue mutations (TAMs) among subtype C ART-treated adults, however, is of concern. To improve the understanding of the dynamics of TDR in Botswana, consistent longitudinal monitoring of actual prevalence of TDR and acquired drug resistance rates is needed. In addition, transmitted viral strains need to be characterized in regard to replicative capacity and the presence of minor variants.

In contrast to acquired drug resistance, transmitted drug resistance needs to be analyzed as close to the transmission time as possible as the drug-resistant virus can gradually be replaced by drug-sensitive wild type virus until ultimately drug-resistant variants will not be detected by conventional population sequencing while persisting as archived virus [38,39]. It is, however, a significant challenge, especially in resource-limited settings, to identify routinely individuals with acute/ recent HIV-1 infections. The mandatory HIVDR-TS eligibility criteria recommend the inclusion of young women (less than 25 years of age) in their first pregnancy based on the assumption that these women will be early in the course of their HIV-1 infection. The WHO protocol, however, also recommends that additional criteria can be used to avoid inclusion of persons with acquired drug resistance and/or with long-established HIV infection. Such additional criteria, where routinely available, include history of ARV medication exposure and criteria that assist in estimating the duration of HIV infection, e.g. laboratory, immunological and/or clinical data.

As the 2007 Botswana antenatal sentinel survey had incorporated the HIVDR-TS while collecting additional information on ARV medication exposure and HIV-1 incidence, we wanted to explore the effect of these additional measures on the number of eligible women and determine the degree of agreement between these methods. The relatively poor concordance of the "mandatory" age/parity criteria with either of the two laboratory-based methods, namely BED-CEIA and LS-EIA, was partly explained by the intrinsic difference in case definition and in population size between these criteria. The case definition for laboratory-based criteria is determined by the relatively narrow time interval inherent in the assay methodology (window period) while the case definition for demographic criteria

extends to all young (less than age 25) HIV-1-infected women experiencing their first pregnancy irrespective of the duration of infection. The population size which is screened by these criteria also differs, e.g. laboratory-based screen all HIV-positive cases while demographic restrict eligible cases to a subpopulation defined by age and parity. Our results show that the number of eligible samples did not significantly change when persons were enrolled based on laboratory criteria compared to the mandatory WHO criteria .

This could be expected as a recent infection by laboratory standard refers to a relatively smaller time interval, i.e. largely the window period of the assay, compared to a potentially "much less recent" (established) infection that is identified by sociodemographic definition (i.e., age less than 25 years and first pregnancy). Of note, the laboratory-based methods detected a substantial number of recent infections that were not identified using mandatory criteria only. Interestingly, an acute HIV-1 infection study done in Botswana in 2007 [19] found that women seroconverted at a median age of 26.0 years, which suggests that focusing on women less than 25 years of age in our setting may preclude the proper identification of a significant number of recently infected females. Overall, laboratory-based criteria did not alter the number of eligible samples for the HIVDR-TS, but these samples were probably more informative for the estimation of transmitted drug resistance as they were derived from a population of recent infections.

The accuracy of the laboratory-based methods used in this study, namely BED-CEIA and LS-EIA, is affected by a number of factors including the individual variation in the immune response, the impact of late disease and antiretroviral therapy on anti-HIV antibody concentrations [40-43], and the effect of the HIV subtype on the antibody response [44,45]. For the identification of individuals with recent infections no single currently available test is sufficient and additional information excluding a long-standing infection is necessary [46,47]. For HIV incidence estimation at population level the application of correction factors [48-50] have been proposed to adjust for the small proportion of infections in the population that are misclassified as 'recent' by the BED-CEIA . Latest advances in the field suggest and support the use of algorithms based on multiple methods

and/or including confirmatory steps for refining a screening result [45,51-53]. One of the main advantages of the HIVDR-TS is the relatively small number of eligible participants (34–47 successfully genotyped samples) who, according to protocol, should be recruited from a small, circumscribed geographical area [1]. Botswana and possibly other countries in the region which are actively scaling up public ART programs yet have small, highly mobile and geographically dispersed populations face significant limitations and may find it difficult to identify sufficient numbers of cases. According to the Botswana 2007 sentinel survey, only one sentinel survey site could enroll a sufficient number of eligible women for the HIVDR-TS protocol. Similar observations were made during the Botswana 2005 sentinel survey despite the fact that young ages were oversampled, i.e. enrollment of 15–24-year-olds continued during the surveillance period even after reaching the expected sampling size [15,54] . This sample size problem will be even further aggravated in the future as successful regional national ART programs decrease overall HIV-1 transmission rates at the population level and thereby lead to even greater reductions in the numbers of acutely/recently infected individuals [55,56] . Our experiences with TDR surveillance among a low-density population with high mobility such as Botswana suggest that an alternative drug resistance surveillance approach be considered which (1) uses available and accepted lab-based methodology/algorithm for the identification of recent HIV-1 infections and (2) considers all nationally identified recent infection samples as a basis to calculate the overall prevalence of transmitted drug resistance. Alternative algorithms including confirmatory tests and laboratory analysis for widely used ARVs might be necessary for reliable monitoring of HIV-1 TDR. Adopting such an approach may be more costly due to the higher number of genotypic resistance tests that need to be performed, but this can be justified given that it will generate important information on the evolution of TDR in the country.

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Chapter 12

General discussion

General discussion

Combination Antiretroviral Therapy (cART) has revolutionized the management of HIV infection by changing a disease with an inevitably fatal outcome in the absence of ART into a treatable although not eradicable infection. In recent years these benefits have been made available to millions of individuals in resource-limited countries especially in Africa, where the vast majority of HIV infected people live - an achievement that is regarded as a major public health triumph. The successful introduction of cART in settings with fragile health care infrastructures is the result of an intensive and broad dialogue between scientists and policy makers. The development from the first uncertain steps of treating HIV- infected individuals in unprepared health systems to the establishment of a mature public ART program occurred on a steep learning curve culminating in the availability of affordable, safe and convenient drug regimens that can be delivered and monitored in a sustainable fashion in these settings. Botswana has been a pioneer in this endeavour and has created a widely acclaimed model based on scientific evidence coupled with strong political leadership, and flexibility and openness of policy makers. The thesis uses selected research findings that informed the implementation of a national ART program in Botswana.

A hallmark of HIV pathogenesis is the decline in CD4 T-lymphocytes (CD4 cells), and the enumeration of these cells have become one of the cornerstones of HIV monitoring [143]. One important aspect of CD4 enumeration is the variability of CD4 cell counts between and within populations attributed to biological factors such as genetic factors, gender [144, 145], age [146, 147, 148], diurnal influence[149], smoking[150], co-morbid conditions and lab- methodological issues [151]. As described in **Chapter 2**, our study of CD4 cell levels among healthy Batswana placed our population at the lower end of reported reference CD4 cell levels[151]. Our study design did not allow us to stratify by age groups, gender and presence of influencing factors such as smoking nor were we able to enumerate naïve and memory T cell phenotypes. Further studies are needed to establish unbiased age- and gender-stratified reference values for our population. The value of CD4 as predictor for disease progression and/or for response to ART has become a topic of debate[152]. Of note, immune activation is a key pathogenetic event in chronic HIV infection and markers of T cell activation[79] are

even stronger predictors of disease progression and of immune recovery following ART initiation than CD4 cell counts and plasma viral load. Recent advances in immunophenotyping of T- lymphocytes[153] have identified distinct subsets which are related by development, but have different functions and anatomic distributions [154]. Also new, recent research methods have characterized T cells according to their functional capability, e.g. expression of cytokines, or chemokines and a polyfunctional lymphocyte subset has been postulated that is associated with the outcome of HIV infection [155]. While the absolute CD4 cell count remains the recommended marker of the immune status of an HIV infected person, it will be important to better understand the clinical relevance of additional T cell markers such as those of maturational state (naïve, memory), activation, proliferation and senescence [154, 156] in order to improve monitoring of HIV disease and treatment.

Early indication that the predominant HIV strain in southern Africa, HIV-1C, has distinct virologic and biologic properties came from investigations of the HIV-1 isolates from Botswana. Novitsky [157] showed the high genomic diversity of HIV-1C when compared to other subtypes and Montano [158] later described the unique landscape of the promoter region in the long terminal repeat (LTR) of the HIV-1 virion, namely the existence of an additional NF-kappa B binding site in the majority of HIV-1C isolates. Montano and collegues went on to demonstrate that viral strains with 3 NF-kappa B sites showed an enhanced viral replication when activated by TNF-alpha, suggesting a gain of function, thereby enhancing viral fitness [159]. Also, analysis of Botswana HIV 1C isolates showed subtype-specific patterns of drug resistance [160, 161,162]. In order to describe the HIV-1 C drug resistance background among HIV infected Batswana before the large-scale national dissemination of ARV drugs, we studied the prevalent viral strains circulating in Botswana as part of the 2001 Botswana national HIV-1 seroprevalence survey (**Chapter 3**)[163]. Our results provided support for offering protease inhibitor (PI)-sparing (dual NRTI, single NNRTI) ART as a first-line treatment in Botswana. Specifically, we did not find any mutations known to cause primary resistance to NRTI/NNRTI; we did, however, describe the frequent occurrence of minor PI mutations and polymorphic sites within the protease enzyme which may suggest an increased susceptibility of the HIV-1 subtype C virus to develop PI resistance under selective drug pressure. In addition, we did not find any regional clustering of distinct HIV-1 strains which we

hypothezised based on the geography of Botswana and the resulting trans-national migratory patterns of populations in the north (via active border crossings to and from Zambia and Zimbabwe) and south (population influx mainly from the Republic of South Africa).

Before the advent of ART, prevention and management of HIV-associated opportunistic infections dominated HIV care. In developing countries patients often presented with multiple co-morbidities. While diagnosis and management strategies existed for active disease such as tuberculosis, enteritis, Kaposi sarcoma, genital infections, the frequency of other co-infections such as hepatitis B, hepatitis C, cytomegalovirus (CMV) infection, toxoplasmosis, and syphilis were unknown as specific diagnostic tests were not recommended or not routinely available. To better guide patient management we studied the distributions of these often latent infections among a patient cohort attending the ART clinic (**Chapter 4**)[164]. The study results emphasized the importance of continuing syphilis testing among HIV-positive individuals and revealed a relatively high proportion of individuals with chronic hepatitis B infection, a fact that has importance as some NRTIs have anti-hepatitis activity and withdrawal could flare up hepatitis and also considering the potential emergence of new drug resistance and immune-escape hepatitis B mutants[165]. We did not find serological evidence for HCV infection, an infection that is emerging as a STI among MSM causing life-threatening liver disease[166]. Of note in a large Nigerian study HCV was identified in approximately 5% of HIV infected persons [167]. Our study was done among HIV infected adults and may not reflect population trends. The study provides a rationale for conducting periodic sero-surveillance of HBV and HCV and underscores the importance to include CMV infection and Toxoplasmosis in the differential diagnosis of patients with manifestation of CNS or ocular disease.

Botswana was the first country in sub-Saharan Africa to offer ART as a public health program. Important clinical questions, as well as issues concerning infrastructure, operational adjustments, and training had to be addressed preceding the launch of the national ART program. The challenges associated with the establishment of the first public ART clinic, the Infectious Disease Care Clinic (IDCC) at the Princess Marina Hospital (PMH), Gaborone, are summarized in **Chapter 5**[168]. Important

lessons could be learnt and could guide the setting up of ART initiatives in other countries. Besides the strong political will as repeatedly expressed by the country's president and the Minister of Health and collaboration and support of international partners and donors, the design and implementation of a standardized training program for different cadres of health care workers was tremendously important for the quality of the program [169]. The provision of adequate space for clinic, pharmacy and laboratory, the recruitment and training of sufficient number of staff and the upgrading of the laboratory infrastructure and monitoring tools had to be coordinated. The report also highlighted the need to decentralize the service with the aim to bring patient care closer to their homes and to decongest clinics thereby providing opportunities for more personalized care. We also advocated exploring the effectiveness of shifting routine ART care from physicians to specially trained nurses. The report had a strong influence on policy makers to plan and implement the roll-out of the national program. Of note, as result of decentralization of ART services the PMH IDCC patient load decreased from over 15 000 patients in 2004 to approximately 9000 patients in 2010 while at the same time national ART coverage had tripled.

In order to study the benefits and risks of different ARV regimens among HIV-1C infected persons we conducted a randomized clinical trial (RCT) called the Adult Antiretroviral Treatment and Drug Resistance ("Tshepo") study where we compared 6 different ART regimens and two randomized adherence strategies among HIV-1C infected persons in a typical urban African setting. The Tshepo study was one of the first large-scale randomized clinical trials in Botswana and Southern Africa. The excellent patient retention and the high quality of collected data demonstrated the feasibility of RCT in our setting. Within two years of the study opening, an interim efficacy analysis prompted our data safety and monitoring board (DSMB) to recommend discontinuation of the ZDV/ddI-containing arms as it became evident that HIV-1 infected adults randomized to this dual NRTI combination had inferior virologic outcomes when compared to persons receiving arms containing the dual NRTI backbones of d4T/3TC and ZDV/3TC (**Chapter 6**)[170]. At two years, the interim study analysis demonstrated an excellent virologic response with an overall virologic failure rate with genotypic resistance of 5.8% (2.0% in the non-

ZDV/ddI containing arms), as well as death (4.6%) and loss to follow up (LTFU) rates (4.1%), which compared very favorably to outcomes data from resource-rich settings. Treatment modifying toxicities developed in 18.2 % of patients which was low compared to reports from the region [171, 172,173], in part due to the different drug regimens and also due to more patient-centered care in our clinical trial setting. A new opportunistic infection, mainly tuberculosis and extensive herpes zoster infection, developed in 16% of participants. Half of these cases occurred within the first 6 months of therapy initiation, and some cases might actually have been an immune reconstitution inflammatory syndrome (IRIS) which we could not exclude on clinical grounds alone[174]. Importantly seven cases of cancer were diagnosed. Medication adherence, defined as greater than 90% taking of medication, had been excellent in the first year (89.8%) with a slight decline in the second year (81.2%), and men having a shorter time to non-adherence. Overall, the study provided important information on the efficacy and tolerability of different ARV regimens which influenced the country's choice on formulating national guidelines [175]. Long-term toxicities e.g. lipodystrophy and metabolic complications and non-AIDS malignancies[176] as well as long term adherence will be essential areas of ongoing research.

Restoration of health and longevity among ART-treated women is often accompanied by childwish as demonstrated by high pregnancy rates in the 'Tshepo' study (**Chapter 7**)[177]. Our study on pregnancy rates and birth outcomes address two important issues namely (i) the need to adjust existing reproductive health services in our setting to new emerging needs and (ii) the safety of ART in pregnancy, especially the use of EFV in early pregnancy. The high rate of unintended pregnancies found among women who were willing not to conceive while taking study medication underlined the gap in adequately addressing the reproductive health needs of HIV-infected women and made policy planners aware of unmet needs. As a result, the national KITSO training program has built in a module on reproductive health which covers the specific family planning needs of HIV affected couples. This issue has also been reported from other sites [178]. Our data on EFV teratogenicity contributed to the accumulating evidence on EFV safety in first-semester pregnancy and have been included in several metaanalyses [180, 179].

Chapter 8 reviews recent ART initiatives in SSA [181]. In recent years many ART initiatives have been implemented in SSA. From 2002 to 2007 approximately 2 million of an estimated 7 million Africans in need were receiving ART. We have summarized the Botswana experiences regarding the provision of public ART and have compared these findings with reports from other sites. We described the status of ART coverage, the preferred regimens, medication adherence issues, common toxicities, drug resistance mutations associated with specific regimens, the occurrence of OIs and survival as of 2007 in SSA. While most initiatives experienced favorable clinical outcomes and excellent adherence rates, these programs also face a number of serious challenges. Program cost and patient retention rates are important indicators which influence long-term program sustainability. The optimal management of virologic failure including the diagnostic approach and the choice of second line regimens is still extensively debated. Other challenges include (i) the need for task shifting among health care professionals in order to most efficiently use scarce resources without compromising quality, and (ii) the increasing occurrence of non-AIDS related conditions which require to adapt diagnostic services to effectively manage these complications.

Monitoring and evaluation (M&E) of the rapidly scaling up of the ART program was of paramount importance for its success and sustainability. Important indicators include the prescription choice and rates of medication uptake, virologic failure, patient retention in care and mortality. A crucial element of M&E is the design of a reliable, accurate and operator-friendly monitoring tool that captures essential data reflecting the program activities. We developed and piloted a data capturing instrument which linked paper-based, provider-friendly data capturing with electronic optical character recognition (OCR) technology and allowed instant documentation of relevant medical data of individual patients (**Chapter 9**) [182]. Key patient level information was collected on a concise one-page visit form from which essential program indicators could be derived. The country eventually decided to implement two separate monitoring systems, one for the larger hospital sites where a propriety hospital information management system is used and one for the smaller sites where data is captured electronically into a Microsoft Access-based application. Unfortunately, up to the present it has proven extremely difficult to

integrate patient-level data from all sites due to the extensive clinical information that is captured, the incompleteness of captured data, often interrupted technical support, and the difficulties of merging the two monitoring systems [183].

While the efficacy and tolerability of Botswana's first-line ARV regimens had been documented in a randomized clinical trial [170], we were also interested to study the long term outcome of patients initiated through the public ART program (**Chapter 10**) [184], especially mortality, LTFU, virologic failure rates, and medication adherence. We analyzed the first 633 patients that received cART in 2002 through the national ART program. By 2007, these patients had been on ART for a median of 5 years. Over the 5 years 21% of patients had died. The majority of deaths occurred within the first year of ART with a 2.3 fold increase of deaths among patients with baseline CD4 less than 50 cells/mm^3 compared to those with CD4 of 50-200 cells/ mm^3. Similar experiences were reported from other cohorts that followed severely immunosuppressed patients in Africa. [185, 186, 187, 188]. The estimated rate of acquired drug resistance, namely 47 (16.2%) of 290 patients followed in care over 5 years is based on the occurrence of complete regimen switch at the time of virologic failure, in the absence of genotypic testing. These projected virologic failure rates compare favorably with data from industrialized countries and reflect the excellent adherence rates that have been reported from Botswana and the region [189, 190, 191, 192, 193]. The analysis of the patient return rate for medication refill in our study suggests an excellent adherence rate in the population, which most probably results from a strong emphasis on patient education and counseling within the Botswana national program. The 1, 3, and 5 years LTFU rate in our study, which includes those patients that were transferred to another treatment site, was 8.9%, 15.4%, and 21.8%. These data compare favorably with those reported from other ART initiatives in SSA. According to a systematic review of patient retention in sub-Saharan Africa [194] on average only 60% of patients are retained in care after two years. It will be important for service providers to develop operational definitions for LTFU and explore the reasons for LTFU in order to improve the long term success of ART programs. Of note, the high rate of incomplete outcome information presumably masks a significant proportion of unreported deaths and virologic failure. The occurrence of ARV drug resistance has serious implications for program sustainability. In addition

to the high cost of second-line regimens, acquired drug resistance also poses the risk of transmitting resistant HIV-1 strains (primary drug resistance) with the subsequent loss of the first-line regimen efficacy in the newly infected person. In resource-rich settings transmitted ARV drug resistance (TDR) is reported in 5-14% of new infections[195, 196, 197, 198, 199, 200] which to a large extent has resulted from use of regimens with inferior potency in the early years of ART care.

The high primary drug resistance prevalence has led to the recommendation for the US and Europe that genotypic drug resistance testing be performed in all individuals prior to HAART initiation and before regimen switches for virologic failure [123, 201]. In contrast, in most resource- limited settings the prevalence of TDR drug resistance is still very low [202 203 204 205 206] due to high medication adherence and the use of potent regimens. A public health approach for the delivery of ART i.e. the use of standardized first- and second-line regimens is still considered appropriate. As large-scale dissemination of ARV drugs, however, will inevitably lead to the spread of drug resistant viruses, the emergence of transmitted drug resistance needs to be closely monitored. WHO has developed a cost-effective, standardized approach for resource-limited countries to estimate the occurrence of transmitted drug resistance i.e. a threshold survey (HIV Drug Resistance Threshold Survey, HIVDR-TS), which categorizes TDR as low (less than 5%), medium (5-15%), or high (greater than 15%)[207, 208]. The survey methodology has an essential drawback for low population density countries like Botswana as it is difficult to accrue the required number of eligible persons in any of the individual ART sites (**Chapter 11**) [209], an experience that has also been reported from other sub-Saharan countries[210]. Another limitation of the survey methodology stems from the assumption that young (less than 25 years of age) women in their first pregnancy are a proxy for recently infected individuals. We could show for our setting a very low concordance of this criteria with lab-based markers of recent infections; in fact close to 50% of recent infections were missed using HIVDR-TS protocol criteria. Our proposal for Botswana to use all recent infections identified via lab-based methods during the sentinel survey will also allow to estimate TDR more accurately (instead of using a threshold) and make better inference on the emergence and dynamic of TDR [211, 212] in the country.

Further research

The concept, that ART can be beneficially implemented in resource-limited settings as a public health approach, has been proven. To secure the success and sustainability of ART programs, important operational and scientific questions need to be addressed including (i) the most cost effective model of care delivery, e.g. integrating the ART program into general health care delivery, (ii) the long-term retention of individuals in care, (iii) the monitoring of acquired and transmitted drug resistance, (iv) the decision on introducing new drug classes for first- and second-line ART regimens, and (v) the timing of ART initiation.

Based on Botswana's present achievements in her efforts to mitigate the epidemic, it is timely to investigate the use of ART for prevention as well. The effect of ART in reducing HIV transmission has recently received considerable attention. Given the facts that (i) a disproportionate number of HIV transmissions are associated with high plasma viral load [213] and (ii) that ART dramatically reduces viral load [214, 215, 216], it is reasonable to consider using treatment of HIV infected individuals as a means to prevent transmission. Such a concept has been proven in PMTCT where ART blocks HIV transmission most efficaciously [127], and recently also through the HPTN 052 study among serodiscordant couples where the earlier start of ART reduced the risk of HIV infection to the uninfected partner by at least 96% [217]. Based on recent findings that persons with acute HIV-1C infections in Botswana have a prolonged high plasma viral load (for up to one year) [218, 219], our research group presently studies the concept of a modified 'test and treat' approach where ART is offered to all high viral load carriers (HVLC) i.e. individuals with plasma viral load >50 000 copies/ml in addition to those qualifying for ART based on CD4 criteria or presence of disease, an approach that promises to be feasible in resource-limited settings with high HIV prevalence.

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Summary

Summary

Since the emergence of HIV in the human population in the first half 20th century and the first clinical description of HIV disease in 1981, the HIV/AIDS pandemic has grown into one of the most devastating public health threats of our time. HIV has become one of the most studied infectious diseases, having broadened our knowledge in basic sciences, including immunology, viral pathogenesis and pharmacology, and in behavioral sciences, but as of now neither cure nor effective preventative public health method has been found.

Combination antiretroviral therapy (cART), first introduced as a treatment option for HIV infection in 1996, has revolutionized the management of HIV infection by changing a disease with inevitably fatal outcome in the absence of ART into a treatable although not eradicable infection. In recent years these benefits have also been made available to millions of individuals in resource-limited countries especially in Africa where the vast majority of HIV infected people live. The successful introduction of cART in these settings with fragile health care infrastructures - an achievement that is regarded as a major public health triumph - is the result of a lively, intensive and broad dialogue between scientists and policy makers. The development from the first uncertain steps of treating HIV infected individuals in unprepared health systems to the establishment of a mature public ART program occurred on a steep learning curve culminating in the availability of affordable, safe and convenient drug regimens that can be delivered and monitored in a sustainable fashion in these settings. Botswana has been a pioneer in this endeavor and has created a widely acclaimed model based on scientific evidence coupled with strong political leadership, and timely responsiveness by policy makers.

The aim of the thesis is to describe the evidence–based approach in guiding public health policy to control HIV infection in a generalized epidemic by using selected research findings that informed the implementation of a national ART program in Botswana.

Preceding the establishment of the public cART program, important information on clinical and immunological characteristics of the population and on the prevalent

circulating virus was essential. The CD4-lymphocyte count plays a crucial role in the management and monitoring of HIV infection. Variability in the normal values of white blood cells and of the CD4 cell subsets had been reported among different populations in Africa. In **Chapter 2** of the thesis it is shown that CD4-lymphocytes of healthy non-HIV infected adults in Botswana are at the lower bound of reported reference values from other populations; the study could not, however, conclude how this affects the course of HIV infection.

Knowledge of antiretroviral drug resistance to HIV-1 subtype C (HIV-1C), the most prevalent virus in sub-Saharan Africa (SSA), was sparse when Botswana considered to publically offer ART. In **Chapter 3** we report on the analysis of the prevalent viral strain in Botswana specifically the presence of primary HIV drug resistance before large-scale introduction of antiretroviral drugs. We concluded that a protease inhibitor (PI)-sparing (dual NRTI, single NNRTI) regimen would be a potent first-line treatment option in Botswana.

The clinical spectrum of major HIV/AIDS- related illnesses in Botswana such as tuberculosis, enteritis, Kaposi's sarcoma and genital infections had been well described in the pre-ART era and management strategies existed for these active diseases. The frequency of other co-infections such as hepatitis B (HBV), hepatitis C (HCV), cytomegalovirus (CMV) infections, syphilis and toxoplasmosis were unknown as specific diagnostic tests were not routinely available. To better guide patient management we studied the distribution of these often latent infections among a patient cohort attending the ART clinic (**Chapter 4**). The study results showed a high prevalence of HBV infection and syphilis in the HIV-infected population, and led to important recommendations, namely to continue syphilis testing in this population and to be vigilant when using certain NRTIs with anti-hepatitis activity (lamivudine, emtricitabine and tenofovir) in individuals with chronic hepatitis B infection, as withdrawal of these drugs could induce a hepatitis flare up.

The provision of ART through the public health care system necessitated multiple adaptations of the existing health care infrastructure. **Chapter 5** describes the challenges and lessons learnt from establishing the first public ART clinic in Botswana.

The article highlights the importance of standardized training, decentralization, and task-shifting as essential steps to ensure program sustainability.

Chapter 6 provides the results of a randomized clinical trial to compare the efficacy, tolerability and optimal adherence strategies of six different ARV regimens for populations in SSA, particularly those infected with HIV-1C. The study showed remarkable immunologic and virologic responses to first-line PI-sparing cART regimens, excellent cohort retention rates and low rates of treatment modifying toxicities.

An additional important finding of the study was the high rate of unintentional pregnancies among women on ART including among women on EFV containing regimens (**Chapter 7**). The study revealed significant gaps in the national family planning services for the HIV-infected population. Also, importantly, we did not find any EFV-associated adverse birth outcomes. As our sample size was too small for a definitive conclusion, we submitted our findings to the international Antiretroviral Pregnancy Registry where data on ART exposure in pregnancy are compiled and reviewed.

Chapter 8 is a review article on the opportunities and challenges for cART programs in SSA as of 2009.

A key component to successfully manage an ART program is the monitoring and evaluation of program goals and indicators. **Chapter 9** provides a model for affordable patient-level ART monitoring which can be used for program evaluation. It is based on a minimum key data set that is captured largely in a physician-independent way and which uses optical character recognition (ORC) technology for data transfer to a central office/server.

While cART initiatives in many SSA countries show early benefits, to date, few results are available concerning long-term clinical outcomes in these treatment programs. **Chapter 10** analyzes the long-term treatment outcomes of the first patients who initiated public cART in Botswana. This initial group of adults on ART in urban

Botswana had excellent sustained immunologic, virologic, and clinical outcomes for up to five years of follow-up with low mortality among those surviving into the second year of antiretroviral treatment. The 5-year lost to follow up rate of 21.8% compared favorably with those from other ART initiatives in SSA.

Chapter 11 assesses the level of transmitted (primary) ARV drug resistance in Botswana five years into the national cART program. The low level of transmitted drug resistance across the country is a reassuring indicator that the public cART program has been successfully implemented . The article also provides a critical review of the methodology to monitor transmitted drug resistance proposed by WHO.

The studies presented here have greatly contributed to the establishment and successful rollout of a public cART program in a Botswana, a program that is widely seen as a model for the region. The successful implementation of cART for treating established HIV disease warrants further exploration of whether cART could also be used as a strategy to prevent new HIV infections by extending the treatment qualifying criteria to adults with high viral loads who are most likely to transmit HIV to others but do not qualify for the drugs based on their disease status.

Samenvatting

Samenvatting

Sinds de introductie van het HIV in de bevolking in het begin van de 20ste eeuw en de eerste klinische beschrijving van de ziekte in 1981, is de HIV/ AIDS-pandemie uitgegroeid tot een van de meest verwoestende bedreigingen van de volksgezondheid van deze tijd. Het HIV is een van de meest bestudeerde infectieziektes geworden. Daardoor is onze wetenschappelijke kennis op het gebied van immunologie, virale pathologie, farmacologie en de gedragswetenschappen aanzienlijk uitgebreid. Er is echter nog steeds geen genezing of effectieve preventieve methode voor de openbare gezondheidzorg gevonden.

De Combinatie Antiretrovirale Therapie (cART), die voor het eerst als behandeling van HIV-besmettingen is geïntroduceerd in 1996, heeft een revolutie teweeggebracht in het management van HIV-infecties. Dankzij cART is een ziekte met een onontkoombare fatale uitkomst door het ontbreken van ART veranderd in een behandelbare ziekte, al is de infectie zelf onuitroeibaar. In de afgelopen jaren zijn deze mogelijkheden ook toegankelijk gemaakt voor miljoenen individuen in de minder bedeelde landen, speciaal in Afrika, waar de overgrote meerderheid van de met HIV besmette mensen woont. De succesvolle introductie van cART in deze minder bedeelde landen met fragiele gezondheidsinfrastructuren - een prestatie die gezien wordt als een grootse overwinning van de openbare gezondheidszorg - is het resultaat van een levendige, intensieve en brede dialoog tussen wetenschappers en beleidsmakers. De ontwikkeling vanaf de eerste onzekere stappen in de behandeling van individuen besmet met HIV in daarop niet voorbereide gezondheidszorgsystemen tot aan de totstandkoming van volgroeide ART programma's in de openbare gezondheidszorg zorgde voor het doorlopen van een intensief leertraject. Dat leertraject is uitgemond in de beschikbaarheid van betaalbare, veilige en gemakkelijk te gebruiken medicatieregimes, die duurzaam kunnen worden geleverd en gecontroleerd binnen deze specifieke omgeving. Botswana is pionier geweest op dit gebied. Het heeft een inmiddels algemeen erkend model ontwikkeld, gebaseerd op wetenschappelijke bewijsvoering gecombineerd met sterk politiek leiderschap en snelle respons van geëngageerde beleidsontwikkelaars.

Het doel van deze thesis is het beschrijven van de door de maatschappelijke gezondheidszorg gebruikte aanpak om een op wetenschappelijk onderzoek gebaseerd antiretroviraal behandelingsprogramma op te zetten in een land met

beperkte middelen. De aanpak van Botswana wordt als voorbeeld gebruikt.

Voorafgaand aan het opzetten van het openbare cART programma was het essentieel om voldoende informatie in te zamelen over de klinische en immunologische parameters van de bevolking en het meest voorkomende virus. De CD4-lymphocietentelling speelt een cruciale rol in de behandeling en het monitoren van HIV-infecties. Variabiliteit in de normale waardes van de witte bloedlichaampjes en van de CD4 deelverzamelingen onder de verschillende populaties in Afrika was gerapporteerd. In **hoofdstuk 2** van de thesis tonen wij aan dat CD4-lymphocieten van gezonde, niet HIV-geïnfecteerde volwassenen in Botswana op de ondergrenzen van de gerapporteerde referentiewaardes van andere populaties zitten. Deze studie kon niet aantonen hoe dit het verloop van de HIV infectie beïnvloedt.

Kennis van resistentie tegen antiretrovirale mediacatie voor HIV-1 subtype C (HIV-1C), het meest voorkomende virus in sub-Sahara Afrika, was schaars toen Botswana overwoog om ART behandeling aan de bevolking aan te bieden. In **hoofdstuk** 3 behandelen we de analyse van het meest voorkomende virus in Botswana. We gaan specifiek in op de aanwezigheid van primaire resistentie tegen HIV-medicijnen voor de grootschalige introductie van antiretrovirale medicijnen. Wij concludeerden dat het protease inhibitor (PI)-sparing (dual NRTI, single NNRTI) regime in Botswana een krachtig eerstelijns behandeling zou vormen.

Het klinische spectrum van de meest voorkomende ziektes gerelateerd aan HIV/AIDS in Botswana, waaronder tuberculose, enteritis, Kaposi's sarcoma en infecties van de genitaliën, is goed beschreven in de pre-ART en management strategieën van deze actieve ziektes. De frequentie van andere co-infecties, zoals hepatitis B (HBV), hepatitis C (HCV), cytomegalovirus (CMV) infecties, syfilis en toxoplasmosis waren onbekend omdat specifieke tests om deze ziektes te diagnosticeren niet algemeen verkrijgbaar waren. Om deze patiënten beter te kunnen behandelen, bestudeerden wij het voorkomen van deze vaak latente infecties onder een patiëntencohort die de ART kliniek bezocht (**hoofdstuk 4**). Dit onderzoek toonde aan dat HBV-infectie en syfilis veel voorkomt onder de met HIV geïnfecteerde populatie. Deze resultaten leidden tot twee belangrijke aanbevelingen: zet het testen op syfilis in deze populatie voort, en wees waakzaam bij het gebruik van bepaalde NRTIs bij antihepatitis activiteit (lamivudine, emtricitabine, tenofovir) in patiënten met een chronische hepatitis B infectie, omdat het stoppen van deze medicijnen een

opvlamming van de hepatitis kan veroorzaken.

De voorziening van ART via het openbare gezondheidssysteem maakte het noodzakelijk om de huidige gezondheidsinfrastructuur op meerder fronten aan te passen. **Hoofdstuk 5** beschrijft de uitdagingen en de lessen die we hebben geleerd bij het tot stand komen van de eerste openbare ART kliniek in Botswana. Het artikel benadrukt het belang van gestandaardiseerde training, decentralisatie en taakverschuiving als essentiële stappen voor de duurzaamheid van het programma.

Hoofdstuk 6 behandelt de resultaten van een willekeurige klinische proef om de werkzaamheid, tolerantie en optimale adherence strategieën te vergelijken van zes verschillende ARV regimes voor populaties in sub-Sahara Arika, speciaal van mensen geïnfecteerd met HIV-1C. Uit het onderzoek blijkt dat er opvallende immunologische en virologische reacties zijn op eerstelijns PI-sparende cART regimes, dat de cohort retentiewaarden uitstekend zijn, en dat vergiftigingsverschijnselen die de behandeling modificeren weinig voorkomen.

Een bijkomend belangrijk resultaat van de studie was de grote hoeveelheid ongeplande zwangerschappen onder vrouwen die ART gebruiken, inclusief vrouwen op regimes die EFV bevatten (**hoofdstuk 7**). De studie bracht verscheidene hiaten aan het licht in de nationale anticonceptievoorzieningen voor de met HIV geinfecteerde populatie. Belangrijk is dat wij geen EFV-geassocieerde negatieve geboorteresultaten hebben gevonden. Omdat onze steekproef te klein was tot een definitieve conclusie te komen, hebben we onze resultaten overgedragen aan de internationale Antiretroviral Pregnancy Registry, die gegevens over blootstelling aan ART tijdens zwangerschap verzamelt en bestudeert.

Op verzoek van de uitgever hebben wij de kansen en uitdagingen bekeken voor cART programma's in sub-Sahara Afrika (**hoofdstuk 8**).

Een belangrijk onderdeel van het managen van een ART programma is het monitoren en evalueren van de doelstellingen en indicatoren van het programma. **Hoofdstuk 9** draagt een model aan voor een betaalbaar monitorsysteem op patiëntniveau dat gebruikt kan worden voor het evalueren van programma's. Het is gebaseerd op een minimale kerndataset die verzameld wordt op een artsonafhankelijke manier en die OCR (Optical Character Recognition) gebruikt voor het oversturen van de data naar een centrale server.

In veel sub-Sahara Afrikaanse landen blijken cART initiatieven al snel voordelen op te leveren. Tot op heden zijn echter weinig gegevens beschikbaar over de resultaten van deze behandelingsprogramma's op de lange termijn. In **hoofdstuk 10** worden de lange termijn behandelingsresultaten geanalyseerd van de eerste patiënten die cART in Botswana gebruikten. Deze eerste groep van volwassenen die ART gebruikten in stedelijke gebieden van Botswana liet uitstekende blijvende immunologische, virologische en klinische resultaten zien voor minstens vijf opeenvolgende jaren met een laag sterftecijfer van patiënten die het eerste jaar van antiretrovirale therapie overleefden. Vergeleken met andere ART initiatieven is de 5-jaar verlies : opvolging ratio van 21,8 % is gunstig.

In **hoofdstuk 11** wordt een schatting gedaan naar het niveau van overgedragen (primaire) ARV medicijnresistentie in Botswana vijf jaar na het beging van het nationale cART programma. Het lage niveau van overdraagbare medicijnresistentie in het hele land is een indicator van de succesvolle implementatie van het openbare cART programma. Het artikel geeft ook een kritische beoordeling van de methodes voorgesteld door de Wereld Gezondheids Organisatie (WHO).

De hier gepresenteerde onderzoeken hebben veel bijgedragen aan het tot stand komen van een succesvolle introductie van een openbaar cART programma in minder bedeelde landen in zuidelijk Afrika; een programma dat op grote schaal gezien wordt als een voorbeeld voor de regio. De succesvolle implementatie van cART voor de behandeling van vastgestelde HIV-ziektebeelden rechtvaardigt een onderzoek naar de toepasbaarheid van cART voor de preventie van HIV-besmetting, bijvoorbeeld door behandeling van volwassenen met hoge virale waardes die hoogstwaarschijnlijk het virus kunnen overbrengen naar anderen, maar nog niet kwalificeren voor medicatie op basis van de status van hun ziekte.

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I have known André van der Ven since 1985, when we both worked as 'medical officers' in different district hospitals in Botswana. We met again in Botswana in 2003, jointly contributing to the estabishment of the national ART program. André has always been a friend and colleague who understood best the challenges and facination of my work in Botswana. I always value his encouragement and support as well as his open-minded and optimistic approach.

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Curriculum Vitae

Curriculum Vitae

Hermann Bussmann was born in Oberhausen, Germany, on 28 January 1953. After completion of the 'Abitur' at the Heinrich Heine Gymnasium in Oberhausen, he studied medicine at the University of Heidelberg and the Université Paris VI, Broussais-Hotel Dieu, with a scholarship of the 'Studienstiftung des Deutschen Volkes' from 1971 to 1978. Subsequently, he spent 3 months at the Children's Hospital, Yorkhill, University of Glasgow and prepared for his social service as volunteer doctor by doing residencies at the Department of Anaesthesiology, University of Heidelberg, Germany, (1978); Department of Surgery, University of Heidelberg, Salem Hospital, Germany (1979-81); Department of Obstetrics, Queen Mother's Hospital, Glasgow, Great Britain, (1981/1982); and the Department of Internal Medicine, University of Heidelberg, Germany. From 1983-87 he did his volunteer service with 'Dienste in Uebersee' at the DRM Hospital, Mochudi, Botswana as an attending physician in charge of the pediatric, surgical and maternity wards. In 1988 he achieved the Diploma in Obstetrics and Gynaecology at the Royal College of Obstetrics and Gynecology (DRCOG), London, UK. Between 1988 and 1993 he completed his fellowship in pediatrics at the Pediatrics hospital, University Hospital Heidelberg, Germany, with focus on Gastroenterology, Neurology, Neonatology and Intensive Care and acted as head of the tropical pediatric clinic. From 1993 to 1998 he worked as a specialist in pediatrics at the DRM Hospital, Mochudi, Botswana. He established a clinic for chronically-ill children focusing on children with AIDS and disabilities. During this time he established a self-support group for HIV-infected mothers, introduced the 'Kangaroo care' method to raise neonates with low birth weights, planned and raised funds for refurbishment and extension of the children's ward. He designed and coordinated a research project on 'the appropriateness of diagnostic ultrasound at district health care level' which was funded by a research grant of the `Life Sciences and Technology for Developing Countries (STD3)' program of the Commission of the European Communities. Between 1995 and 1998 he took over the position of medical superintendent of DRM Hospital, Botswana. In 1998 he moved to Boston, MA, where he took up a research fellowship at the Department of Immunology and Infectious Diseases, Harvard School of Public Health and completed his Master of Public Health in International Health at the Harvard School of Public Health in 2000.

Since 2000 he has worked as a Senior Research Associate of the Harvard School of Public Health AIDS Initiative in Botswana in collaboration with the Botswana-Harvard AIDS Institute Partnership, Gaborone, Botswana. He was a co-principal investigator and co-ordinating study physician of the (i) Adult Antiretroviral Treatment and Resistance Study, (ii) The Natural History of HIV-1 Subtype C Disease Progression Study and (iii) The Study Evaluating the Efficacy and Tolerability of Tenofovir and Emtricitabine (Truvada™) in HIV-1C Infected Adults. Since September 2009 he is the site leader of a community-wide modified test-and-treat project to reduce HIV transmission in a Botswana community funded by the National Institutes of Health, Bethesda, Maryland, USA.

Herman Bussmann has been a member of the National Standing Committee on Drugs, Ministry of Health, Botswana (1995-1998), the Clinical Subcommittee on AIDS, Ministry of Health, Botswana (1996-1998), the Technical Working Group on HIV Incidence and Drug Resistance, and the Technical Working Group on Sentinel Surveillance, Ministry of Health, Botswana (2005 to present) and the Botswana National HIV/AIDS Treatment Guidelines Committee. He is also a senior faculty member and lecturer in KITSO AIDS training program, Botswana.

He is married to Christine Bussmann and they have two children Benjamin Mothibe and Paul Louis.

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